Recovery Pattern of Behavioral Responses in Female Zebrafish to Short-term Amitriptyline Hydrochloride Exposure

LI, Lixia
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

DONG, Kejun
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

CHEN, Chen
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

CHEN, Kun
Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University

他

https://doi.org/10.5109/4797825

出版情報：九州大学大学院農学研究院紀要. 67 (2), pp.181-187, 2022-09. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **67** (2), 181–187 (2022)

Recovery Pattern of Behavioral Responses in Female Zebrafish to Short–term Amitriptyline Hydrochloride Exposure

Lixia LI[1], Kejun DONG[1], Chen CHEN[1], Kun CHEN[1], Yanhong SHI[1], Xuchun QIU[1,2]*, Yohei SHIMASAKI and Yuji OSHIMA

Laboratory of Marine Environmental Science, Faculty of Agriculture, Kyushu University, Fukuoka 819–0395, Japan

(*Received May 9, 2022 and accepted May 10, 2022*)

Amitriptyline (AMI) is a tricyclic antidepressant (TCA) that has been widely used to treat depression. The widespread use has caused AMI to enter the environmental waters with a maximum concentration of μg/L. However, knowledge of its persistent toxic effects on fish is still limited. Therefore, this study exposed female zebrafish to 10 μg/L of AMI for seven days, followed by a 21–day recovery in AMI–free water. The results showed that AMI could induce persistent or delayed hypoactivity and abnormal social behavior in zebrafish. AMI exposure also significantly altered the brain levels of 5–HT, 5–HIAA, and ACH. Correlation analysis revealed that 5–HT and ACH were negatively correlated with locomotor activity, and 5–HT was also positively correlated with social interaction. Our finding demonstrated that AMI could cause persistent abnormal behavior in zebrafish and alter the brain neurotransmitter levels in zebrafish, suggesting that those long–term impact should not be ignored.

Key words: Amitriptyline; Zebrafish; Neurotransmitter levels; Behavior; Recovery

INTRODUCTION

Amitriptyline (AMI) is a kind of tricyclic antidepressant (TCAs) that has been widely used to treat depression (Kaur and Malik 2013). The TCAs can achieve antidepressant effects by inhibiting serotonin and norepinephrine reuptake, and topical application of AMI also can relieve neuropathic pain (Abdollahi and Mostafalou 2014; Thompson and Brooks 2015; Chang *et al*., 2021). The widespread use has caused TCAs to enter the environmental waters through human excretion, disposal of unused medicines, medical effluent discharges, and others (Li *et al*., 2013; Choi *et al*., 2018; Chang *et al*., 2021). As a result, the TCAs residues in water may lead to continuous exposure of aquatic organisms to the active ingredients of such drugs, which pose potential risks to aquatic systems (Lajeunesse *et al*., 2011).

Antidepressants can affect brain neurotransmitter levels in fish, thereby influencing a series of behaviors even at the environmentally relevant concentration (David *et al*., 2018; Qiu *et al*., 2022a). Previous studies have reported that exposure to antidepressants could affect the activity, socialization, survival, growth, predator, learning, and social interactions of aquatic organisms (Yang *et al*., 2014; Eisenreich and Szalda–Petree 2015; Qiu *et al*., 2022a). In mammals, discontinuation of antidepressants could induce various withdrawn syndromes (Cosci and Chouinard 2020). Similarly, exposure to fluoxetine for 6 days resulted in a decreased serotonin level and impaired hunting ability of bass, and the serotonin level did not return to the control levels during the 6–day recovery period (Gaworecki and Klaine 2008). Exposure to venlafaxine (250 and 500 μg/L) caused an increase in predatory behavior and a significant decrease in brain serotonin levels in bass during exposure, with the serotonin levels returning to normal during the 6–days recovery period (Bisesi *et al*., 2014). Short–term exposure to antidepressants could have long–term adverse effects on fish.

Zebrafish is a model organism and has been widely used in ecotoxicological studies (Grabicova *et al*., 2014; Arnnok *et al*., 2017; Melvin 2017). This model organism has previously been used to study behavioral changes caused by various neurotoxic substances (Bisesi *et al*., 2014; Demin *et al*., 2017; Chen *et al*., 2021). In a previous study, we reported that short–term AMI exposure at 40 μg/L induced long–term hypoactivity and abnormal schooling behavior and found that the upregulated brain 5–HT may serve as the central modulator of the above persistent behavioral impacts (Qiu *et al*., 2022a). Considering that AMI residues can be up to 6.7 μg/L in wastewaters (Kasprzyk–Hordern *et al*., 2009; Richmond *et al*., 2018), fish may temporarily be exposed to AMI at relatively high concentrations due to periodic emissions from wastewater treatment plants. Thus, more insights into the recovery pattern of behavioral responses to short–term AMI exposure are critical for comprehensively assessing their risks.

Therefore, in the present study, we exposed zebrafish to AMI at 10 μg/L (near the environmentally relevant concentration) for 7 days and then transferred zebrafish to AMI–free water to recover for 21 days. Locomotor activity and social behavior of zebrafish were examined at the end of the exposure and on days 7 and 21 of the recovery period. The brain neurotransmitter was also measured to evaluate their relationship to behavior. This

[1] Institute of Environmental Health and Ecological Security, School of the Environment and Safety Engineering, Jiangsu University, Zhenjiang, Jiangsu 212013, China
[2] Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment, Suzhou University of Science and Technology, Suzhou 215009, China
* Corresponding author: e–mail: xuchunqiu@ujs.edu.cn (Xuchun QIU)

study aimed to investigate the recovery pattern of female zebrafish to short–term AMI exposure and the role of brain neurotransmitters in those processes.

MATERIALS AND METHODS

Test organism

Female zebrafish (AB–strain, six months after hatching) were maintained in two 16–L circular glass aquariums (40 fish per tank) containing 12 L of dechlorinated tap water (conductivity at 0.50–0.53 mS/cm; 27±1°C; light: dark cycle=14 L:10 D h). The fish were fed with *Artemia nauplii* (<24 h after hatching) twice per day, and half of the water volume was changed every 24 h.

Chemicals

Amitriptyline hydrochloride (CAS No. 439–14–5) was purchased from the Dalian Meilun Biotechnology Co., Ltd. (Dalian, China). Other reagents at analytical grade were purchased from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Exposure and recovery

The test solution of AMI (10 μg/L) was prepared by pipetting calculated amounts of the amitriptyline hydrochloride stock solution into dechlorinated tap water. In the control group, only dechlorinated tap water was used. For the exposure experiment, 10 healthy individuals were introduced into 3–L glass cylindrical tanks that contained 2.5 L of a test solution, with three replicates (n=3). Thus, a total of 60 female zebrafish were used in this study. The exposure was conducted for 7 days (not feeding), and half of the test solutions were renewed every 24 h. After the 7–day exposure, half of the zebrafish (i.e., 5 fish of each replicate) were transferred into dechlorinated tap water to recover for 21 days. The recovery test was conducted under the same conditions mentioned in section 2.1, and fish were fed with *A. nauplii* (<24 h after hatching) during the recovery period.

Behavioral assay

The behavioral assay was conducted on day 0 (R–0, i.e., immediately after the 7–day exposure), 7 (R–7), and 21 (R–21) of the recovery period. For this assay, five zebrafish in each tank (as one group) and transferred to an aquarium (20×9×10 cm) containing 1000 mL of dechlorinated tap water. After a 10–min acclimation, fish behavioral traits behavior was tracked for 10 min using the DanioVision system (Noldus, Wageningen, Netherlands) and then analyzed using the EthoVision XT software (Vison 11.5; Noldus). The fish locomotor activity was judged based on the threshold values reported previously (Chen *et al*., 2021), and the shoaling behavior is defined as all five individuals staying together in an area with a diameter of 7 cm (i.e., the distance between an individual and the other individuals is ≤3.5 cm) (Miller and Gerlai 2012).

Brain neurotransmitter levels

At R–0 (i.e., the end of the 7–day exposure period) and R–21 (i.e., the end of the 21–day recovery period), five fish in each tank were placed into an ice water bath (0–4°C) for euthanasia (Wallace *et al*., 2018). Subsequently, the whole brain was extracted and stored at –80°C. For assaying brain neurotransmitter levels, 4 individuals were pooled as one sample, and 3 replicates were used for each treatment group. The homogenate and supernatant for those assays were prepared following the method described by Qiu *et al*. (2022b). Briefly, each sample was homogenized with 9 vol (w/v) phosphate buffer (10 mM, pH 7.2–7.4). Then, the homogenate was centrifuged at 6000× g for 5 min, and the supernatant was collected. The concentrations of serotonin (5–HT), 5–Hydroxyindoleacetic acid (5–HIAA), acetylcholine (ACH), and acetylcholinesterase (ACHE) in each sample were conducted using the corresponding ELISA Kit (Bomei Biotechnology Co., Ltd., Hefei, Anhui, China). The units of each parameter were normalized by the total protein amount (/mg–protein).

Statistical Analysis

A generalized linear model (GzLM) was used to analyze the combined effects of AMI and sampling time on the behavioral traits and the neurotransmitter levels. Following the GzLM analysis, a simple effects analysis was conducted to examine the difference between exposure and control within either time point. For the correlation analysis, data were first normalized to percentages respective to the control, and the correlations of neurotransmitter levels with those of behavioral traits were tested for significance using Pearson correlation analysis. All statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL).

RESULTS

Impacts on the behavior related to locomotor activity

No mortality was observed in all the experimental groups during the four–day exposure. As shown in Fig. 1, significantly altered behavioral traits related to locomotor activity were usually observed on R–7 and R–21. The GzLM model evaluated that AMI exhibited a significant main effect on the duration of low mobility (DLM), while the interaction between AMI and sampling time (ST) exhibited significant effects on the DLM (Table 1). Although no significant difference in the average swimming velocity (ASV) (Fig. 1A) and frequency of low mobility (FLM) (Fig. 1C) was observed at any time point, the maximum swimming velocity (MSV) of zebrafish in the exposure group significantly increased on R–7 (Fig. 1B, p<0.01). The DLM of zebrafish in the exposure group did not significantly change on R–0, but significantly increased to 244% (p<0.01) and 230 % (p< 0.01) of that in the control group on R–7 and R–21, respectively (Fig. 1D). Thus, short–term exposure to AMI could induce delayed hypoactivity in zebrafish.

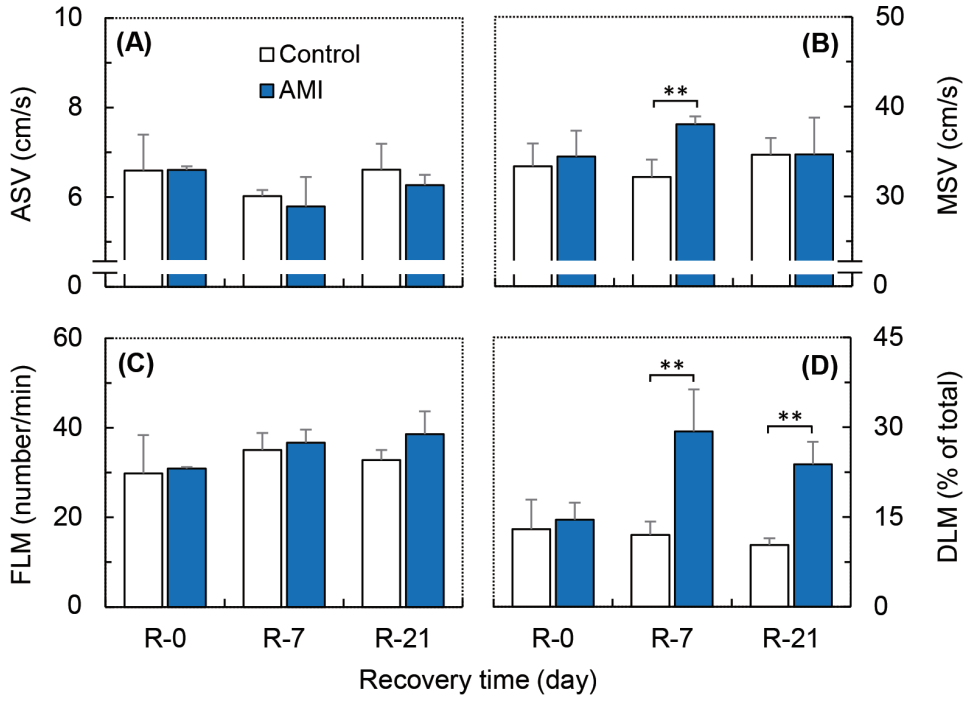

Fig. 1. Variations in the average swimming velocity (ASV, A), maximum swimming velocity (MSV, B), frequency of low mobility (FLM, C), and duration of low mobility (DLM, D) of zebrafish (*Danio rerio*) exposed to amitriptyline at 10 μg/L. Data are mean±SE (n=3), and asterisks indicate significant differences (**p<0.01).

Table 1. Summary of generalized linear model testing the statistical significance for the effect of AMI, sampling time (ST), and their interaction (AMI×ST) on the behavioral traits of zebrafish (*Danio rerio*)

	Locomotor activity				Social interaction			
	ASV	MSV	FLM	DLM	ADI	FBC	DSH	DSO
AMI (*df*=1)	0.50	2.78	1.30	23.1**	12.3**	3.20	0.19	12.0**
ST (*df*=2)	4.76	0.67	3.11	5.07	32.6**	10.4**	0.37	19.8**
AMI×ST (*df*=2)	0.28	4.77	0.88	8.65*	15.9**	8.0*	5.26	19.5**

[a] Wald Chi–Square is listed; *df*: degree of freedom; asterisks indicate statistical significance (*p<0.05; **p<0.01).

Impacts on the behavior related to social interaction

As shown in Fig. 2, significant alteration in the behavioral traits related to social interaction could be observed on R–0, R–7, and R–21. The GzLM model evaluated that AMI exhibited significant main effects on the average distance between individuals (ADI) and duration of solitary (DSO); the ST exhibited significant main effects on the ADI, FBC, and DSO; and their interaction significantly affected the ADI, frequency of body contact (FBC), and DSO (Table 1). The ADI of zebrafish in the exposure group did not significantly change on R–0, but significantly decreased on R–7 (p<0.01) and R–21 (p<0.01), respectively (Fig. 2A). The FBC of zebrafish in the exposure group significantly decreased on R–0 (p<0.01) but did not significantly change on R–7 and R–21 (Fig. 2B). The duration of shoaling (DSH) of zebrafish in the exposure group significantly decreased on R–0 (p<0.05) but significantly increased on R–7 (p<0.05) (Fig. 2C). The DSO of zebrafish in the exposure group did not significantly change on R–0, but significantly decreased on R–7 (p<0.01) and R–21 (p<0.01), respectively (Fig. 2D).

Impacts on the brain neurotransmitter levels

As shown in Fig. 3, significantly altered brain neurotransmitter levels could be observed on both R–0 and R–21. Serotonin (5–HT) in the exposure group did not significantly change on R–0, but significantly increased on R–21 (Fig. 3A, p<0.01). 5–Hydroxyindoleacetic acid (5–HIAA) in the exposure group significantly decreased on R–0 (Fig. 3B, p<0.01) but not on R–21. Acetylcholine (ACH) in the exposure group significantly increased on both R–0 and R–21 (Fig. 3C, p<0.01). Acetylcholinesterase (ACHE) in the exposure group did not significantly change on R–0 and R–21.

Correlations analysis

As shown in Table 2, the 5–HT level was significantly correlated with DLM (positive, p<0.05), ADI (negative,

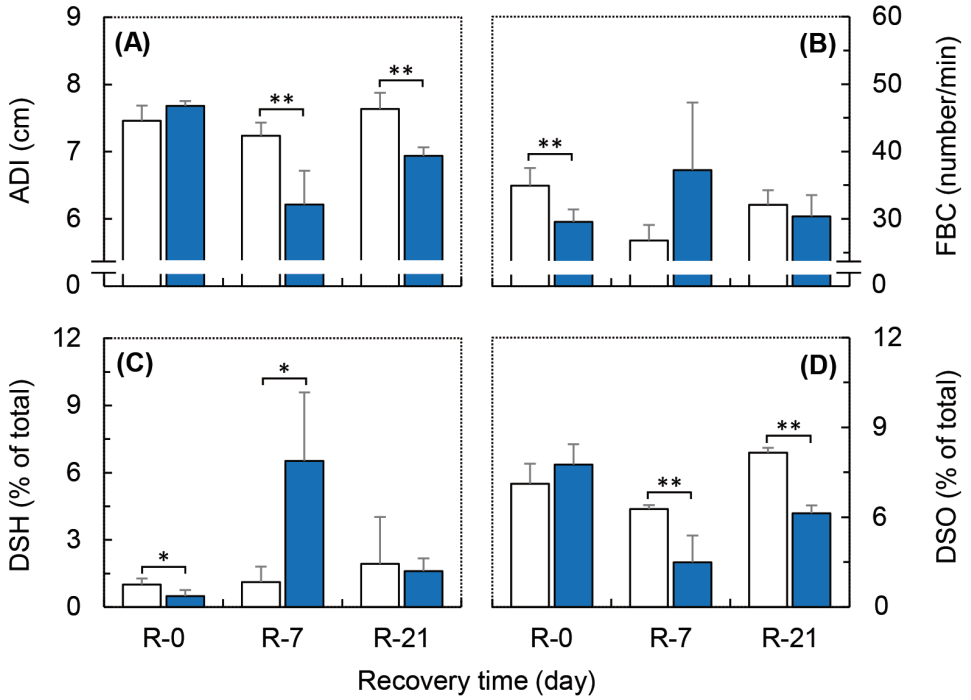

Fig. 2. Variations in the average distance between individuals (ADI, A), frequency of body contact (FBC, B), duration of shoaling (DSH, C), and duration of solitary (DSO, D) of zebrafish (*Danio rerio*) exposed to amitriptyline at 10 μg/L. Data are mean±SE (n=3), and asterisks indicate significant differences (*p<0.05; **p<0.01).

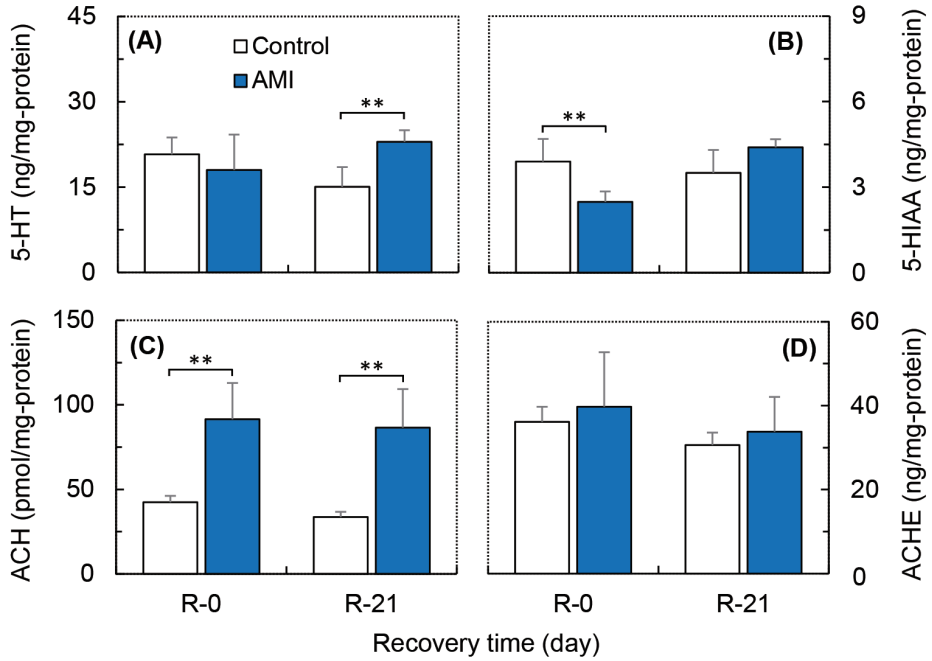

Fig. 3. Variations in the brain neurotransmitter levels of juvenile and adult zebrafish (*Danio rerio*) exposed to amitriptyline at 0 (control)and 10 μg/L for seven days. (A) Serotonin (5–HT); (B) 5–Hydroxyindoleacetic acid (5–HIAA); (C) Acetylcholine (ACH); (D) Acetylcholinesterase (ACHE). Data are mean±SE (n=3), and asterisks indicate significant differences (**p<0.01).

p<0.05), and DSO (negative, p<0.01), and the ACH level was significantly and positively correlated with DLM (p<0.05). However, no significant correlation was observed between the 5–HAA (or ACHE) and the behavioral parameters.

DISCUSSION

Our results demonstrated that 7–days exposure to AMI induced delayed hypoactivity and increased social interaction in zebrafish. Our previous study showed that

Table 2. Pearson correlation between the brain neurotransmitter levels and behavioral parameters of zebrafish (*Danio rerio*)

	Locomotor activity				Social interaction			
	ASV	MSV	FLM	DLM	ADI	FBC	DSH	DSO
5–HT	0.016	0.267	0.101	0.648*	–0.547*	0.132	–0.164	–0.666**
5–HIAA	0.109	0.064	0.018	0.439	–0.416	0.243	–0.161	–0.492
ACH	–0.146	–0.092	0.357	0.601*	–0.388	–0.139	0.023	–0.220
ACHE	–0.054	–0.031	0.017	0.203	–0.128	–0.386	–0.034	0.115

Asterisks indicate significant correlation (*$p<0.05$; ** $p<0.01$).

exposure of zebrafish to AMI (at 40 μg/L) reduced locomotor activity and increased schooling behavior, both of which were reduced after 21 days recovery period (Qiu *et al*., 2022a). Similar to our findings, Sehonova *et al*. (2019) demonstrated that exposure to AMI (at 300 μg/L) could reduce the swimming distance of zebrafish in the dark, and Bisesi *et al*. (2014) found that venlafaxine exposure could increase the time for the bass to feed, while the time spent by the bass feeding gradually decreased with the recovery period. Thus, insights into the recovery pattern of behavioral responses to short-term AMI exposure are critical for comprehensively assessing their risks.

The locomotor activity integrates physiological abilities that enable the fish to generate and coordinate the energy needed for basic functions such as migrating or avoiding predators (Gaworecki and Klaine 2008; Eisenreich and Szalda–Petree 2015). Previous studies have demonstrated that antidepressants can reduce feeding rates of juvenile Eurasian perch (*Perca fluviatilis*) at both low and high prey densities, and such effects may alter the stability of the predator–prey system and the community structure (Hedgespeth *et al*., 2014). On the other hand, shoaling behavior is an aggregation behavior, and the groups among multiple individuals can reduce the risk of predation (Buske and Gerlai 2012; Gerlai 2014). These behavioral changes may further affect fish populations by influencing foraging efficiency and predator avoidance (Demin *et al*., 2017; Meshalkina *et al*., 2018). Therefore, the effects of AMI on zebrafish causing delayed hypoactivity and abnormal schooling behavior should not be ignored.

To evaluate the possible mechanisms involved in the behavioral change of zebrafish to AMI, we assessed the levels of 5–HT, 5–HIAA, ACH, and ACHE in the brain of zebrafish. Our results showed that altered DLM was significantly associated with increased 5–HT in the ACH, and altered social behavior was significantly associated with 5–HT.

The increased brain 5–HT level in zebrafish is consistent with the mode of action of AMI, which exhibits an inhibitory effect on 5–HT reuptake transporters, resulting in the increased 5–HT levels (Abdollahi and Mostafalou 2014). As a monoamine neurotransmitter, 5–HT is a major modulator of other excitatory and inhibitory neurotransmitter systems in the central nervous system, which has been associated with stress response, feeding, motor activity, social interaction, and migration in fish (Bisesi *et al*., 2016; Horzmann and Freeman 2016). For example, Mennigen *et al*. (2011) reported that antidepressants could increase serotonin concentrations and alter 5–HT receptor function through chronic exposure. On the other hand, 5–HIAA is a product of 5–HT, and the level of 5–HIAA is often used to measure 5–HT neuronal activity (Horzmann and Freeman 2016; Mcdonald 2017). Previously, Meshalkina *et al*. (2018) found that zebrafish exposed to AMI (50 μg/L) for a fortnight showed reduced serotonin turnover (5–HIAA/5–HT ratio), which led to significantly reduced locomotor activity. Consistent with our results, acute fluoxetine (an antidepressant) exposure also reduced 5–HIAA concentrations in the zebrafish brain, which may have contributed to their decreased social interaction (Winberg and Thörnqvist 2016). Thus, our findings supported that the serotonergic system plays an important role in modulating fish behavioral responses to antidepressants.

We also found that AMI affected the locomotor activity of zebrafish and was significantly associated with changes in the level of ACH. Acetylcholine (ACH) is the predominant neurotransmitter in the parasympathetic nervous system, and its neurotransmission is widespread in the central nervous system, helps regulate the release of other neurotransmitters, and is associated with learning and memory (Horzmann and Freeman 2016). Furthermore, The significantly elevated brain ACH levels may be explained by negative feedback to the AMI, as all TCAs can block the action of ACH (Abdollahi and Mostafalou 2014). Previous studies have reported that abnormal ACH levels led to reduced locomotor activity in zebrafish (Mennigen *et al*., 2011; Sarasamma *et al*., 2018). Chinese minnow exposed to psychotropic drugs showed a significant increase in anxiety–type behavior, accompanied by a significant increase in ACH level (Gao and Yang 2022). Considering that ACH is a typical target of TCAs (Abdollahi and Mostafalou 2014), its mechanisms in mediating behavioral responses to those antidepressants should be addressed in future studies.

AUTHOR CONTRIBUTIONS

L. LI, K. DONG and C. CHEN performed the exposure test, analyzed the data, and wrote the paper. K. CHEN, Y. SHI, and C. CHEN performed the behavioral experiments and participated in the data analysis. X. QIU designed the study, supervised the work, wrote the paper, and provided facilities and resources. Y.

SHIMASAKI and Y. OSHIMA designed the study, wrote the paper, and provided resources. All authors assisted in editing the manuscript and approved the final version.

ACKNOWLEDGMENTS

This work was supported by the Natural Science Foundation of Jiangsu Province of China (Grant No. BK20191433). Thanks for the support of Jiangsu Collaborative Innovation Center of Technology and Material of Water Treatment, Suzhou University of Science and Technology Suzhou 215009, China.

REFERENCES

Abdollahi, M. and S. Mostafalou (2014). Tricyclic antidepressants. In *Encyclopedia of Toxicology (Third Edition)* (Ed P. Wexler), pp. 838–845. Oxford: Academic Press

Arnnok, P., R. R. Singh, R. Burakham, A. Pérez-Fuentetaja and D. S. Aga 2017 Selective uptake and bioaccumulation of antidepressants in fish from effluent-impacted niagara river. *Environ. Sci. Technol*. **51**: 10652–10662

Bisesi, J. H., Jr., W. Bridges and S. J. Klaine 2014 Reprint of: effects of the antidepressant venlafaxine on fish brain serotonin and predation behavior. *Aquat. Toxicol*. **151**: 88–96

Bisesi, J. H., Jr., L. E. Sweet, P. Van Den Hurk and S. J. Klaine 2016 Effects of an antidepressant mixture on the brain serotonin and predation behavior of hybrid striped bass. *Environ. Toxicol. Chem*. **35**: 938–945

Buske, C. and R. Gerlai 2012 Maturation of shoaling behavior is accompanied by changes in the dopaminergic and serotoninergic systems in zebrafish. *Dev. Psychobiol*. **54**: 28–35

Chang, P. H., P. Liu, B. Sarkar, R. Mukhopadhyay, Q. Y. Yang, Y. M. Tzou, B. Zhong, X. Li, *et al*., 2021 Unravelling the mechanism of amitriptyline removal from water by natural montmorillonite through batch adsorption, molecular simulation and adsorbent characterization studies. *J. Colloid. Interface. Sci*. **598**: 379–387

Chen, K., M. Wu, C. Chen, H. Xu, X. Wu and X. Qiu 2021 Impacts of chronic exposure to sublethal diazepam on behavioral traits of female and male zebrafish (*Danio rerio*). *Ecotoxicol. Environ. Saf*. ***208***: 111747

Choi, J. W., Y. Zhao, J. K. Bediako, C. W. Cho and Y. S. Yun 2018 Estimating environmental fate of tricyclic antidepressants in wastewater treatment plant. *Sci. Total. Environ*. **634**: 52–58

Cosci, F. and G. Chouinard 2020 Acute and persistent withdrawal syndromes following discontinuation of psychotropic medications. *Psychother. Psychosom*. **89**: 283–306

David, A., A. Lange, C. R. Tyler and E. M. Hill 2018 Concentrating mixtures of neuroactive pharmaceuticals and altered neurotransmitter levels in the brain of fish exposed to a wastewater effluent. *Sci. Total Environ*. **621**: 782–790

Demin, K. A., T. O. Kolesnikova, S. L. Khatsko, D. A. Meshalkina, E. V. Efimova, Y. Y. Morzherin and A. V. Kalueff 2017 Acute effects of amitriptyline on adult zebrafish: Potential relevance to antidepressant drug screening and modeling human toxidromes. *Neurotoxicol. Teratol*. **62**: 27–33

Eisenreich, B. R. and A. J. B. p. Szalda–Petree 2015 Behavioral effects of fluoxetine on aggression and associative learning in siamese fighting fish (*Betta splendens*). *Behav. Process*. **121**: 37–42

Gao, S. and F. Yang 2022 Behavioral changes and neurochemical responses in Chinese rare minnow exposed to four psychoactive substances. *Sci. Total. Environ*. **808**: 152100

Gaworecki, K. M. and S. J. Klaine 2008 Behavioral and biochemical responses of hybrid striped bass during and after fluoxetine exposure. *Aquat. Toxicol*. *88*: 207–213

Gerlai, R. 2014 Social behavior of zebrafish: from synthetic images to biological mechanisms of shoaling. *J. Neurosci. Methods* **234**: 59–65

Grabicova, K., R. H. Lindberg, M. stman, R. Grabic, T. Randak, D. G. Joakim Larsson and J. Fick 2014 Tissue–specific bioconcentration of antidepressants in fish exposed to effluent from a municipal sewage treatment plant. *Sci. Total. Environ*. **488–489**: 46–50

Hedgespeth, M. L., P. A. Nilsson and O. Berglund 2014 Ecological implications of altered fish foraging after exposure to an antidepressant pharmaceutical. *Aquat. Toxicol*. **151**: 84–87

Horzmann, K. A. and J. L. Freeman 2016 Zebrafish get connected: investigating neurotransmission targets and alterations in chemical toxicity. *Toxics*. 4

Kasprzyk–Hordern, B., R. M. Dinsdale and A. J. Guwy 2009 The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res*. **43**: 363–380

Kaur, K. and A. K. Malik 2013 Study on the fluorescence quenching reaction of amitriptyline and clomipramine hydrochlorides with eosin Y and its analytical application. *J. Fluoresc*. **23**: 533–542

Lajeunesse, A., C. Gagnon, F. Gagne, S. Louis, P. Cejka and S. Sauve 2011 Distribution of antidepressants and their metabolites in brook trout exposed to municipal wastewaters before and after ozone treatment–evidence of biological effects. *Chemosphere* **83**: 564–571

Li, H., M. W. Sumarah and E. Topp 2013 Persistence of the tricyclic antidepressant drugs amitriptyline and nortriptyline in agriculture soils. *Environ. Toxicol. Chem*. **32**: 509–516

Mcdonald, M. D. 2017 An AOP analysis of selective serotonin reuptake inhibitors (SSRIs) for fish. *Comp. Biochem. Phys. C* **197**: 19–31

Melvin, S. D. 2017 Effect of antidepressants on circadian rhythms in fish: Insights and implications regarding the design of behavioural toxicity tests. *Aquat. Toxicol*. **182**: 20–30

Mennigen, J. A., P. Stroud, J. M. Zamora, T. W. Moon and V. L. Trudeau 2011 Pharmaceuticals as neuroendocrine disruptors: lessons learned from fish on prozac. *J. Toxicol. Environ. Health. B. Crit. Rev*. **14**: 387–412

Meshalkina, D. A., E. V. Kysil, K. A. Antonova, K. A. Demin, T. O. Kolesnikova, S. L. Khatsko, R. R. Gainetdinov, P. A. Alekseeva, *et al*., 2018 The effects of chronic amitriptyline on zebrafish behavior and monoamine neurochemistry. *Neurochem. Res*. **43**: 1191–1199

Miller, N. and R. Gerlai 2012 From schooling to shoaling: patterns of collective motion in zebrafish (*Danio rerio*). *PLOS ONE* **7**: e48865

Qiu, X., C. Chen, Y. Shi, K. Chen, M. Li, H. Xu, X. Wu, Y. Takai, *et al*., 2022a Persistent impact of amitriptyline on the behavior, brain neurotransmitter, and transcriptional profile of zebrafish (*Danio rerio*). *Aquat. Toxicol*. **245**: 106129

Qiu, X., M. S. Tengbe, X. Xia, K. Dong, C. Chen, Y. Shi, M. Li, H. Xu, *et al*., 2022b Impacts of cetylpyridinium chloride on the survival, development, behavior, and oxidative stress of early–life–stage zebrafish (*Danio rerio*). *Antioxidants* **11**: 11040676

Richmond, E. K., E. J. Rosi, D. M. Walters, J. Fick, S. K. Hamilton, T. Brodin, A. Sundelin and M. R. Grace 2018 A diverse suite of pharmaceuticals contaminates stream and riparian food webs. *Nat. Commun*. **9**: 4991

Sarasamma, S., G. Audira, S. Juniardi, B. P. Sampurna, S. T. Liang, E. Hao, Y. H. Lai and C. D. Hsiao 2018 Zinc chloride exposure inhibits brain acetylcholine levels, produces neurotoxic signatures, and diminishes memory and motor activities in adult zebrafish. *Int. J. Mol. Sci*. **19**: 3195

Sehonova, P., N. Hodkovicova, M. Urbanova, S. Orn, J. Blahova, Z. Svobodova, M. Faldyna, P. Chloupek, *et al*., 2019 Effects of antidepressants with different modes of action on early life stages of fish and amphibians. *Environ. Pollut*. **254**: 112999

Thompson, D. F. and K. G. Brooks 2015 Systematic review of topical amitriptyline for the treatment of neuropathic pain. *J. Clin. Pharm. Ther*. **40**: 496–503

Wallace, C. K., L. A. Bright, J. O. Marx, R. P. Andersen, M. C. Mullins and A. J. Carty 2018 Effectiveness of rapid cooling as

a method of euthanasia for young zebrafish (*Danio rerio*). *J. Am. Assoc. Lab. Anim. Sci*. **57**: 58–63

Winberg, S. and P.-O. Thörnqvist 2016 Role of brain serotonin in modulating fish behavior. *Curr. Zool*. **62**: 317–323

Yang, M., W. Qiu, J. Chen, J. Zhan, C. Pan, X. Lei and M. Wu 2014 Growth inhibition and coordinated physiological regulation of zebrafish (*Danio rerio*) embryos upon sublethal exposure to antidepressant amitriptyline. *Aquat. Toxicol*. **151**: 68–76