Chapter 22

Assessing Startle Responses and Their Habituation in Adult Zebrafish

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Abstract

Zebrafish is rapidly becoming a popular model species for neurobehavioral and psychopharmacological research. The startle response represents the instinctive, evolutionarily conserved reaction of an organism to novel unexpected and/or aversive stimuli. While startle testing is a well-established assay to study anxiety-like behaviors in different species, screening of the startle response and its habituation in zebrafish is also an important direction of translational biomedical research. Complementing rich literature on zebrafish startle, this chapter outlines a brief and simple protocol to assess the tapping-induced startle response and its inter- and intra-trial habituation in adult zebrafish.

Key words: Adult zebrafish, Startle response, Within-trial (intra-session) habituation, Between-trial (inter-session) habituation, Anxiety, Behavioral testing, Neurophenotyping

1. Introduction

The startle response is an autonomous reflex evoked by a sudden exposure to unexpected, most commonly aversive stimuli (1, 2). It represents an evolutionarily conserved instinctive behavior which is observed in multiple species including humans (3–10) and enables an organism to quickly react to perceived threats, avoid harm, and initiate adaptive fight-or-flight responses (11).

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The startle assays are commonly used in neurobehavioral and psychopharmacological research. While anxiolytic drugs generally reduce startle responses, anxiogenic agents typically increase startle responses (3, 12, 13). Furthermore, startle response is based on cognitive processing of sensory information, which is highly relevant to modeling cognitive deficits, especially psychotic-like pathogenesis (12, 13). For example, antipsychotic drugs commonly diminish and psychotogenic agents augment startle responses (9, 14–16).

Startle responses have previously been examined in fish species, including adult (17–19) and larval zebrafish (Table 1) (20, 21). In fish, startling stimuli evoke a typical stereotyped "fast start" behavior consisting of a rapid turn and swimming with high velocity away from the stimulus (22). This chapter describes a simple protocol used in our laboratory to assess startle response in adult zebrafish. Habituation of the startle response is another important brain phenomenon manifested in the reduction of behavioral responses to startling stimuli over time (2, 10, 23, 24). Like any other type of habituation, startle habituation reflects cognitive functions and can be assessed in zebrafish in conjunction with the present protocol using both inter- and intra-trial paradigms (see a related chapter on habituation to novelty in this book).

Importantly, there are some differences in behavioral endpoints in startle studies between larval and adult zebrafish (e.g., (25)). Table 1 summarizes some endpoints used for both types of models. Note that the larval fish startle is generally shorter (starting <15 ms after the stimulus and lasting less than 100 ms, depending on the stimulus) compared to adult zebrafish (showing a longer latency and duration of their startle response, Fig. 2; (25)). The larval fish behavior is also more prominently expressed in turning endpoints

Table 1
Example of startle assays in adult and larval zebrafish reported in the literature

Model	Endpoints	References
Adult zebrafish		
Tapping-induced stimulation	Distance traveled, swim velocity (m/s)	(17)
Tapping-induced stimulation	% Animals showing avoidance	(19)
Larval zebrafish		
Acoustic stimulation	Distance traveled (m)	(37)
Mild electrical stimulation	Heart rate (bpm)	(20)
Vibrational stimulation	Bend angle (°) and maximum angular velocity (°/ms)	(18)
	Distance traveled (pixels)	(28)
Visual stimulation	Swim speed (mm/min)	(27)
	Distance traveled (pixels)	(28)

The method of startle and endpoints measured vary in the experiments (see references listed for details), and the behavior of both adult and larval zebrafish can be assessed

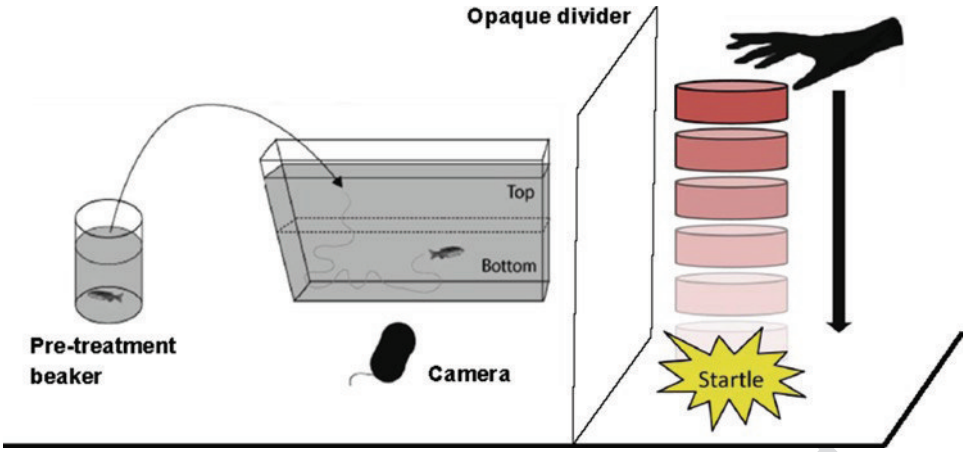

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Fig. 1. Experimental set-up to assess tapping-induced startle responses in adult zebrafish. The novel tank set up with a side-view camera, a pretreatment beaker to hold fish prior to testing, and the startle stimulus, separated from the tank by a vertical plastic opaque divider.

(e.g., characteristics of body curvature (18, 25, 26)) for which the top-view position of the video-camera, traditionally used in larval studies, seems to be appropriate (see (18, 22, 25) for detailed review of larval zebrafish startle).

However, the focus of this chapter is on behavioral characterization of *adult* zebrafish startle responses. Although they can also be recorded using the top-view camera (e.g., (17)), we developed a sensitive method based on video-recording of startle in the novel tank test using the *side-view* camera (Fig. 1) and examining multiple sensitive locomotory endpoints (Table 1, Fig. 2). Furthermore, using shallow arenas (e.g., Petri dishes) may be ideal for testing startle in small organisms, such as larval fish (whose behavior is less complex when compared and occurs in lateral dimensions (25, 27, 28)). Behavioral responses to startle stimuli in *adult* zebrafish are more complex in terms of their dimensionality, occurring in both horizontal and vertical planes. Therefore, deeper tanks (such as the novel tank test used here, Fig. 1) may be more useful, to enable a better focus on adult zebrafish locomotion and their sensitive vertical behavior. Utilizing a side-view camera and deeper testing tanks, as described in this protocol, provides a valid and reliable method to assess startle response and its habituation in adult zebrafish.

2. Materials and Methods

2.1. Animal Housing

Adult zebrafish (e.g., 3–5 months old; ~50:50 male:female ratio) can be raised in-house or obtained from a commercial distributor. The animals should be given sufficient time (e.g., 14 days) to

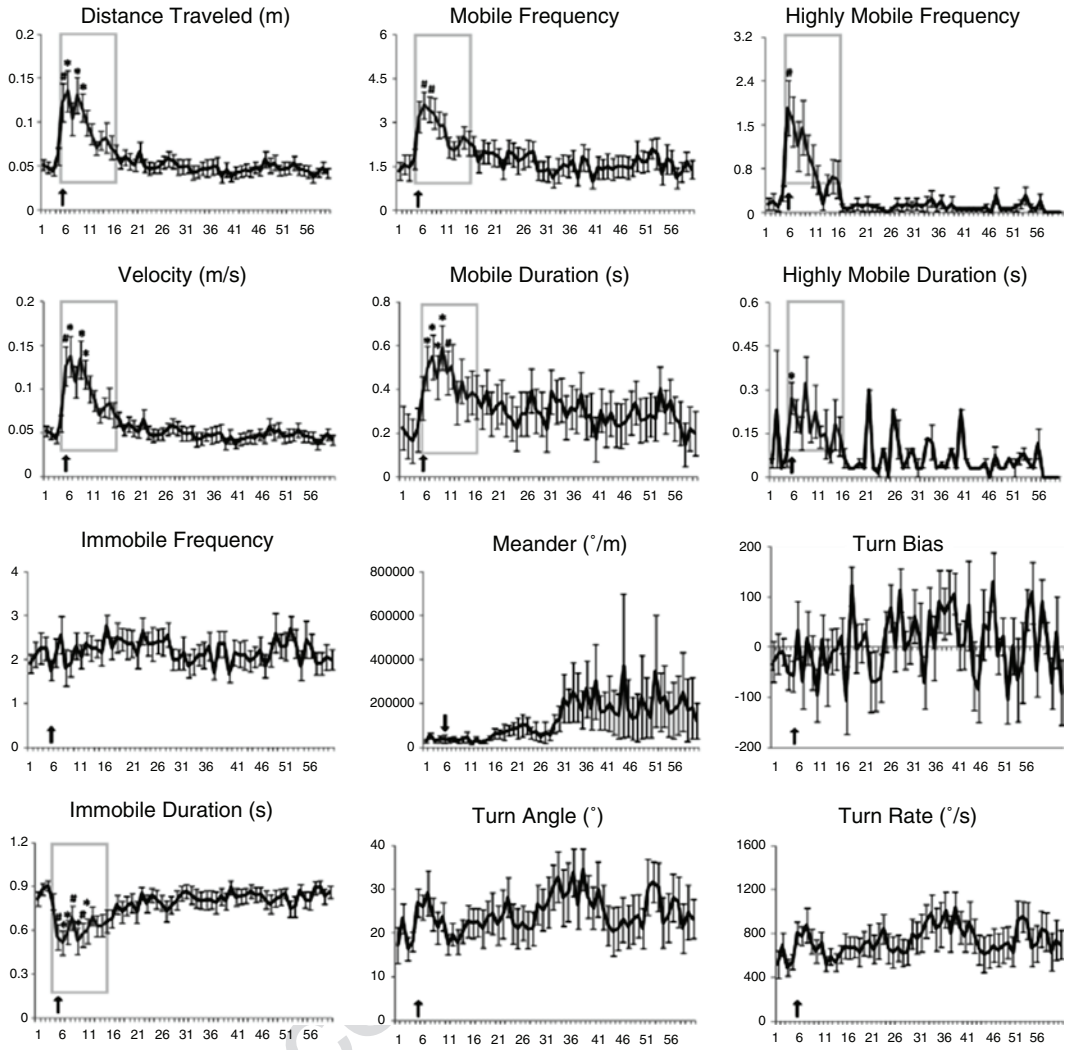

Fig. 2. Examples of intra-session habituation of startle responses in adult zebrafish exposed to a single startle stimulus (trial 1). Startle was applied at second 5, and is denoted by the *arrows*. *Horizontal axis* represents time (s). *$P<0.05$, $^{\#}P=0.05$–0.1, paired *U*-test with Bonferroni correction for each poststartle second vs. prestartle baseline activity (at second 4).

acclimate to the laboratory environment, and can be housed in groups of 20–30 fish per 40-L tank. The tanks can be filled with filtered facility water maintained at 25–27°C and a pH of 7.0–8.0. Illumination (e.g., 1,000–1,100 lux) can be provided by ceiling mounted fluorescent light tubes on a 12:12-h (or 14:10-h) cycle according to the standards of zebrafish care protocols (e.g., (2)).

2.2. Apparatus

While the exact apparatuses to assess zebrafish startle can vary, the standard small novel tank (24) described in several chapters of this book will suffice for capturing phenotypically robust startle

responses. In this experimental setup, the 1.5-L trapezoidal novel tank (15 cm height × 7 cm width × 28 cm top × 23 cm bottom length; Aquatic Habitats, Apopka, FL) faces the video-analysis web-camera and is filled with room temperature filtered facility water (Fig. 1).

2.3. Experimental Setup and Materials

- 1 Roll Scotch masking tape (e.g., 8–12 cm in diameter) weighing approximately 180 g (locally purchased).
- Opaque plastic screen (e.g., 50 × 50 cm, 0.5 cm thick, positioned (as in Fig. 1)) between the tank and the startle stimulus.
- 1.5-L novel tank (Aquatic Habitats, Apopka, FL) as described above.
- Video-tracking software (e.g., Ethovision XT7, Noldus IT, Wageningen, The Netherlands).
- Digital timing device (e.g., Fisher Scientific, Pittsburgh, PA).

The stimulus used in our laboratory to evoke a startle response from adult zebrafish is produced with a roll of Scotch masking tape described above. The object is raised to the height of the tank (15 cm) and released by hand onto a hard, flat surface on which the novel tanks rested, creating both an acoustic and vibrational stimulus that evoked an overt startle response in fish (Fig. 1). Note that other standardized stimuli to evoke a startle may include releasing a rubber hammer from a standard height onto a flat surface, or utilizing any other vibrational "tapping" stimulus that could adequately alert the zebrafish.

2.4. Computer-Aided Analysis of Data

Analysis of recorded trials can be performed on- or off-line using commercially available video-tracking software (e.g., Ethovision XT7, Noldus IT (29–32)). Simultaneously recording two or more tanks can reduce experiment duration without changing the stimulus, as long as it is administered uniformly to all zebrafish tested. Video-recording and its settings can be similar to those used in other zebrafish swimming behavioral tracking protocols (33, 34). Note that the location of the camera (side or top) could influence the results of the experiment. For example, many zebrafish startle studies use open–field-like tanks and top-view cameras (17, 27), especially common in high-throughput larval assays (17, 21). However, our own experiments in adult zebrafish showed that startled zebrafish swim very actively in a vertical direction immediately after the tapping stimulation. While a camera oriented on the top of the tank would also record the startle response (similar to startle assays in larval zebrafish), a side-view camera used here (Fig. 1) may be best able to capture adult animal movements occurring mostly in a vertical plane.

3. Procedure

3.1. Experimental Protocol

As already mentioned, due to the broad nature of a startle response, various procedures can elicit a startle response in adult zebrafish, including electrical, visual, or tapping-induced stimuli (Table 1). An easy, inexpensive, and practical stimulus to use in startle studies in zebrafish is tapping, such as tapping on the novel tank or the table on which the tank rests. The following protocol can be used to produce a standardized physical stimulus to evoke a startle response in the zebrafish.

1. Collect naïve zebrafish with a net (12–15 fish per group may usually suffice, but animal numbers can be increased depending on the experimental task) and place them in a preexperiment container filled with room temperature facility water for acclimation for 1–2 h. Keep this container far away (e.g., in a separate room) from the startle response testing area, so that acoustic and vibrational stimuli do not interfere with the naive animals (see troubleshooting notes further). If using a pretreatment exposure to various drugs in the experiment, use a pretreatment container (e.g., 3-L plastic square opaque beaker) to expose fish individually to drugs for a desired amount of time prior to testing.
2. Following acclimation (and, if necessary, pretreatment), transfer the zebrafish individually into the testing tank, and allow a 3-min acclimation period prior to the first startle session. At the end of this acclimation period, begin recording the video. Note that the acclimation period can be longer, if necessary, depending on experimental design and baseline anxiety levels of the specific zebrafish strain tested.
3. After the first 5 s of baseline video-recording, produce the stimulus (tapping as described above) and continue video-recording of fish activity for 1 min.
4. If assessing habituation of startle response, repeat this stimulus once every minute for 10 min with continuous video-recording, and stop recording after all trials are completed.

3.2. Habituation

Habituation is the attenuation of responses after repeated exposures to the same stimulus and can be tested in zebrafish to assess their ability to adapt to a novel environment (24). Analyzing zebrafish habituation to a startle stimulus is highly relevant to studying anxiety-related and cognitive phenotypes. The initial reaction to the startle stimulus represents various anxiety-like avoidance behaviors, such as increased distance traveled, higher velocity, and a characteristic "fast start" behavior involving a rapid turn away from the startle source (22). In the period following the

initial startle reaction, these anxiety-like behaviors gradually decrease (as the fish demonstrates intra-session habituation to the startle stimulus, and the behavioral endpoints return to normal prestartle levels; Fig. 2).

Repeated presentation of the startle stimulus (e.g., a series of 10 startles) targets another aspect of habituation in zebrafish—inter-session habituation. This type of habituation represents a more gradual decrease in anxiety-like behaviors each time the startle is evoked. Note that both intra-session and inter-session habituation only occur for certain endpoints, and that some endpoints show no habituation at all. For example, distance traveled or velocity may habituate consistently after one startle production or across many stimuli, while turn bias or meander is likely to show no habituation (Figs. 2 and 3).

3.3. Data Analysis

Use the Mann–Whitney *U*-test (with or without Bonferroni correction, where appropriate) for comparing two groups (Student's *t*-test can also be used for normally distributed data). For more than two groups, use analysis of variance (ANOVA), followed by an appropriate post hoc test (e.g., Tukey, Dunn, Newman–Keuls, or Dunnet test). A general n-way ANOVA can be used, with common factors being treatment, dose, sex, strain, time, trial, or age (34). To assess startle habituation, use post-hoc tests to compare baseline and startle-evoked activity (e.g., prestartle second 4 vs. each individual poststartle second of the test for intra-session habituation, Fig. 2; or Trial 1 with each individual subsequent trial for inter-session habituation, Fig. 3).

4. Typical Results

The results for the startle experiment using our protocol are generally robust and highly reproducible (see Fig. 2 for typical results for the startle response assay and its intra-session habituation, and Fig. 3 for its inter-session habituation). Based on our experience, the startle response can be observed in the critical 10–15-s window following the startle stimulus (Fig. 2). Per-second distribution of startle-related endpoints over the period of 60 s post-stimulus reflects inter-session habituation (Fig. 2), while inter-trial habituation can be assessed by measuring startle behaviors during the 15-s window and comparing their change across all ten 15-s post-startle windows (Fig. 3).

4.1. Startle Response and Its Dynamics

Our protocol yields phenotypically robust startle behaviors that can be recorded from the side view capturing numerous sensitive endpoints (Table 2) using video-tracking software (Fig. 1).

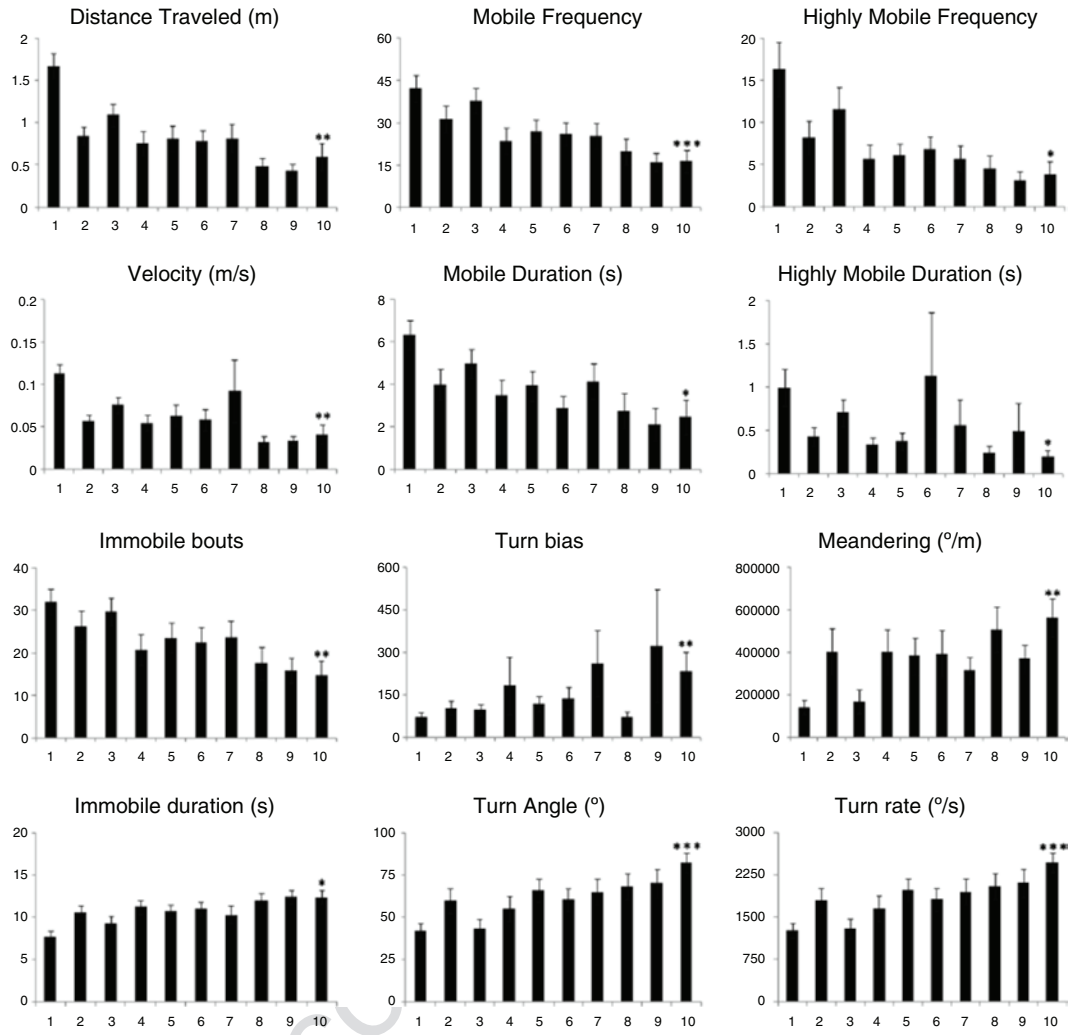

Fig. 3. Representative examples of inter-session habituation of some startle responses observed in adult zebrafish exposed to tapping-induced startle stimulus every 60 s for a period of 10 min (total 10 trials). *Horizontal axis* represents consecutive trials (each *bar* represents cumulative scores generated for the 15-s post-startle window for each trial). $^{*}P<0.01$, $^{**}P<0.005$, $^{***}P<0.0005$, paired *U*-test (trial 10 vs. trial 1) with Bonferroni correction.

Data analysis revealed that distance traveled, velocity, and highly mobile frequency all display high sensitivity to the startle behavior. As shown in Fig. 2, within the 10–15-s period after the startle stimulus, these endpoints show a marked change followed by its gradual return to normal. When the aversive stimulus is encountered by zebrafish, the animal attempts to escape or avoid it, leading to rapid swimming away from the source of the startle. This behavior is logically reflected in altered distance traveled, highly mobile frequency, and velocity, which all show marked increase upon presentation of a startle stimulus followed by a relatively fast return to normal levels (Fig. 2).

Table 2
A brief summary of behavioral endpoints used in startle analysis and their habituation

Endpoint	Explanation	Startle	Habituation	
			Intra-session	Inter-session
Distance traveled, m	Total distance the zebrafish traveled within the novel tank	+	+	+
Velocity, m/s	Distance traveled by the subject per unit time (s)	+	+	+
Turn angle, °	Total turning angle between consecutive frames (recorded at 30 fps)	–	–	+
Turn rate (absolute angular velocity)	Absolute change in direction of movement between consecutive frames (recorded at 30 fps) calculated per unit time (s)	–	–	+
Turn bias (relative angular velocity)	Relative change in direction of body between two consecutive frames calculated between consecutive frames (recorded at 30 fps) per unit time (s)	–	–	+
Meander, °/m	The absolute change in direction of movement of a subject relative to the distance traveled	–	–	+
Highly mobile frequency	Number of times the subject's body area is displaced by >80% between frames	+	+	+
Highly mobile duration, s	Total time spent highly mobile locomotion	+	+	+
Mobile frequency	Number of times the subject's body area is displaced by 20–80% between frames	+	+	+
Mobile duration, s	Total time spent mobile	+	+	+
Immobile frequency	Number of times the subject's body area is displaced by <20% between frames	–	–	+
Immobile duration, s	Total time spent immobile	+	+	+
Transitions to upper half	The number of crosses from the defined bottom portion to the top of the novel tank	–	–	
Time in upper half, s	Total time spent in top portion of the novel tank	–	–	

Plus sign indicates presence of startle behavior or its habituation, and minus sign denotes their absence. A brief definition for each endpoint outlines the behavior assessed, based on (33, 38). Note that some endpoints show good inter-session, but not intra-session, habituation. This difference merits further studies, but may reflect differential roles that long-term and short-term memory (as well as their modulation by anxiety-related mechanisms) plays in adult zebrafish startle responses

4.2. Habituation (Intra-/Inter-Session) of the Startle Responses

In addition to startle, our protocol generates prominent results for the intra-session habituation of the startle response in zebrafish (Fig. 2), using an approach conceptually similar to screening habituation in novel arenas (described in detail in a separate chapter by Raymond et al. in this book). Furthermore, by measuring the data for various endpoints within the 10–15-s window, and comparing them across all 10 startle stimuli (1 startle every 60 s), zebrafish inter-session habituation to aversive stimuli can also be evaluated (Fig. 3). Our findings indicate that zebrafish display overt habituation of several startle-related behavioral endpoints, including distance traveled and velocity, as well as highly mobile frequency and duration (also see Table 2 for details). While all of these endpoints show a marked increase within the 10–15-s window (intra-session habituation; Fig. 2), their gradual decrease over the course of 10 repeated startle sessions demonstrates robust *inter-session* (between-trial) habituation (Fig. 3).

5. Notes (Troubleshooting)

5.1. Standardization of the Stimulus

An important and common methodological problem with startle research is standardization of the stimulus used to evoke startle responses. Based on our experience, releasing a roll of masking tape onto a flat surface or striking a rubber hammer against a flat surface both represent adequate stimuli to evoke startle responses. However, their standardization may prove somewhat difficult, since experimenters may handle the hammer or tape differently, resulting in varying stimuli. If using the roll of tape, it is important to release it from a fixed height with the flat side facing down, to produce the most consistent stimulus. The best way to standardize the vibrational startle stimuli is to utilize a machine or computer to perform a consistent and reproducible "tapping" action (e.g., (17)). The automated production of the "tapping" stimulus reduces human error due to a more standardized stimulus and less chance of visual or acoustic interference, because the experimenters need not be in the immediate testing area during the experiment (see further). Nevertheless, the automated method may be more expensive, and therefore the simple mechanical method described here (Fig. 1) may suffice to evoke sufficient startle responses in zebrafish (Figs. 2 and 3). Alternatively, consider using other types of stimuli (see Table 1 for details) to evoke zebrafish startle.

5.2. Avoiding Preexposure to Stimulus

Prior to the experiment, the fish must be netted and placed in a holding container (similar to the startle observation tank described earlier) filled with room temperature filtered facility water for an acclimation period of 1–2 h. Due to the nature of the startle stimulus, this holding container should be kept in a separate room for the

duration of the experiment. Note that placing the holding container in the vicinity of the testing tanks (where the experiment is being performed) risks prematurely exposing the naive fish to the stimulus. This may lead to the situation when untested fish may begin to habituate to the stimulus, thus invalidating their naivety upon testing.

5.3. Minimizing Testing Arena Interference

Another possible problem in the startle testing procedure is visual and acoustic interference in the arena of the experiment. The best way to avoid this problem is to program a machine or computer for a consistent "tapping" (17, 26), so that the experimenters may be absent from the vicinity of the testing tank. The presence of the investigators in the testing area may startle the fish due to sudden movements, approaching the novel tank during stimulus production, or due to acoustic disturbances in the vicinity. Another practical way to reduce interference (especially if testing multiple zebrafish simultaneously in the same area) is to place white or opaque screens (see Fig. 1) around the novel tank to prevent the fish from being visually startled by other fish being tested or by movements of the experimenters in the testing vicinity (17). Additionally, since water temperature interferes with zebrafish startle response (25), ensure that the temperature of the water in the preexposure beaker and the novel tank is maintained at 25–27°C.

5.4. Selecting Correct Endpoints

While distance traveled or velocity after startle (Table 1) are considered to be reliable startle endpoints (17, 35, 36), our data show that other computer-generated parameters may be useful to assess startle in adult zebrafish, including mobile and highly mobile frequencies and durations (Table 2). Upon receiving a startle stimulus, zebrafish show marked increase in all of these behaviors, whereas several less sensitive endpoints (e.g., turn bias or meander) would usually remain unaltered (Fig. 2).

5.5. Optimizing Animal Detection

Assessing zebrafish startle endpoints is performed by video-tracking software, which can be prone to misdetection of animals. This problem is common for novel tank and similar paradigms, and various ways to optimize the detection settings have been comprehensively described in the literature (34). Furthermore, the amount of data collected per trial can vary depending on the nature of the study. For example, we typically use 10–30 frames per second (fps) video-recording and analyze data using 1-s time bins (Fig. 2). However, using high-frame videography and/or shorter time bins may generate more data points and represent fish startle phenotypes more accurately. In the published literature, various fps ranges and shorter time bins (e.g., (22, 25)) have been successfully applied to zebrafish startle research. While such a degree of detail may not be necessary for strong startle responses (e.g., as in (22) and our studies presented in Figs. 2 and 3), less clear-cut and more subtle startle phenotypes (e.g., if impaired under certain experimental conditions) may require in-depth characterization using more

sensitive high-fps and/or shorter time bins, which can be optimized and adjusted to specific research needs.

5.6. Additional Endpoints

It is also possible to expect that some additional endpoints, such as the "amplitude" of startle responses (e.g., difference between pre- and startle-induced behaviors, as a measure of startle magnitude) can be used to characterize startle responses. This possibility merits further studies and validation in adult zebrafish models.

6. Summary

This chapter provides a brief and simple protocol for assessing the startle response and its habituation in adult zebrafish. The methods described here are fast, simple, easily reproducible, and require inexpensive materials. The endpoints measured are based on computer analysis (rather than manual human recording), thereby further standardizing the procedure, increasing its throughput and making it less prone to bias. The specific endpoints selected here are highly sensitive to startle stimuli and can be used in parallel to characterize the startle response behavior in the adult zebrafish. Another advantage of this protocol is that it tests both startle and habituation in one experiment, adding an additional (cognitive) domain to affective phenotypes traditionally assessed using startle paradigms. Collectively, this increases the translational value of adult zebrafish startle responses (17), with multiple applications from screening genetic mutations and novel pharmacological agents to modeling complex affective or psychotic disorders.

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References

1. Schachinger H (2003) The startle reaction in emotion research. Praxis (Bern 1994) 92(38):1584–1586
2. Westerfield M (2007) The zebrafish book. A guide for the laboratory use of zebrafish (*Danio rerio*), 5th edn. University of Oregon Press, Eugene, OR
3. Hale ME (2000) Startle responses of fish without Mauthner neurons: escape behavior of the lumpfish (*Cyclopterus lumpus*). Biol Bull 199(2):180–182
4. Plappert CF, Pilz PK (2001) The acoustic startle response as an effective model for elucidating the effect of genes on the neural

mechanism of behavior in mice. Behav Brain Res 125(1–2):183–188

5. Laming PR (1987) Behavioural arousal and its habituation in the squirrel fish, *Holocentrus rufus*: the role of the telecephalon. Behav Neural Biol 47(1):80–104
6. Marino-Neto J, Sabbatini RM (1983) Discrete telencephalic lesions accelerate the habituation rate of behavioral arousal responses in Siamese fighting fish (*Betta splendens*). Braz J Med Biol Res 16(3):271–278
7. Maschke M et al (2000) Involvement of the human medial cerebellum in long-term habituation of the acoustic startle response. Exp Brain Res 133(3):359–367
8. Nieuwenhuijzen PH et al (2000) Modulation of the startle response during human gait. J Neurophysiol 84(1):65–74
9. Hammer TB et al (2011) Stability of prepulse inhibition and habituation of the startle reflex in schizophrenia: a 6-year follow-up study of initially antipsychotic-naive, first-episode schizophrenia patients. Int J Neuropsychopharmacol 14(7):913–925
10. Quednow BB et al (2006) Normal prepulse inhibition and habituation of acoustic startle response in suicidal depressive patients without psychotic symptoms. J Affect Disord 92(2–3): 299–303
11. Gotz T, Janik VM (2011) Repeated elicitation of the acoustic startle reflex leads to sensitisation in subsequent avoidance behaviour and induces fear conditioning. BMC Neurosci 12:30
12. Joordens RJ, Hijzen TH, Olivier B (1998) The anxiolytic effect on the fear-potentiated startle is not due to a non-specific disruption. Life Sci 63(25):2227–2232
13. Hasenkamp W et al (2011) Lack of relationship between acoustic startle and cognitive variables in schizophrenia and control subjects. Psychiatry Res 187(3):324–328
14. Kumari V et al (2007) Startle gating in antipsychotic-naive first episode schizophrenia patients: one ear is better than two. Psychiatry Res 151(1–2):21–28
15. Frau R et al (2008) Sleep deprivation disrupts prepulse inhibition of the startle reflex: reversal by antipsychotic drugs. Int J Neuropsychopharmacol 11(7):947–955
16. Ouagazzal AM, Jenck F, Moreau JL (2001) Drug-induced potentiation of prepulse inhibition of acoustic startle reflex in mice: a model for detecting antipsychotic activity? Psychopharmacology (Berl) 156(2–3):273–283
17. Eddins D et al (2010) Zebrafish provide a sensitive model of persisting neurobehavioral effects of developmental chlorpyrifos exposure: comparison with nicotine and pilocarpine effects and relationship to dopamine deficits. Neurotoxicol Teratol 32(1):99–108
18. Burgess HA, Johnson SL, Granato M (2009) Unidirectional startle responses and disrupted left-right co-ordination of motor behaviors in robo3 mutant zebrafish. Genes Brain Behav 8(5):500–511
19. Carvan MJ 3rd et al (2004) Ethanol effects on the developing zebrafish: neurobehavior and skeletal morphogenesis. Neurotoxicol Teratol 26(6):757–768
20. Mann KD et al (2010) Cardiac response to startle stimuli in larval zebrafish: sympathetic and parasympathetic components. Am J Physiol Regul Integr Comp Physiol 298(5):R1288–R1297
21. Colwill RM, Creton R (2011) Imaging escape and avoidance behavior in zebrafish larvae. Rev Neurosci 22(1):63–73
22. Orger MB et al (2004) Behavioral screening assays in zebrafish. Methods Cell Biol 77:53–68
23. Meliska JA, Meliska CJ (1976) Effects of habituation on threat display and dominance establishment in the Siamese fighting fish, *Betta splendens*. Anim Learn Behav 4(2):167–171
24. Wong K et al (2010) Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav Brain Res 208(2):450–457
25. Burgess HA, Granato M (2007) Sensorimotor gating in larval zebrafish. J Neurosci 27(18):4984–4994
26. Eaton RC, Bombardieri RA, Meyer DL (1977) The Mauthner-initiated startle response in teleost fish. J Exp Biol 66(1):65–81
27. Pelkowski SD et al (2011) A novel high-throughput imaging system for automated analyses of avoidance behavior in zebrafish larvae. Behav Brain Res 223(1):135–144
28. Griffith BB (2011) A larval zebrafish model of glucocorticoid-serotonin interactions in behavioral stress reactivity (Master's Thesis), Humboldt State University: Arcata, CA, p 70
29. Delcourt J et al (2006) Comparing the EthoVision 2.3 system and a new computerized multitracking prototype system to measure the swimming behavior in fry fish. Behav Res Methods 38(4):704–710
30. Pham J et al (2009) Automated scoring of fear-related behavior using EthoVision software. J Neurosci Methods 178(2):323–326
31. Noldus LP, Spink AJ, Tegelenbosch RA (2001) EthoVision: a versatile video tracking system for automation of behavioral experiments.

Behav Res Methods Instrum Comput 33(3): 398–414

32. Marechal JP et al (2004) Settlement behaviour of marine invertebrate larvae measured by EthoVision 3.0. Biofouling 20(4–5): 211–217
33. Cachat J et al (2011) Three-dimensional neurophenotyping of adult zebrafish behavior. PLoS One 6(3):e17597
34. Cachat J et al (2010) Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Protoc 5(11):1786–1799
35. Dlugos CA, Rabin RA (2003) Ethanol effects on three strains of zebrafish: model system for genetic investigations. Pharmacol Biochem Behav 74(2):471–480
36. Zeddies DG, Fay RR (2005) Development of the acoustically evoked behavioral response in zebrafish to pure tones. J Exp Biol 208(Pt 7):1363–1372
37. Zurn J, Falls WA, Motai Y (2006) Detecting startle responses in the zebrafish using novel digital imaging techniques. Neuroscience Meeting Planner. Society for Neuroscience, SanDiego, CA
38. Grieco F et al (2009) Noldus Ethovision XT7 reference manual. Noldus Information Technology, Wageningen, The Netherlands