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Social stress alters the efficacy of 8-OH-DPAT in zebrafish tests of anxiety-like behavior

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Abstract

Social hierarchy can lead to a series of distressing states and neurobehavioral adjustments that can result in symptoms of anxiety and depression. Social stress paradigms have been used to model anxiety and distress in non-human models, and have been shown to produce changes in the serotonergic system. Here, zebrafish (*Danio rerio* Hamilton 1822), a model teleost fish that enters dominance-subordinate hierarchies in the wild, were used to model the effects of these hierarchies on anxiety-like behavior and to investigate the roles of the 5-HT_{1A} receptor in these effects. 85 adult zebrafish were used, distributed into six groups formed by combining pharmacological treatment with 8-OH-DPAT (0.3 mg/kg) or vehicle and the experimental hierarchy condition. After status determination, the animals underwent behavioral assessments in the Novel Tank Test and Light/Dark Test. Subordinate animals exhibited greater geotaxis and scototaxis compared to the control and dominant groups, an effect that was reversed by the administration of 8-OH-DPAT. 8-OH-DPAT had no behavioral effect in control and dominant animals. Lipid peroxidation in brain tissue was higher in subordinate animals, an effect that was reversed by treatment with 8-OH-DPAT. The effects of 8-OH-DPAT on behavior and oxidative stress in zebrafish are modulated according to social position in the hierarchy, suggesting desensitization of autoreceptors and sensitization of heteroreceptors.

Introduction

Social stress is a potent source of distress and a risk factor for the development of various mental disorders, including anxiety and depression (Björkqvist, 2001; Creel et al., 2013; Ganzel et al., 2010; Smail, 2015). Social hierarchies can lead to a series of distressing states and neurobehavioral adjustments, especially to subordinate individuals, that mediate symptoms of anxiety and depression (Backström & Winberg, 2017; Chaouloff, 2013; Masis-Calvo et al., 2018). As such, social stress paradigms using hierarchy formation and social defeat have been used to model anxiety and distress in non-human animals (Chaouloff, 2013; Gilbert & Allan, 1998; Masis-Calvo et al., 2018). A structural equation model (Gilbert et al., 2002) links social defeat, entrapment, social comparison, and shame to decreased positive affect and higher anxiety, interacting to produce social withdrawal, passivity, low exploratory behavior, and low positive affect.

These behavioral effects are likely mediated by a plethora of neurobiological mechanisms, including neuroplasticity (Davidson & McEwen, 2012), neuroendocrine adjustments (Cacioppo, 1994), and changes in neurotransmitter homeostasis (Soares et al., 2018). The term “social plasticity” – the ability of organisms to adaptively modify behavior and physiology to specific situations in a complex and variable social world (Cardoso et al., 2015; Maruska et al., 2019) – has been used to describe some of these effects, especially in teleost fish. Given the increasing use of fish as models in neurobehavioral research, either to uncover how stress impacts animal welfare (White et al., 2017) or to illuminate hypothetical mechanisms for human psychopathology (Stewart et al., 2014), it is not surprising that much of the discovery in the field now includes fish species.

The serotonergic system plays a key role in coordinating physiological, behavioral, and vegetative responses to stress across species (Maximino, 2012). In fish, this system is impacted by social interactions and likely represent an interface between stress and sociality (Backström & Winberg, 2017; Soares et al., 2018). Losing fights for social dominance and being subordinate has important effects on the behavior and physiology of fish, some of which are likely to be mediated by the serotonergic system (Backström & Winberg, 2017). Subordinate animals show reduced aggression, appetite suppresion, decreased reproductive output, and increased anxiety-like behavior (Best et al., 2023; Bozi et al., 2021; Filby et al., 2010; Paull et al., 2010). In zebrafish, subordinate stress leads to increased anxiety-like behavior associated with increased oxidative stress in the brain, suggesting a mechanism in which redox balance mediates the effects of stress (Lima-Maximino et al., 2025). In Arctic charr (*Salvelinus alpinus* L.), subordinate animals show increased serotonergic activity (5-HIAA/5-HT ratios) in the telencephalon and brainstem (Winberg et al., 1991, 1992), an effect that is dependent on the most recent experience instead of being present before hierarchy formation (Winberg et al., 1992). Similar effects are observed in zebrafish (Dahlbom et al., 2012). These effects might be related to social defeat, since losing a fight increases serotonin levels in the optic tectum of zebrafish, while winners show higher serotonin levels in the telencephalon (Teles et al., 2013). Moreover, social descent (i.e., becoming subordinate) increases serotonergic activity in the telencephalon and brainstem of Arctic charr irrespective of previous status (Winberg et al., 1992). The increases observed in the brains of dominant animals are transient, while the changes observed in the brains of subordinates are sustained: in Arctic charr, the latter effects are observed up to 21 days of social interaction (Winberg & Nilsson, 1993).

The long-term effects of increasing serotonergic activity in the brainstem are likely to produce adjustments in the expression of genes of the serotonergic pathway, given that the brainstem houses serotonergic nuclei across vertebrates (Herculano & Maximino, 2014). In subordinate female zebrafish, an increase is observed in the expression of *zmao* (monoamine oxidase), *tph1a* (tryptophan hydroxylase), and *slc6a4a* (serotonin transporter) in the hypothalamus in relation to the beginning of hierarchy formation (Filby et al., 2010); in subordinate males, on the other hand, there is a decrease in the expression of *zmao* and *slc6a4a* in the hypothalamus, and *zmao* and *tph1b* in the forebrain (Filby et al., 2010). Dominant females, on the other hand, show an increased expression of *slc6a4a* in the forebrain, while dominant males show increased expression of *zmao* and *htr1a* (5-HT_{1A} receptor) in the hypothalamus (Filby et al., 2010). In African cichlids (*Astalotilapia burtoni*),

subordinate animals show increased sensitivity to the pharmacological manipulation of 5-HT_2-type receptors in both fast-starts and in the electrophysiological properties of the Mauthner cell, an important sensory integrator located in the brainstem that initiates escape responses in fish (Whitaker et al., 2011). Interestingly, in zebrafish 5-HT_2 receptors are also involved in fear, anxiety, and sociality (de Moura et al., 2023; do Carmo Silva et al., 2021; Nathan et al., 2015). Importantly, the effects of 5-HT_2 drugs on anxiety-like behavior are also modulated by social status in zebrafish: a 5-HT_{2C} selective agonist produces stronger anxiogenic-like effects in subordinate animals, and an antagonist produces an anxiolytic-like effect in dominants and an anxiogenic-like effect in subordinates (De Oliveira et al., 2025). This suggests that increased serotonin levels in the brainstem raphe of subordinate individuals sensitize 5-HT_{2C} receptors in postsynaptic sites.

5-HT_{1A} receptors are strongly expressed in presynaptic neurons in all serotonergic nuclei of the zebrafish brain (Norton et al., 2008), and there is evidence of a receptor reserve for the anxiolytic-like effects of 5-HT_{1A} receptor activation (Bruce de Souza et al., 2025). In the presence of a receptor reserve, low doses of a full antagonist preferentially activate autoreceptors, inhibiting serotonin release from serotonergic nerve endings, while higher doses both decrease serotonin availability at other non-5HT_{1A} receptors and activate 5-HT_{1A} heteroreceptors (Sprouse & Aghajanian, 1988). In a situation in which serotonin activity is increased in the brainstem, as appears to be the case with subordinate individuals, 5-HT_{1A} receptors are likely to be downregulated If that is the case, full agonists such as 8-OH-DPAT would exert their effects mainly by acting at heteroreceptors, and higher doses would be needed to produce an anxiolytic-like effect in subordinate animals.

The present work tests this hypothesis by subjecting animals to a social stress paradigm and evaluating the effects of 8-OH-DPAT on anxiety-like behavior in controls, dominant and subordinate animals. Moreover, to understand whether 5-HT_{1A} receptors also mediate the effects on social stress on oxidative stress, we also measured brain lipid peroxidation. Contrary to our hypothesis, a relatively low dose of 8-OH-DPAT (0.3 mg/kg) decreased anxiety-like behavior and blocked lipid peroxidation only in subordinate animals.

Materials and methods

Animals and housing

85 adult wild-type zebrafish (> 4 months old) from the longfin phenotype were used in the present experiments. Sample size calculations were based on a power analysis for a 2-way ANOVA with interaction effects, with $\alpha = 0.05$, $\beta = 0.8$, and expected effect size $f = 0.68$ for each independent variable (IV); effect sizes used for estimating sample sizes were based on a meta-analysis of the effects of buspirone on zebrafish behavioral models that can be found at https://github.com/lanec-unifesspa/5HT/blob/main/5HT1A/zf_metanalysis_buspirone.pdf. A sample size of 12 animals/group was established; given the subchronic character of the experimental manipulation, we sought to maximize sample size to compensate for possible losses during the experiment; final sample sizes varied between 12 and 18 animals for a given group, and are presented in Figure legends.

Animals were obtained from a colony resulting from crosses between animals purchased from specialized stores (Power Fish Fisheries, Itaguaí/RJ, Brazil). At the end of the experiments, after sacrifice, sex was determined by gonadal histomorphology, following Siegfried & Steinfeld (2021), and it was found that 69.84% of animals of the sample were female. All animals were kept in 50L tanks (density of 2 animals/L) and acclimatized in the laboratory for a period of 14 days before the onset of experiments. The tanks were kept constant at a temperature of 26 ± 2ºC and pH 7.2, oxygenation, 14/10 light-dark cycle provided by fluorescent lamps, according to standards of care for zebrafish (Lawrence, 2007). Animals were fed twice a day with commercial flocked feed. Animal density adopted was in accordance with the protocol adopted in the Brazilian guideline for the care of zebrafish in the laboratory (Diretriz brasileira para o cuidado e a utilização de animais para fins científicos e didáticos - DBCA. Anexo I. Peixes mantidos em instalações de instituições de ensino ou pesquisa científica, 2017). At the end of the experiments, animals were humanely sacrificed through rapid cooling (water at 2–3 ºC), followed by rapid decapitation (Ferreira et al., 2022); gonads (for sex determination) and brains were quickly dissected. The project was carried out with the approval of the Animal Use Ethics Committee of UNIFESSPA (Universidade Federal do Sul e do Sudeste do Pará) protocol number 23479.019576/2022-43.

Hierarchy formation

The protocol to establish a dominant-subordinate hierarchy in subchronic dyadic encounters was based on previous work with zebrafish (Bozi et al., 2021; De Oliveira et al., 2025; Paull et al., 2010). Before starting experiments, on day 1, each animal was briefly and lightly anesthesized in ice-cold water (12–14 ºC; Kinkel et al., 2010), weighted, and photographed for morphometric analysis. On day 1, at 8 am, individuals were randomly moved in pairs to 6 experimental containers (10 cm high x 15 cm in diameter) and left overnight to acclimatize to the container, recover from stress, and return to normal behavior. They remained in these containers for the next 5 consecutive days so that hierarchies could be established. This time interval, based on previous studies (Bozi et al., 2021; Filby et al., 2010; Paull et al., 2010), was

established as sufficient for the establishment of hierarchies in this species. During the 5-day period, the individuals were observed for possible physical injuries resulting from interactions or any other signs of health problems. While a protocol was created to remove injured individuals for treatment (removing the pair from the experiments and replacing it with another pair), no replacements were necessary during the course of the experiments. On the morning of the fifth day, all individuals were visually analyzed by three different observers, and the experimental tanks were videotaped for five minutes to quantify aggressive and territorial behaviors: 1) Chasing - representing the number of direct attacks by one individual toward another during the 5-minute recording period (Paull et al., 2010); 2) Bottom-dwelling, defined as the time the fish remained in the lower third of the experimental tank (Bozi et al., 2021); 3) Retreats - characterized by swimming rapidly away from the opponent in response to an attack (Oliveira et al., 2011); and 4) Attack - encompassing the number of agonistic events, with or without association with biting (Paull et al., 2010). X-Plo-Rat (Tejada et al., 2018) was used to evaluate these behaviors to determine dominant and subordinate status, with dominant individuals displaying higher chasing and attacks, and subordinate animals displaying geotaxis and freezing. The fish in the control groups were not involved in dyadic encounters and were transferred directly to the NTT and then NTT.

Drugs and treatments

8-OH-DPAT (8-hydroxy-2-(dipropylamino)tetralin; CAS#87394-87-4) from Sigma-Aldrich (St Louis, USA) and dissolved in Cortland's salt solution (NaCl 124.1 mM, KCl 5.1 mM, Na2HPO4 2.9 Mm, MgSO4 1.9 mM, CaCl2 1.4 mM, NaHCO3 11.9 mM, 1000 units heparin; Perry et al., 1984). For all treatments, the solutions were injected intraperitoneally into cold-anesthetized, and once anesthetized, they were positioned on a sponge-based surgical bed for injection according to the method described by Kinkel et al. (2010). For both treatments, the injection was carried out using a microsyringe (Hamilton 701N, 26 gauge needle, 200 conical tip, 10 µL), with a dose proportional to the animal's body weight (0.3 mg/kg) and an injection volume of 5 µL. The dose was based on a previous work on zebrafish (Bruce de Souza et al., 2025). Experimenters were blind to treatment through the use of coded vials; only the principal investigator had access to the codes before data analysis. Treatment order was randomized within sessions by using a random number generator (https://www.randomizer.org/). A scheme of the experimental design can be found on Fig. 1.

Behavioral assays

Animals were subjected to behavioral assays for anxiety-like behavior that are well-established for this species, light/dark test (LDT) and the novel tank test (NTT). Light/dark preference in zebrafish, characterized by a preference for either dark or light environments, has been described and validated as a model for evaluating anxiety-like behavior for the species (Maximino et al., 2010; Serra et al., 1999). Preference for either light or dark environments have been described in the literature, depending on developmental stage (e.g., more preference for light environments in larvae and juvenile zebrafish; Chen et al., 2015; Steenbergen et al., 2011) and light levels (e.g., more preference for dark/black environments under bright light; Araujo et al., 2012; Blaser & Peñalosa, 2011; Facciol et al., 2017, 2019). In both cases, anxiolytic drugs, including buspirone, have been shown to reduce preference (Maximino et al., 2011; Steenbergen et al., 2011). The novel tank test aims to evaluate zebrafish vertical locomotion/exploration as an index of defensive responses related to the response to novelty (Bencan et al., 2009; Egan et al., 2009; Levin et al., 2007). Anxiolytic drugs have been shown to reduce bottom-dwelling and/or increase top time (Cachat et al., 2011). However, stimulus control appears to differ in both tests, with scototaxis (dark preference) in the LDT being controlled mainly by an approach/avoidance conflict, and geotaxis (bottom-dwelling) in the NTT being controlled mainly by escape from the top (Maximino et al., 2012). Moreover, the effects of some serotonergic drugs, including 8-OH-DPAT, appear to differ in these tests (Bruce de Souza et al., 2025; Maximino et al., 2013). Animals were reused in both behavioral assays. Immediately after hierarchy formation, the animal received the intraperitoneal injection, the fish were relocated with a fish net to a recovery tank, where they remained for 30 minutes; After the recovery time, they were reallocated to NTT and then to the phototaxis assay (Fig. 1A); thus, each experimental unit took 51 minutes to end. (Fig. 1B).

Table 1 summarizes the endpoints assessed in both assays. 1) Chasing - representing the number of swimming by one individual toward another, causing it to increase its speed while actively pursuing the other fish, during the 5-minute recording period (Paull et al., 2010); 2) Bottom-dwelling, defined as the time the fish remained in the lower third of the experimental tank (Bozi et al., 2021); 3) Retreats - characterized by swimming rapidly away from the opponent in response to an attack (Oliveira et al., 2011); and 4) Attack - encompassing the number of agonistic events, with or without association with biting (Paull et al., 2010)

Table 1
Behavioral endpoints assessed in the novel tank test (NTT) and light/dark test (LDT).

Test	Behavior	ZBC code (Kalueff et al., 2013)	Description	Operationalization
Status determination	Chasing	1.28	Fish swims directly toward the other animal, causing it to increase its speed and actively pursuing the other fish	Number of chases (N)
	Bottom-dwelling	1.46	Preference for the bottom of the tank, avoidance of the top	Time spent in the bottom third of the tank (s)
	Retreat	1.129	Swimming rapidly away from the opponent in response to an attack	Number of retreats (N)
	Attack	1.10	Short bouts of fast swimming di- rected at an opponent, accompanied by the mouth opening behavior, with or without association withbiting	Number of attacks
NTT	Geotaxis (main endpoint)	1.46	Preference for the bottom of the tank, avoidance of the top	Time spent in the bottom third of the tank (s)
	Erratic swimming	1.51	Complex behavior in which the swim has no defined direction and the speed changes abruptly	Absolute turn angle (º)
	Freezing	1.68	Complete cessation of movement (except gills and eyes)	Total time swimming at speeds < 0.5 cm/s
	Hypolocomotion	1.81	Abnormally slow swimming for a sustained period of time, typically related to sedation or neuromotor deficits	Swimming speed (cm/s)
	Hyperlocomotion	1.79	Abnormally fast swimming for a sustained period of time, typically related to psychostimulant action	Swimming speed (cm/s)
Light/dark test	Scototaxis (main endpoint)	1.137	Preference for the dark part of the tank	Time spent in the black compartment (s)
	Erratic swimming	1.51	Complex behavior in which the swim has no defined direction and the speed changes abruptly	Absolute turn angle (º)
	Freezing	1.68	Complete cessation of movement (except gills and eyes)	Total time swimming at speeds < 0.5 cm/s
	Hypolocomotion	1.81	Abnormally slow swimming for a sustained period of time, typically related to sedation or neuromotor deficits	Swimming speed (cm/s)
	Hyperlocomotion	1.79	Abnormally fast swimming for a sustained period of time, typically related to psychostimulant action	Swimming speed (cm/s)

Light/dark test

After either recovery from drug treatment or exposure to the NTT, the fish was transferred to an apparatus (15 cm x 10 cm x 45 cm; height, width, length) divided into a white compartment and a black compartment, with hatches in the middle where the fish is placed for acclimatization for 3 min. After this, the hatches were removed and the fish was free to explore the apparatus for 15 min. The protocol established in the research group (https://dx.doi.org/10.17504/protocols.io.puydnxw) was used. Behavioral endpoints (Table 1) were extracted using TheRealFishTracker (v. 0.4.0; https://www.dgp.toronto.edu/~mccrae/projects/FishTracker/).

Novel tank test

Behavioral assessment of the animals was carried out according to the methodology previously described in works by the research group (https://dx.doi.org/10.17504/protocols.io.bp2l6x45klqe/v1), in which parameters related to locomotion and the vertical position occupied by

the animal in the tank (15 cm x 25 cm x 20 cm; width, length, height) were evaluated. Dechlorinated water was used for both the recovery tank and the test tank. Using the fish net, the fish was transferred to the test tank, where it remained for the 6-min recording period, during which time the fish's behavior was recorded, generating a spatio-temporal exploratory profile pattern. The behavioral endpoints (Table 1) were transcribed using The Real Fish Tracker software (v. 0.4.0; https://www.dgp.toronto.edu/~mccrae/projects/FishTracker/).

Lipid peroxidation assay

Immediately after the end of the experiments, animals were cryoanesthesized and sacrificed through spinal bisection, and their brains were dissected, homogenized, and frozen. After thawing samples, the protocol described by Sachett et al. (2020) to adapt the TBARS (thiobarbituric acid reactive substances) assay for zebrafish. The test quantifies the production of malondialdehyde (MDA), a byproduct generated by lipid oxidation in samples. The concentrations of TBARS were adjusted according to the amount of protein present in each sample, determined by the Bradford method (Zor & Selinger, 1996).

Statistical analysis

Analysis of differences in agonistic behavior in days 1 and 5 of the dyadic encounters were assessed using 2 (intra-subject factor, day) x 2 (inter-subject factor, status) repeated measures analyses of variance (RMANOVA). Analysis of differences in morphometric variables between males and females were made using unpaired t-tests. The relationship between morphometric variables and social status were made using a logistic regression model. The effects of social status and drug treatment on anxiety-like behavior and lipid peroxidation were analyzed using 2 (status, with levels “CTRL”, “DOM”, and “SUB”) x 2 (drug, with levels “VEH” and “DPAT”) analyses of variance (ANOVAs). In all cases, differences were considered significant when $p < 0.001$.

Results

Hierarchy establishment

All dyads resulted in clear dominant-subordinate hierarchies, as assessed by bottom-dwelling (Fig. 2A), attacks (Fig. 2B), chasing (Fig. 2C), and retreats (Fig. 2D) in observations made during day 1 and day 5 of dyadic interactions. A repeated measures ANOVA (RMANOVA) found significant effects of day ($F_{[1, 8]} = 17.764$, $p = 0.003$, $\eta^2p = 0.689$) and day X status interaction ($F_{[1, 8]} = 7.843$, $p = 0.023$, $\eta^2p = 0.495$) on time on bottom, with dominants spending more time patrolling the bottom on day 5 (DOM day 1 vs. DOM day 5: $d = -2.459$, $p = 0.007$; DOM day 5 vs. SUB day 5: $d = -2.264$, $p = 0.05$). RMANOVA found a significant effect of day on attacks ($F_{[1, 8]} = 9.281$, $p = 0.016$, $\eta^2p = 0.537$), but not a significant interaction ($F_{[1, 8]} = 0.439$, $p = 0.526$, $\eta^2p = 0.052$), with both dominant and subordinate animals increasing attacks on day 5 ($d = -1.362$, $p = 0.016$). RMANOVA did not find significant effects of day on chasing ($F_{[1, 8]} = 5.295$, $p = 0.07$, $\eta^2p = 0.514$), but a significant day X status interaction was found ($F_{[1, 8]} = 8.077$, $p = 0.036$, $\eta^2p = 0.618$), with dominant animals performing more chases in day 5 (DOM day 1 vs. DOM day 5: $d = -1.555$, $p = 0.077$; DOM day 5 vs. SUB day 5: $d = -3.068$, $p = 0.005$). Finally, RMANOVA found significant effects of day ($F_{[1, 8]} = 6.207$, $p = 0.037$, $\eta^2p = 0.437$) and day X status interaction ($F_{[1, 8]} = 5.289$, $p = 0.05$, $\eta^2p = 0.398$) on retreats, with subordinates performing more retreats on both day 1 (DOM day 1 vs. SUB day 1: $d = 2.022$, $p = 0.016$) and day 5 (SUB day 1 vs. SUB day 5: $d = -2.181$, $p = 0.029$; DOM day 5 vs. SUB day 5: $d = -4.116$, $p = 0.003$).

A logistic regression of morphometric data on status (dominant vs. subordinate) found that only maximum body depth predicted status (model statistics: $\Delta\chi^2_{[df = 33]} = 14.487$, $p = 0.152$; for maximum body depth: estimate = -9.535, odds ratio = 7.231×10^{-5}; $p = 0.046$); dominant individuals had higher maximum body depth (DOM: 0.708 ± 0.147 cm; SUB: 0.56 ± 0.17 cm). Sex information was available for 63 of the 85 animals used in the experiment. The proportion of dominant (66.67%) and subordinate (61.9%) females was similar to the overall proportion of females in the total sample (69.84%), and therefore no association between sex and status were found ($\chi^2_{[df = 2]} = 2.298$, $p = 0.317$, $n = 63$). Morphometric differences likely do not account for this lack of effect, since females showed lower body weight (□: 36.19± 108.024 mg; □: 138.576± 144.08 mg), larger head length (□: 0.577± 0.15 cm; □: 0.44± 0.1 cm), and larger body width (□: 0.421± 0.105 cm; □: 0.344± 0.1 cm), but not maximum body depth (□: 0.674± 0.186; □: 0.575± 0.129 mg), in relation to males (t-test, $p < 0.02$ for all significant variables; full data can be found at https://github.com/lanec-unifesspa/5HT/tree/main/5HT1A/SocialStress).

Effects of 8-OH-DPAT on the light/dark test

A significant main effect of status ($F_{[2, 79]} = 5.987$, $p = 0.004$, $\eta^2p = 0.132$), but not drug ($F_{[1, 79]} = 3.052$, $p = 0.085$, $\eta^2p = 0.037$), was found for scototaxis (Fig. 3A). A significant interaciton effect was also found ($F_{[2, 79]} = 3.213$, $p = 0.046$, $\eta^2p = 0.075$). Post-hoc tests found that subordinate stress increased scototaxis (CTRL + VEH vs. SUB + VEH: $d = 1.09$, $p = 0.043$; DOM + VEH vs. SUB + VEH: $d = 1.536$, $p = 0.003$), and treatment with 8-OH-DPAT reverted this effect in subordinate animals (SUB + VEH vs. SUB + DPAT: $d = -1.159$, $p = 0.039$). 8-OH-DPAT did not

affect behavior in controls (CTRL + VEH vs. CTRL + DPAT: d = -0.062, p = 1) or dominant animals (DOM + VEH vs. DOM + DPAT: d = 0.072, p = 1).

A significant main effect of status ($F_{[2, 79]}$ = 9.072, p < 0.001, η^2p = 0.187), but not drug ($F_{[1, 79]}$ = 0.433, p = 0.512, η^2p = 0.005), was found for erratic swimming (Fig. 3B). No interaction was found between status and drug ($F_{[1, 79]}$ = 1.154, p = 0.321, η^2p = 0.028). Post-hoc tests found that, irrespective of drug, subordinate animals showed higher erratic swimming (CTRL vs. SUB: d = -0.733, p = 0.02; DOM vs. SUB: d = -1.12, p < 0.001).

No main effects of status ($F_{[2, 79]}$ = 0.759, p = 0.473, η^2p = 0.026) nor drug ($F_{[1, 79]}$ = 0.541, p = 0.465, η^2p = 0.009) were found for freezing (Fig. 3C); interaction effects were also absent ($F_{[2, 79]}$ = 0.12, p = 0.887, η^2p = 0.004). Finally, no main effects of status ($F_{[2, 79]}$ = 0.089, p = 0.915, η^2p = 0.002) or drug ($F_{[1, 79]}$ = 0.428, p = 0.515, η^2p = 0.005), nor an interaction effect ($F_{[2, 79]}$ = 0.647, p = 0.527, η^2p = 0.016), were found for swimming speed (Fig. 3D).

Effects of 8-OH-DPAT on the novel tank test

No significant main effects of status ($F_{[2, 65]}$ = 2.451, p = 0.094, η^2p = 0.07) or drug ($F_{[1, 65]}$ = 0.282, p = 0.597, η^2p = 0.004) were found for geotaxis (Fig. 4A). A significant interaction effect, however, was found ($F_{[2, 65]}$ = 7.407, p = 0.001, η^2p = 0.186). Post-hoc tests found that subordinate individuals displayed more geotaxis than controls (d = -1.179, p = 0.036) and dominant animals (d = -1.709, p = 0.001), and 8-OH-DPAT partially reversed this effect (SUB + VEH vs. SUB + DPAT: d = 1.169, p = 0.05). No significant main or interaction effects were found for erratic swimming (Fig. 4B; status: $F_{[2, 65]}$ = 0.544, p = 0.583, η^2p = 0.016; drug: $F_{[1, 65]}$ = 0.991, p = 0.323, η^2p = 0.015; interaction: $F_{[2, 65]}$ = 0.067, p = 0.936, η^2p = 0.002). Similar results were observed for freezing (Fig. 4C; status: $F_{[2, 65]}$ = 0.065, p = 0.937, η^2p = 0.002; drug: $F_{[1, 65]}$ = 0.39, p = 0.535, η^2p = 0.006; interaction: $F_{[2, 65]}$ = 0.819, p = 0.445, η^2p = 0.025) and swimming speed (Fig. 4D; status: $F_{[2, 65]}$ = 0.379, p = 0.686, η^2p = 0.012; drug: $F_{[1, 65]}$ = 0.231, p = 0.632, η^2p = 0.004; interaction: $F_{[2, 65]}$ = 0.365, p = 0.696, η^2p = 0.011).

Effects of 8-OH-DPAT on oxidative stress

Main effects of status ($F_{[2, 119]}$ = 6.605, p = 0.002, η^2p = 0.1) and drug ($F_{[1, 119]}$ = 6.604, p = 0.011, η^2p = 0.053) were found for brain lipid peroxidation (Fig. 5). A significant interaction was also found ($F_{[2, 119]}$ = 12.263, p < 0.001, η^2p = 0.171). Post-hoc tests found that subordinate stress increased lipid peroxidation (CTRL + VEH vs. SUB + VEH: d =−1.531, p < 0.001; DOM + VEH vs. SUB + VEH: d =−1.625, p < 0.001), and 8-OH-DPAT reversed this effect (SUB + VEH vs. SUB + DPAT: d = 1.687, p < 0.001). 8-OH-DPAT did not affect lipid peroxidation in controls (CTRL + VEH vs. CTRL + DPAT: d =−0.135, p = 0.99) or dominant animals (DOM + VEH vs. DOM + DPAT: d =−0.15, p = 0.996).

Discussion

The present work investigated whether 8-OH-DPAT produces social status-dependent effects on anxiety-like behavior and oxidative stress in zebrafish. It was found that subordinate animals showed higher scototaxis and erratic swimming in the light/dark test (LDT) and higher geotaxis in the novel tank test (NTT), and that 8-OH-DPAT partially reversed these effects in subordinate animals, but had no behavioral effect on dominants and controls. Subordinate animals also displayed higher lipid peroxidation levels in their brains, an effect that was also partially reversed by 8-OH-DPAT treatment.

The results of dyadic encounters for 5 days produced a clear dominant-subordinate hierarchy in animals which were subjected to the procedure, with dominant animals patrolling the bottom, attacking, and chasing, while subordinate animals freezing or retreating from attacks. This is consistent with previous work showing changes in aggressive behavior in zebrafish with similar protocols (Dahlbom et al., 2012; Filby et al., 2010; Paull et al., 2010). Animals that would be classified as dominants at day 5 already displayed high aggression levels at day 1, which is consistent with the role of aggression in securing territories and establishing dominance, and is also observed in other experiments (Dahlbom et al., 2012). While the specific morphometric parameters that predicted status were different from those assessed by Paull et al. (2010), dominant individuals had higher maximum body depth than subordinate individuals, suggesting that larger individuals are more likely to become dominant. Finally, agreeing with Bozi et al. (2021), male and female individuals were likely to occupy any status in the hierarchy, irrespective of whether the opponent was same-sex or different-sex.

Importantly, in the present experiments, all dependent variables used to measure anxiety-like behavior did not show a reversed effect neither in the novel tank nor in the light-dark preference test, except for the increase in geotaxis in the novel tank and scototaxis in the light-dark test. Both of these were reversed by 8-OH-DPAT. These endpoints indicated a decrease in anxiety-like behavior after administration of 8-OH-DPAT; although the anxiogenic-like effect was only reversed in case of subordinate stress, it did not have a behavioral effect on dominant or subordinate animals. Filby et al. (2010) found sex-specific adjustments in the expression of genes involved in 5-HT synthesis, metabolism, and transport in the hypothalamus and forebrain of subordinate and dominant animals; since 5-HT activity appear to be increased in the

brainstem of subordinate animals (Dahlbom et al., 2012). Those results were contrary to what was expected by the hypothesis, it can indicate a long-term inhibition of autoreceptors in subordinates.

It is reasonable to suppose that these adjustments are related to an increased serotonergic tone on the raphe, which should lead to a desensitization of autoreceptors. However, if that was simply the case, the effects of 8-OH-DPAT should be smaller or absent in subordinate animals. Given the receptor reserve that is observed for anxiety-like behavior in zebrafish (Bruce de Souza et al., 2025), however, it seems likely that the effects of 8-OH-DPAT that were observed in subordinate animals in the present work are mediated by heteroreceptors. In the zebrafish brain, autoreceptor activity occurs at the pretectal diencephalic cluster, superior raphe nucleus, paraventricular organ, and the caudal zone of the periventricular hypothalamus (Norton et al., 2008). Heteroreceptor sites include the ventral nucleus of the ventral telencephalic area (homologous to the septum: Ganz et al., 2012), the dorsal nucleus of the ventral telencephalic area (homologous to the striatum: Ganz et al., 2012), the anterior thalamic nucleus, periventricular gray zone, central posterior thalamic nucleus, periventricular nucleus of the posterior tuberculum, and ventral zone of periventricular hypothalamus (Norton et al., 2008). Some of these regions – particularly the ventral nucleus of the ventral telencephalic area (Vv) and the preoptic area – are activated by agonistic encounters in both winner and loser zebrafish (Teles et al., 2015); however, winners of an encounter (which are more likely to become dominant), but not losers, show increased connectivity between Vv and preoptic area (Teles et al., 2015). This suggests that the effects of 8-OH-DPAT are mediated by 5-HT_{1A} receptors located in other areas (Fig. 6).

Similar results were observed with the 5-HT_{2C} agonist MK-212, which produced a larger anxiogenic-like effect in subordinates than dominants or controls (De Oliveira et al., 2025). This suggests a similar mechanism of sensitization of behavioral responses to 5-HT agonists at the heteroreceptor level, and a dual mechanism through which 5-HT_{2C} receptors increase anxiety-like behavior while 5-HT_{1A} receptors decrease it in a social status-dependent way. Further studies are necessary to understand whether this is mediated by changes in receptor expression levels and the brain region(s) that mediate these effects.

In addition to the behavioral effects of subordinate stress, an increase in oxidative stress in the brains of these animals was also observed. This is consistent with previous findings with this species (Lima-Maximino et al., 2025), which show that different stressors (including subordinate stress) produce different patterns of behavioral adjustments and brain lipid peroxidation. 8-OH-DPAT was also able to revert these effects, suggesting that oxidative stress is a downstream mediator of anxiety-like behavior. 8-OH-DPAT has been shown to inhibit the effects of oxidative stress in mammalian cells *in vitro* (Lee et al., 2005; Thampi et al., 2012), suggesting another mechanism by which activation of the 5-HT_{1A} receptor can mediate neurobehavioral effects. A likely candidate to initiate oxidative stress is glutamate, since not only activation of glutamate receptors can lead to redox imbalance (Pereira & Oliveira, 2000), it has also been shown to interact with serotonergic signaling to mediate anxiety-like behavior in zebrafish (Herculano et al., 2015).

An important limitation of the present study is that anxiety-like behavior of dominant and subordinate animals was not assessed prior to hierarchy formation, and neither were the effects of 8-OH-DPAT. While animals that would be later classified as dominant showed higher aggression already at day 1, and the 5-HT1A antagonist WAY 100,635 was shown to increase aggression in dominant males (Filby et al., 2010), it is currently not known whether (future) dominant and subordinate individuals already show differences in anxiety-like behavior before hierarchy establishment, and whether this is mediated by already-present differences in the serotonergic system. While the first seems possible, given that “boldness” (asssessed in an open field test, a roof test, and the novel object test) predicts dominant status in zebrafish (Dahlbom et al., 2011), the latter is unlikely, given that 5-HT1A receptors appear to be overexpressed in the hypothalamus of *dominant*, but not subordinate zebrafish, on day 1 of dyadic encounters (Filby et al., 2010). Nonetheless, more experiments are needed to confirm this, which would help understand whether presently observed differences are due to subordinate status or are already-present before social conflict.

Declarations

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Conflict of interests statement

The authors declare no conflict of interest.

Data availability statement

Data for the experiments reported in the manuscript can be found at https://github.com/lanec-unifesspa/5HT/tree/main/5HT1A/SocialStress

Author contributions

CRediT (https://credit.niso.org/): Conceptualization: ARBP, CM; Data curation: MLM, CM; Formal analysis: ARBP, TRRR, CM; Investigation: ARBP, TRRR, AOSdM, BPDdC, JAH, CLDdS; Methodology: ARBP, MLM, CM; Project administration: MLM, CM; Resources: CM; Supervision: CM; Validation: MLM, CM; Visualization: ARBP, TRRR, AOSdM, BPDdC, JAH, CLDdS, MLM, CM; Writing – original draft: ARBP, TRRR, AOSdM, BPDdC, JAH, CLDdS, MLM, CM; Writing – review & editing: MLM, CM.

Ethical statement

All protocols for the present experiments were made in accordance with the protocol adopted in the Brazilian guideline for the care of zebrafish in the laboratory (Diretriz brasileira para o cuidado e a utilização de animais para fins científicos e didáticos - DBCA. Anexo I. Peixes mantidos em instalações de instituições de ensino ou pesquisa científica, 2017), and were approved by the Animal Use Ethics Committee of UNIFESSPA (Universidade Federal do Sul e do Sudeste do Pará) protocol number 23479.019576/2022-43.

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Figures

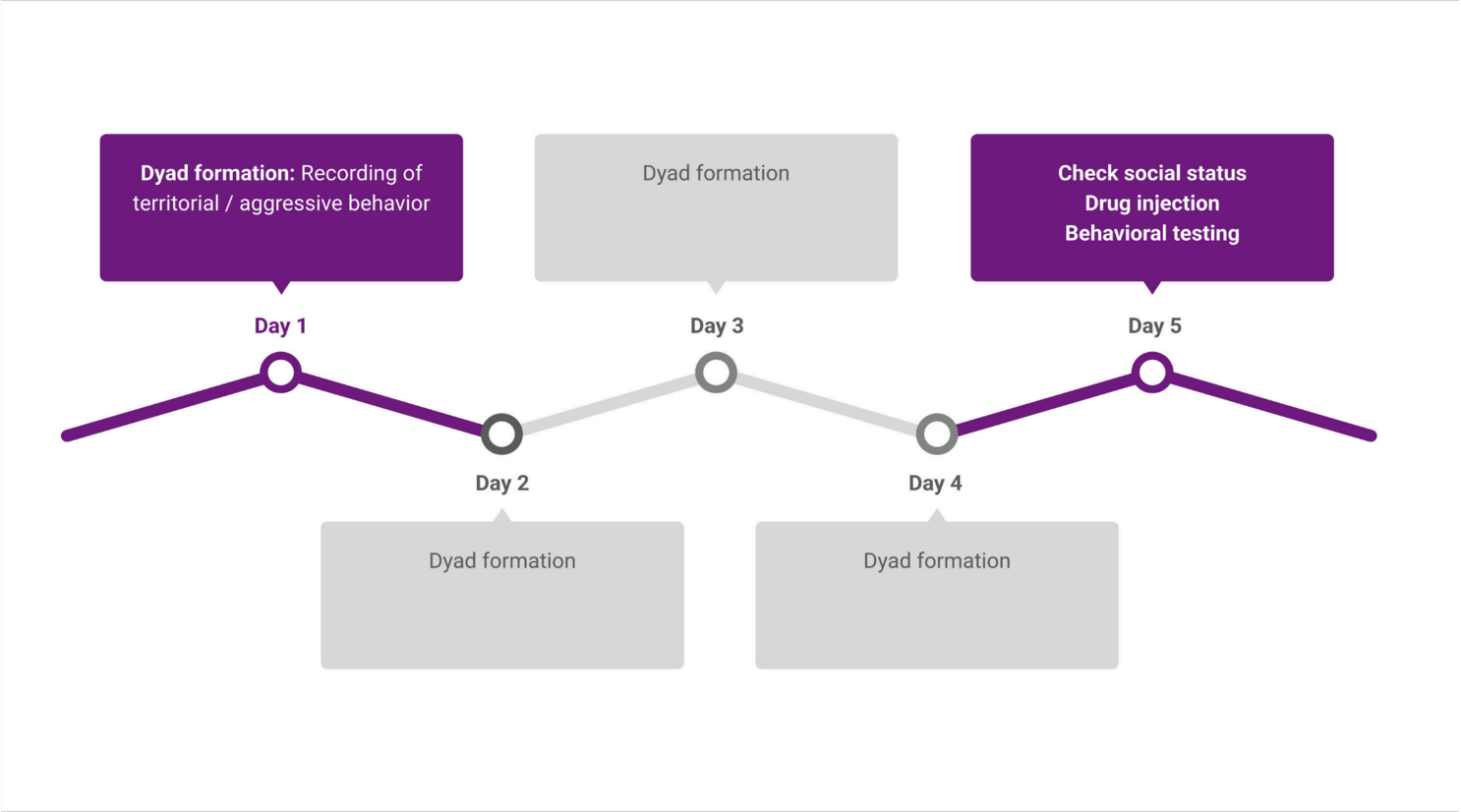


Figure 1

Experimental timeline. Animals were separated in dyads in Day 1, and their territorial / aggressive behavior was recorded, with the units described in Table 1. On Days 2-4, animals were kept in dyadic interaction full-time, and checked for potential injuries. On Day 5, status was determined by observing territorial / aggressive behavior, and both dominant and subordinate animals were injected with either vehicle or 8-OH-DPAT; injections were randomly determined. After injection, animals were tested in the light/dark test and the novel tank test. Immediately after the end of tests, animals were sacrificed and their brains and gonads were dissected.

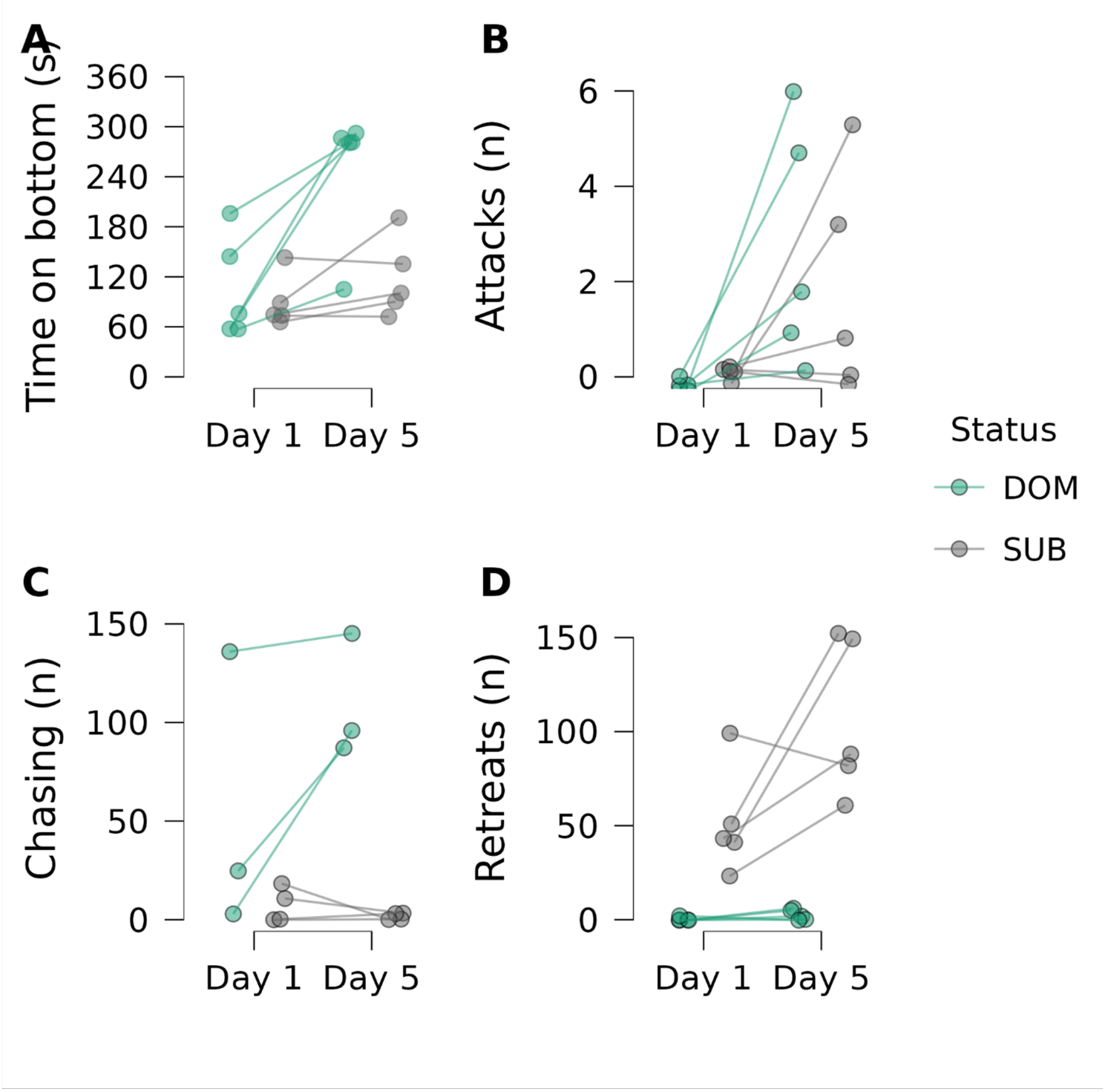


Figure 2

Changes in (A) Bottom-dwelling, (B) attacks, (C) chasing, and (D) retreats in dominant (DOM, green) and subordinate (SUB, grey) zebrafish between days 1 and 5 of the dyadic encounter. The raw data is plotted; each paired set of observations is connected by a line.

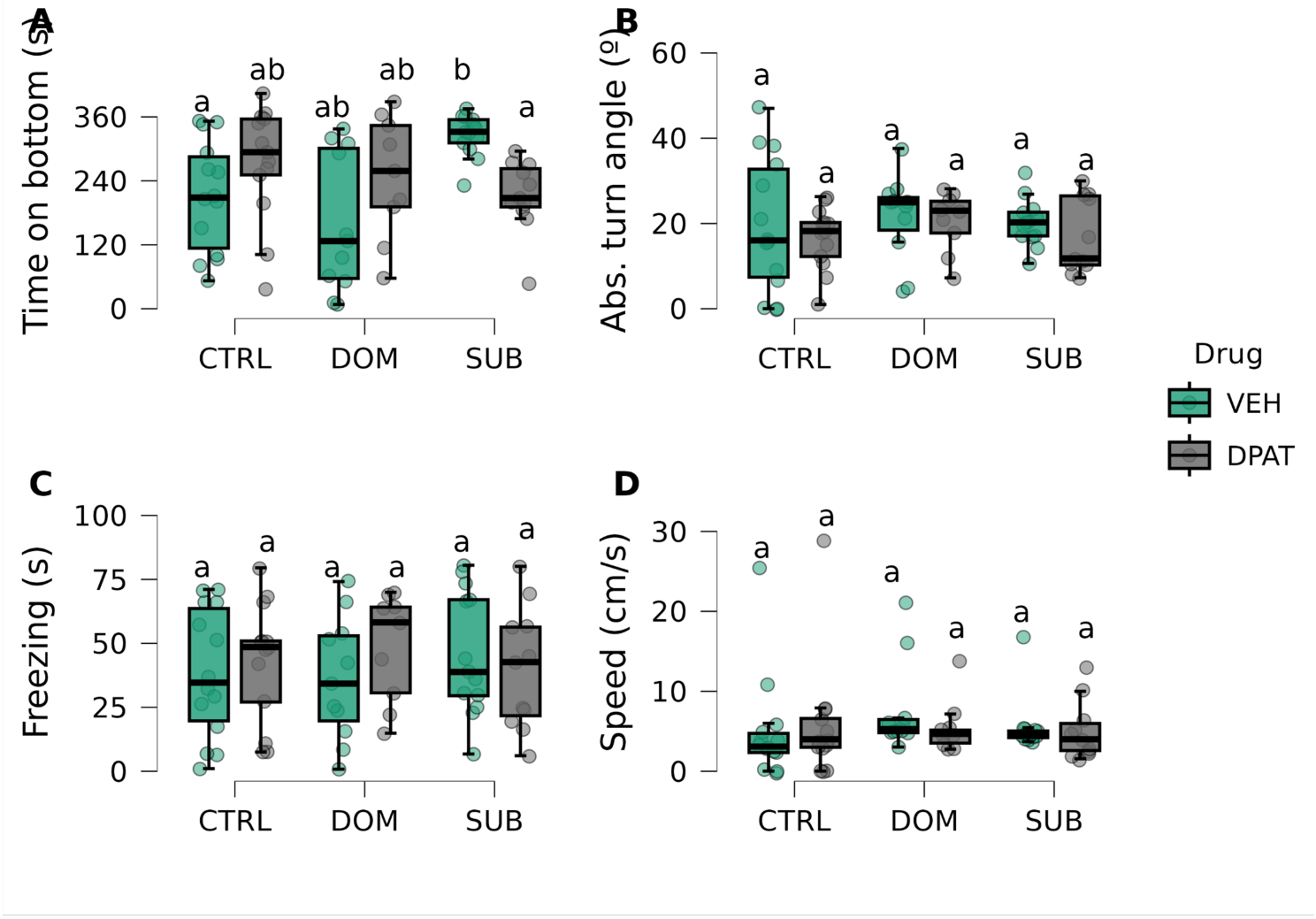


Figure 3

Changes in (A) Scototaxis, (B) erratic swimming, (C) freezing, and (D) swimming speed in controls (CTRL), dominant (DOM), and subordinate (SUB) zebrafish treated with vehicle (VEH, green) or 8-OH-DPAT (DPAT, grey) before exposure to the light/dark test (LDT). Each dot represents an individual animal; boxplots represent the group. Letters above the boxplots represent statistically significant differences at the $p < 0.05$ level; boxes followed by a common letter are not significantly different by the HSD-test at the 5% level of significance. HSD = Tukey's honestly significant difference at 5% level of significance.

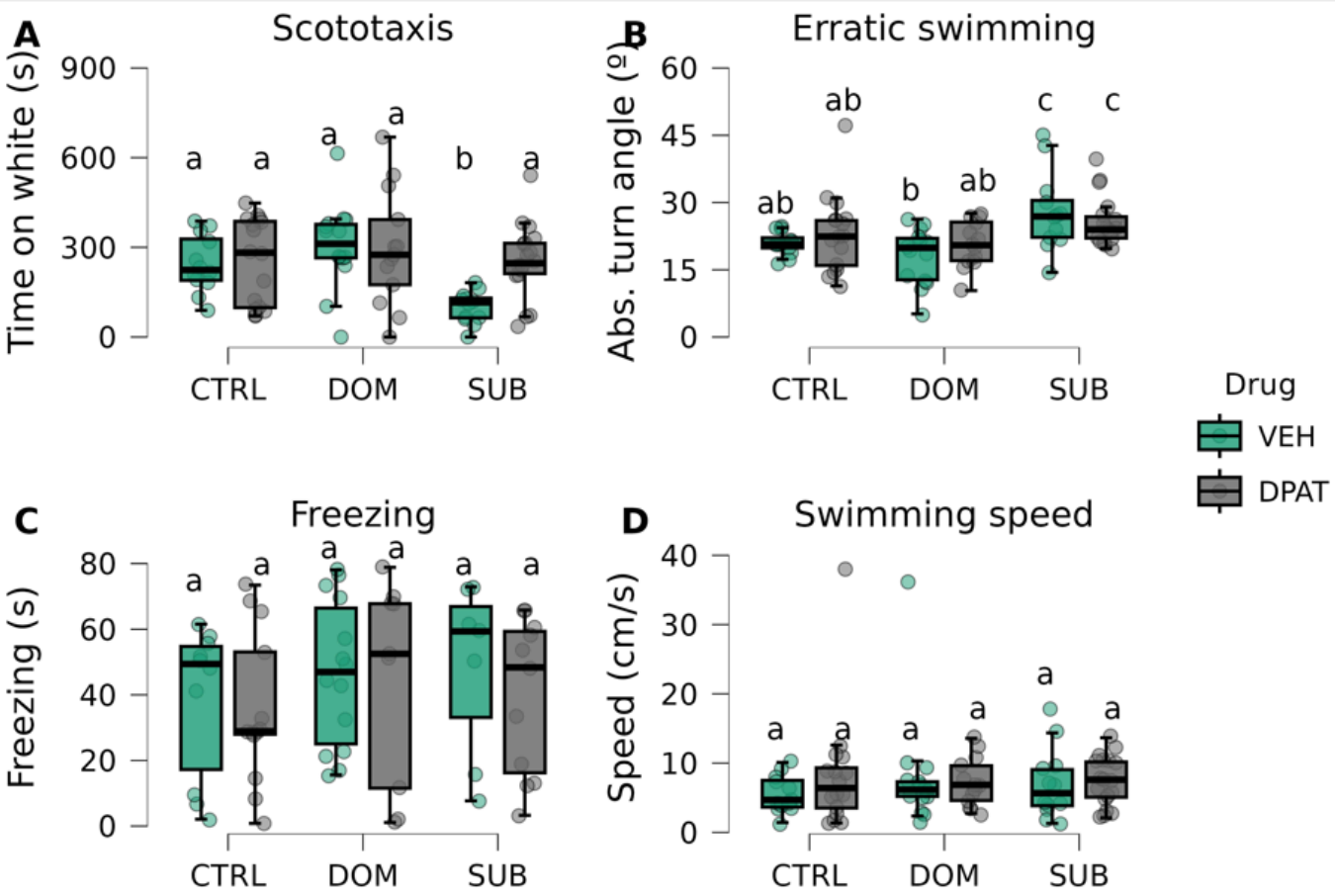


Figure 4

Changes in (A) Geotaxis, (B) erratic swimming, (C) freezing, and (D) swimming speed in controls (CTRL), dominant (DOM), and subordinate (SUB) zebrafish treated with vehicle (VEH, green) or 8-OH-DPAT (DPAT, grey) before exposure to the novel tank test (NTT). Each dot represents an individual animal; boxplots represent the group. Letters above the boxplots represent statistically significant differences at the $p < 0.05$ level; boxes followed by a common letter are not significantly different by the HSD-test at the 5% level of significance. HSD = Tukey's honestly significant difference at 5% level of significance.

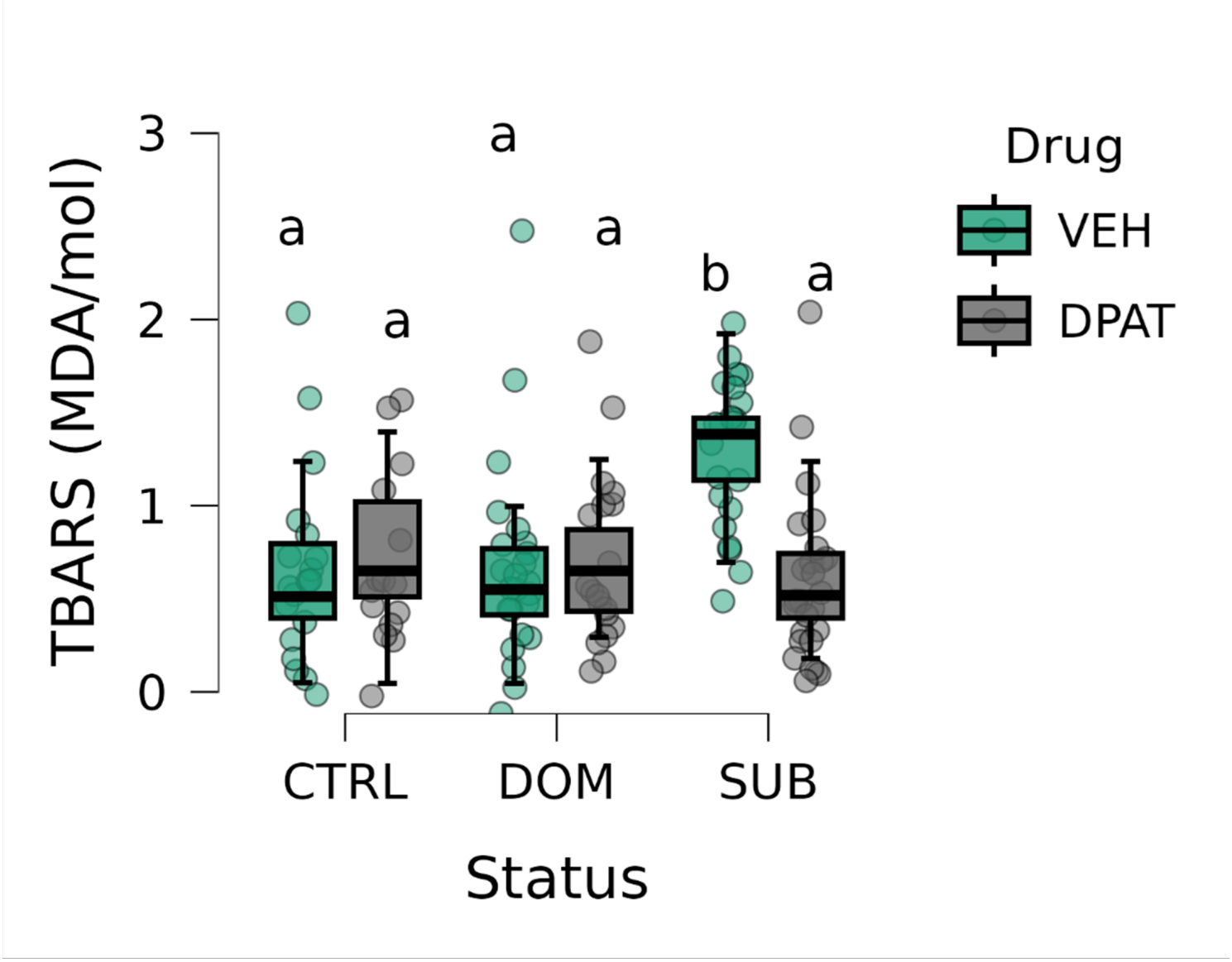


Figure 5

Changes in brain lipid peroxidation in in controls (CTRL), dominant (DOM), and subordinate (SUB) zebrafish treated with vehicle (VEH, green) or 8-OH-DPAT (DPAT, grey). Each dot represents an individual animal; boxplots represent the group. Letters above the boxplots represent statistically significant differences at the $p < 0.05$ level; boxes followed by a common letter are not significantly different by the HSD-test at the 5% level of significance. HSD = Tukey's honestly significant difference at 5% level of significance.

Image not available with this version

Figure 6

Suggested mechanisms of social plasticity in 8-OH-DPAT efficacy. 8-OH-DPAT produced anxiolytic-like effects in subordinate, but not dominant or control zebrafish. This is likely due to desensitization of autoreceptors at 5-HT neurons (in the superior raphe, periventricular

organ, ventral hypothalamus, and/or pretectal diencephalic cluster) and sensitization of heteroreceptors at postsynaptic targets (in the dorsal or ventral nuclei of the ventral telencephalic area, parvocellular preoptic region, periventricular gray zone, and/or periventricular nucleus of posterior tuberculum).

Supplementary Files

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