Chapter 21

Assessing Habituation Phenotypes in Adult Zebrafish: Intra- and Inter-Trial Habituation in the Novel Tank Test

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Abstract

Although adult zebrafish are increasingly utilized as a model organism in neurobehavioral research, their habituation responses have only recently been evaluated in detail. When exposed to a novel environment, zebrafish demonstrate marked habituation responses, similar to the behavioral response of rodents. Representing an adaptive response to novelty and a simple form of spatial memory, both intra- and inter-session habituation can be easily assessed in adult zebrafish using novelty-based paradigms, such as the novel tank test. Alterations in zebrafish habituation can also be evoked by pharmacological manipulations, collectively representing a useful tool for drug screening and behavioral phenotyping. Here, we outline a simple protocol for evaluating zebrafish intra- and inter-session habituation to novelty in the novel tank test.

Key words: Zebrafish, Intra-session habituation, Inter-session, Behavioral phenotyping, Novel tank test

1. Introduction

Habituation is an important adaptive behavior (1, 2) representing a reduction of responses to novelty over time (3). As the simplest form of learning (3), habituation has been extensively assessed in numerous species from invertebrates to rodents and humans (4–8). Due to various internal and external factors affecting behavior, there is considerable variation in habituation responses among

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Allan V. Kalueff and Adam Michael Stewart (eds.), *Zebrafish Protocols for Neurobehavioral Research*, Neuromethods, vol. 66,
DOI 10.1007/978-1-61779-597-8_21, © Springer Science+Business Media New York 2012

different species (9–11). In rodents, e.g., habituation is commonly measured by alterations in distance traveled and horizontal or vertical beam breaks over time (9, 12–16).

Zebrafish have become increasingly popular in biomedical research due to their low maintenance costs, rapid reproductive cycle, ease of acclimation, and robust behavioral phenotypes (17–21). Zebrafish behavior was initially thought to lack higher cognitive ability and to display predominantly instinctively driven escape reactions (rather than active exploration of new environments) (3). However, recent studies have revealed the greater complexity of adult zebrafish behavior, as they are capable of creating spatial memories (20, 22, 23) and exhibit robust habituation responses (3) (also see habituation in larval models (24)).

The present protocol outlines a simple method for studying two types of habituation in adult zebrafish: intra-session (within-trial) and inter-session (between-trial) habituation, which reflects short-term and longer-term memory, respectively. Depending on the study design, different experimental (e.g., pharmacological) manipulations may also be used to modify zebrafish habituation phenotypes.

2. Materials and Methods

2.1. Animal Housing

Adult zebrafish (e.g., 3–5 months old, ~50:50 male:female ratio) can be obtained from a commercial vendor or raised in-house. Fish can be separated by sex in order to assess sex differences in behavioral testing or pharmacological treatment. Fish can be housed in commercial aquatic systems (e.g., Aquatic Habitats, Apopka, FL) or in groups of 20–30 per 40-L tank, and should be given approximately 20 days to acclimate. The zebrafish are kept in filtered facility water at room temperature (~25°C) with pH maintained at 7.0–8.0. Ceiling-mounted fluorescent light tubes can provide illumination in the holding and testing rooms. Animals are typically fed twice a day (e.g., Tetramin Tropical Flakes, Petco Inc., San Diego, CA) and are kept on a 14:10 h schedule (e.g., light on at 6:00 h; off at 20:00 h).

2.2. Apparatus

Testing can be performed in the trapezoidal novel tank (e.g., 15 height × 28 top × 22 bottom × 7 cm width; Aquatic Habitats, Apopka, FL) resting on a level surface with the tank maximally filled with water (see Fig. 1a) (25, 26). A horizontal line is drawn across the middle of the tank to divide it into two equal sections (3, 26). Importantly, when assessing inter-session habituation over a period of several days, the apparatus should remain in the same location to ensure consistent lighting conditions (17). Habituation assays can be performed under normal lighting conditions of the holding and testing rooms (see above).

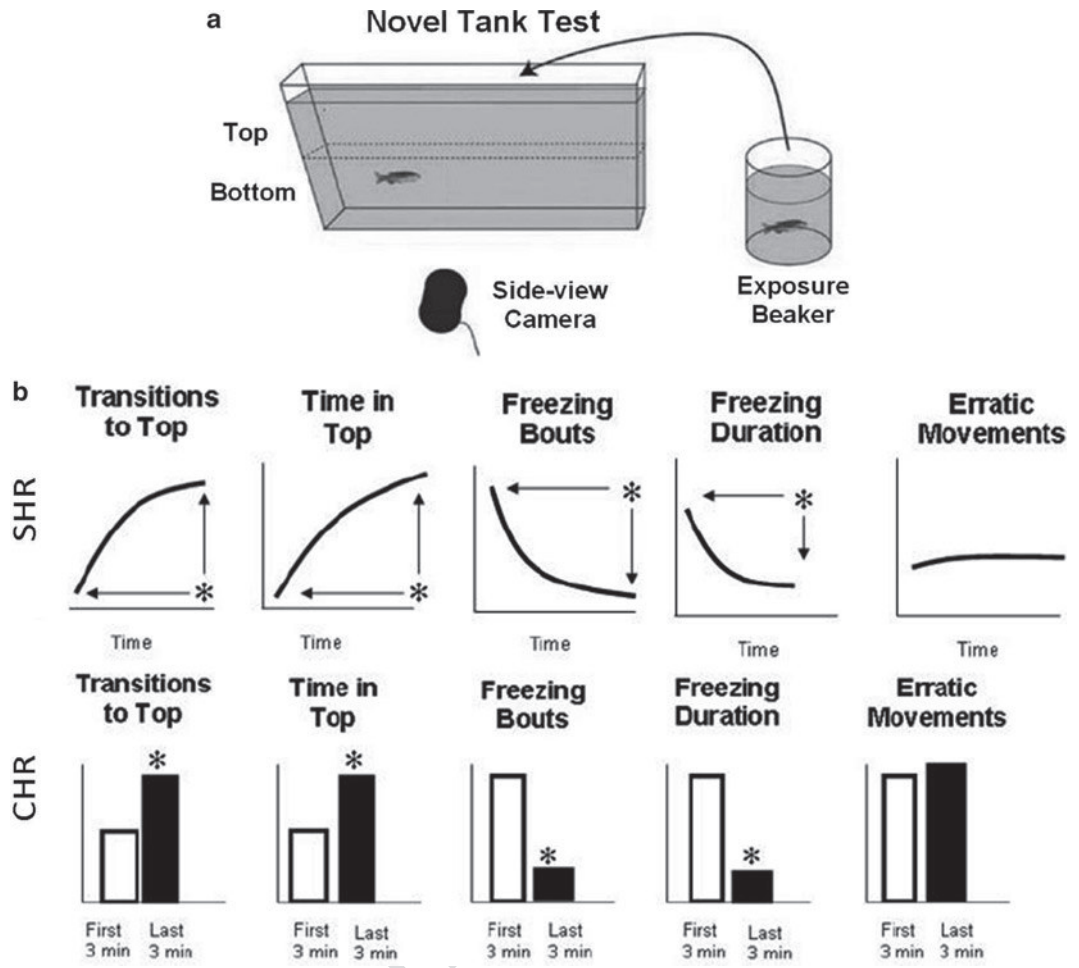

Fig. 1. The experimental set-up (**a**) and typical results (**b**) for a 6-min novel tank intra-session habituation experiment. (**a**) The novel tank apparatus, the exposure beaker (where pharmacological treatment occurs), and the side-view web-camera. (**b**) Typical habituation responses in the novel tank test (endpoints are given in relative units, for a better visual representation); *asterisks* denotes significant habituation over time as assessed by the single-minute habituation ratio (SHR; *top row*) or the cumulative habituation ratio (CHR, *bottom row*; paired *U*-test). Note the lack of significant differences in erratic movements over time (based on (3); also see Tables 1–2).

2.3. Experimental Manipulations

Zebrafish habituation can be studied using various experimental manipulations. Tables 1 and 2 summarize examples of habituation responses in zebrafish to several drugs, including ethanol, morphine, caffeine, fluoxetine, and pentylenetetrazole (PTZ). Anxiogenic responses may be evoked with caffeine and PTZ, while anxiolytic effects may be tested with ethanol, morphine, and fluoxetine (26). Other psychotropic drugs (such as a memory-enhancing agent piracetam, see (27)) can also be tested in this model, and their doses and exposure time can be based on previous published literature or pilot studies.

t1.1 **Table 1**

t1.2 **Examples of the effects on intra-session habituation in adult zebrafish (compared to control groups) produced by the**

t1.3 **anxiogenic drugs caffeine and pentylenetetrazole (PTZ) in the 6-min novel tank test**

	Habituation (see the definition of the endpoints in the methods section)					
Drug (dose and exposure time)	Transitions to top		Time spent in top		Erratic movements	
	SHR	CHR	SHR	CHR	SHR	CHR
Caffeine (100 mg/L for 15 min)	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Habituation is absent in controls, and impaired in experimental group; decreased SHR	Habituation is absent in controls, and impaired in experimental group; decreased CHR
Pentylene-tetrazole (900 mg/L for 10 min)	Habituation is absent in experimental, but not control group; no difference in SHR	Habituation is absent in experimental, but not control group; no difference in CHR	Habituation is absent in experimental, but not control group; no difference in SHR	Habituation is absent in experimental, but not control group; no difference in CHR	Habituation is absent in controls, and facilitated in experimental group; increased SHR	Habituation is absent in controls, and facilitated in experimental group; increased CHR

t1.21 Single-minute habituation ratio (SHR) is defined as (min 1):(min 6) ratio for each individual endpoint; cumulative habituation ratio (CHR) is defined as the
t1.22 sum of (min 1–3):sum of (min 4–6) scores for each individual endpoint (e.g., see Fig. 1b), based on (3)

t2.1 **Table 2**

t2.2 **Examples of the effects on intra-session habituation in adult zebrafish (compared to control groups) produced by various**

t2.3 **anxiolytic agents in the 6-min novel tank test**

t2.4 Drug (dose and exposure time)	Habituation					
t2.5	Transitions to top		Time spent in top		Erratic movements	
t2.6	SHR	CHR	SHR	CHR	SHR	CHR
t2.7 t2.8 t2.9 t2.10 t2.11 t2.12 Acute ethanol (0.3% for 5 min)	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Habituation is absent in both experimental and control groups; no difference in SHR	Habituation is absent in both experimental and control groups; no difference in CHR
t2.13 t2.14 t2.15 t2.16 t2.17 Chronic ethanol (0.2% for 14 days)	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Habituation is absent in both experimental and control groups; no difference in SHR	Habituation is absent in controls, and facilitated in increased CHR
t2.18 t2.19 t2.20 t2.21 t2.22 t2.23 t2.24 Chronic flouxetine (100 µg/L for 14 days)	Habituation is facilitated in experimental group when compared to controls; increased SHR	Similar habituation in both groups; no difference in CHR	Habituation is facilitated in experimental group when compared to controls; increased SHR	Similar habituation in both groups; no difference in CHR	Habituation is absent in both experimental and control groups; no difference in SHR	Habituation is absent in both experimental and control groups; no difference in CHR
t2.25 t2.26 t2.27 t2.28 t2.29 Morphine (2 mg/L for 15 min)	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Similar habituation in both groups; no difference in SHR	Similar habituation in both groups; no difference in CHR	Habituation is absent in both experimental and control groups; no difference in SHR	Habituation is absent in both experimental and control groups; no difference in CHR

t2.30 Legend as in Table 1; based on (3)

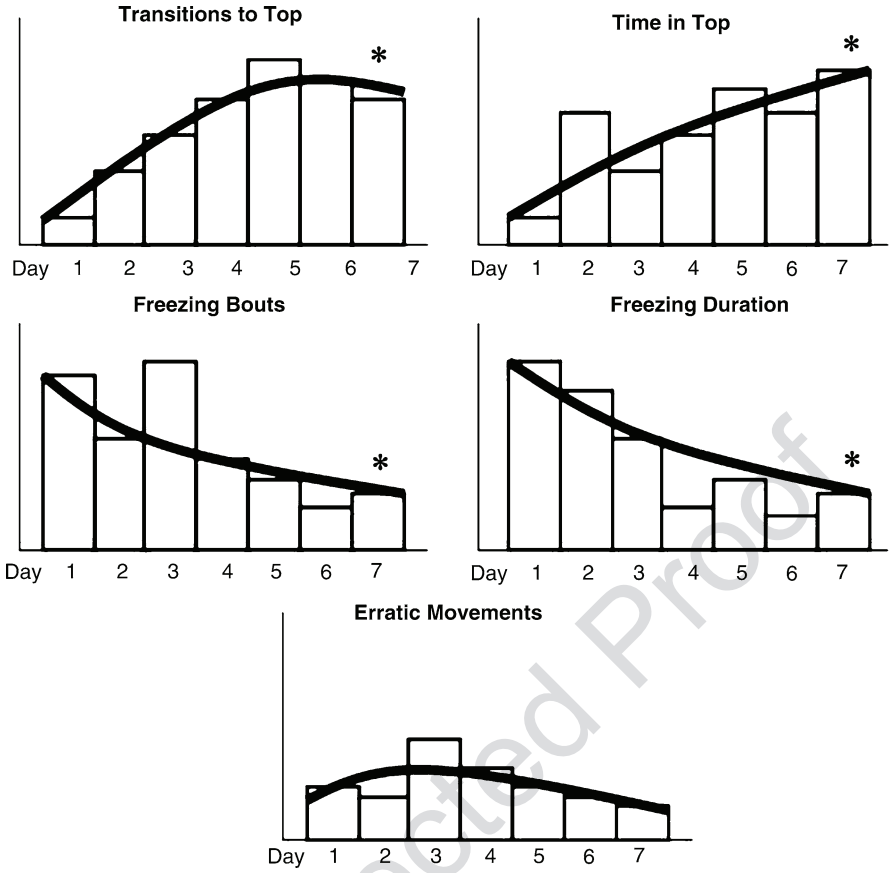

Fig. 2. Typical results for 7-day inter-session habituation experiment in the novel tank test (on 6-min trial per day). The *solid line* indicates alterations in habituation over time (endpoints are given in relative units, for a better visual representation). There was no significant habituation in erratic movements over time (*asterisks* denotes a statistically significant difference of day 1 vs. day 7 by paired *U*-test), based on (3).

2.4. Intra- and Inter-Session Habituation

For both the control and experimental groups, either an intra- or inter-session assay can be performed. Intra-session assay examines the habituation profiles of fish within a single trial (e.g., 6 min). Inter-session paradigm assesses long-term habituation over a series of 6 min novel tank trials repeated daily (e.g., for 7 days). The same endpoints can be used for both types of habituation tests (Fig. 2; see Sect. 4.4 for troubleshooting).

3. Procedure

3.1. Acclimation and Pretreatment

1. Transport animals from the holding room to the experimental room 1 h prior to testing using nets and a preexperimental container that is isothermal with the home tank. Be sure to

minimize handling during transport, because this may cause undesired increases in baseline anxiety levels in the fish. The water used in the novel tank must be at the same temperature as that of the home tank and of the preexperimental container. Note that facility water may be drawn the night before to allow proper acclimation to room temperature.

2. Depending on the experiment's objectives, fish may be treated with pharmacological agents acutely or chronically prior to testing. Acutely exposed zebrafish can be placed in a plastic beaker (e.g., 1–3 L, Fig. 1a) for a specific pretreatment time (e.g., 5–15 min). Chronically exposed zebrafish can be treated with the drug in the home tank for 1–2 weeks. Note that since some drugs may hydrolyze in water (e.g., fluoxetine), exposure tanks may need to be changed and re-dosed every 2–3 days during chronic treatment. Drug treatments are prepared by researchers separate from the experiment (so that the experimenters are blind to treatment). A good inter-rater and intra-rater reliability for the observers is usually set out >0.85, as assessed by Spearman correlation coefficient.

3.2. Novel Tank Testing

1. Following pretreatment, gently introduce the fish into the novel tank test apparatus. The fish is observed for 6 min, manually scoring transitions to the top of the tank, time spent in the top of the tank (s), freezing bouts (absence of movement except for gills for at least 2 s), freezing duration (s), and erratic movement (abrupt changes in direction or speed). Additionally, video-tracking software (e.g., Ethovision XT7, Noldus IT, Netherlands) can be used in this test to complement manual observations, further assessing endpoints such as distance traveled, average velocity, turning angle, and angular velocity (28, 29). If assessing inter-session habituation, novel tank testing is performed daily for several days (e.g., 7 days), at the same time each day. After testing, return fish to their respective holding tanks.

3.3. Habituation Analysis

1. Intra-session habituation is assessed for every endpoint (Fig. 1b) by comparing the first minute and the last minute (single-minute habituation ratio, SHR) as well as the first 3 min and the last 3 min (cumulative habituation ratio, CHR) of each trial (3). It is advantageous to assess habituation using both SHR and CHR indices, because these two measurements together minimize the errors in habituation data. While SHR is a more robust and sensitive measure, it is also more prone to skewing the data. For example, if a disturbance in the testing area or any behavioral irregularity occurs during the first or last minute, the SHR is likely to be affected. Using CHR in parallel minimizes this risk by ensuring data collection from several minutes, and although CHR is less sensitive than SHR, it is less likely to skew the data due to an artifact. Similar to intra-session

habituation, inter-session habituation is evaluated by comparing the first trial (e.g., Day 1) and the last trial (e.g., Day 7) (see Sect. 4.1 for locomotion troubleshooting) (3).

3.4. Statistical Analysis

1. For a single-cohort study, in order to globally assess the presence or absence of habituation, the data can be analyzed with a two-sample unpaired or paired Wilcoxon *U*-test for significance either between the groups or vs. the initial observation time (e.g., min 1 vs. min 6; Fig. 1b). Two-way ANOVA (factors: time, group) or one-way ANOVA with repeated measures (time or trials) can be used more universally, for the intra- and inter-session habituation analyses in studies using several different cohorts, followed by a post-hoc *U*-test (with Bonferroni correction) or any other appropriate post-hoc test.

4. Notes

4.1. Zebrafish Locomotion Is Abnormally Low or High

Ensure that zebrafish have had adequate time to acclimate to testing room. Other factors, such as differences in water temperature or excessive net stress prior to testing, can markedly reduce fish locomotion. Increased locomotion is also possible, e.g., if the zebrafish are nonanxious or hyperactive. If this becomes a recurring problem, consider a different strain of zebrafish for the experiment, as differing levels of baseline motor activity exist between strains. For example, high-anxiety zebrafish strains (e.g., leopard strain (26)) demonstrate heightened freezing behavior and reduced exploration and therefore may exhibit decreased locomotion.

4.2. High Variability of Habituation Responses

While zebrafish habituation is a typical natural response, high data variability is rather common in biobehavioral research (10, 11), including habituation studies. Genetic influences, animal stress, and testing room conditions (e.g., temperature, soundproofing, or lighting) must be taken into account and standardized throughout the experiments. Increasing the cohort size could also reduce data variability. A recommended cohort size for acquiring statistically significant data using this protocol is 12–15 adult zebrafish, although the sample size may be increased to 20–25 fish, if needed.

4.3. Lack of Habituation Responses

High anxiety strains or certain pharmacological manipulations may require a longer trial duration to reveal habituation responses. For example, extending the trial to 30–60 min may be helpful to solve this problem. Factors that may confound the trial should also be considered. Specifically, excessive handling stress or rapid movements and loud noise made by the experimenter during testing may startle the fish and cause excessive freezing and/or positive

geotaxis (the preference for the bottom of the tank) which would confound habituation responses.

4.4. Zebrafish Show Robust Inter-Session Habituation, But Fail to Exhibit Intra-Session Response (or Vice Versa)

While this may be a normal phenotype depending on the drug or battery of tests used, care should be taken to rule out stressful factors. For example, in addition to robust habituation responses, zebrafish also possess adequate learning and memory, and can recall training for up to 10 days (18). Therefore, it is possible that fish may habituate very quickly within a single trial (intra-session habituation), but will demonstrate minimal responses with subsequent testing. Extending the trial duration (e.g., 30 min) or increasing the sample size may improve the assay sensitivity (this can be especially relevant when testing the effects on memory by nootropic drugs, or other drugs with cognition-enhancing capabilities; e.g., (27)). Conversely, fish may exhibit an overt inter-session response, but fail to habituate within a single trial. While this may be an accurate response (e.g., specific impairment of spatial working memory) to a particular experimental manipulation, it is recommended to demonstrate that this phenotype is not due to a heightened baseline anxiety (e.g., by using an additional low/moderate-anxiety strain such as wild-type/long-fin fish).

4.5. Drug Treatments (Rather Than Habituation) Nonspecifically Affect Behavior and Locomotion

While habituation is measured by change in locomotor activity, pharmacological treatments may affect animal locomotion, motor control, and/or buoyancy. To minimize the chance of drug treatments distorting habituation behavior, precise and appropriate doses must be determined from pilot studies or established literature. These doses should have minimal effects on motor control and buoyancy, and should be appropriate for assessing various behavioral endpoints. Habituation is a learning process that shows gradual change across (or within) trials, so a sharp change in behavioral results may indicate a problem with pharmacological treatment in the experiment.

4.6. An Alternative Approach: Using Control Groups to Assess Learning

Although control groups are utilized in all experiments in this protocol that involve pharmacological treatments, another type of control may also be used. The control and drug-treated fish in our protocol are both placed in the novel tank test to measure change in behavior, which is then assessed as habituation. Including a control group that does not undergo the novel tank test, and measuring the change in behavior of this group, may show change due to development or naturally occurring phenomena (as opposed to behavioral testing and/or pharmacological treatment) (see (30) for details). By including a control group that received no experimental or pharmacological treatment, baseline learning conditions can be assessed and compared with learning conditions of the tested zebrafish, thereby providing further distinction between different behavioral domains in question.

4.7. Labeling and Recognizing Fish When Testing Over Multiple Days

When testing for habituation in adult zebrafish across multiple days, it is crucial that specific cohorts or individuals be recognizable, so that testing may proceed with the same organisms as previously. When using medium- to large-sized groups (e.g., $n = 12$ or $n = 25$), each cohort exposed to a specific pharmacological treatment or behavioral test must be housed together in an appropriately labeled tank for easy identification. If using smaller groups, it may be possible to label and identify *individual* zebrafish as separate from each other. The most obvious method is to house fish individually. However, this would require multiple tanks (which is impractical) and may also induce an unwanted isolation stress. Alternatively, fin-clipping may be used, involving severing, removing or marking the dorsal, caudal or anal fins for identification (larger fins usually regenerate following amputation (31)). Note that while demonstrating habituation in individual organisms may yield important findings, fin-clipping and any other methods that isolate or disturb individual zebrafish will likely affect locomotion and/or increase anxiety, thereby confounding habituation testing results.

5. Typical Results

5.1. Habituation Responses Over Specific Time

Throughout the 6-min intra-session habituation trials in the novel tank test, a significant increase in exploratory behavior and decrease in freezing behavior is typically observed (Fig. 1b). Erratic movements generally show no significant changes over time, suggesting that erratic behavior does not habituate. The 7-day inter-session trials usually show similar results, with gradual increases in exploratory behavior and decreases in freezing behavior (Fig. 2).

5.2. Habituation Responses to Anxiogenic Drug Treatment

To observe the effects of anxiogenic drugs on habituation patterns, zebrafish can be exposed to agents, such as caffeine and PTZ. Caffeine-treated zebrafish show similar habituation (vs. controls) for transitions to top and time in top, and impaired habituation of erratic movements, with decreased SHR and CHR scores for this endpoint. The latter phenotype is strongly consistent with an anxiogenic profile, since the erratic behavior not only failed to habituate (as it does in controls) but also showed an increase over time, demonstrating caffeine-induced impairment of habituation. In contrast, PTZ-treated zebrafish (unlike controls) exhibit impaired habituation for transitions to top and time in top, also showing more erratic movements (Table 1).

5.3. Habituation Responses to Anxiolytic Drug Treatment

The effects of anxiolytic drugs on zebrafish habituation can be tested with acute ethanol, chronic ethanol, fluoxetine, and morphine treatments (Table 2). Acute ethanol can lead to unaltered habituation behavior, while chronic ethanol can lead to an increase

in habituation and CHR for the erratic movements endpoint. Fluoxetine causes an increase in habituation for transitions to top and time in top (and SHR). In contrast, like acute ethanol, morphine at doses tested did not elicit marked changes in zebrafish habituation (Table 2), despite being effective in reducing anxiety responses (3).

6. Summary

Here, we have outlined a simple method to assess habituation to novelty in adult zebrafish. As the testing time elapses, zebrafish generally increase their exploration and reduce freezing behavior. In contrast, erratic behavior has not been shown to habituate in adult zebrafish. The habituation response of adult zebrafish is also sensitive to pharmacological manipulations, including both anxiolytic and anxiogenic agents (Tables 1 and 2), producing results as effectively as current testing methods traditionally used to study habitation in rodents (3).

Overall, the in-depth assessment of habituation profiles can be used to study the effects of pharmacological agents to determine whether various manipulations improve or hinder habituation. Similar to rodents (9, 32–34), impaired habituation in zebrafish can be viewed as a failure to adapt to a novel environment, which is relevant to anxiety (2) and other complex disorders, such as schizophrenia (35), depression (36), or cognitive deficits (3). Such analyses can also be useful for testing various inbred and mutant zebrafish strains (which may display aberrant habituation), offering a simple method to foster the discovery of novel anxiolytic and/or memory-modulating treatments.

Acknowledgements

The study was supported by Tulane University Intramural funds, Tulane Neurophenotyping Platform, Tulane University Pilot, and the Newcomb Fellows grants.

References

1. Salomons AR et al (2010) Behavioural habituation to novelty and brain area specific immediate early gene expression in female mice of two inbred strains. Behav Brain Res 215(1):95–101
2. Salomons AR et al (2010) Identifying emotional adaptation: behavioural habituation to novelty and immediate early gene expression in two inbred mouse strains. Genes Brain Behav 9(1):1–10
3. Wong K et al (2010) Analyzing habituation responses to novelty in zebrafish (*Danio rerio*). Behav Brain Res 208(2):450–457
4. File SE, Mabbutt PS (1990) Long-lasting effects on habituation and passive avoidance

performance of a period of chronic ethanol administration in the rat. Behav Brain Res 36(1–2):171–178

5. Thompson RF, Spencer WA (1966) Habituation: a model phenomenon for the study of neuronal substrates of behavior. Psychol Rev 73(1):16–43
6. Zucker RS (1972) Crayfish escape behavior and central synapses. II. Physiological mechanisms underlying behavioral habituation. J Neurophysiol 35(5):621–637
7. Davis M (1970) Effects of interstimulus interval length and variability on startle-response habituation in the rat. J Comp Physiol Psychol 72(2):177–192
8. Davis M, Wagner AR (1969) Habituation of startle response under incremental sequence of stimulus intensities. J Comp Physiol Psychol 67(4):486–492
9. Leussis MP, Bolivar VJ (2006) Habituation in rodents: a review of behavior, neurobiology, and genetics. Neurosci Biobehav Rev 30(7):1045–1064
10. Crusio WE, Schwegler H (1987) Hippocampal mossy fiber distribution covaries with open-field habituation in the mouse. Behav Brain Res 26(2–3):153–158
11. Glowa JR, Hansen CT (1994) Differences in response to an acoustic startle stimulus among forty-six rat strains. Behav Genet 24(1): 79–84
12. Bolivar VJ (2009) Intrasession and intersession habituation in mice: from inbred strain variability to linkage analysis. Neurobiol Learn Mem 92(2):206–214
13. Glickman SE, Hartz KE (1964) Exploratory behavior in several species of rodents. J Comp Physiol Psychol 58:101–104
14. Bolivar VJ et al (2000) Habituation of activity in an open field: a survey of inbred strains and F1 hybrids. Behav Genet 30(4):285–293
15. Bolivar VJ, Manley K, Messer A (2004) Early exploratory behavior abnormalities in R6/1 Huntington's disease transgenic mice. Brain Res 1005(1–2):29–35
16. Gunn RK, Keenan ME, Brown RE (2011) Analysis of sensory, motor and cognitive functions of the coloboma (C3Sn.Cg-Cm/J) mutant mouse. Genes Brain Behav 10(5): 579–588
17. Cachat J et al (2010) Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. Nat Protoc 5(11): 1786–1799
18. Spence R et al (2008) The behaviour and ecology of the zebrafish, *Danio rerio*. Biol Rev Camb Philos Soc 83(1):13–34
19. Gerlai R, Lee V, Blaser R (2006) Effects of acute and chronic ethanol exposure on the behavior of adult zebrafish (*Danio rerio*). Pharmacol Biochem Behav 85(4):752–761
20. Blaser R, Gerlai R (2006) Behavioral phenotyping in zebrafish: comparison of three behavioral quantification methods. Behav Res Methods 38(3):456–469
21. Bencan Z, Sledge D, Levin ED (2009) Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. Pharmacol Biochem Behav 94(1):75–80
22. Stewart A et al (2010) Homebase behavior of zebrafish in novelty-based paradigms. Behav Processes 85(2):198–203
23. Rosemberg DB et al (2011) Differences in spatio-temporal behavior of zebrafish in the open tank paradigm after a short-period confinement into dark and bright environments. PLoS One 6(5):e19397
24. Best JD et al (2008) Non-associative learning in larval zebrafish. Neuropsychopharmacology 33(5):1206–1215
25. Levin ED, Bencan Z, Cerutti DT (2007) Anxiolytic effects of nicotine in zebrafish. Physiol Behav 90(1):54–58
26. Egan RJ et al (2009) Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behav Brain Res 205(1):38–44
27. Grossman L et al (2011) Effects of piracetam on behavior and memory in adult zebrafish. Brain Res Bull 85(1–2):58–63
28. Cachat J et al (2010) Deconstructing adult zebrafish behavior with swim trace visualizations. In: Kalueff AV, Cachat J (eds) Zebrafish neurobehavioral protocols. Humana Press, New York, pp 191–201
29. Cachat J et al (2011) Three-dimensional neurophenotyping of adult zebrafish behavior. PLoS One 6(3):e17597
30. Rescorla RA (1988) Behavioral studies of pavlovian conditioning. Annu Rev Neurosci 11:329–352
31. Shao J et al (2011) Tissue regeneration after injury in adult zebrafish: the regenerative potential of the caudal fin. Dev Dyn 240(5): 1271–1277
32. Hughes RN (1982) A review of atropinic drug effects on exploratory choice behavior in laboratory rodents. Behav Neural Biol 34(1): 5–41
33. Klamer D et al (2004) Habituation of acoustic startle is disrupted by psychotomimetic drugs: differential dependence on dopaminergic and nitric oxide modulatory mechanisms.

Psychopharmacology (Berl) 176(3–4): 440–450

34. Alleva E, Bignami G (1985) Development of mouse activity, stimulus reactivity, habituation, and response to amphetamine and scopolamine. Physiol Behav 34(4): 519–523

35. Morris JA (2009) Zebrafish: a model system to examine the neurodevelopmental basis of schizophrenia. Prog Brain Res 179:97–106

36. Monson CA, Sadler KC (2010) Inbreeding depression and outbreeding depression are evident in wild-type zebrafish lines. Zebrafish 7(2):189–197