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RESEARCH ARTICLE

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Moderate early life stress improves adult zebrafish (*Danio rerio*) working memory but does not affect social and anxiety-like responses

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Abstract

Early life stress (ELS) is defined as a short or chronic period of trauma, environmental or social deprivation, which can affect different neurochemical and behavioral patterns during adulthood. Zebrafish (*Danio rerio*) have been widely used as a model system to understand human neurodevelopmental disorders and display translationally relevant behavioral and stress-regulating systems. In this study, we aimed to investigate the effects of moderate ELS by exposing young animals (6-weeks postfertilization), for 3 consecutive days, to three stressors, and analyzing the impact of this on adult zebrafish behavior (16-week postfertilization). The ELS impact in adults was assessed through analysis of performance on tests of unconditioned memory (free movement pattern Y-maze test), exploratory and anxiety-related task (novel tank diving test), and social cohesion (shoaling test). Here, we show for the first time that moderate ELS increases the number of alternations in turn-direction compared to repetitions in the unconditioned Y-maze task, suggesting increased working memory, but has no effect on shoal cohesion, locomotor profile, or anxiety-like behavior. Overall, our data suggest that moderate ELS may be linked to adaptive flexibility which contributes to build "resilience" in adult zebrafish by improving working memory performance.

KEYWORDS

adaptive flexibility, FMP Y-maze, moderate-stress, novel tank, resilience, shoal test

1 | INTRODUCTION

Early life experiences are often linked to changes in cognitive and behavioral aspects in humans (Pechtel & Pizzagalli, 2011). In animal models, early life stress (ELS) is shown to directly cause long-term changes in several brain functions (Molet, Maras, Avishai-Eliner, & Baram, 2014; Nishi, Horii-Hayashi, Sasagawa, & Matsunaga, 2013; Spinelli et al., 2009). ELS can occur prenatally or

postnatally (or both), and can affect both neurological and physiological development (Maniam, Antoniadis, & Morris, 2014; Pechtel & Pizzagalli, 2011). The neurochemical and hormonal changes induced by ELS are associated with emotional and cognitive processes, and ELS is a risk factor for neuropsychiatric disturbances such as depression (Coffino, 2009; Heim & Nemeroff, 2001; Kaufman, Plotsky, Nemeroff, & Charney, 2000), posttraumatic stress disorder, schizophrenia, substance abuse (Scheller-Gilkey, Moynes, Cooper, Kant, & Miller, 2004), attention-deficit disorder (Heim & Nemeroff, 2001), eating disorders (Gilbert et al., 2009), and an abnormal stress response (Kajantie & Raikkonen, 2010; Kaufman et al., 2000) in adult life.

ELS can be assessed behaviorally and biologically in rodents by using protocols such as maternal separation, postweaning social isolation, and peripubertal stress (Dunphy-Doherty et al., 2018; Lyons, Parker, & Schatzberg, 2010; Ouchi et al., 2018; Tsuda, Yamaguchi, & Ogawa, 2011). Although high or chronic levels of stress may disturb brain development and affect behavior, acute activation of the body's stress response systems can adapt and increase chances of survival (Anda et al., 2006; De Bellis et al., 1999; Lupien, McEwen, Gunnar, & Heim, 2009; Maniglio, 2009; Pechtel & Pizzagalli, 2011; Spataro, Mullen, Burgess, Wells, & Moss, 2004). For example, *predictable* chronic stress increases "resilience" (i.e. positive outcomes regardless of adverse environment or negative experiences) in rats by increasing hippocampal neurogenesis and memory (Parihar, Hattiangady, Kuruba, Shuai, & Shetty, 2011), whereas *unpredictable* chronic stress may impair learning and memory processes (Rice, Sandman, Lenjavi, & Baram, 2008). Moreover, ELS can change other behavioral domains such as stress reactivity (O'Mahony et al., 2009) and impulsivity (Lovallo, 2013). In general, because neuropsychiatric studies depict only deleterious outcomes following ELS (Grassi-Oliveira, Ashy, & Stein, 2008; Teicher et al., 2003; Teicher, Tomoda, & Andersen, 2006; Weber & Reynolds, 2004), relatively little attention has been devoted to evaluating positive cognitive outcomes of ELS. Thus, a better understanding of the long-term consequences of ELS could improve prevention strategies for mental disorders, particularly for those that are affected by stress.

Zebrafish (*Danio rerio*) are widely utilized as a translational and complementary model to better understand human neurodevelopmental-related disorders (Sakai, Ijaz, & Hoffman, 2018). This usage is primarily due to high genetic (70% of zebrafish genes have at least one human orthologue; Howe et al., 2013) and physiological homology (Holzschuh, Ryu, Aberger, & Driever, 2001; MacRae & Peterson, 2015; Rico et al., 2011) between fish and other vertebrates. Although there are several differences between human and zebrafish stress-regulating systems, there is conservation of organization and functioning in terms of anatomy, connectivity, and molecular constituents, making zebrafish an important model to study stress-related responses (Alsop & Vijayan, 2008, 2009a, 2009b; Grunwald & Eisen, 2002). Zebrafish display a broadly conserved behavioral repertoire (Kalueff et al., 2013), including different domains such as learning (Cognato Gde et al., 2012; Valente, Huang, Portugues, & Engert, 2012), aggressiveness (Ariyomo, Carter, & Watt, 2013; Norton, 2018), anxiety-like behavior (Blaser & Rosemberg, 2012), and social behavior (De Polavieja & Orger, 2018; Dreosti, Lopes, Kampff, & Wilson, 2015; Paull et al., 2010). Like mammals, zebrafish respond to a variety of stressors such as handling, social isolation, rapid temperature changes, overcrowding, and novel environments, operationally defined through increases in abnormal behaviors such as decreased locomotion and social interactions (Fulcher, Tran, Shams, Chatterjee, & Gerlai, 2017; Pavlidis et al., 2013; Piato et al., 2011). Overall, zebrafish have great potential as a translational model for studying ELS. Here, we investigated the effects of moderate ELS by exposing young animals (6-week postfertilization), for 3 consecutive days, to three stressors, and analyzing the impact of this on adult zebrafish behavior (16-week postfertilization) through analysis of performance on tests of unconditioned memory (free movement pattern (FMP) Y-maze test), exploratory and anxiety-related task (novel tank diving test), and social cohesion (shoaling test).

2 | MATERIALS AND METHODS

2.1 | Animals, blinding, and randomization

Experiments were carried out using Tubingen (TU) zebrafish bred in house following breeding from multiple pairs of fish. Fish were sorted into groups of 30–40, and grown to 6-weeks postfertilization in a recirculating nursery system (Tecniplast). At this point, the ELS protocol was carried out (see below for details). All fish were then grown to 4-months postfertilization in groups of ~20, and were housed in groups according to stress protocol (ELS versus handling control; 5 × groups of each condition). The experimental and technical teams were blind as to treatment allocation during rearing. Adult (4-month postfertilization) animals were tested on one of the three behavioral procedures to ascertain perseveration/working memory (FMP Y-maze test), exploratory and anxiety-related task (novel tank diving test), and sociability (shoaling test). Animals were maintained on a 14/10-hr light/dark cycle (lights on at 9:00 a.m.), pH 7.1, at ~28.5°C (±1°C) and were fed three times per day with a mixture of live brine shrimp and flake food, except during the weekend, when they were fed once per day. Each animal was tested in only one behavioral protocol to avoid potential effects of multiple testing. Animals were euthanized using 2-phenoxyethanol (Aqua-Sed™, Vetark) and all experiments were carried out following scrutiny by the University of Portsmouth Animal Welfare and Ethical Review Board and under license from the UK Home Office (Animals [Scientific Procedures] Act, 1986) (PPL: P9D87106F) All stressors and behavioral tasks were carried out in a fully blinded manner and once all data were collected and screened for extreme outliers (e.g., fish freezing and returning values of "0" for behavioral parameters indicating nonengagement), group allocation (control or ELS) was revealed and data were analyzed in full. Final sample sizes for all behavioral tests were established following power

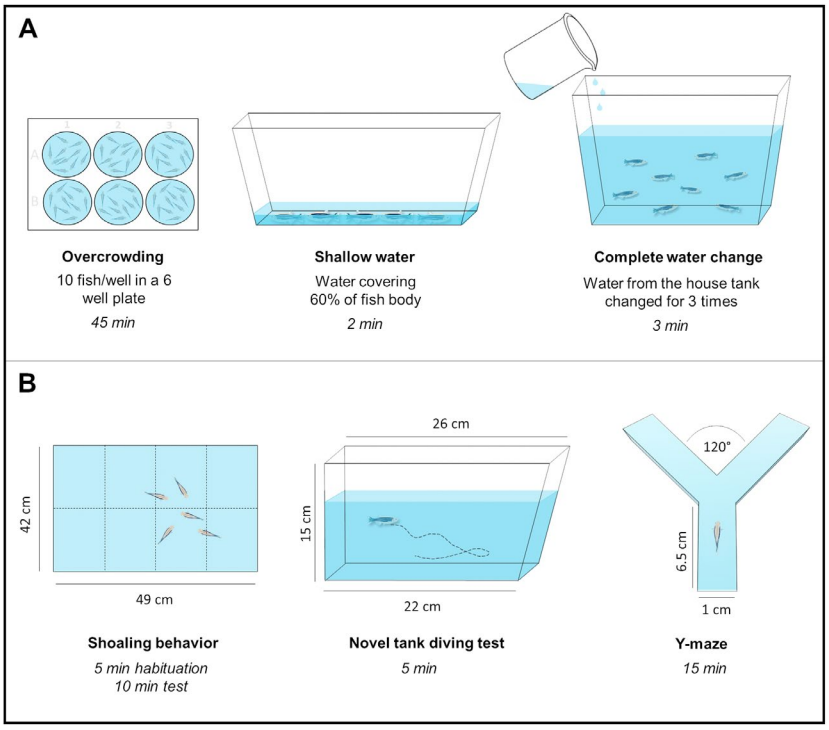

FIGURE 1 Schematic representation of the (A) stressors and (B) behavioral tasks used to assess ELS in adult zebrafish

analyses calculated with pilot experiments/prior publications from our group.

2.2 | Short-term ELS protocol

The stressors were applied each day for 3 consecutive days. During this time, the fish were exposed to one of three stressors on each day. These included "water change" (water from tank house changed for three times), "shallow water" (2 min in shallow water; 60% of body exposure to the air), and "overcrowding" (10 fish/well in a 6-well plates for 45 min; Figure 1a). The three stressors used here were adapted from previous studies where water change, shallow water, and overcrowding induced behavioral and cortisol changes (Clark, Boczek, & Ekker, 2011; Ramsay et al., 2006). Furthermore, all the three stressors were separately tested to investigate if they were able to modulate cortisol levels in 6-week-old animals. The randomization schedule for exposure to stressors is displayed in Table 1.

TABLE 1 Procedure of mild stress protocol in larvae zebrafish

Group	*N*	Day 1	Day 2	Day 3
S1	25	O, S, W	S, O, W	W, O, S
S2	25	W, O, S	O, S, W	S, O, W
S3	26	S, O, W	W, O, S	O, S, W
C1	25	–	–	–
C2	25	–	–	–
C3	25	–	–	–

Abbreviations: Overcrowding (O); Shallow Water (S); Water change (W).

2.3 | Novel tank diving test

The novel tank diving test is commonly used for analyzing exploratory and locomotor profiles. Moreover, this task is often used to analyze anxiety-like behavior being sensitive to a range of anxiogenic and anxiolytic drugs in the absence or presence of different aversive stimulus (Egan et al., 2009; Maximino et al., 2018; Mezzomo, Silveira, Giuliani, Quadros, & Rosemberg, 2016; Wong et al., 2010). Zebrafish (n = 23) were placed individually in a novel tank (22–26 cm length × 15 cm height × 9 cm width; Figure 1b). Behavioral activity was recorded in front of the tank using a webcam for 5 min to analyze diving response (Egan et al., 2009; Parker, Millington, Combe, & Brennan, 2012; Rosemberg et al., 2012). The tank was virtually divided into three areas (bottom, middle, and top), and time at the bottom was used to assess anxiety-like phenotypes. The total distance traveled was used to evaluate differences in locomotor profile. All behaviors were measured with automated video-tracking software (EthoVision, Noldus Information Technology Inc.) at a rate of 60 frames/s.

2.4 | Shoaling test

Zebrafish is a social species that presents a natural preference for conspecifics in neutral and mildly stressful situations (De Polavieja & Orger, 2018; Paull et al., 2010; Saverino & Gerlai, 2008). Social cohesion (n = 80, organized into 16 groups of n = 5) was tested in a shoaling assay. Sixteen groups of five fish from each treatment (ELS versus no stress) were placed in a white opaque tank (49 cm length × 15 cm height × 42 cm width) filled with 6 L of

aquarium treated water (Figure 1b). Animals were left to habituate for 5 min, then filmed for 10 min from above using a webcam. The unit of replication was shoal (i.e., group of five fish) nested in housing group (n = 8 shoals per treatment). Data analysis was based on previous work (Parker et al., 2014; Parker, Brock, Millington, & Brennan, 2013). Briefly, the arena was split into eight equal sections and the number of fishes in each square (as a function of total number of squares occupied), at any one time, was ascertained every 10 s to calculate a dispersion index. If a fish was between squares, the square containing >50% of the fish was recorded as the occupied square. Shoaling behavior was measured using the automated video-tracking software (EthoVision, Noldus Information Technology Inc.) at a rate of 60 frames/s.

2.5 | FMP Y-maze

The FMP Y-maze is distinct from a previously used "Y-maze" protocols, in which animals would explore three spatially distinct arms and the analysis would be based (typically over a short period of time, such as 5 min, or a discrete number of "choices"—"some refs") on the spatial location of the animals' choices. Critically, the FMP Y-maze is conceptually more comparable to a series of discrete choices as would be the case in a series of trials in a T-maze, or in a two-choice guessing task (Cleal & Parker, 2018; Fontana, Cleal, Clay, & Parker, 2019; Fontana, Cleal, & Parker, 2019). We have demonstrated that alternations in turn-direction are reduced following administration of memory-impairing drugs (e.g., MK801 and scopolamine), suggesting that alternations are indicative of working memory (Cleal et al., 2020). Fish (n = 48) were placed, individually, in a Y-shaped maze (6.5 cm length × 1 cm width; three identical arms at a 120° angle from each other) with white opaque walls (Figure 1b) and recorded for 15 min. During this time, the fish could either turn left or right at each choice point. Ambient light allowed some visibility in the maze, but no explicit intra-maze cues were added to the environment. All responses were non-reinforced. Turn choices were organized in blocks of four trials, and within each four-trial block, the fish could either show pure alternation (rlrl, lrlr) or pure repetition (llll, rrrr), or any combination (16 in total). The use of the overlapping tetragrams (four choices) was used as it gives a higher chance of identifying random search patterns than using sequences of two or three choices (Frith & Done 1983). Therefore, FMP Y-maze behavior was analyzed through 16 possible four-trial outcomes (tetragrams) for each fish as a proportion of the total number of turns to examine, in detail, their behavior patterns (Cleal et al., 2020; Cleal & Parker, 2018; Gross, Engel, Richter, Garner, & Wurbel, 2011). If behavior was fully random, it would be predicted that each choice would be equally likely (i.e., the predicted frequencies would be equal for each tetragram). However, if behavior was biased toward alternation, or repetition, it would be predicted that the alternation and repetition tetragrams would be significantly different from one another.

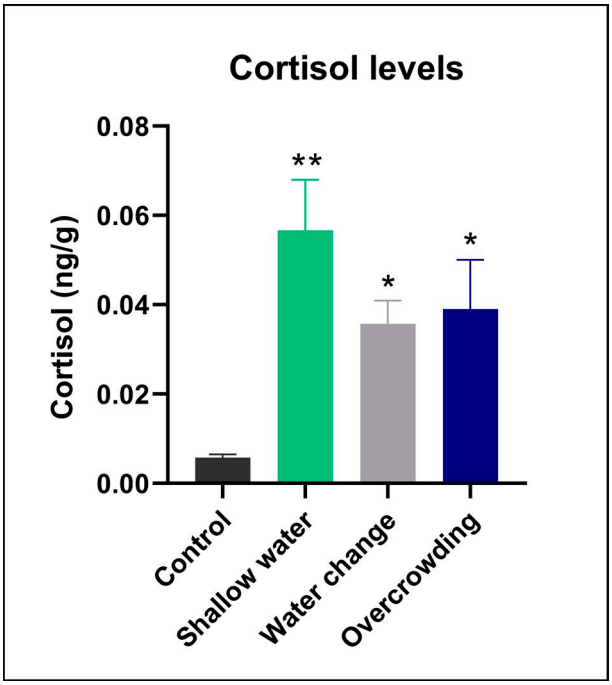

FIGURE 2 Cortisol levels for non-stressed (control) and stressed animals. Data were represented as mean ± S.E.M and analyzed by one-way ANOVA, followed by Tukey's multiple comparison test. Asterisks indicate statistical differences compared to control group (*P < .05 and **P < .01, n = 3 –4 per group)

2.6 | Cortisol levels

For the analysis of cortisol levels, fish (6-week old) were acutely exposed to water change, shallow water, or overcrowding using the conditions previously described. Due to their small size, animals were pooled in 12 per sample. Cortisol levels were assessed using a human salivary cortisol ELISA kit (Salimetrics Salivary Cortisol ELISA, Stratech) as previously described (Cachat et al., 2010; Parker et al., 2012). Fish were killed by immersion in ice-water and the whole bodies were snap-frozen in liquid nitrogen and kept at −80°C until assay. Samples were homogenized in 5-ml ice-cold PBS. Five milliliter of diethyl ether was added, and samples were centrifuged (3,500 ***g***) for 30 min, and the top (organic) layer was removed. This process was repeated three times, and then the diethyl ether was evaporated overnight. The resulting cortisol was reconstituted in 1-mL ice-cold phosphate-buffered saline (PBS) and the ELISA was then performed in 96-well plates as per the manufacturer's instructions. Cortisol concentrations (ng/g) were determined from optical density readings compared against manufacturer's standards. All samples were run in duplicate and the inter- and intra-assay coefficients of variation were determined.

2.7 | Statistics

Data were analyzed in IBM SPSS® Statistics and results were expressed as means ± standard error of the mean (S.E.M). To assess

whether there were any effects of stress on total turns, alternations (lrlr + rlrl), repetitions (rrrr + llll), right turns, left turns, distance traveled, time at the bottom, and shoaling ratio, we used Student's *t*-tests. Additionally, to investigate the stress effects on behavioral tetragrams, we fitted a generalized linear mixed effects model (Poisson distribution, log link), with stress and block choice as fixed factors, and ID as a random effect (to account for non-independence of replicates). Cortisol levels were analyzed using one-way ANOVA. When appropriate, Tukey's test was used as post hoc analysis, and results were considered significant when $P \leq .05$.

3 | RESULTS

3.1 | Cortisol levels are increased in stressed young animals

Initially, we exposed small groups of 6-week-old fish to the three stressors (shallow water, water change, and overcrowding) and examined the cortisol response to the exposures. A one-way ANOVA confirmed that the treatments were all invoking a stress response, with a significant difference between groups for cortisol levels ($F_{(3, 8)} = 10.67$; $P = .0036$). Post hoc tests revealed that cortisol levels were significantly increased when acutely exposing 6-weeks animals to each of the three stressors as compared to no treatment controls: control < shallow water ($P < .005$), control < water change ($P < .05$), and control < overcrowding ($P < .05$; Figure 2). All samples were run in duplicate and the inter- and intra-assay coefficients of variation were < 4%.

3.2 | Anxiety and social-related phenotypes are not modulated by short-term ELS

We examined the effects of ELS on anxiety using the novel tank test, and social behavior using a social cohesion assay. Student's *t*-tests (two-tailed) were performed for the analyses of anxiety-like behavior and shoal responses. We observed that ELS did not change any locomotor nor anxiety-related patterns such as distance travelled ($t_{(54)} = 0.868$; $P = .388$) and time spent in the bottom section ($t_{(54)} = 0.166$; $P = .86$; Figure 3a). There was also no difference in the social

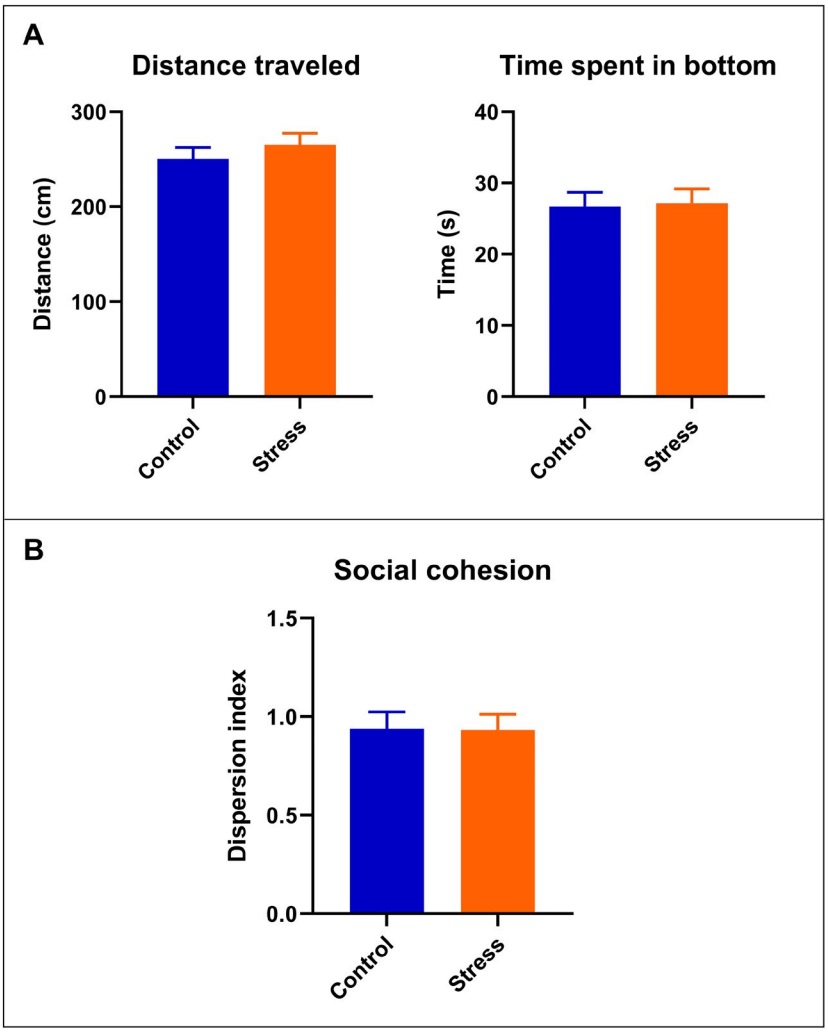

FIGURE 3 Effects of ELS in stress reactivity and social responses. (A) ELS do not change distance traveled and time spent in bottom in the novel tank diving task (n = 11–12 per group). (B) Shoal cohesion is not affected by 3 days of ELS in adult zebrafish (n = 8 per group). Data were represented as mean ± SEM and analyzed by Student's *t*-test

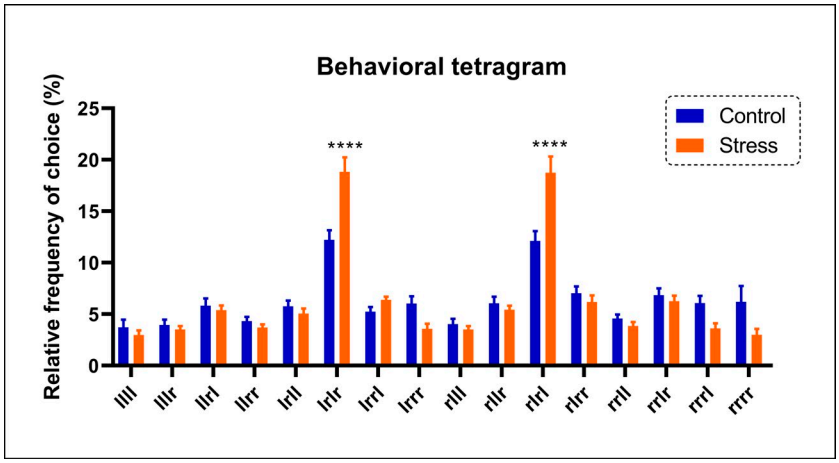

FIGURE 4 Y-maze tetragrams showing the behavioral phenotype of control and ELS groups. Data were represented as mean ± SEM and analyzed by linear mixed effects, followed by Tukey's multiple comparison test. Asterisks indicate statistical differences compared to non-biased group or between biased groups (*P < .05, **P < .01, ***P < .001 and *P < .0001, n = 24 per group)

cohesion ratio ($t_{(54)}$ = 0.060; P = .952) observed for animals exposed to ELS compared to the control group (Figure 3b).

3.3 | Working memory is enhanced in adult zebrafish exposed to ELS

Finally, we analyzed the effects of ELS on working memory by examining turn choices in the FMP Y-maze exploration task. Through analysis of overall turn choices, we found that ELS affected FMP Y-maze performance. Figure 4 depicts the behavioral 16-choice tetragrams for control versus ELS-exposed animals. There was no main effect of ELS on tetragram analysis ($F_{(1, 336)}$ = 0.00; P > .999). There was, however, a main effect of block choice ($F_{(15, 336)}$ = 54.75; P < .0001), and a treatment × block-choice interaction ($F_{(15, 336)}$ = 8.724; P < .0001). Post hoc Tukey tests revealed that ELS significantly increased the rlrl (P < .0001) and lrlr (P < .0001) block choices. Examining alternations and repetitions in turn-direction in more detail revealed that ELS significantly decreased the number of total turns ($t_{(21)}$ = 2.846; P = .009) and number of repetitions ($t_{(21)}$ = 3.055; P = .006) while increasing the number of alternations ($t_{(21)}$ = 3.055; P = .0012) in the FMP Y-maze test (Figure 5). ELS did not alter the animals' preference for left ($t_{(21)}$ = 0.533; P = .599) or right turns ($t_{(21)}$ = 0.533; P = .599).

4 | DISCUSSION

In this study, we evaluated the effects of exposing young zebrafish (16-day old) to unpredictable moderate stress for 3 days on adult behavior (4-month old), through the analysis of three different domains: locomotor and anxiety (novel tank diving test), sociality (shoal cohesion test), and memory (FMP Y-maze test). We showed, for the first time, that 3 days of ELS improves zebrafish working memory by increasing alternations and decreasing repetitions in turn-direction in the FMP Y-maze test. Although learning and memory process were affected by short-term ELS, stress did not modulate social behavior and anxiety-like behavior in adult zebrafish. Collectively, these data support previous evidence that early life exposure to moderate stress can build resilience and have an important adaptive role for different species, including humans. Our study has highlighted

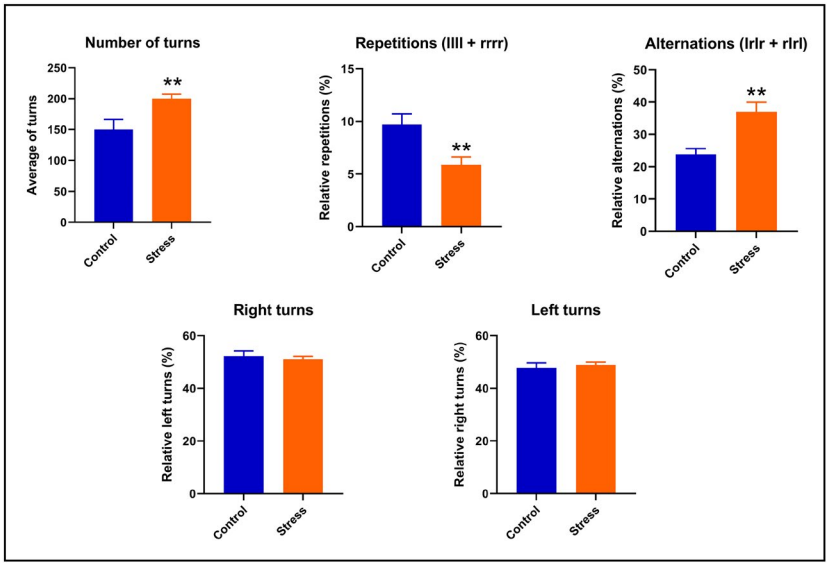

FIGURE 5 ELS increases alternations and decreases repetition in turn-direction without changing the left or right preference of adult zebrafish. Data were represented as mean ± SEM and analyzed by Student's t-test. Asterisks indicate statistical differences compared to control group (*P < .05 and **P < .01, n = 24 per group)

that zebrafish is an important animal model for elucidating the developmental processes underlying ELS, and how this impacts adult behavior.

Early life experiences regulate individual differences through both neural plasticity and epigenetic modifications, and these modifications later determine the capacity for flexible and adaptive behaviors in adulthood (McEwen, Bowles, et al., 2015; McEwen, Gray, & Nasca, 2015). Stressful stimuli can exert both positive and negative impacts on neurophysiological aspects being balanced to give the best fitness outcome (Bogdan & Pizzagalli, 2006; Gluckman, Hanson, & Beedle, 2007; Maier, Amat, Baratta, Paul, & Watkins, 2006; Zannas & West, 2014). To confirm if shallow water, water changes, and overcrowding changed zebrafish stress-regulating systems, we analyzed the effects of these three stressors separately in 6-week-old animals. As previously observed in adults (Pavlidis et al., 2013; Song et al., 2018), the acute stressors increased cortisol levels in young animals.

The impact on adult life of ELS follows an inverted U-shaped curve, where exposure to mild/moderate stress during development results in positive/beneficial outcomes such as resilience, and either too little or too much stress exposure (or too high an intensity) leads to negative outcomes (Russo, Murrough, Han, Charney, & Nestler, 2012; Sapolsky, 2015). Concerning this, chronic ELS is related to different behavioral outcomes in different species, including enhanced emotional- and stress reactivity, which are frequently linked to anxiety disorders (Coplan et al., 1996; Nugent, Tyrka, Carpenter, & Price, 2011), aggressiveness (Veenema, 2009; Veenema, Blume, Niederle, Buwalda, & Neumann, 2006), impulsiveness (Lovallo, 2013), and antisocial behavior (Haller, Harold, Sandi, & Neumann, 2014; Kohl et al., 2015). Meanwhile, adolescents exposed to moderately severe ELS events showed blunted depressive symptom responses to changes in proximal stressful events in the previous 9 months, compared to those with fewer ELS events (Shapero et al., 2015). Here, we showed that short-term ELS did not affect adult zebrafish anxiety-like profile nor social cohesion in both the novel tank diving test and shoal cohesion test. However, ELS increased the number of alternations in turn-direction and decreased the number of repetitions in the FMP Y-maze task.

The FMP Y-maze task comprises three identical arms, where the animal is introduced to the center of the maze and allowed to freely explore. In this task, over the course of multiple arm entries, animals have a natural tendency to enter the recently visited arm less frequently, thus increasing the number of alternations in turn-direction across the test (Drew, Miller, & Baugh, 1973; Hughes, 2004; Kokkinidis & Anisman, 1976; Swonger & Rech, 1972). In general, various configurations of the Y-maze task have been used to study learning and processes in animal models, including rodents (Fu et al., 2017; Ghafouri et al., 2016; Luine, 2015) and fish (Aoki, Tsuboi, & Okamoto, 2015; Cleal & Parker, 2018; Cognato Gde et al., 2012). Both decreased alternations and increased repetitions are affected pharmacologically by muscarinic and NMDA-receptor antagonists, and additionally by β-amyloid peptides in rodents (Cunha et al., 2008; Hiramatsu & Inoue, 2000; Park et al., 2010; Walker & Gold, 1992). Recently, we also observed that zebrafish presented decreased alternations in turn-direction when exposed to moderate alcohol during early nervous system developmental (Cleal & Parker, 2018). Although the FMP Y-maze responses can be influenced by novelty-seeking (Fontana, Cleal, Clay, et al., 2019), we observed here that ELS did not change exploratory responses of animals at the novel tank diving task. Thus, it seems likely, that increased alternations in turn-direction and a coincidental decrease in repetitions are indicative that the ELS animals may have an improvement in learning and memory adaptive flexibility, thus suggesting increased "resilience" as a result of the ELS exposure.

Memory is a highly dynamic process that is built from initial encoding to the new and fragile memory trace that is stabilized during consolidation and reactivated during memory retrieval (Abel & Lattal, 2001; Bisaz, Travaglia, & Alberini, 2014). Memory can also return to an unstable state in which reconsolidation is needed to stabilize it (Dudai, 2006; Lv, 2015). Stress has been shown to affect all phases of memory (encoding, consolidation, memory retrieval, and reconsolidation); however, how stress influences memory depends on *when* an individual is stressed, and what *frequency* and *intensity* of that stress are (Schwabe & Wolf, 2012). When looking at resilience induced by stress, it seems that recovery from stress-inducing changes in neural architecture is not simply a reversal, but instead is a form of neuroplastic adaptation (McEwen, Gray, et al., 2015). Although neural plasticity and epigenetic factors are known to underlie the mechanisms responsible for ELS inducing resilience in adults (Cadet, 2016; Kentner, Cryan, & Brummelte, 2018; McEwen, Gray, et al., 2015), there remains no clear understanding of the mechanisms involved in these processes and how they are functionally associated.

5 | CONCLUSION

Here, we show for the first time that adult zebrafish exposed to moderate ELS can build resilience as evidenced by increases in working memory, but without changing anxiety-like behavior or social behavior patterns. We suggest that this protocol could serve as a useful model to understand: (a) translational genetic and physiological aspects are associated with memory adaptive flexibility; (b) the evolutionary and conserved characteristics that are common between zebrafish and humans correspond to stress-induced changes in memory and learning. Overall, future studies should investigate how moderate ELS can modulate neuroplastic adaptation and epigenetics in zebrafish adaptive flexibility and resilience.

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CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

DATA AVAILABILITY STATEMENT

All raw data from this study are openly available in the University of Portsmouth Pure repository, at researchportal.port.ac.uk/portal/

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REFERENCES

Abel, T., & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Current Opinion in Neurobiology*, *11*(2), 180–187. https://doi.org/10.1016/S0959-4388(00)00194-X

Alsop, D., & Vijayan, M. M. (2008). Development of the corticosteroid stress axis and receptor expression in zebrafish. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology*, *294*(3), R711–719. https://doi.org/10.1152/ajpregu.00671.2007

Alsop, D., & Vijayan, M. (2009). The zebrafish stress axis: Molecular fallout from the teleost-specific genome duplication event. *General and Comparative Endocrinology*, *161*(1), 62–66. https://doi.org/10.1016/j.ygcen.2008.09.011

Alsop, D., & Vijayan, M. M. (2009). Molecular programming of the corticosteroid stress axis during zebrafish development. *Comparative Biochemistry and Physiology Part A Molecular Integrative Physiology*, *153*(1), 49–54. https://doi.org/10.1016/j.cbpa.2008.12.008

Anda, R. F., Felitti, V. J., Bremner, J. D., Walker, J. D., Whitfield, C. H., Perry, B. D., … Giles, W. H. (2006). The enduring effects of abuse and related adverse experiences in childhood. A convergence of evidence from neurobiology and epidemiology. *European Archives of Psychiatry and Clinical Neuroscience*, *256*(3), 174–186. https://doi.org/10.1007/s00406-005-0624-4

Aoki, R., Tsuboi, T., & Okamoto, H. (2015). Y-maze avoidance: An automated and rapid associative learning paradigm in zebrafish. *Neuroscience Research*, *91*, 69–72. https://doi.org/10.1016/j.neures.2014.10.012

Ariyomo, T. O., Carter, M., & Watt, P. J. (2013). Heritability of boldness and aggressiveness in the zebrafish. *Behavior Genetics*, *43*(2), 161–167. https://doi.org/10.1007/s10519-013-9585-y

De Bellis, M. D., Keshavan, M. S., Clark, D. B., Casey, B. J., Giedd, J. N., Boring, A. M., Ryan, N. D. (1999). A.E. Bennett research award. Developmental traumatology. Part II: Brain development. *Biological Psychiatry*, *45*(10), 1271–1284.

Bisaz, R., Travaglia, A., & Alberini, C. M. (2014). The neurobiological bases of memory formation: From physiological conditions to psychopathology. *Psychopathology*, *47*(6), 347–356. https://doi.org/10.1159/000363702

Blaser, R. E., & Rosemberg, D. B. (2012). Measures of anxiety in zebrafish (*Danio rerio*): Dissociation of black/white preference and novel tank test. *PLoS ONE*, *7*(5), e36931. https://doi.org/10.1371/journal.pone.0036931

Bogdan, R., & Pizzagalli, D. A. (2006). Acute stress reduces reward responsiveness: Implications for depression. *Biological Psychiatry*, *60*(10), 1147–1154. https://doi.org/10.1016/j.biopsych.2006.03.037

Cachat, J., Stewart, A., Grossman, L., Gaikwad, S., Kadri, F., Chung, K. M., … Kalueff, A. V. (2010). Measuring behavioral and endocrine responses to novelty stress in adult zebrafish. *Nature Protocols*, *5*(11), 1786–1799. https://doi.org/10.1038/nprot.2010.140

Cadet, J. L. (2016). Epigenetics of Stress, Addiction, and Resilience: Therapeutic Implications. *Molecular Neurobiology*, *53*(1), 545–560. https://doi.org/10.1007/s12035-014-9040-y

Clark, K. J., Boczek, N. J., & Ekker, S. C. (2011). Stressing zebrafish for behavioral genetics. *Reviews in the Neurosciences*, *22*(1), 49–62. https://doi.org/10.1515/RNS.2011.007

Cleal, F. B. D., Ranson, D. C., McBride, S. D., Swinny, J. D., Redhead, E. S., & Parker, M. O. (2020). A test of memory: The fish, the mouse, the fly and the human. *bioRxiv*, *[Preprint]*, https://doi.org/10.1101/2020.02.15.950816

Cleal, M., & Parker, M. O. (2018). Moderate developmental alcohol exposure reduces repetitive alternation in a zebrafish model of fetal alcohol spectrum disorders. *Neurotoxicology and Teratology*, *70*, 1–9. https://doi.org/10.1016/j.ntt.2018.09.001

Coffino, B. (2009). The role of childhood parent figure loss in the etiology of adult depression: Findings from a prospective longitudinal study. *Attachment & Human Development*, *11*(5), 445–470. https://doi.org/10.1080/14616730903135993

Cognato Gde, P., Bortolotto, J. W., Blazina, A. R., Christoff, R. R., Lara, D. R., Vianna, M. R., & Bonan, C. D. (2012). Y-Maze memory task in zebrafish (*Danio rerio*): The role of glutamatergic and cholinergic systems on the acquisition and consolidation periods. *Neurobiology of Learning and Memory*, *98*(4), 321–328. https://doi.org/10.1016/j.nlm.2012.09.008

Coplan, J. D., Andrews, M. W., Rosenblum, L. A., Owens, M. J., Friedman, S., Gorman, J. M., & Nemeroff, C. B. (1996). Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and anxiety disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(4), 1619–1623. https://doi.org/10.1073/pnas.93.4.1619

Cunha, G. M., Canas, P. M., Melo, C. S., Hockemeyer, J., Muller, C. E., Oliveira, C. R., & Cunha, R. A. (2008). Adenosine A2A receptor blockade prevents memory dysfunction caused by beta-amyloid peptides but not by scopolamine or MK-801. *Experimental Neurology*, *210*(2), 776–781. https://doi.org/10.1016/j.expneurol.2007.11.013

De Polavieja, G. G., & Orger, M. B. (2018). Social behavior: A neural circuit for social behavior in Zebrafish. *Current Biology*, *28*(15), R828–R830. https://doi.org/10.1016/j.cub.2018.06.065

Dreosti, E., Lopes, G., Kampff, A. R., & Wilson, S. W. (2015). Development of social behavior in young zebrafish. *Frontiers in Neural Circuits*, *9*, 39. https://doi.org/10.3389/fncir.2015.00039

Drew, W. G., Miller, L. L., & Baugh, E. L. (1973). Effects of delta9-THC, LSD-25 and scopolamine on continuous, spontaneous alternation in the Y-maze. *Psychopharmacologia*, *32*(2), 171–182.

Dudai, Y. (2006). Reconsolidation: The advantage of being refocused. *Current Opinion in Neurobiology*, *16*(2), 174–178. https://doi.org/10.1016/j.conb.2006.03.010

Dunphy-Doherty, F., O'Mahony, S. M., Peterson, V. L., O'Sullivan, O., Crispie, F., Cotter, P. D., … Fone, K. C. F. (2018). Post-weaning social isolation of rats leads to long-term disruption of the gut

microbiota-immune-brain axis. *Brain, Behavior, and Immunity*, *68*, 261–273. https://doi.org/10.1016/j.bbi.2017.10.024

Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., … Kalueff, A. V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioral Brain Research*, *205*(1), 38–44. https://doi.org/10.1016/j.bbr.2009.06.022

Fontana, B. D., Cleal, M., Clay, J. M., & Parker, M. O. (2019). Zebrafish (*Danio rerio*) behavioral laterality predicts increased short-term avoidance memory but not stress-reactivity responses. *Animal Cognition*, *22*(6), 1051–1061. https://doi.org/10.1007/s10071-019-01296-9

Fontana, B. D., Cleal, M., & Parker, M. O. (2019). Female adult zebrafish (*Danio rerio*) show higher levels of anxiety-like behavior than males, but do not differ in learning and memory capacity. *European Journal of Neuroscience*, *00*, 1–10. https://doi.org/10.1111/ejn.14588

Frith, C. D., & Done, D. J. (1983). Stereotyped responding by schizophrenic patients on a two-choiceguessing task. *Psychological medicine*, *13*(4), 779–786.

Fu, Y., Chen, Y., Li, L., Wang, Y., Kong, X., & Wang, J. (2017). Food restriction affects Y-maze spatial recognition memory in developing mice. *International Journal of Developmental Neuroscience*, *60*, 8–15. https://doi.org/10.1016/j.ijdevneu.2017.03.010

Fulcher, N., Tran, S., Shams, S., Chatterjee, D., & Gerlai, R. (2017). Neurochemical and behavioral responses to unpredictable chronic mild stress following developmental isolation: The zebrafish as a model for major depression. *Zebrafish*, *14*(1), 23–34. https://doi.org/10.1089/zeb.2016.1295

Ghafouri, S., Fathollahi, Y., Javan, M., Shojaei, A., Asgari, A., & Mirnajafi-Zadeh, J. (2016). Effect of low frequency stimulation on impaired spontaneous alternation behavior of kindled rats in Y-maze test. *Epilepsy Research*, *126*, 37–44. https://doi.org/10.1016/j.eplepsyres.2016.06.010

Gilbert, R., Widom, C. S., Browne, K., Fergusson, D., Webb, E., & Janson, S. (2009). Burden and consequences of child maltreatment in high-income countries. *Lancet*, *373*(9657), 68–81. https://doi.org/10.1016/S0140-6736(08)61706-7

Gluckman, P. D., Hanson, M. A., & Beedle, A. S. (2007). Early life events and their consequences for later disease: A life history and evolutionary perspective. *American Journal of Human Biology*, *19*(1), 1–19. https://doi.org/10.1002/ajhb.20590

Grassi-Oliveira, R., Ashy, M., & Stein, L. M. (2008). Psychobiology of childhood maltreatment: Effects of allostatic load? *Brazilian Journal of Psychiatry*, *30*(1), 60–68. https://doi.org/10.1590/S1516-44462008000100012

Gross, A. N., Engel, A. K., Richter, S. H., Garner, J. P., & Wurbel, H. (2011). Cage-induced stereotypies in female ICR CD-1 mice do not correlate with recurrent perseveration. *Behavioral Brain Research*, *216*(2), 613–620. https://doi.org/10.1016/j.bbr.2010.09.003

Grunwald, D. J., & Eisen, J. S. (2002). Headwaters of the zebrafish – Emergence of a new model vertebrate. *Nature Reviews Genetics*, *3*(9), 717–724. https://doi.org/10.1038/nrg892

Haller, J., Harold, G., Sandi, C., & Neumann, I. D. (2014). Effects of adverse early-life events on aggression and anti-social behaviours in animals and humans. *Journal of Neuroendocrinology*, *26*(10), 724–738. https://doi.org/10.1111/jne.12182

Heim, C., & Nemeroff, C. B. (2001). The role of childhood trauma in the neurobiology of mood and anxiety disorders: Preclinical and clinical studies. *Biological Psychiatry*, *49*(12), 1023–1039. https://doi.org/10.1016/S0006-3223(01)01157-X

Hiramatsu, M., & Inoue, K. (2000). Improvement by low doses of nociceptin on scopolamine-induced impairment of learning and/or memory. *European Journal of Pharmacology*, *395*(2), 149–156. https://doi.org/10.1016/S0014-2999(00)00162-X

Holzschuh, J., Ryu, S., Aberger, F., & Driever, W. (2001). Dopamine transporter expression distinguishes dopaminergic neurons from other catecholaminergic neurons in the developing zebrafish embryo. *Mechanisms of Development*, *101*(1–2), 237–243. https://doi.org/10.1016/S0925-4773(01)00287-8

Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., … Stemple, D. L. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, *496*(7446), 498–503. https://doi.org/10.1038/nature12111

Hughes, R. N. (2004). The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. *Neuroscience and Biobehavioral Reviews*, *28*(5), 497–505. https://doi.org/10.1016/j.neubiorev.2004.06.006

Kajantie, E., & Raikkonen, K. (2010). Early life predictors of the physiological stress response later in life. *Neuroscience and Biobehavioral Reviews*, *35*(1), 23–32. https://doi.org/10.1016/j.neubiorev.2009.11.013

Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., & Chawla, J. S. … Zebrafish Neuroscience Research, C. (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish*, *10*(1), 70–86. doi:https://doi.org/10.1089/zeb.2012.0861

Kaufman, J., Plotsky, P. M., Nemeroff, C. B., & Charney, D. S. (2000). Effects of early adverse experiences on brain structure and function: Clinical implications. *Biological Psychiatry*, *48*(8), 778–790. https://doi.org/10.1016/S0006-3223(00)00998-7

Kentner, A. C., Cryan, J. F., & Brummelte, S. (2018). Resilience priming: Translational models for understanding resiliency and adaptation to early life adversity. *Developmental Psychobiology*, *61*(3), 350–375. https://doi.org/10.1002/dev.21775

Kohl, C., Wang, X.-D., Grosse, J., Fournier, C., Harbich, D., Westerholz, S., … Schmidt, M. V. (2015). Hippocampal neuroligin-2 links early-life stress with impaired social recognition and increased aggression in adult mice. *Psychoneuroendocrinology*, *55*, 128–143. https://doi.org/10.1016/j.psyneuen.2015.02.016

Kokkinidis, L., & Anisman, H. (1976). Interaction between cholinergic and catecholaminergic agents in a spontaneous alternation task. *Psychopharmacology (Berl)*, *48*(3), 261–270. https://doi.org/10.1007/BF00496859

Lovallo, W. R. (2013). Early life adversity reduces stress reactivity and enhances impulsive behavior: Implications for health behaviors. *International Journal of Psychophysiology*, *90*(1), 8–16. https://doi.org/10.1016/j.ijpsycho.2012.10.006

Luine, V. (2015). Recognition memory tasks in neuroendocrine research. *Behavioral Brain Research*, *285*, 158–164. https://doi.org/10.1016/j.bbr.2014.04.032

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445. https://doi.org/10.1038/nrn2639

Lv, K. (2015). The involvement of working memory and inhibition functions in the different phases of insight problem solving. *Memory & Cognition*, *43*(5), 709–722. https://doi.org/10.3758/s13421-014-0498-7

Lyons, D. M., Parker, K. J., & Schatzberg, A. F. (2010). Animal models of early life stress: Implications for understanding resilience. *Developmental Psychobiology*, *52*(7), 616–624. https://doi.org/10.1002/dev.20500

MacRae, C. A., & Peterson, R. T. (2015). Zebrafish as tools for drug discovery. *Nature Reviews Drug Discovery*, *14*(10), 721–731. https://doi.org/10.1038/nrd4627

Maier, S. F., Amat, J., Baratta, M. V., Paul, E., & Watkins, L. R. (2006). Behavioral control, the medial prefrontal cortex, and resilience. *Dialogues in Clinical Neuroscience*, *8*(4), 397–406.

Maniam, J., Antoniadis, C., & Morris, M. J. (2014). Early-life stress, HPA axis adaptation, and mechanisms contributing to later health outcomes. *Frontiers in Endocrinology*, *5*, 73. https://doi.org/10.3389/fendo.2014.00073

Maniglio, R. (2009). The impact of child sexual abuse on health: A systematic review of reviews. *Clinical Psychology Review*, *29*(7), 647–657. https://doi.org/10.1016/j.cpr.2009.08.003

Maximino, C., Meinerz, D. L., Fontana, B. D., Mezzomo, N. J., Stefanello, F. V., de S. Prestes, A., … Rosemberg, D. B. (2018). Extending the analysis of zebrafish behavioral endophenotypes for modeling psychiatric disorders: Fear conditioning to conspecific alarm response. *Behavioural Processes*, *149*, 35–42. https://doi.org/10.1016/j.beproc.2018.01.020

McEwen, B. S., Bowles, N. P., Gray, J. D., Hill, M. N., Hunter, R. G., Karatsoreos, I. N., & Nasca, C. (2015). Mechanisms of stress in the brain. *Nature Neuroscience*, *18*(10), 1353–1363. https://doi.org/10.1038/nn.4086

McEwen, B. S., Gray, J., & Nasca, C. (2015). Recognizing resilience: Learning from the effects of stress on the brain. *Neurobiology of Stress*, *1*, 1–11. https://doi.org/10.1016/j.ynstr.2014.09.001

Mezzomo, N. J., Silveira, A., Giuliani, G. S., Quadros, V. A., & Rosemberg, D. B. (2016). The role of taurine on anxiety-like behaviors in zebrafish: A comparative study using the novel tank and the light-dark tasks. *Neuroscience Letters*, *613*, 19–24. https://doi.org/10.1016/j.neulet.2015.12.037

Molet, J., Maras, P. M., Avishai-Eliner, S., & Baram, T. Z. (2014). Naturalistic rodent models of chronic early-life stress. *Developmental Psychobiology*, *56*(8), 1675–1688. https://doi.org/10.1002/dev.21230

Nishi, M., Horii-Hayashi, N., Sasagawa, T., & Matsunaga, W. (2013). Effects of early life stress on brain activity: Implications from maternal separation model in rodents. *General and Comparative Endocrinology*, *181*, 306–309. https://doi.org/10.1016/j.ygcen.2012.09.024

Norton, W. H. J. (2018). Screening for drugs to reduce aggression in zebrafish. *Neuropharmacology*, *156*, 107394. https://doi.org/10.1016/j.neuropharm.2018.10.023

Nugent, N. R., Tyrka, A. R., Carpenter, L. L., & Price, L. H. (2011). Gene-environment interactions: Early life stress and risk for depressive and anxiety disorders. *Psychopharmacology (Berl)*, *214*(1), 175–196. https://doi.org/10.1007/s00213-010-2151-x

O'Mahony, S. M., Marchesi, J. R., Scully, P., Codling, C., Ceolho, A.-M., Quigley, E. M. M., … Dinan, T. G. (2009). Early life stress alters behavior, immunity, and microbiota in rats: Implications for irritable bowel syndrome and psychiatric illnesses. *Biological Psychiatry*, *65*(3), 263–267. https://doi.org/10.1016/j.biopsych.2008.06.026

Ouchi, R., Kawano, T., Yoshida, H., Ishii, M., Miyasaka, T., Ohkawara, Y., … Ohno, I. (2018). Maternal separation as early-life stress causes enhanced allergic airway responses by inhibiting respiratory tolerance in mice. *Tohoku Journal of Experimental Medicine*, *246*(3), 155–165. https://doi.org/10.1620/tjem.246.155

Parihar, V. K., Hattiangady, B., Kuruba, R., Shuai, B., & Shetty, A. K. (2011). Predictable chronic mild stress improves mood, hippocampal neurogenesis and memory. *Molecular Psychiatry*, *16*(2), 171–183. https://doi.org/10.1038/mp.2009.130

Park, S. J., Kim, D. H., Lee, I. K., Jung, W. Y., Park, D. H., Kim, J. M., … Ryu, J. H. (2010). The ameliorating effect of the extract of the flower of *Prunella vulgaris* var. lilacina on drug-induced memory impairments in mice. *Food and Chemical Toxicology*, *48*(6), 1671–1676. https://doi.org/10.1016/j.fct.2010.03.042

Parker, M. O., Annan, L. V., Kanellopoulos, A. H., Brock, A. J., Combe, F. J., Baiamonte, M., … Brennan, C. H. (2014). The utility of zebrafish to study the mechanisms by which ethanol affects social behavior and anxiety during early brain development. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *55*, 94–100. https://doi.org/10.1016/j.pnpbp.2014.03.011

Parker, M. O., Brock, A. J., Millington, M. E., & Brennan, C. H. (2013). Behavioural phenotyping of casper mutant and 1-pheny-2-thiourea treated adult zebrafish. *Zebrafish*, *10*(4), 466–471. https://doi.org/10.1089/zeb.2013.0878

Parker, M. O., Millington, M. E., Combe, F. J., & Brennan, C. H. (2012). Housing conditions differentially affect physiological and behavioural stress responses of zebrafish, as well as the response to anxiolytics. *PLoS ONE*, *7*(4), e34992. https://doi.org/10.1371/journal.pone.0034992

Paull, G. C., Filby, A. L., Giddins, H. G., Coe, T. S., Hamilton, P. B., & Tyler, C. R. (2010). Dominance hierarchies in zebrafish (*Danio rerio*) and their relationship with reproductive success. *Zebrafish*, *7*(1), 109–117. https://doi.org/10.1089/zeb.2009.0618

Pavlidis, M., Digka, N., Theodoridi, A., Campo, A., Barsakis, K., Skouradakis, G., … Tsalafouta, A. (2013). Husbandry of zebrafish, Danio rerio, and the cortisol stress response. *Zebrafish*, *10*(4), 524–531. https://doi.org/10.1089/zeb.2012.0819

Pechtel, P., & Pizzagalli, D. A. (2011). Effects of early life stress on cognitive and affective function: An integrated review of human literature. *Psychopharmacology (Berl)*, *214*(1), 55–70. https://doi.org/10.1007/s00213-010-2009-2

Piato, Â. L., Capiotti, K. M., Tamborski, A. R., Oses, J. P., Barcellos, L. J. G., Bogo, M. R., … Bonan, C. D. (2011). Unpredictable chronic stress model in zebrafish (Danio rerio): Behavioral and physiological responses. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*(2), 561–567. https://doi.org/10.1016/j.pnpbp.2010.12.018

Ramsay, J. M., Feist, W. G., Varga, Z. M., Westerfield, M., Kentgen, L. M., & Schrecka, C. B. (2006). Whole-body cortisol is an indicator of crowding stress in adult zebrafish, *Danio rerio*. *Aquaculture*, *258*(1–4), 565–574. https://doi.org/10.1016/j.aquaculture.2006.04.020

Rice, C. J., Sandman, C. A., Lenjavi, M. R., & Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology*, *149*(10), 4892–4900. https://doi.org/10.1210/en.2008-0633

Rico, E. P., Rosemberg, D. B., Seibt, K. J., Capiotti, K. M., Da Silva, R. S., & Bonan, C. D. (2011). Zebrafish neurotransmitter systems as potential pharmacological and toxicological targets. *Neurotoxicology and Teratology*, *33*(6), 608–617. https://doi.org/10.1016/j.ntt.2011.07.007

Rosemberg, D. B., Braga, M. M., Rico, E. P., Loss, C. M., Córdova, S. D., Mussulini, B. H. M., … & Calcagnotto, M. E. (2012). Behavioral effects of taurine pretreatment in zebrafish acutely exposed to ethanol. *Neuropharmacology*, *63*(4), 613–623. https://doi.org/10.1016/j.neuropharm.2012.05.009

Russo, S. J., Murrough, J. W., Han, M. H., Charney, D. S., & Nestler, E. J. (2012). Neurobiology of resilience. *Nature Neuroscience*, *15*(11), 1475–1484. https://doi.org/10.1038/nn.3234

Sakai, C., Ijaz, S., & Hoffman, E. J. (2018). Zebrafish models of neurodevelopmental disorders: Past, present, and future. *Frontiers in Molecular Neuroscience*, *11*, 294. https://doi.org/10.3389/fnmol.2018.00294

Sapolsky, R. M. (2015). Stress and the brain: Individual variability and the inverted-U. *Nature Neuroscience*, *18*(10), 1344–1346. https://doi.org/10.1038/nn.4109

Saverino, C., & Gerlai, R. (2008). The social zebrafish: Behavioral responses to conspecific, heterospecific, and computer animated fish. *Behavioral Brain Research*, *191*(1), 77–87. https://doi.org/10.1016/j.bbr.2008.03.013

Scheller-Gilkey, G., Moynes, K., Cooper, I., Kant, C., & Miller, A. H. (2004). Early life stress and PTSD symptoms in patients with comorbid schizophrenia and substance abuse. *Schizophrenia Research*, *69*(2–3), 167–174. https://doi.org/10.1016/S0920-9964(03)00188-9

Schwabe, L., & Wolf, O. T. (2012). Stress modulates the engagement of multiple memory systems in classification learning. *Journal of Neuroscience*, *32*(32), 11042–11049. https://doi.org/10.1523/JNEUROSCI.1484-12.2012

Shapero, B. G., Hamilton, J. L., Stange, J. P., Liu, R. T., Abramson, L. Y., & Alloy, L. B. (2015). Moderate childhood stress buffers against depressive response to proximal stressors: A multi-wave prospective study

of early adolescents. *Journal of Abnormal Child Psychology*, *43*(8), 1403–1413. https://doi.org/10.1007/s10802-015-0021-z

Song, C., Liu, B.-P., Zhang, Y.-P., Peng, Z., Wang, J. J., Collier, A. D., … Kalueff, A. V. (2018). Modeling consequences of prolonged strong unpredictable stress in zebrafish: Complex effects on behavior and physiology. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *81*, 384–394. https://doi.org/10.1016/j.pnpbp.2017.08.021

Spataro, J., Mullen, P. E., Burgess, P. M., Wells, D. L., & Moss, S. A. (2004). Impact of child sexual abuse on mental health: Prospective study in males and females. *British Journal of Psychiatry*, *184*, 416–421. https://doi.org/10.1192/bjp.184.5.416

Spinelli, S., Chefer, S., Suomi, S. J., Higley, J. D., Barr, C. S., & Stein, E. (2009). Early-life stress induces long-term morphologic changes in primate brain. *Archives of General Psychiatry*, *66*(6), 658–665. https://doi.org/10.1001/archgenpsychiatry.2009.52

Swonger, A. K., & Rech, R. H. (1972). Serotonergic and cholinergic involvement in habituation of activity and spontaneous alternation of rats in a Y maze. *Journal of Comparative and Physiological Psychology*, *81*(3), 509–522.

Teicher, M. H., Andersen, S. L., Polcari, A., Anderson, C. M., Navalta, C. P., & Kim, D. M. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience and Biobehavioral Reviews*, *27*(1–2), 33–44. https://doi.org/10.1016/S0149-7634(03)00007-1

Teicher, M. H., Tomoda, A., & Andersen, S. L. (2006). Neurobiological consequences of early stress and childhood maltreatment: Are results from human and animal studies comparable? *Annals of the New York Academy of Sciences*, *1071*, 313–323. https://doi.org/10.1196/annals.1364.024

Tsuda, M. C., Yamaguchi, N., & Ogawa, S. (2011). Early life stress disrupts peripubertal development of aggression in male mice. *NeuroReport*, *22*(6), 259–263. https://doi.org/10.1097/WNR.0b013e328344495a

Valente, A., Huang, K. H., Portugues, R., & Engert, F. (2012). Ontogeny of classical and operant learning behaviors in zebrafish. *Learning & Memory*, *19*(4), 170–177. https://doi.org/10.1101/lm.025668.112

Veenema, A. H. (2009). Early life stress, the development of aggression and neuroendocrine and neurobiological correlates: What can we learn from animal models? *Frontiers in Neuroendocrinology*, *30*(4), 497–518. https://doi.org/10.1016/j.yfrne.2009.03.003

Veenema, A. H., Blume, A., Niederle, D., Buwalda, B., & Neumann, I. D. (2006). Effects of early life stress on adult male aggression and hypothalamic vasopressin and serotonin. *European Journal of Neuroscience*, *24*(6), 1711–1720. https://doi.org/10.1111/j.1460-9568.2006.05045.x

Walker, D. L., & Gold, P. E. (1992). Impairment of spontaneous alternation performance by an NMDA antagonist: Attenuation with non-NMDA treatments. *Behavioral and Neural Biology*, *58*(1), 69–71. https://doi.org/10.1016/0163-1047(92)90952-Z

Weber, D. A., & Reynolds, C. R. (2004). Clinical perspectives on neurobiological effects of psychological trauma. *Neuropsychology Review*, *14*(2), 115–129. https://doi.org/10.1023/B:NERV.0000028082.13778.14

Wong, K., Elegante, M., Bartels, B., Elkhayat, S., Tien, D., Roy, S., … Kalueff, A. V. (2010). Analyzing habituation responses to novelty in zebrafish (Danio rerio). *Behavioral Brain Research*, *208*(2), 450–457. https://doi.org/10.1016/j.bbr.2009.12.023

Zannas, A. S., & West, A. E. (2014). Epigenetics and the regulation of stress vulnerability and resilience. *Neuroscience*, *264*, 157–170. https://doi.org/10.1016/j.neuroscience.2013.12.003

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