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Embryological exposure to valproic acid induces social interaction deficits in zebrafish (*Danio rerio*): A developmental behavior analysis

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ABSTRACT

Changes in social behavior are associated with brain disorders, including mood disorders, stress, schizophrenia, Alzheimer's disease, and autism spectrum disorders (ASD). Autism is a complex neurodevelopmental disorder characterized by deficits in social interaction, impaired communication, anxiety, hyperactivity, and the presence of restricted interests. Zebrafish is one of the most social vertebrates used as a model in biomedical research, contributing to an understanding of the mechanisms that underlie social behavior. Valproic acid (VPA) is used as an anti-epileptic drug and mood stabilizer; however, prenatal VPA exposure in humans has been associated with an increased incidence of autism and it can also affect fetal brain development. Therefore, we conducted a behavioral screening at different periods of zebrafish development at 6, 30, 70, and 120 dpf (days postfertilization) after VPA exposure in the early development stage to investigate social behavior, locomotion, aggression, and anxiety. VPA (48 μM) exposure during the first 48 hpf (hours postfertilization) did not promote changes on survival, morphology, and hatching rate at 24 hpf, 48 hpf, and 72 hpf. The behavioral patterns suggest that VPA exposure induces changes in locomotor activity and anxiety at different developmental periods in zebrafish. Furthermore, a social interaction deficit is present at 70 dpf and 120 dpf. VPA exposure did not affect aggression in the adult stage at 70 dpf and 120 dpf. This is the first study that demonstrated zebrafish exposed to VPA during the first 48 h of development exhibit deficits in social interaction, anxiety, and hyperactivity at different developmental periods.

1. Introduction

Several psychiatric and neurological disorders are characterized by alterations in the social domain (Kennedy and Adolphs, 2012). Impairments in social behavior abilities are considered features associated to mood disorders, stress, anxiety, schizophrenia, Alzheimer's disease, and autism spectrum disorders (American Psychiatric Association, 2013; Burns, 2006; Djukic and McDermott, 2012; Mahoney et al., 2014; Pelphrey et al., 2011; Rapin and Tuchman, 2008).

Autism spectrum disorders (ASD) comprise a heterogeneous group of neurodevelopmental disorders, which include Asperger's disorder, pervasive developmental disorder, childhood disintegrative disorder, Rett's disorder, and autism disorder (Grzadzinski et al., 2013). Autism is a complex neurodevelopmental disorder characterized by core symptoms related to stereotyped movements of behavior, deficits in social interaction, impaired communication, anxiety, hyperactivity, and the presence of restricted interests (Canitano, 2014; Schneider and Przewłocki, 2005). ASD has several forms and levels of severity; the clinical presentation of core symptoms can be heterogeneous because autistic patients exhibit moderate to severe symptoms, as well as different intellectual (Charman et al., 2011) and language profiles (Tager-Flusberg and Caronna, 2007). ASD are highly heritable (~90%) and are considered the most heritable brain disorder in humans (Constantino et al., 2013; Zafeiriou et al., 2013). Multiple genes, several cellular pathways and disordered molecular pathways (Pinto et al., 2014) have been implicated in the development of autism. Furthermore, environmental factors may also contribute to the risk of ASD (Banerjee et al., 2014; Persico and Bourgeron, 2006; Raff, 2014).

Currently, the neurological, behavioral, and genetic bases of autism represent a major problem for researchers because of the uncertainty of the origin of this neuropathology. Animal models of brain disorders are essential for the investigation of neuropathology development and

Abbreviations: ASD, autism spectrum disorders; dpf, days postfertilization; 5-HTT, serotonin transporter; VPA, valproic acid.

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provide a basis for new therapeutic approaches. The most commonly studied animal models use rodents; however, data indicate that several aspects of almost all brain disorders that can be studied in rodents can be modeled in zebrafish because this teleost is an excellent tool not only for basic research but also for the study of translational neurosciences (Kalueff et al., 2014; Miller and Gerlai, 2011). Zebrafish have been proposed as a model of Alzheimer's disease (Nery et al., 2014; Newman et al., 2014), schizophrenia (Seibt et al., 2011), drug abuse (Collier et al., 2014), and other brain disorders (Kalueff et al., 2014; Siebel et al., 2013). Zebrafish represent an useful model because they are one of the most social vertebrates used in biomedical research (Saverino and Gerlai, 2008). A remarkable feature of their behavior is the interdependence of the group members, which exhibits ordered social relationships and social interaction (Kalueff et al., 2014; Gerlai, 2014). Gerlai et al. (2000) identified preferences of animals for a group of animals of the same species. It is important to consider that these behaviors can change throughout zebrafish development (Buske and Gerlai, 2011). Furthermore, some behavioral assays for complex social interactions are easier to perform in zebrafish (Maaswinkel et al., 2013; Mahabir et al., 2013; Miller and Gerlai, 2011). Therefore, zebrafish represent an animal model that can significantly contribute toward understanding the mechanisms that underlie social behavior (Grossman et al., 1997; Morrison and Bellack, 1987).

Valproic acid (VPA) is extensively used as an antiepileptic and mood stabilizing drug; however, prenatal VPA exposure in humans has been associated with an increased incidence of autism, and it can also affect fetal brain development and decrease social interaction in rodents (Kim et al., 2011; Markram et al., 2007; Schneider and Przewłocki, 2005). VPA exposure produces similar patterns of abnormal development across species (Menegola et al., 1998; Petrere et al., 1986). This is a well-established animal model used to induce autism because it causes morphological and behavioral changes associated with the pathophysiology of autism (Arndt et al., 2005; Markram et al., 2007; Rodier et al., 1997; Schneider and Przewłocki, 2005); these symptoms predominately include alterations in social behavior, such as deficits in social interaction and anxiety symptoms.

Therefore, we conducted a behavioral screening at different periods of zebrafish development at 6, 30, 70, and 120 dpf (days postfertilization) after VPA exposure in the early development stage to investigate social behavior, locomotion, aggressiveness, and anxiety.

2. Materials and methods

2.1. Animals

Adult wild type zebrafish were maintained and bred according to standard procedures in an automated re-circulating system (Tecniplast, Buguggiate, VA, Italy) at a density of 1.5 fish per liter with a constant light–dark cycle (14–10 h) (Westerfield, 2000). For breeding, females and male (1:2) were placed in breeding tanks overnight and were separated by a transparent barrier that was removed after the lights were turned on the following morning. Embryos were collected after 15 min and transferred to sterile 6-well cell culture plates (20 embryos per well); the embryos were maintained in incubators at 28.5 °C with a controlled 14:10 h light–dark cycle. The embryos were maintained on Biochemical Oxygen Demand (BOD) incubators until 7 dpf at a density of 7 ml per larva. They were then immediately transferred to a tank with a density of one larva per 60 mL. When the animals reached the age of 30 dpf, they were maintained in a density of one animal per 200 mL until adulthood. The light and temperature control was performed in accordance with the previously described parameters (Westerfield, 2000). Survival assessments and general morphology were analyzed daily by visual inspection of the embryo under a dissection scope monitored under an inverted stereomicroscope.

2.2. Pharmacological treatment

Valproic acid (Sigma Aldrich, St. Louis, MO, USA) at a concentration of 48 μM diluted in water was administered in selected embryos from 0 to 48 h postfertilization (48 hpf). For the treatment, we used six wells that contained 15 embryos per well in 12 mL of VPA (treated group) or water (control) plates.

2.3. Analysis of VPA levels in treatment medium by ICP-MS

The levels of VPA in treatment medium for 48 h were assessed by inductively coupled plasma mass spectrometry (ICP-MS). The water of treatments were diluted (20×) with saline solution and filtrated for analysis (0.22 μm filter). The chromatographic parameters used were based on the method described by Gao et al. (2011). Briefly, 5 μl of diluted samples was injected into the UHPLC 1290/MS 6460 TQQQ — Agilent (all UHPLC components and software MassHunter were from Agilent Technologies®, Santa Clara, CA, USA). Chromatographic separation was performed using a Zorbax SB-C18 (4.6 × 50 mm 2.1 μm column). The flow rate of methanol:10 mM ammonium formate containing 0.1% formic acid (80:20, v/v) was 0.4 ml/min with a column temperature at 35 °C. VPA was detected using electrospray positive ionization and selected ion monitoring (SIM) for m/z = 143.1.

2.4. Behavioral assessment

2.4.1. Locomotion and anxiety

The locomotor activity and anxiety of the animals were evaluated at 6 dpf, 30 dpf, 70 dpf, and 120 dpf. The 6-dpf larvae were individually placed in a 24-well plate filled with 3 ml of system water for locomotor performance analysis during a 5-min session following 1-min of acclimation. The performance was video recorded using a digital HD webcam (Logitech, Newark, CA, USA) for automated analysis (ANY-Maze, Stoelting Co., Wood Dale, IL, USA). The total distance traveled, mean speed, number of crossings, and absolute turn angle were considered the main parameters of locomotion, whereas the entries in the outer area represented a parameter of anxiety. At 30 dpf, 70 dpf, and 120 dpf, the animals were individually placed in the test tank (30 cm × 15 cm × 10 cm, length × height × width) and maintained for 30 s prior to the video recording as previously described (Gerlai et al., 2000). The locomotor activity was video recorded for 5 min after

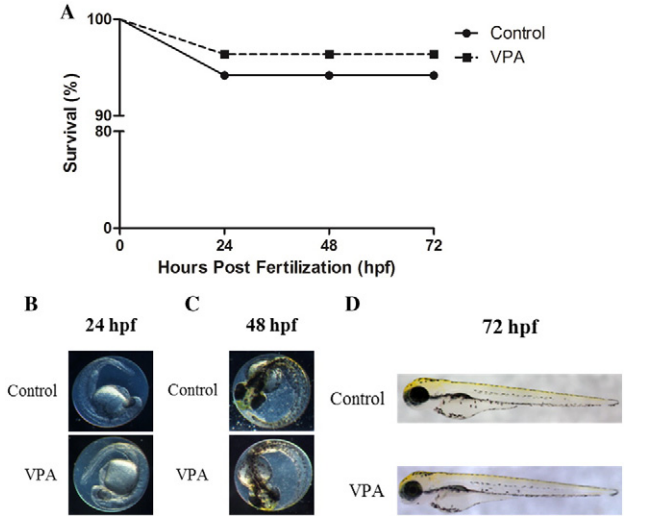

Fig. 1. Effect of VPA exposure on zebrafish embryos. Kaplan–Meier survival comparison for groups throughout the experiment showed no effects at 24 hpf, 48 hpf, and 72 hpf (log-rank (Mantel–Cox) test) that were not statistically significant when individual comparisons were performed (A) and no morphological changes at 24 hpf (B), 48 hpf (C) and 72 hpf (D).

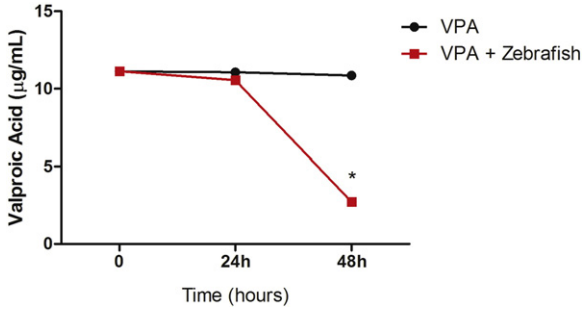

Fig. 2. VPA levels in treatment medium over 48 h of exposure. The asterisk (*) indicates a significant difference compared with the control group ($p < 0.05$). Statistical comparison of the data was performed by one-way ANOVA followed by Tukey's test.

the habituation period and simultaneously analyzed using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA). The tank was divided into equal sections with four vertical lines and one horizontal line, and the following behavior patterns were measured: the number of crossings (vertical and horizontal lines), distance traveled and mean speed. The time spent in each tank position (bottom vs. upper levels) was considered an index of anxiety. This task exploits the natural tendency of zebrafish to spend most of the time at the bottom when introduced to a novel environment and then gradually extend the swimming range, over a period of minutes, to include the upper portions of the test tank (Levin et al., 2007). A longer time spent in the bottom and less time spent in the top part of the tank indicates increased anxiety (Levin et al., 2007).

2.4.2. Social interaction

The social interaction of the animals was evaluated at 70 and 120 dpf because the zebrafish exhibit preference for its conspecifics. Each fish was placed in an experimental tank (30 cm × 15 cm × 10 cm, length × height × width). On one side of the experimental tank, an empty fish tank was placed; on the other side, a tank of identical size held 15 zebrafish, which were designed the "stimulus fish". The experimental fish was allowed to acclimate to the experimental tank for a 30 s period, after which behavior was video recorded over a period of 5 min. To quantify fish preference between the "stimulus fish" side of their tank in detriment of the empty tank, the experimental fish tank was divided in four equal sections. The zones 1 and 2 of the tank correspond to the segments closer to the conspecific school and the zones 3 and 4 are considered to the segments closer to the empty tank. The amount of time the experimental fish spent on each zone was measured using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA) (Gerlai et al., 2000).

2.4.3. Aggression

The aggression of the animals was evaluated on 70 dpf and 120 dpf. The mirror test was used to measure aggression (Gerlai et al., 2000; Moretz et al., 2007). Each fish was placed in an experimental tank (30 cm × 15 cm × 10 cm, length × height × width). A mirror (45 cm × 38 cm) was placed at the side of the tank at an angle of 22.5° to the back wall of the tank so that the left vertical edge of the mirror touched the side of the tank and the right edge was further away. Thus, when the experimental fish swam to the left side of the tank, their mirror image appeared closer to them. A test fish was added to the tank and was allowed acclimate for 60 s; the aggressive behaviors that a fish conducted toward its mirror image were subsequently recorded over a period of 5 min. The vertical lines divided the tank into four equal sections and allowed the number of entries to each section made by the fish to be counted. Entry to the left-most segment indicated preference for proximity to the "opponent", whereas entry to the right-most segments implied avoidance. The amount of time the experimental fish spent in each segment was measured using ANY-Maze recording software (Stoelting Co., Wood Dale, IL, USA).

2.5. Statistical analysis

Survival throughout the first 72 h was analyzed by Kaplan–Meier test. The ICP-MS data were analyzed by one-way ANOVA followed by Tukey's test. The behavioral assessment data were expressed as the mean ± standard error of the mean (S.E.M.) and analyzed by independent Student's t-tests, Multivariate analysis of variance (MANOVA) and analysis of variance (ANOVA). The α value determined is 5% to indicate different means between groups.

3. Results

We investigated the effect of VPA (48 μM) exposure during the first 48 h of development (48 hpf) on survival, morphology, and hatching rate at 24 hpf, 48 hpf, and 72 hpf. Survival rates were analyzed by Kaplan–Meier test and results indicated no significant difference on survival rate when groups were compared (log-rank (Mantel–Cox) test $p > 0.05$ $n = 85$) (Fig. 1A). VPA exposure did not promote significant changes in the morphology at 24 hpf, 48 hpf, and 72 hpf, respectively (Fig. 1B–D). We observed the normal appearance of the animals treated with VPA when compared to the control group, suggesting that perceived behavioral effects are not due to morphological changes. We observed that there was no significant alteration in the hatching rate of animals treated with VPA and the control group (data not shown).

In order to investigate if VPA levels alter during the exposure we analyzed VPA levels at treatment medium at 0 h, 24 h, and 48 h (hours) of exposure. As demonstrated in Fig. 2, the VPA levels decreased at 48 h of the treatment, suggesting that VPA was absorbed by the embryos.

Table 1
Locomotion parameters for VPA-treated and control groups at different ages.

Age	Parameters of locomotor activity	Control group		VPA group		p value
		Means ± S.E.M.	n	Means ± S.E.M.	n	
6 dpf	Distance traveled	0.7 ± 0.1 m	38	1.6 ± 0.4 m	38	$p < 0.05$
	Mean speed	0.2 ± 0.03 cm/s		0.5 ± 0.1 cm/s		
	Number of line crossings	28.5 ± 3.5		70 ± 14.3		
30 dpf	Distance traveled	1.6 ± 0.1 m	17	2.2 ± 0.2 m	17	$p > 0.05$
	Mean speed	0.5 ± 0.04 cm/s		0.7 ± 0.1 cm/s		
	Number of line crossings	137.6 ± 10.7		169.8 ± 11.1		
70 dpf	Distance traveled	3 ± 0.2 m	32	2.7 ± 0.2 m	32	$p > 0.05$
	Mean speed	1 ± 0.08 cm/s		0.9 ± 0.07 cm/s		
	Number of line crossings	233.6 ± 14.52		214.8 ± 14.58		
120 dpf	Distance traveled	2.6 ± 0.1 m	26	2.5 ± 0.2 m	26	$p > 0.05$
	Mean speed	0.8 ± 0.04 cm/s		0.8 ± 0.07 cm/s		
	Number of line crossings	202 ± 14.1		186 ± 16.2		

Data are expressed as mean ± S.E.M.

3.1. VPA exposure results in differential changes in locomotor activity and anxiety expressed at different ages in zebrafish

VPA (48 μM) exposure during the first 48 h of development (48 hpf) induced significant changes in the parameters of locomotor activity and anxiety behavior at different developmental periods: 6 dpf, 70 dpf, and 120 dpf. Different parameters of zebrafish locomotor activity were evaluated and measured in the tank test. The same tank used to measure locomotor activity was also used to identify the index of anxiety, which was determined by the time spent in the bottom portion of the test tank.

MANOVA was performed in order to analyze the parameters related to locomotor activity (distance traveled, mean speed, and number of line crossings) simultaneously, and investigate the correlation between these parameters. At 6 dpf, the results of MANOVA presented a significant correlation between parameters of locomotor activity ($F = 2.62$, num df = 3, den df = 74, $p < 0.05$) (Table 1). The locomotion at 6 dpf of the animals treated with VPA exhibited an increase in the distance traveled (1.6 ± 0.4 m) compared with the control animals (0.7 ± 0.1 m) (Table 1). There was also an increase in the mean speed (0.5 ± 0.1 cm/s) compared with the control animals (0.2 ± 0.03 cm/s) (Table 1). The number of line crossings increased (70 ± 14.3) compared with the control animals (28.5 ± 3.5) (Table 1). Regarding to anxiety-related parameter, ANOVA showed that there were significant differences in the means between groups ($F = 5.56$, num df = 1, den df = 76, $p < 0.05$) (Table 2). The entries in the outer area also increased (35.8 ± 7.2) compared with the control group (15.1 ± 1.7) (Table 2). However, we did not identify changes in the absolute turn angle or rotations (data not shown).

At 30 dpf and 70 dpf, the results of MANOVA showed no significant correlations between the parameters of locomotor activity ($F = 1.35$, num df = 3, den df = 32, $p > 0.05$ and $F = 0.32$, num df = 3, den df = 58, $p > 0.05$, respectively) (Table 1). Furthermore, the anxiety-related parameter showed no significant differences at 30 dpf, in the mean between the groups ($F = 3.75$, num df = 1, den df = 34, $p > 0.05$ (Table 2). However, at 70 dpf, it has been observed differences in the means between groups ($F = 5.05$, num df = 1, den df = 60, $p < 0.05$) (Table 2). The time spent in the bottom portion of the test tank increased (268.5 ± 4.0 s) compared with the control group (248.9 ± 7.9 s) (Table 2).

At 120 dpf, the results of MANOVA did not present a significant correlation between parameters of locomotor activity ($F = 2.60$, num df = 3, den df = 48, $p > 0.05$) (Table 1). Regarding to anxiety-related parameter, ANOVA showed that there were significant differences in the means between groups ($F = 3.77$, num df = 1, den df = 50, $p < 0.05$) (Table 2). The time spent in the bottom portion of the test tank decreased (235.3 ± 10.9 s) compared with the control group (266.1 ± 12.6 s) (Table 2).

These results demonstrated that VPA exposure in embryos produced changes in parameters of locomotor activity at 6 dpf, which were demonstrated by the changes in the distance traveled, mean speed, and number of crossings compared with the control group. At 30 dpf, 70 dpf and 120 dpf, there were no significant differences in locomotion. The early exposure to VPA induced an increase in anxiety behavior at 6 dpf and 70 dpf, which may be interpreted as an indicator of anxiogenic behavior; however, this pattern changed at 120 dpf and indicated VPA exposure promoted anxiolytic behavior.

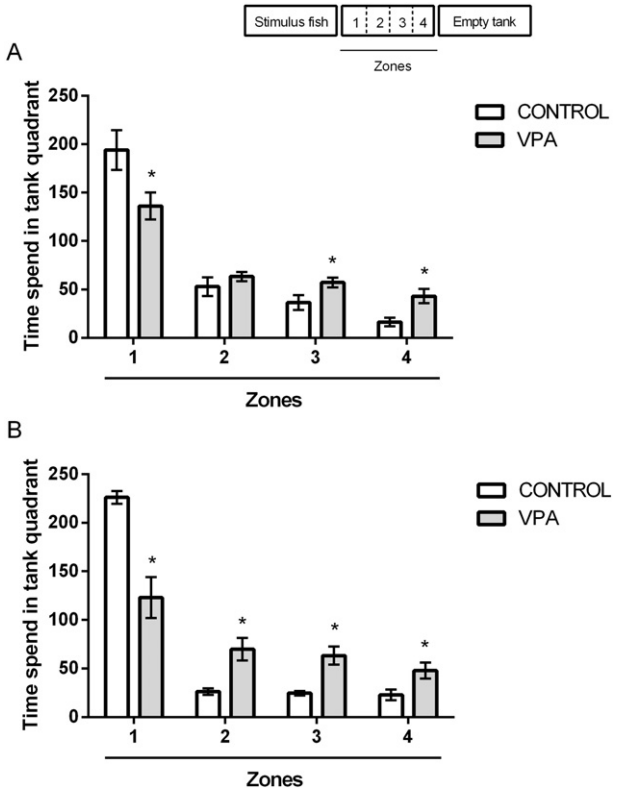

Fig. 3. Social interaction at 70 dpf (A) and 120 dpf (B) in the animals treated with VPA, which was determined during 5 min of video recording in the tank test. The inset of the figure demonstrates the segments of the test tank: the zone 1 of the tank corresponds to the segment closer to the conspecific school and the zone 4 is considered to the segment closer to the empty tank. The amount of time the experimental fish spent on each zone was measured using ANY-Maze recording software. The data were expressed as the mean ± S.E.M. The asterisk (*) indicates a significant difference compared with the control group ($p < 0.05$).

3.2. Social interaction deficits at different developmental periods in zebrafish exposed to VPA

Social interaction was assessed only at 70 and 120 dpf because at 6 and 30 dpf, the social behavior of zebrafish is not completely developed (Buske and Gerlai, 2012). Fig. 3 indicates that VPA induced impairment in social interaction in zebrafish at 70 and 120 dpf. We identified a significant ($p < 0.05$, n = 18) decrease in the time spent in the segment closest to the conspecific school (Zone 1; 136.2 ± 13.7 s) compared to the control group at 70 dpf (194 ± 20.6 s) (Fig. 3A). In addition, VPA treatment induced a significant increase ($p < 0.01$, n = 18) in the time spent in the zone 4 (43.1 ± 7.3 s), which is closest to the empty tank, when compared to control group (16.5 ± 4.4 s) (Fig. 3A). Similarly, at 120 dpf, we identified a significant ($p < 0.01$, n = 10) decrease in the

Table 2
Anxiety parameters for VPA-treated and control groups at different ages.

Age	Anxiety parameter	Control group		VPA group		p value
		Means ± S.E.M.	n	Means ± S.E.M.	n	
6 dpf	Entries in the outer area	15.1 ± 1.7 s	38	35.8 ± 7.2 s	38	$p < 0.05$
30 dpf	Time spent in the bottom portion	160.6 ± 12.6 s	17	197.1 ± 10.9 s	17	$p > 0.05$
70 dpf	Time spent in the bottom portion	248.9 ± 7.9 s	32	268.5 ± 4.0 s	32	$p < 0.05$
120 dpf	Time spent in the bottom portion	266.1 ± 12.6 s	26	235.3 ± 10.9 s	26	$p < 0.05$

Data are expressed as means ± S.E.M.

time spent in the segment closest to the conspecific school (Zone 1; 123.0 ± 21 s) compared to the control group at 70 dpf (226.1 ± 6.6 s) (Fig. 3B). Furthermore, VPA treatment induced a significant increase ($p < 0.05$, $n = 10$) in the time spent in the zone 4 (47.9 ± 8.1 s), which is closest to the empty tank, when compared to control (22.9 ± 5.5 s). Thus, VPA induced a social interaction deficit in zebrafish at both ages (Fig. 3B).

3.3. VPA does not induce changes in aggressive behavior at different developmental periods in zebrafish

Aggressive behavior was evaluated at 70 dpf and 120 dpf. The embryological VPA exposure did not promote significant changes in the time spent in the segment nearest to the mirror image at 70 dpf and 120 dpf. Thus, in these conditions, VPA most likely did not affect the aggression of the animals.

4. Discussion

This study performed a behavioral screening at different development periods in zebrafish at 6 dpf, 30 dpf, 70 dpf, and 120 dpf to establish a model of social interaction deficits induced by VPA. Our results indicated the normal appearance of the animals treated with VPA, suggesting that perceived behavioral effects are not due to morphological changes. We observed that there was no significant alteration in the survival and hatching rate of animals treated with VPA. Likewise, Zellner et al. (2011) demonstrated the exposure to 48 μM VPA during 0–48 hpf without affecting the death or malformations. The behavioral patterns identified in our experiments suggest that VPA exposure results in a profile of hyperactivity in the early development stages in zebrafish, i.e., 6 dpf, because there was an increase in locomotion parameters, such as the total distance traveled, mean speed and number of line crossings. In addition, the animals exhibited a marked anxiety based on the increased entries in the outer area of the well. De Esch et al. (2012) demonstrated that zebrafish larvae can act as an alternative animal model to test toxicity; however, it is important to consider the age (and the brain stage of development) because it can generate changes in behavior. Studies have demonstrated that age influences the pattern of motor activity in zebrafish larvae (Colwill and Creton, 2011; Padilla et al., 2011). In addition, high doses of VPA caused histopathological alterations in zebrafish larvae at 72 hpf and 96 hpf (Beker van Woudenberg et al., 2014). These authors demonstrated that low doses of VPA (60 μM VPA) induced minimal disruption of normal brain structure, which was characterized by small regions of reduced cellularity. However, at 150 μM VPA, all larvae exhibited mild disruption of normal brain structure, which was characterized by large areas of reduced cellularity, in particular, in the developing preoptic region and tectum opticum. In addition, 60 μM VPA did not affect motor activity, whereas 150 μM VPA induced a reduction in total distance traveled (Beker van Woudenberg et al., 2014). However, Zellner et al. (2011) demonstrated that exposure to 48 μM VPA during 0–48 hpf promoted marked hyperactivity at 6 dpf without changes in neurotoxicological parameters. Therefore, differences in VPA concentrations and treatment times may influence the behavioral patterns observed in zebrafish.

Throughout development, the hyperactivity of 6 dpf zebrafish presented after embryological VPA treatment was attenuated. The locomotion parameters of 30 dpf, 70 dpf and 120 dpf were similar to the control animals. The anxiety index at 70 dpf remained increased; however, at 120 dpf, there was a decrease in anxiety. To support these results, it is important to consider that VPA exposure may affect brain development. Several studies have demonstrated that VPA can regulate signaling pathways and gene expression throughout brain development in rodents thereby interfering with critical windows of vulnerability (Almeida et al., 2014; Bartkowska et al., 2007; Kolozsi et al., 2009; Stodgell et al., 2006). Our results also demonstrated that animals treated with VPA during the first 48 h exhibited a significant deficit in social interaction at 70 dpf, and this effect was maintained throughout development at 120 dpf, suggesting that VPA can modulate differentially behavioral patterns throughout brain development. We also suggest that the profile of hyperactivity and anxiety may change throughout development and indicates neurochemical alterations in animals exposed to VPA at an early age. The serotoninergic system during early life can produce different behavioral responses, including changes in social behavior in zebrafish (Buske and Gerlai, 2011; Mahabir et al., 2013). It has been reported that anxiety and social interaction deficits are related to changes in the serotonergic system and these alterations are extremely relevant for several psychiatric disorders (Homberg et al., 2007; Homberg, 2013).

Several neuropsychiatric disorders are characterized by impaired social interactions and anxiety. Silverman et al. (2010) demonstrated that rodent models of autism exhibit deficits in both sociability and anxiety behaviors. These findings are consistent with our results at 70 dpf. However, at 120 dpf, a decrease in anxiety was identified. The literature shows the controversial results between social interaction and anxiety. For example, social interaction deficits can occur without affecting anxiety. Some autistic mouse models have deficits in social interaction but do not exhibit changes in anxiety behaviors (Liu and Smith, 2009; McFarlane et al., 2008). Schneider and Przewłocki (2005) demonstrated that the altered behaviors in rodents induced by VPA appeared prior to puberty. However, the social structure in the early developmental stage is primitive in zebrafish. Young larvae do not exhibit shoaling behavior, which develops later with age (Buske and Gerlai, 2012). Based on this finding, the results related to the anxiety index have different responses when evaluated at different periods of development; this is most likely because of changes in the behavioral response maturity. According to Buske and Gerlai (2012), changes in the serotonergic and dopaminergic systems may explain the age-dependent behavioral changes.

According to Roullet et al. (2013), behavioral studies have indicated that VPA in both rat and mouse models induced symptoms of social interaction deficits, stereotyped movements, disability in communication, and anxiety. However, aggression is poorly studied. In our study, we verified that VPA exposure in the embryonic phase did not affect aggression in the adult stage at 70 dpf and 120 dpf in zebrafish. Aggression in zebrafish may have several important functions (Gerlai, 2003), and this behavior is often used to maintain dominance (Larson et al., 2006; Spence and Smith, 2006). The quantification of this behavior could provide information regarding whether VPA and aggressive tendencies are related. However, our results indicated that animals treated with VPA at an early age may exhibit behavioral responses without expressing a domain of territory, and there is no direct association between a deficit in social interaction and aggression.

In summary, the present study evaluated a behavioral screening at different periods of development in zebrafish and established a model of social interaction deficit induced by VPA. This is the first study to demonstrate that zebrafish exposed to VPA during the first 48 h of life exhibit deficits in social interaction, anxiety, and locomotor changes at different periods of development. Together, these results highlight the importance of behavioral research for understanding the toxicological action of drugs, such as VPA; these drugs can have a significant impact on the behavioral neurodevelopment associated with neuropsychiatric disorders evaluated in a new model of social interaction deficits in zebrafish.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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