Roles of the 5-HT1A receptor in zebrafish responses to potential threat and in sociality

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ABSTRACT

Anxiety is a normal emotion representing a reaction to potential danger, whereas fear can be defined as a reaction to real, explicit danger. Anxiety-like behavior in animal models has been associated with differences in the serotonergic system. Treatment of zebrafish cohorts with 8-OH-DPAT, a full agonist at the 5-HT_{1A} receptor, decreased anxiety-like behavior in the novel tank test, but increased it in the light-dark preference test, both considered assays

for anxiety-like behavior for this species. The same treatment decreased social approach in both the social investigation and social novelty phases of the social preference test. These effects suggest a participation of the 5-HT_{1A} heteroreceptors in zebrafish anxiety and social preference, decreasing both. Thus, the study of this receptor is important for a better understanding of anxiety-like behavior in zebrafish and its relationship with similar phenomena in vertebrates.

Keywords: zebrafish; model organism; heteroreceptor; vertebrates; defensive behavior

1. Introduction

Under situations of potential threat, humans and non-human animals adjust their behavior to cautious exploration, risk assessment, and behavioral inhibition, sometimes described as “anxiety-like behavior” (McNaughton & Corr, 2004). This pattern of defensive behavior is conceptually related to a fear-like state, but fear usually involves a response to an actual threat, whether that threat is proximal or distal (Perusini & Fanselow, 2015). While both are part of a taxonomy of defensive behavior, there is growing evidence to show that anxiety and fear are distinct emotions in terms of behavioral responses, physiological settings, and neural bases (Fanselow & Lester, 1988; McNaughton & Corr, 2022; McNaughton & Zangrossi Jr, 2008; Perusini & Fanselow, 2015).

The relationship between the neurobehavioral responses to threat and the serotonergic system points to a complex regulation, in which different subpopulations of serotonergic neurons in the dorsal raphe nucleus and the medial raphe nucleus are uniquely involved in the pathophysiology of anxiety and affective disorders (Deakin & Graeff, 1991; Maximino, 2012; Paul et al., 2014). This control is thought to be mediated by topographically organized projections to different areas of the limbic forebrain and midbrain (Abrams et al., 2004, 2005; Paul & Lowry, 2013). Serotonergic projections to forebrain regions, such as the amygaloid nuclei and the ventral hippocampus, increase behavioral responses to potential threat when activated, possibly via 5-HT2 and 5-HT3 receptors (Graeff et al., 1997; Maximino, 2012; Olivier et al., 2000; Quesseveur et al., 2012). In the midbrain – especially in the periaqueductal gray area –, activation of 5-HT1A and 5-HT2 receptors lead to an inhibition

of neurobehavioral responses to actual threat (Castilho et al., 2002; Gomes & Nunes-de-Souza, 2009; Graeff, 2004; Graeff et al., 1997; Lima-Maximino et al., 2020; Paul et al., 2014). Thus, the Deakin-Graeff theory postulates not only the apparently paradoxical "double role" of serotonin on fear-like and anxiety-like behaviors in relation to different parts of the neuraxis, but also the differential participation of serotonin receptors in these effects.

The 5-HT1A receptor (5-HT1AR) is one of the most abundant and widely expressed receptors in the brain and the main inhibitory serotonergic receptor (Akimova et al., 2009; Albert & Vahid-Ansari, 2019). This receptor is found in two populations, the presynaptic autoreceptors (which promote local inhibitory control of neuronal firing rates and 5-HT synthesis in serotonergic neurons), which are located in the cell body and dendrites of 5-HT neurons; and postsynaptic heteroreceptors (which modulate the release of other neurotransmitters in non-serotoninergic neurons), which are located in the dendrites and cell body of non-serotoninergic neurons in 5-HT projection areas (Albert & Vahid-Ansari, 2019).

There is some evidence for a large receptor reserve for autoreceptors in opposition to heteroreceptors (Cox et al., 1993; Meller et al., 1990), which could explain why azapirones, partial agonists of the 5-HT1AR with anxiolytic-like activity in clinical settings (Chessick et al., 2006), act as antagonists at heteroreceptor sites and full agonists in autoreceptors (Newman-Tancredi et al., 2019). Low doses of 8-OH-DPAT, a full agonist, and buspirone, a partial agonist, preferentially activate 5-HT1A autoreceptors, and consequently, the release of 5-HT from serotonergic nerve endings decreases. Therefore, 5-HT synthesis is reduced as a feedback mechanism. From a systemic point of view, therefore, the effect of buspirone leads to inhibition of the activity of serotonergic neurons (and consequent decrease in the release of

5-HT) associated with the blockade of 5-HT1A postsynaptic receptors. Full agonists, such as 8-OH-DPAT, appear to act as full agonists on both autoreceptors and heteroreceptors (Sprouse & Aghajanian, 1988), decreasing the availability of 5-HT in other receptor subtypes at the same time that post-synaptic 5-HT1ARs are activated. This discrepancy could explain why both partial agonists and antagonists of the 5-HT1AR decrease defensive behavior to potential threat (Cao & Rodgers, 1997; Maximino et al., 2013; Rodgers & Cao, 1997). The putative differential effects of drugs on auto- and heteroreceptors lead to the necessity of understanding the roles of these different sites in the control of defensive behavior. Studies using mice lacking 5-HT1A auto- or heteroreceptors (Richardson-Jones et al., 2011), 5-HT1AR knockouts with expression rescued in pre- or post-synaptic sites (Gross et al., 2002; Piszczek et al., 2013), intact animals treated with biased agonists that activate pre- or postsynaptic 5-HT1ARs *in vivo* (Jastrzębska-Więsek et al., 2018), or microinjection of agonists in the raphe or in postsynaptic sites (File et al., 1996; File & Gonzalez, 1996), produced conflicting results.

The roles of 5-HT1ARs were also investigated in non-mammalian model organisms, including zebrafish (*Danio rerio* Hamilton 1822). This model organism has increasingly been used in neuroscience and behavioral pharmacology to understand the mechanisms of defensive behavior (Cueto-Escobedo et al., 2022; Gerlai, 2020, 2023; MacRae & Peterson, 2023). While there is considerable conservation of the serotonergic system is in zebrafish, there are important differences in the degree of neuroanatomical and genomic conservation (Herculano & Maximino, 2014). For example, zebrafish present extra serotonergic nuclei in the brain in addition to the raphe (Kaslin & Panula, 2001; Lillesaar et al., 2009), and a

genomic duplication event led to multiple copies of genes involved in serotonin synthesis, transport, and metabolism, as well as of serotonin receptors (including two copies of the 5-HT1AR) (Norton et al., 2008; Sourbron et al., 2016). Despite this, the role of the serotonergic system appears to be relatively conserved in zebrafish (Herculano & Maximino, 2014). Some evidence suggests a role for 5-HT1A receptors in anxiety-like behavior in zebrafish; for example, buspirone produces consistent type-anxiolytic effects in behavioral assays with zebrafish, regardless of the route of administration, dose, or test (Abozaid & Gerlai, 2022; Bencan et al., 2009; Connors et al., 2014; Facchin et al., 2015; Gebauer et al., 2011; Hawkey et al., 2021; Lau et al., 2011; Maaswinkel et al., 2012, 2013; Maximino et al., 2011, 2014).

Maximino et al. (2013) evaluated behavior characterized by scototaxis (preference for dark environments over light environments) and geotaxis (the measurement of time at the top or bottom of the apparatus). They observed anxiety-like behavior correlated positively with extracellular levels of 5-HT in the brain in the novel tank test (NTT), while the correlation was negative in the light-dark test (LDT). This suggests that 5-HT may regulate zebrafish behavior differently in the NTT and LDT tests. Nonetheless, buspirone produced an anxiolytic-like effect in both the NTT and the LDT. Puzzlingly, WAY 100635, a full 5-HT_{1A} receptor antagonist, also produced anxiolytic-like effects in both tests. Nowicki et al. (2014), on the other hand, observed an anxiogenic-like effect on the NTT after treatment with p-MPPF, another 5-HT_{1A} receptor antagonist. The similar effects of buspirone, a partial agonist, and WAY 100,635, a full antagonist, suggest the existence of a receptor reserve in presynaptic sites. Furthermore, there are no studies with full agonists in this species. Thus,

the aim of the present study is to evaluate the effect of treatment with 8-OH-DPAT, a full agonist of the 5-HT_{1A} receptor, on behaviors relevant to anxiety and sociability in zebrafish.

2. Materials and methods

2.1 Animals and housing

48 adult zebrafish (*Danio rerio*) (>4 months old) from the shortfin phenotype were used in the experiments. Adult wild-caught zebrafish (4-6 months old) of both sexes from the strain were obtained from a colony resulting from cross-breeding between animals purchased in specialized stores. All animals were kept in 50L tanks 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 (Conselho Nacional de Controle de Experimentação Animal - CONCEA, 2017). 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.

2.2 Drugs and treatments

The 5-HT1A receptor agonist 8-OH-DPAT (8-hydroxy-2-(dipropylamino)tetralin; CAS#87394-87-4) was brought from Sigma-Aldrich (St Louis, USA) and was 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.

2.3. Experiment 1 : Novel tank test

In general, this task 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). 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. 12 adult zebrafish were used per group; the same 24 animals were also used in Experiment 2. Immediately after the intraperitoneal injection, the fish were relocated with a fish net to a recovery tank, where they remained for 30 minutes. Then, using

the fish net, the fish was transferred to the test tank, where it remained for the 6-min habituation period, during which time the fish's behavior was recorded, generating a spatio-temporal exploratory profile pattern. The behavioral endpoints were transcribed using The Real Fish Tracker software (v. 0.4.0; https://www.dgp.toronto.edu/~mccrae/projects/FishTracker/). Table 1 summarizes the endpoints assessed in Experiment 1.

Table 1

Behavioral endpoints assessed in the novel tank test (NTT), light/dark test (LDT), and social preference.

Test	**Behavior**	**ZBC code** (Kalueff et al., 2013)	**Description**	**Operationalization**
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)

	Top-dwelling	1.176	Dwelling of the animal near the surface of a novel tank; can include surfacing (ZBC1.164), with aberrant swimming very close to the water surface	Time spent in the top 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)
	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)
LDT	Scototaxis (main endpoint)	1.137	Preference for the dark part of the tank	Time spent in the black compartment (s)
	Risk assessment	n/a	Cautious exploration of the environment to investigate possible threats	Partial entries (up to half of body length) or full entries of low duration (< 1 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

	Thigmotaxis	1.173	Preference for tank walls	Percent of time, while in the white compartment, spent at a distance of less than 2 cm of a tank wall
	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)
Social preference test	Social approach	1.152	Tendency to spend time close to or inspecting conspecifics	Preference score (time near the conspecific (SI) or “new stranger” (SN) / time near the empty tank + time near the conspecifi)

	Social avoidance	n/a	Tendency to avoid novel conspecifics	Time spent near empty tank (SI) or "old stranger" (SN)
	Erratic swimming	1.51	Complex behavior in which the swim has no defined direction and the speed changes abruptly	Absolute turn angle (º)
	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)

2.4. Experiment 2: Light-dark test

Scototaxis behavior in zebrafish, characterized by a preference for dark environments over light environments, has been described and validated as a model for evaluating anxiety-like behavior for the species (Araujo et al., 2012; Maximino et al., 2010, 2011; Serra et al., 1999). 12 zebrafish were used in each group. Since animals were reused in Experiments 1 and 2, the order in which animals were subjected to either the NTT or the LDT was randomized. Immediately after the intraperitoneal injection, the fish was relocated with an fish net to a recovery tank, where it remained for 30 min. Then, using the fish net, the fish wass 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. Scototaxis, swimming speed, erratic swimming, and freezing were extracted using TheRealFishTracker (v. 0.4.0; https://www.dgp.toronto.edu/~mccrae/projects/FishTracker/), while risk assessment and thigmotaxis were scored manually with the help of X-Plo-Rat (Tejada et al., 2018; https://sites.ffclrp.usp.br/scotty/). Table 1 summarizes the endpoints assessed in Experiment 2.

2.5. Experimental 3: Social preference test

The social preference test was divided in two stages, social interaction and social novelty tests, and carried out based on the protocol described by Barba-Escobedo and Gould

(2012). These tasks aim to analyze the tendency of zebrafish to approach co-specifics and investigate novel social situations. 48 adult zebrafish were used as conspecific stimuli, while 24 adult zebrafish were used as focal fish in the test, where 12 animals were injected intraperitoneally with the vehicle solution (Cortland's saline solution), and 12 animals with 8-OH-DPAT. Animals from this experiment were not reused from Experiments 1 and 2, due to the longer extension of the test; exposing animals to three behavioral tests would risk the drug having been eliminated by the end of the third task. Conspecifics were drawn from different tanks than the hometank of the focal fish, and therefore focal fish had no prior contact with them. Immediately after intraperitoneal injection, the focal fish was relocated using a fish net to a recovery tank, where it remained for 30 min. Then, using the fish net, the focal fish is transferred to the test tank where it remains for 3 min for acclimatization. For both the social interaction and social novelty tests, three tanks were used: the main tank (15 cm x 25 cm x 20 cm; width, length, height), for the focal animal, and two other tanks for the conspecifics (15 cm x 17 cm x 16 cm; width, length, height). Both sides of the main tank were separated from the lateral tanks with barriers to prevent visual access. Once the fish's acclimatization period was over, the barriers were removed allowing the focal fish visual access to the conspecifics. In the social interaction test (SI), the behavior of 1 focal animal was evaluated for an initial period of 6 min, in which it will have visual access to an unknown conspecific and an empty tank. After that time interval, barriers were lowered, a new conspecific was transferred to the empty tank, and the barriers were again removed to allow the focal fish visual access to the conspecifics. The behavior was observed again for 6 minutes, consisting in the social novelty test (SN). The conspecific that was present in the former stage was labeled as "old stranger", while the conspecific that was introduced in the

empty tank was labeled as "new stranger". In both the social interaction and social novelty stages, behavior was filmed and later analyzed using TheRealFishTracker software (v. 0.4.0; https://www.dgp.toronto.edu/~mccrae/projects/FishTracker/); behavioral endpoints are described in Table 1.

2.6. Statistical analysis

Results obtained from the behavioral tests were analyzed using unpaired t-tests or Wilcoxon rank sum test with continuity correction for variables which violated the assumptions of parametric statistics. The data was represented as Gardner-Altman plots (Ho et al., 2019), with individual datapoints plotted on the upper axes. On the lower axes, each effect size (Cohen's d; Cohen, 1988) was plotted as a bootstrap sampling distribution. Bootstrap resamples were taken using Efron's (1987) method; the confidence interval is bias-corrected and accelerated. Plots were made using R 4.1.2, using the dabestr package (v. 2023.9.12; https://github.com/ACCLAB/dabestr).

2.7. Open science practices

Hypotheses were not formally preregistered. Data packages and analysis scripts can be found on our GitHub repository (https://github.com/lanec-unifesspa/5HT/tree/main/5HT1A).

3. Results

3.1. Novel tank test

8-OH-DPAT decreased geotaxis ($t_{[df = 19.484]} = 5.222$, $p < 0.001$; Figure 1A) and increased top-dwelling ($t_{[df = 16.386]} = -4.009$, $p < 0.001$; Figure 1B). No effects were observed on erratic swimming ($t_{[df = 21.998]} = -0.159$, $p = 0.875$; Figure 1C), freezing ($t_{[df = 17.214]} = -1.555$, $p = 0.138$; Figure 1D), or swimming speed ($t_{[df = 20.107]} = -0.971$, $p = 0.343$; Figure 1E).

3.2. Light/dark preference test

8-OH-DPAT increased scototaxis ($t_{[df = 17.361]} = 2.43$, $p = 0.262$; Figure 2A) and increased risk assessment ($W = 33$, $p = 0.024$; Figure 2B). No effect were found for erratic swimming ($t_{[df = 20.671]} = 0.727$, $p = 0.475$; Figure 2C), freezing ($W = 71.5$, $p = 0.99$; Figure 2D), thigmotaxis ($t_{[df = 14.873]} = -1.323$, $p = 0.206$; Figure 2E), or swimming speed ($t_{[df = 21.985]} = 1.71$, $p = 0.101$; Figure 2F).

3.3. Social preference test

During the SI phase, 8-OH-DPAT decreased the preference score ($t_{[df = 13.466]} = 2.981$, $p = 0.0103$; Figure 3A), with no effects on social avoidance ($t_{[df=21.594]} = 0.881$, $p = 0.388$; Figure 3B). No effects were observed on erratic swimming ($t_{[df = 21.392]} = 0.548$, $p = 0.589$; Figure 3C), nor on swimming speed ($t_{[df = 19.036]} = 1.1803$, $p = 0.252$; Figure 3D). During the SN phase 8-OH-DPAT also decreased the preference score ($t_{[df = 21.847]} = 2.667$, $p = 0.014$; Figure 4A), but the time near the “new stranger” was also decreased ($t_{[df = 21.767]} = 2.701$, $p = 0.013$; Figure 4B). No effect was observed on erratic swimming ($t_{[df = 21.785]} = -0.7101$, $p = 0.485$; Figure 4C), nor on swimming speed ($t_{[df = 21.797]} = -1.359$, $p = 0.188$; Figure 4D).

4. Discussion

In the present experiments, differences were observed in anxiety-like behavioral endpoints in the groups treated with the full agonist of the 5-HT1A receptor, 8-OH-DPAT, with opposite outcomes in the novel tank test (NTT) an light-dark test (LDT). While in the first 8-OH-DPAT decreased anxiety-like behavior, in the latter the drug increased it. We also observed decreases in social preference in both the social interaction (SI) and social novelty (SN) tests.

4.1. Effects of 8-OH-DPAT on anxiety tests

One of the most striking findings of this study is the difference in the effects of 8-OH-DPAT between the LDT and the NTT tests. In previous studies with serotonergic drugs in these two tests, it was shown that increasing 5-HT levels with acute fluoxetine increases geotaxis (the main endpoint of NTT) and decreases scototaxis (the main endpoint of the LDT), while decreasing 5-HT levels with pCPA produces the opposite effects (Maximino et al., 2013). On the other hand, both buspirone (partial 5-HT1AR agonist) and WAY 100,635 (full 5-HT1AR antagonist) decreased geotaxis and scotototaxis (Maximino et al., 2013). SB 224,289, an inverse agonist of the 5-HT1B receptor, reduced geotaxis but not scototaxis, an effect similar to that observed in the present experiment. Unlike buspirone, which produces consistent reductions in responses to potential threat in both the LDT and the NTT (Abozaid & Gerlai, 2022; Araujo et al., 2012; Bencan et al., 2009; Connors et al., 2014; Facchin et al., 2015; Gebauer et al., 2011; Hawkey et al., 2021; Lau et al., 2011; Maaswinkel et al., 2012,

2013; Maximino et al., 2011, 2013, 2014), 8-OH-DPAT seemed to produce opposite effects in the NTT and LDT. Despite being 5-HT_{1A} receptor agonists, there are important differences between buspirone and 8-OH-DPAT. In a context of large receptor reserves in presynatic neurons, as is the case with the rodent raphe, buspirone acts as a full agonist at the 5-HT1A autoreceptor, while it acts as an antagonist at the postsynaptic 5-HT1AR, reducing serotonin avaiability at other postynapic sites while simultaneously blocking postsynaptic 5-HT1ARs (Newman-Tancredi et al., 2019). Meanwhile, 8-OH-DPAT is a serotonergic drug that acts as a full agonist at both sites, reducing serotonin availability for other types of receptor in the postsynaptic sites while simultaneously activating postsynaptic 5-HT1ARs. It is therefore possible to assume that this is also the reason why the results differed between the tests.

A related question is why 8-OH-DPAT reduced behavioral responses to potential threat in the NTT and increased it in the LDT. One possibility is that NTT is more sensitive to pharmacological treatment than LDT. However, in a meta-analysis of the effects of drugs on the two tests, Kysil et al. (2017) found no differences in the sensitivity of both assays. A second possibility is that the effect observed on the NTT is not related to a decrease in responsiveness to potential threat, but represents either serotonin toxicity (Stewart et al., 2013) or a psychedelic-like effect (Kyzar & Kalueff, 2016). While 8-OH-DPAT increased top-dwelling in the NTT, which could be interpreted as “surfacing” (an aberrant behavioral pattern of slow swimming close to the water surface that is typically observed under the effects of serotonergic hallucinogens; Kalueff et al., 2013; Kyzar & Kalueff, 2016), animals treated with the drug still spent most of the time in the bottom of the tank, differently from zebrafish treated with psychedelics. Moreover, psychedelics typically produce an

hypolocomotion phenotype in zebrafish, and changes in the swimming speed were not observed in any test in the present experiments. A third possibility is that the two tests model different defensive processes, with opposite modulation of the serotonergic system on both. Blaser et al. (2012) analyzed stimulus control in the two tests, and suggested that geotaxis is mainly controlled by escape from the top (closer, therefore, to fear), while scototaxis is mainly controlled by approach-avoidance conflict (closer, therefore, to anxiety). Thus, the effects of 8-OH-DPAT in the NTT and LDT could be interpreted as decreasing fear and increasing anxiety, respectively. In support of this hypothesis, microinjection of 8-OH-DPAT in forebrain regions such as the hippocampus (Cheeta et al., 2000; File et al., 1996), septum (Cheeta et al., 2000; Martins De Almeida et al., 1998), and amygdala (Nunes-de-Souza et al., 2000) has been found to increase anxiety-like behavior in rodents. Conversely, microinjection of 8-OH-DPAT in the periaqueductal gray (Zangrossi Jr. et al., 2001; Zanoveli et al., 2003, 2010) and dorsomedial hypothalamus (Nascimento et al., 2014), but not amygdala (Zangrossi et al., 1999), impair escape in the elevated T-maze, an effect consistent with decreased fear-like states. Taken together, these results suggest that the differential effects of 8-OH-DPAT on the NTT and LDT mediate different behavioral processes via 5-HT1A heteroreceptors.

4.2. Effects of 8-OH-DPAT on social approach

Different phases of the social preference tests used in the present experiment emphasize the role of social interaction vs. social novelty. Our results with 8-OH-DPAT showed a decrease in SI and SN, suggesting a broader effect on social approach. Barba-Escobedo and Gould (2012) showed that buspirone increases social approach in the SI test

(the opposite of what was observed here with 8-OH-DPAT), with no effect on the SN test. Whether this difference indicates that, as in the case with anxiety-like behavior, the effects of 8-OH-DPAT are likely to be mediated by heteroreceptors is still opened. These results could be interpreted either as an anxiogenic-like effect on social preference, or a specific effect on sociality. These hypotheses are difficult to untangle, since, while no effects on erratic swimming was observed in neither the SI or SN tests, they were also absent in the NTT or LDT. While social approach is sensitive to acute stress in adult zebrafish (Cook et al., 2023), the fact that, differently from lab rodents, zebrafish do not avoid novel conspecifics, and actively spend time investigating them when possible (Barba-Escobedo & Gould, 2012; do Nascimento & Maximino, 2023; Ogi et al., 2021), makes it difficult to understand how threat factors in sociability in this species. The activation of 5-HT2 receptors has been shown to increase sociability in zebrafish (de Moura et al., 2023; Ponzoni et al., 2016), and single-nucleotide polymorphisms of the *htr1aa* gene (which code for one isoform of the 5-HT1A receptor) mediate populational variability in social exploration and sociability in this species (Gonçalves et al., 2022). These results further underscore the role of serotonin in sociability in zebrafish, with opponent modulation by 5-HT1AR and 5-HT2CR agonists.

5. Conclusions

In the present experiments, the full agonist of the 5-HT1A receptor, 8-OH-DPAT, decreased anxiety-like behavior in the novel tank test (NTT), but increased it in the light-dark test (LDT), both considered outcomes of anxiety-like behavior for this species. The same treatment decreased social approach in both the social investigation test and the social

novelty test. In comparison with previous data on the effects of the partial agonist buspirone and the full antagonist WAY 100,635, we suggest that 8-OH-DPAT increases anxiety-like behavior mainly by blocking heteroreceptors.

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References

Abozaid, A., & Gerlai, R. (2022). Behavioral Effects of Buspirone in Juvenile Zebrafish of Two Different Genetic Backgrounds. *Toxics*, *10*, 22. https://doi.org/10.3390/toxics10010022

Abrams, J. K., Johnson, P. L., Hollis, J. H., & Lowry, C. A. (2004). Anatomic and functional topography of the dorsal raphe nucleus. *Annals of the New York Academy of Sciences*, *1018*, 46–57. https://doi.org/10.1196/annals.1296.005

Abrams, J. K., Johnson, P. L., Mikkelsen, J. D., Shekhar, A., & Lowry, C. A. (2005). Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. *Neuroscience*, *133*, 983–997. https://doi.org/10.1016/j.neuroscience.2005.03.025

Akimova, E., Lanzenberger, R., & Kasper, S. (2009). The Serotonin-1A Receptor in Anxiety Disorders. *Biological Psychiatry*, *66*(7), 627–635. https://doi.org/10.1016/j.biopsych.2009.03.012

Albert, P. R., & Vahid-Ansari, F. (2019). The 5-HT1A receptor: Signaling to behavior. *Biochimie*, *161*, 34–45. https://doi.org/10.1016/j.biochi.2018.10.015

Araujo, J., Maximino, C., Brito, T. M. de, Silva, A. W. B. da, Batista, E. de J. O., Oliveira, K. R. M., Morato, S., Herculano, A. M., & Gouveia Jr., A. (2012). Behavioral and pharmacological aspects of anxiety in the light/dark preference test. Em A. V. Kalueff & A. M. Stewart (Orgs.), *Zebrafish Protocols for Neurobehavioral Research*. Humana Press. https://doi.org/10.1007/978-1-61779-597-8_14

Barba-Escobedo, P. A., & Gould, G. G. (2012). Visual social preferences of lone zebrafish in a novel environment: Strain and anxiolytic effects. *Genes, Brain and Behavior*, *11*, 366–373. https://doi.org/10.1111/j.1601-183X.2012.00770.x

Bencan, Z., Sledge, D., & Levin, E. D. (2009). Buspirone, chlordiazepoxide and diazepam effects in a zebrafish model of anxiety. *Pharmacology Biochemistry & Behavior*, *94*(1), 75–80. https://doi.org/10.1016/j.pbb.2009.07.009

Cao, B.-J., & Rodgers, R. J. (1997). Influence of 5-HT1A receptor antagonism on plus-maze behaviour in mice. I. Pindolol enantiomers and pindobind 5-HT1A. *Pharmacology, Biochemistry and Behavior*, *58*, 583–591.

Castilho, V., Macedo, C., & Brandão, M. (2002). Role of benzodiazepine and serotonergic mechanisms in conditioned freezing and antinociception using electrical stimulation of the dorsal periaqueductal gray as unconditioned stimulus in rats. *Psychopharmacology*, *165*(1), 77–85. https://doi.org/10.1007/s00213-002-1246-4

Cheeta, S., Kenny, P. J., & File, S. E. (2000). Hippocampal and septal injections of nicotine and 8-OH-DPAT distinguish among different animal tests of anxiety. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *24*, 1053–1067. https://doi.org/10.1016/S0278-5846(00)00129-9

Chessick, C. A., Allen, M. H., Thase, M. E., Cunha, A. A. B. M. da, Kapczinski, F., Lima, M. S. de, & Souza, J. J. dos S. (2006). Azapirones for generalized anxiety disorder. *Cochrane Database of Systematic Reviews*, CD006115. https://doi.org/10.1002/14651858.CD006115

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences* (2nd ed). Routledge.

Connors, K. A., Valenti, T. W., Lawless, K., Sackerman, J., Onaivi, E. S., Brooks, B. W., & Gould, G. G. (2014). Similar anxiolytic effects of agonists targeting serotonin 5-HT1A or cannabinoid CB receptors on zebrafish behavior in novel environments. *Aquatic Toxicology*, *151*, 105–113. https://doi.org/10.1016/j.aquatox.2013.12.005

Cook, A., Beckmann, H., Azap, R., & Ryu, S. (2023). Acute Stress Modulates Social Approach and Social Maintenance in Adult Zebrafish. *eNeuro*, *10*(9), ENEURO.0491-22.2023. https://doi.org/10.1523/ENEURO.0491-22.2023

Cox, R. F., Meller, E., & Waszczak, B. L. (1993). Electrophysiological evidence for a large receptor reserve for inhibition of dorsal raphe neuronal firing by 5-HT1A agonists. *Synapse*, *14*, 297–304. https://doi.org/10.1002/syn.890140407

Cueto-Escobedo, J., German-Ponciano, L. J., Guillén-Ruiz, G., Soria-Fregozo, C., & Herrera-Huerta, E. V. (2022). Zebrafish as a Useful Tool in the Research of Natural Products With Potential Anxiolytic Effects. *Frontiers in Behavioral Neuroscience*, *15*, 795285. https://doi.org/10.3389/fnbeh.2021.795285

de Moura, L. A., Pyterson, M. P., Pimentel, A. F. N., Araújo, F., de Souza, L. V. X. B., Mendes, C. H. M., Costa, B. P. D., de Siqueira-Silva, D. H., Lima-Maximino, M., & Maximino, C. (2023). Roles of the 5-HT2C receptor on zebrafish sociality. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *125*, 110769. https://doi.org/10.1016/j.pnpbp.2023.110769

Deakin, J. F. W., & Graeff, F. G. (1991). 5-HT and mechanisms of defense. *Journal of Psychopharmacology*, *5*, 305–315.

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,

Resolução Normativa CONCEA nº 34 218 (2017). https://www.mctic.gov.br/mctic/opencms/legislacao/outros_atos/resolucoes/Resolucao_Normativa_CONCEA_n_34_de_27072017.html

do Nascimento, B. G., & Maximino, C. (2023). Social investigation and social novelty in zebrafish: Roles of salience and novelty. *Behavioural Processes*, *210*, 104903. https://doi.org/10.1016/j.beproc.2023.104903

Efron, B. (1987). Better Bootstrap Confidence Intervals. *Journal of the American Statistical Association*, *82*(397), 171–185. https://doi.org/10.1080/01621459.1987.10478410

Egan, R. J., Bergner, C. L., Hart, P. C., Cachat, J. M., Canavello, P. R., Elegante, M. F., Elkhayat, S. I., Bartels, B. K., Tien, A. K., Tien, D. H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., & Kalueff, A. V. (2009). Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research*, *205*(1), 38–44. https://doi.org/10.1016/j.bbr.2009.06.022

Facchin, L., Duboué, E. R., & Halpern, M. E. (2015). Disruption of Epithalamic Left–Right Asymmetry Increases Anxiety in Zebrafish. *Journal of Neuroscience*, *35*, 15847–15859. https://doi.org/10.1523/JNEUROSCI.2593-15.2015

Fanselow, M. S., & Lester, L. (1988). A functional behavioristic approach to aversively motivated behavior: Predatory imminence as a determinant of the topography of defensive behavior. Em R. C. Bolles & M. D. Beecher (Orgs.), *Evolution and Learning* (p. 185–211). Erlbaum.

File, S. E., & Gonzalez, L. E. (1996). Anxiolytic effects in the plus-maze of 5-HT1A-receptor ligands in dorsal raphe and ventral hippocampus. *Pharmacology, Biochemistry and Behavior*, *54*, 123–128.

File, S. E., Gonzalez, L. E., & Andrews, N. (1996). Comparative Study of Pre- and Postsynaptic 5-HT1A Receptor Modulation of Anxiety in Two Ethological Animal Tests. *Journal of Neuroscience*, *16*, 4810–4815. https://doi.org/10.1523/JNEUROSCI.16-15-04810.1996

Gebauer, D. L., Pagnussat, N., Piato, Â. L., Schaefer, I. C., Bonan, C. D., & Lara, D. R. (2011). Effects of anxiolytics in zebrafish: Similarities and differences between benzodiazepines, buspirone and ethanol. *Pharmacology, Biochemistry & Behavior*, *99*, 480–486. https://doi.org/10.1016/j.pbb.2011.04.021

Gerlai, R. (2020). Evolutionary conservation, translational relevance and cognitive function: The future of zebrafish in behavioral neuroscience. *Neuroscience & Biobehavioral Reviews*, *116*, 426–435. https://doi.org/10.1016/j.neubiorev.2020.07.009

Gerlai, R. (2023). Zebrafish (*Danio rerio*): A newcomer with great promise in behavioral neuroscience. *Neuroscience & Biobehavioral Reviews*, *144*, 104978. https://doi.org/10.1016/j.neubiorev.2022.104978

Gomes, K. S., & Nunes-de-Souza, R. L. (2009). Implication of the 5-HT2A and 5-HT2C (but not 5-HT1A) receptors located within the periaqueductal gray in the elevated plus-maze test-retest paradigm in mice. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *33*, 1261–1269. https://doi.org/10.1016/j.pnpbp.2009.07.015

Gonçalves, C., Kareklas, K., Teles, M. C., Varela, S. A. M., Costa, J., Leite, R. B., Paixão, T., & Oliveira, R. F. (2022). Phenotypic architecture of sociality and its associated genetic polymorphisms in zebrafish. *Genes, Brain and Behavior*, *21*(5), e12809. https://doi.org/10.1111/gbb.12809

Graeff, F. G. (2004). Serotonin, the periaqueductal gray and panic. *Neuroscience & Biobehavioral Reviews*, *28*(3), 239–259. https://doi.org/10.1016/j.neubiorev.2003.12.004

Graeff, F. G., Viana, M. B., & Mora, P. O. (1997). Dual Role of 5-HT in Defense and Anxiety. *Science*, *21*(6), 791–799.

Gross, C., Zhuang, X., Stark, K., Ramboz, S., Oosting, R., Kirby, L., Santarelli, L., Beck, S., & Hen, R. (2002). Serotonin1A receptor acts during development to establish normal anxiety-like behaviour in the adult. *Nature*, *416*, 396–400.

Hawkey, A. B., Hoeng, J., Peitsch, M. C., Levin, E. D., & Koshibu, K. (2021). Subchronic effects of plant alkaloids on anxiety-like behavior in zebrafish. *Pharmacology Biochemistry and Behavior*, *207*, 173223. https://doi.org/10.1016/j.pbb.2021.173223

Herculano, A. M., & Maximino, C. (2014). Serotonergic modulation of zebrafish behavior: Towards a paradox. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *55*, 50–66. https://doi.org/10.1016/j.pnpbp.2014.03.008

Ho, J., Tumkaya, T., Aryal, S., Choi, H., & Claridge-Chang, A. (2019). Moving beyond P values: Data analysis with estimation graphics. *Nature Methods*, *16*(7), 565–566. https://doi.org/10.1038/s41592-019-0470-3

Jastrzębska-Więsek, M., Partyka, A., Rychtyk, J., Śniecikowska, J., Kołaczkowski, M., Wesołowska, A., Varney, M. A., & Newman-Tancredi, A. (2018). Activity of Serotonin 5-HT1A Receptor Biased Agonists in Rat: Anxiolytic and Antidepressant-like properties. *ACS Chemical Neuroscience*, *9*(5), 1040–1050. https://doi.org/10.1021/acschemneuro.7b00443

Kalueff, A. V., Gebhardt, M., Stewart, A. M., Cachat, J. M., Brimmer, M., Chawla, J. S., Craddock, C., Kyzar, E. J., Roth, A., Landsman, S., Gaikwad, S., Robinson, K., Baatrup, E., Tierney, K., Shamchuk, A., Norton, W., Miller, N., Nicolson, T., Braubach, O., … Zebrafish Neuroscience Research Consortium. (2013). Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond. *Zebrafish*, *10*, 70–86. https://doi.org/10.1089/zeb.2012.0861

Kaslin, J., & Panula, P. (2001). Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (Danio rerio). *Journal of Comparative Neurology*, *440*, 342–377. https://doi.org/10.1002/cne.1390

Kinkel, M. D., Eames, S. C., Philipson, L. H., & Prince, V. E. (2010). Intraperitoneal injection into adult zebrafish. *Journal of Visualized Experiments*, *42*, 2126. https://doi.org/10.3791/2126

Kysil, E. V., Meshalkina, D. A., Frick, E. E., Echevarria, D. J., Rosemberg, D. B., Maximino, C., Lima, M. G., Abreu, M. S., Giacomini, A. C., Barcellos, L. J. G., Song, C., & Kalueff, A. V.

(2017). Comparative Analyses of Zebrafish Anxiety-Like Behavior Using Conflict-Based Novelty Tests. *Zebrafish*, *14*(3), 197–208. https://doi.org/10.1089/zeb.2016.1415

Kyzar, E. J., & Kalueff, A. V. (2016). Exploring Hallucinogen Pharmacology and Psychedelic Medicine with Zebrafish Models. *Zebrafish*, *13*(5), 379–390. https://doi.org/10.1089/zeb.2016.1251

Lau, B. Y. B., Mathur, P., Gould, G. G., & Guo, S. (2011). Identification of a brain center whose activity discriminates a choice behavior in zebrafish. *Proceedings of the National Academy of Sciences*, *108*, 2581–2586. https://doi.org/10.1073/pnas.1018275108

Lawrence, C. (2007). The husbandry of zebrafish (*Danio rerio*): A review. *Aquaculture*, *269*, 1–20. https://doi.org/10.1016/j.aquaculture.2007.04.077

Levin, E. D., Bencan, Z., & Cerutti, D. T. (2007). Anxiolytic effects of nicotine in zebrafish. *Physiology & Behavior*, *90*, 54–58. https://doi.org/10.1016/j.physbeh.2006.08.026

Lillesaar, C., Stigloher, C., Tannhäuser, B., Wullimann, M. F., & Bally-Cuif, L. (2009). Axonal projections originating from raphe serotonergic neurons in the developing and adult zebrafish, Danio rerio, using transgenics to visualize raphe-specific pet1 expression. *Journal of Comparative Neurology*, *512*(2), 158–182. https://doi.org/10.1002/cne.21887

Lima-Maximino, M., Pyterson, M. P., Silva, R. X. do C., Gomes, G. C. V., Rocha, S. P., Herculano, A. M., Rosemberg, D. B., & Maximino, C. (2020). Phasic and tonic serotonin modulate alarm reactions and post-exposure behavior in zebrafish. *Journal of Neurochemistry*, *153*(4), 495–509. https://doi.org/10.1111/jnc.14978

Maaswinkel, H., Le, X., He, L., Zhu, L., & Weng, W. (2013). Dissociating the effects of habituation, black walls, buspirone and ethanol on anxiety-like behavioral responses in shoaling zebrafish. A 3D approach to social behavior. *Pharmacology, Biochemistry & Behavior*, *108*, 16–27. https://doi.org/10.1016/j.pbb.2013.04.009

Maaswinkel, H., Zhu, L., & Weng, W. (2012). The immediate and the delayed effects of buspirone on zebrafish (*Danio rerio*) in an open field test: A 3-D approach. *Behavioural Brain Research*, *234*, 365–374. https://doi.org/10.1016/j.bbr.2012.07.014

MacRae, C. A., & Peterson, R. T. (2023). Zebrafish as a Mainstream Model for In Vivo Systems Pharmacology and Toxicology. *Annual Review of Pharmacology and Toxicology*, *63*(Volume 63, 2023), 43–64. https://doi.org/10.1146/annurev-pharmtox-051421-105617

Martins De Almeida, R. M., Giovenardi, M., Charchat, H., & Bolten Lucion, A. (1998). 8-OH-DPAT in the median raphe nucleus decreases while in the medial septal area it may increase anxiety in female rats. *Neuroscience & Biobehavioral Reviews*, *23*, 259–264. https://doi.org/10.1016/S0149-7634(98)00026-8

Maximino, C. (2012). *Serotonin and Anxiety: Neuroanatomical, Pharmacological, and Functional Aspects*. Springer.

Maximino, C., da Silva, A. W. B., Gouveia, A., & Herculano, A. M. (2011). Pharmacological analysis of zebrafish (*Danio rerio*) scototaxis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *35*, 624–631. https://doi.org/10.1016/j.pnpbp.2011.01.006

Maximino, C., Marques de Brito, T., Dias, C. A. G. de M., Gouveia, A., & Morato, S. (2010). Scototaxis as anxiety-like behavior in fish. *Nature Protocols*, *5*(2), Artigo 2. https://doi.org/10.1038/nprot.2009.225

Maximino, C., Oliveira, D. L. de, Rosemberg, D. B., Batista, E. de J. O., Herculano, A. M., Oliveira, K. R. M., Benzecry, R., & Blaser, R. (2012). A comparison of the light/dark and novel tank tests in zebrafish. *Behaviour*, *149*(10–12), 1099–1123. https://doi.org/10.1163/1568539X-00003029

Maximino, C., Puty, B., Benzecry, R., Araújo, J., Lima, M. G., de Jesus Oliveira Batista, E., Renata de Matos Oliveira, K., Crespo-Lopez, M. E., & Herculano, A. M. (2013). Role of serotonin in zebrafish (*Danio rerio*) anxiety: Relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and para-chlorophenylalanine (pCPA) in two

behavioral models. *Neuropharmacology*, *71*, 83–97. https://doi.org/10.1016/j.neuropharm.2013.03.006

Maximino, C., Silva, A. W. B. da, Araújo, J., Lima, M. G., Miranda, V., Puty, B., Benzecry, R., Picanço-Diniz, D. L. W., Jr, A. G., Oliveira, K. R. M., & Herculano, A. M. (2014). Fingerprinting of Psychoactive Drugs in Zebrafish Anxiety-Like Behaviors. *PLOS ONE*, *9*(7), e103943. https://doi.org/10.1371/journal.pone.0103943

McNaughton, N., & Corr, P. J. (2004). A two-dimensional neuropsychology of defense: Fear/anxiety and defensive distance. *Neuroscience & Biobehavioral Reviews*, *28*(3), 285–305. https://doi.org/10.1016/j.neubiorev.2004.03.005

McNaughton, N., & Corr, P. J. (2022). The non-human perspective on the neurobiology of temperament, personality, and psychopathology: What's next? *Current Opinion in Behavioral Sciences*, *43*, 255–262. https://doi.org/10.1016/j.cobeha.2021.11.005

McNaughton, N., & Zangrossi Jr, H. (2008). Theoretical approaches to the modeling of anxiety in animals. Em R. J. Blanchard, D. C. Blanchard, G. Griebel, & D. J. Nutt (Orgs.), *Handbook of Anxiety and Fear* (p. 11–27). Elsevier B. V.

Meller, E., Goldstein, M., & Bohmaker, K. (1990). Receptor reserve for 5-hydroxytryptamine1A-mediated inhibition of serotonin synthesis: Possible relationship to anxiolytic properties of 5-hydroxytryptamine1A agonists. *Molecular Pharmacology*, *37*, 231–237.

Nascimento, J. O. G., Kikuchi, L. S., de Bortoli, V. C., Zangrossi Jr., H., & Viana, M. B. (2014). Dorsomedial hypothalamus serotonin 1A receptors mediate a panic-related response in the elevated T-maze. *Brain Research Bulletin*, *109*, 39–45. https://doi.org/10.1016/j.brainresbull.2014.09.011

Newman-Tancredi, A., Cussac, D., Ormière, A.-M., Lestienne, F., Varney, M. A., & Martel, J.-C. (2019). Bell-shaped agonist activation of 5-HT1A receptor-coupled Gαi3 G-proteins: Receptor density-dependent switch in receptor signaling. *Cellular Signalling*, *63*, 109383. https://doi.org/10.1016/j.cellsig.2019.109383

Norton, W. H. J., Folchert, A., & Bally-Cuif, L. (2008). Comparative analysis of serotonin receptor (HTR1A/HTR1B families) and transporter (slc6a4a/b) gene expression in the zebrafish brain. *Journal of Comparative Neurology*, *511*(4), 521–542. https://doi.org/10.1002/cne.21831

Nowicki, M., Tran, S., Muraleetharan, A., Markovic, S., & Gerlai, R. (2014). Serotonin antagonists induce anxiolytic and anxiogenic-like behavior in zebrafish in a receptor-subtype dependent manner. *Pharmacology Biochemistry and Behavior*, *126*, 170–180. https://doi.org/10.1016/j.pbb.2014.09.022

Nunes-de-Souza, R. L., Canto-de-Souza, A., Da-Costa, M., Fornari, R. V., Graeff, F. G., & Pelá, I. R. (2000). Anxiety-induced antinociception in mice: Effects of systemic and intra-amygdala administration of 8-OH-DPAT and midazolam. *Psychopharmacology*, *150*, 300–310.

Ogi, A., Licitra, R., Naef, V., Marchese, M., Fronte, B., Gazzano, A., & Santorelli, F. M. (2021). Social Preference Tests in Zebrafish: A Systematic Review. *Frontiers in Veterinary Science*, *7*, 590057.

Olivier, B., van Wijngaarden, I., & Soudijn, W. (2000). 5-HT3 receptor antagonists and anxiety; a preclinical and clinical review. *European Neuropsychopharmacology*, *10*, 77–95. https://doi.org/10.1016/S0924-977X(99)00065-6

Paul, E. D., Johnson, P. L., Shekhar, A., & Lowry, C. A. (2014). The Deakin/Graeff hypothesis: Focus on serotonergic inhibition of panic. *Neuroscience & Biobehavioral Reviews*, *46*, 379–396. https://doi.org/10.1016/j.neubiorev.2014.03.010

Paul, E. D., & Lowry, C. A. (2013). Functional topography of serotonergic systems supports the Deakin/Graeff hypothesis of anxiety and affective disorders. *Journal of Psychopharmacology*, *27*, 1090–1106. https://doi.org/10.1177/0269881113490328

Perry, S. F., Davie, P. S., Daxboeck, C., Ellis, A. G., & Smith, D. G. (1984). Perfusion methods for the study of gill physiology. Em W. S. Hoar & D. J. Randall (Orgs.), *Fish Physiology, Vol. 10. Gills: Part B. Ion and Water Transfer*. Academic Press.

Perusini, J. N., & Fanselow, M. S. (2015). Neurobehavioral perspectives on the distinction between fear and anxiety. *Learning & Memory*, *22*(9), 417–425. https://doi.org/10.1101/lm.039180.115

Piszczek, L., Schlax, K., Wyrzykowska, A., Piszczek, A., Audero, E., & Gross, C. (2013). Serotonin 1A auto-receptors are not sufficient to modulate anxiety in mice. *European Journal of Neuroscience*. https://doi.org/10.1111/ejn.12260

Ponzoni, L., Sala, M., & Braida, D. (2016). Ritanserin-sensitive receptors modulate the prosocial and the anxiolytic effect of MDMA derivatives, DOB and PMA, in zebrafish. *Behavioural Brain Research*, *314*, 181–189. https://doi.org/10.1016/j.bbr.2016.08.009

Quesseveur, G., Nguyen, H. T., Gardier, A. M., & Guiard, B. P. (2012). 5-HT2 ligands in the treatment of anxiety and depression. *Expert Opinion on Investigational Drugs*, *21*, 1701–1725. https://doi.org/10.1517/13543784.2012.719872

Richardson-Jones, J. W., Craige, C. P., Nguyen, T. H., Kung, H. F., Gardier, A. M., Dranovsky, A., David, D. J., Guiard, B. P., Beck, S. G., Leonardo, E. D., & Hen, R. (2011). Serotonin-1A autoreceptors are necessary and sufficient for the normal formation of circuits underlying innate anxiety. *Journal of Neuroscience*, *31*, 6008–6018. https://doi.org/10.1523/JNEUROSCI.5836-10.2011

Rodgers, R. J., & Cao, B.-J. (1997). Anxiolytic-like profile of p-MPPI, a novel 5HT1A receptor antagonist, in the murine elevated plus-maze. *Psychopharmacology*, *129*, 365–371.

Serra, E. L., Medalha, C. C., & Mattioli, R. (1999). Natural preference of zebrafish (*Danio rerio*) for a dark environment. *Brazilian Journal Of Medical And Biological Research*, *32*, 1551–1553.

Sourbron, J., Schneider, H., Kecskés, A., Liu, Y., Buening, E. M., Lagae, L., Smolders, I., & de Witte, P. (2016). Serotonergic Modulation as Effective Treatment for Dravet Syndrome in a Zebrafish Mutant Model. *ACS Chemical Neuroscience*, *7*(5), 588–598. https://doi.org/10.1021/acschemneuro.5b00342

Sprouse, J. S., & Aghajanian, G. K. (1988). Responses of hippocampal pyramidal cells to putative serotonin 5-HT1A and 5-HT1B agonists: A comparative study with dorsal raphe neurons. *Neuropharmacology*, *27*(7), 707–715. https://doi.org/10.1016/0028-3908(88)90079-2

Stewart, A. M., Cachat, J., Gaikwad, S., Robinson, K. S. L., Gebhardt, M., & Kalueff, A. V. (2013). Perspectives on experimental models of serotonin syndrome in zebrafish. *Neurochemistry International*, *62*, 893–902. https://doi.org/10.1016/j.neuint.2013.02.018

Tejada, J., Chaim, K. T., & Morato, S. (2018). X-PloRat: A Software for Scoring Animal Behavior in Enclosed Spaces. *Psicologia: Teoria e Pesquisa*, *33*. https://doi.org/10.1590/0102.3772e3322

Zangrossi, H., Viana, M. B., & Graeff, F. G. (1999). Anxiolytic effect of intra-amygdala injection of midazolam and 8-hydroxy-2-(di-*n*-propylamino)tetralin in the elevated T-maze. *European Journal of Pharmacology*, *369*(3), 267–270. https://doi.org/10.1016/S0014-2999(99)00075-8

Zangrossi Jr., H., Viana, M. B., Zanoveli, J., Bueno, C., Nogueira, R. L., & Graeff, F. G. (2001). Serotonergic regulation of inhibitory avoidance and one-way escape in the rat elevated T-maze. *Neuroscience & Biobehavioral Reviews*, *25*, 637–645. https://doi.org/10.1016/S0149-7634(01)00047-1

Zanoveli, J. M., Nogueira, R. L., & Zangrossi Jr., H. (2003). Serotonin in the dorsal periaqueductal gray modulates inhibitory avoidance and one-way escape behaviors in the elevated T-maze. *European Journal of Pharmacology*, *473*, 153–161. https://doi.org/10.1016/S0014-2999(03)01970-8

Zanoveli, J. M., Pobbe, R. L. H., de Bortoli, V., Carvalho, M. C., Brandão, M. L., & Zangrossi Jr, H. (2010). Facilitation of 5-HT1A-mediated neurotransmission in dorsal periaqueductal grey matter accounts for the panicolytic-like effect of chronic fluoxetine. *International Journal of Neuropsychopharmacology*, *13*, 1079–1088.

Figure captions

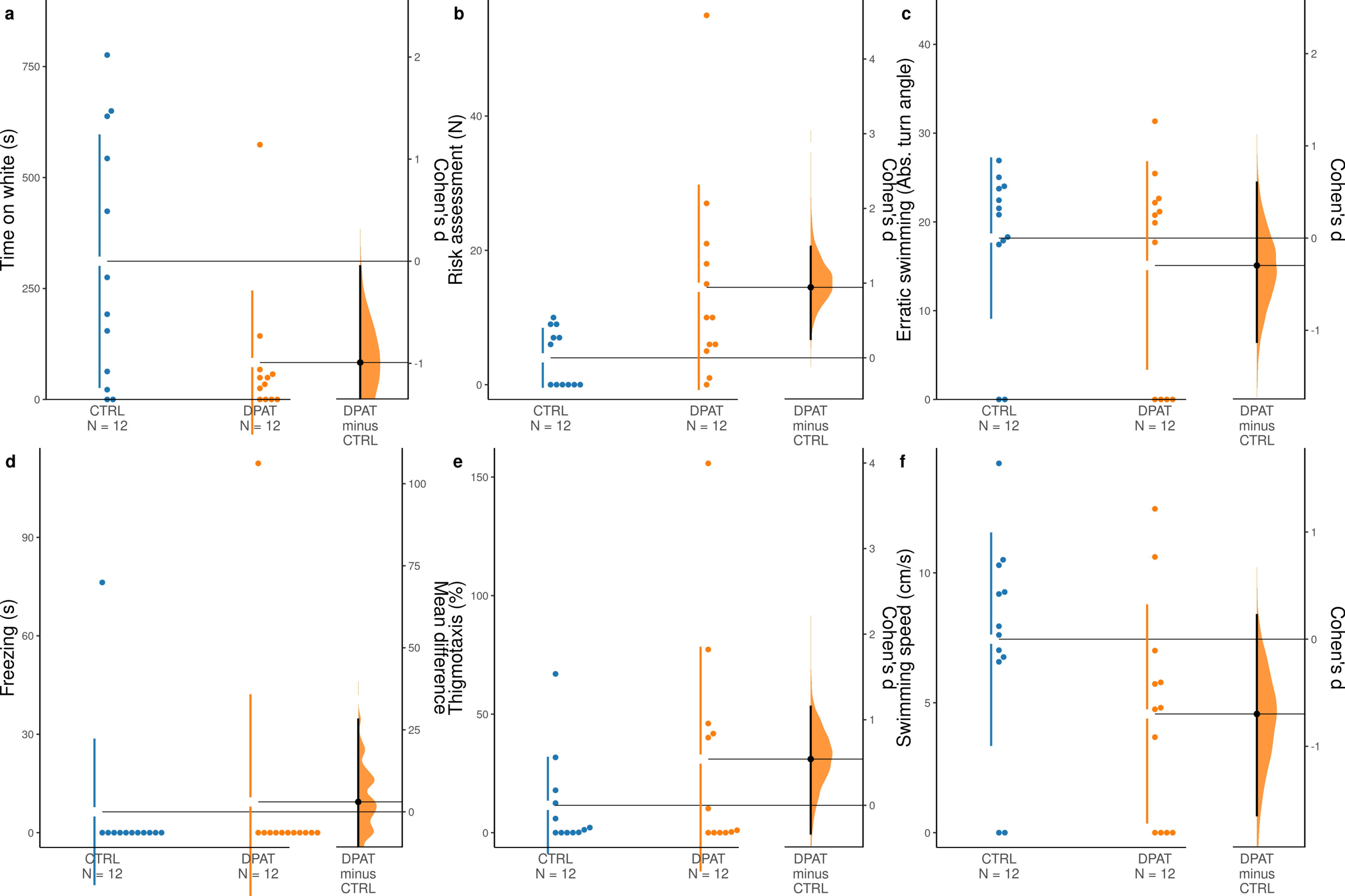
Figure 1 – 8-OH-DPAT increases anxiey-like behavior in the zebrafish light/dark test. (A) Scototaxis. (B) Risk assessment. (C) Erratic swimming. (D) Freezing. (E) Thigmotaxis. (F) Swimming speed. For a description of variables, refer to Table 1. Blue dots represent animals treated with vehicle (CTRL), and orange dots represent animals treated with 8-OH-DPAT (DPAT). The Cohen's *d* between CTRL and DPAT is shown in Gardner-Altman estimation plot. Both groups are plotted on the left axes; the effect size (Cohen's *d*) is plotted on floating axes on the right as a bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

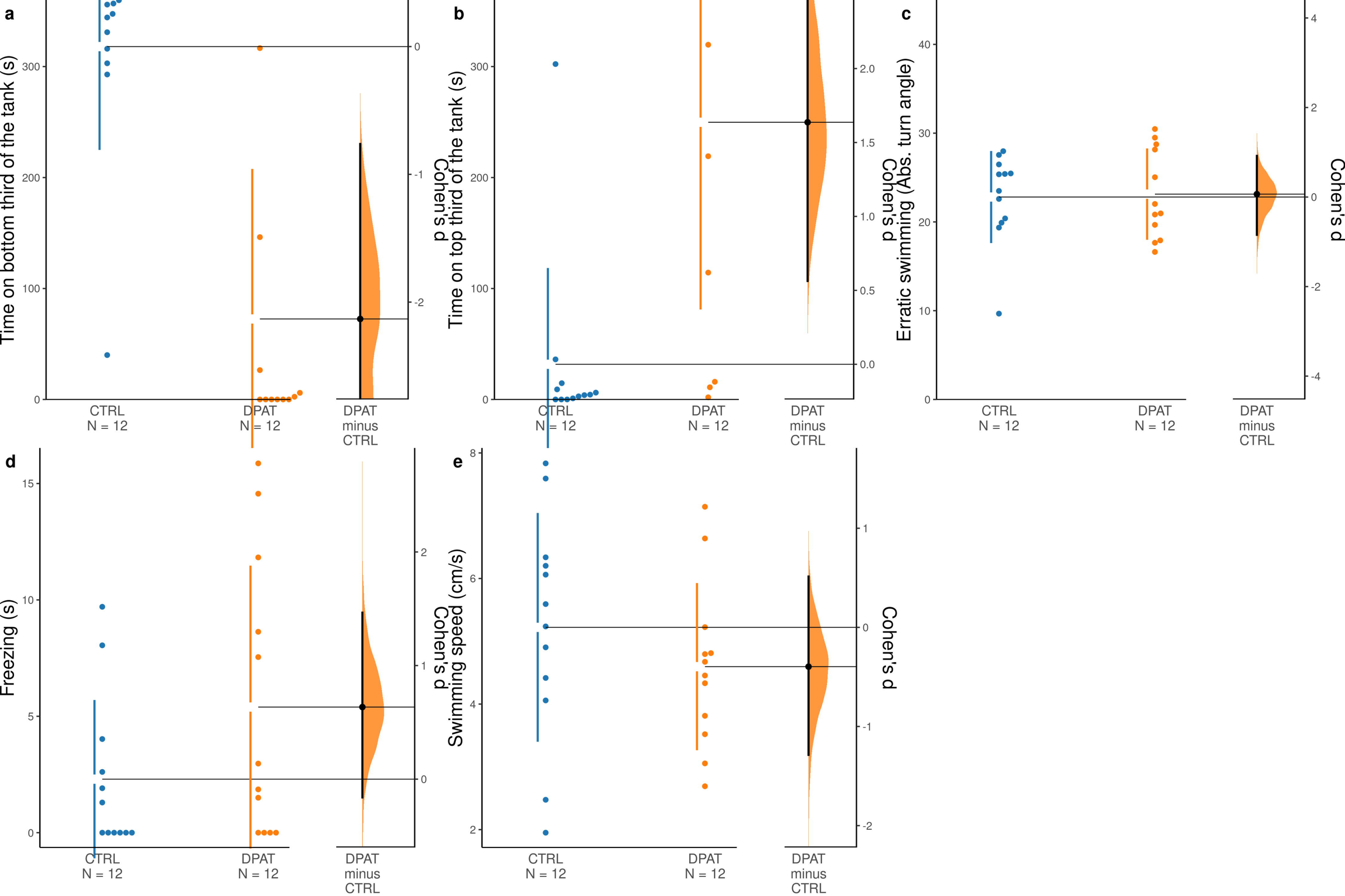
Figure 2 – 8-OH-DPAT decreases anxiety-like behavior in the zebrafish novel tank test. (A) Geotaxis. (B) Top-dwelling. (C) Erratic swimming. (D) Freezing. (E) Swimming speed. For a description of variables, refer to Table 1. Blue dots represent animals treated with vehicle (CTRL), and orange dots represent animals treated with 8-OH-DPAT (DPAT). The Cohen's *d* between CTRL and DPAT is shown in Gardner-Altman estimation plot. Both groups are plotted on the left axes; the effect size (Cohen's *d*) is plotted on floating axes on the right as a bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

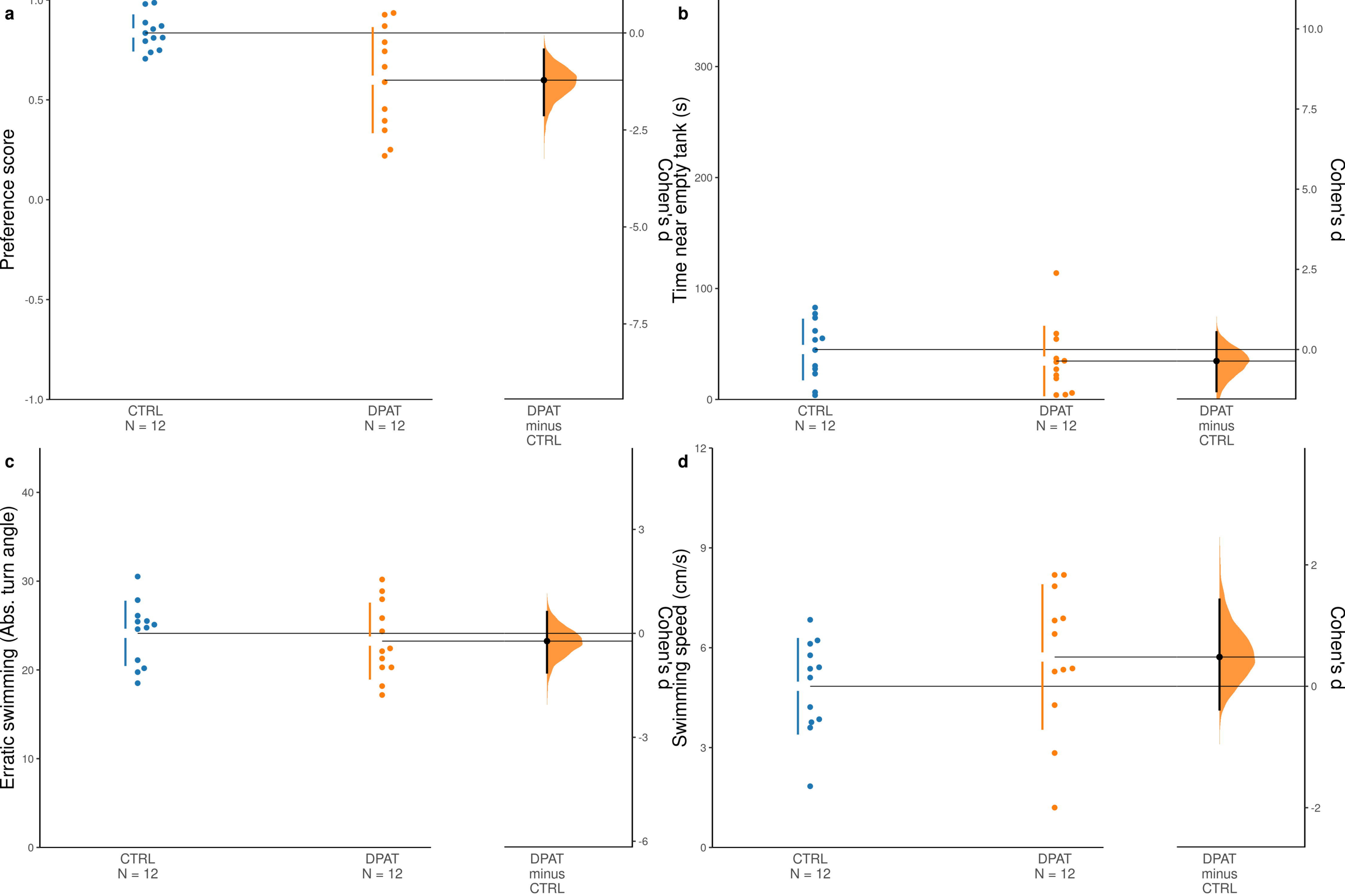
Figure 3 – 8-OH-DPAT decreases social approach in the social investigation test. (A) Preference score. (B) Social avoidance. (C) Erratic swimming. (D) Swimming speed. For a description of variables, refer to Table 1. Blue dots represent animals treated with vehicle (CTRL), and orange dots represent animals treated with 8-OH-DPAT (DPAT). The Cohen's *d* between CTRL and DPAT is shown in Gardner-Altman estimation plot. Both groups are plotted on the left axes; the effect size (Cohen's *d*) is plotted on floating axes on the right as a

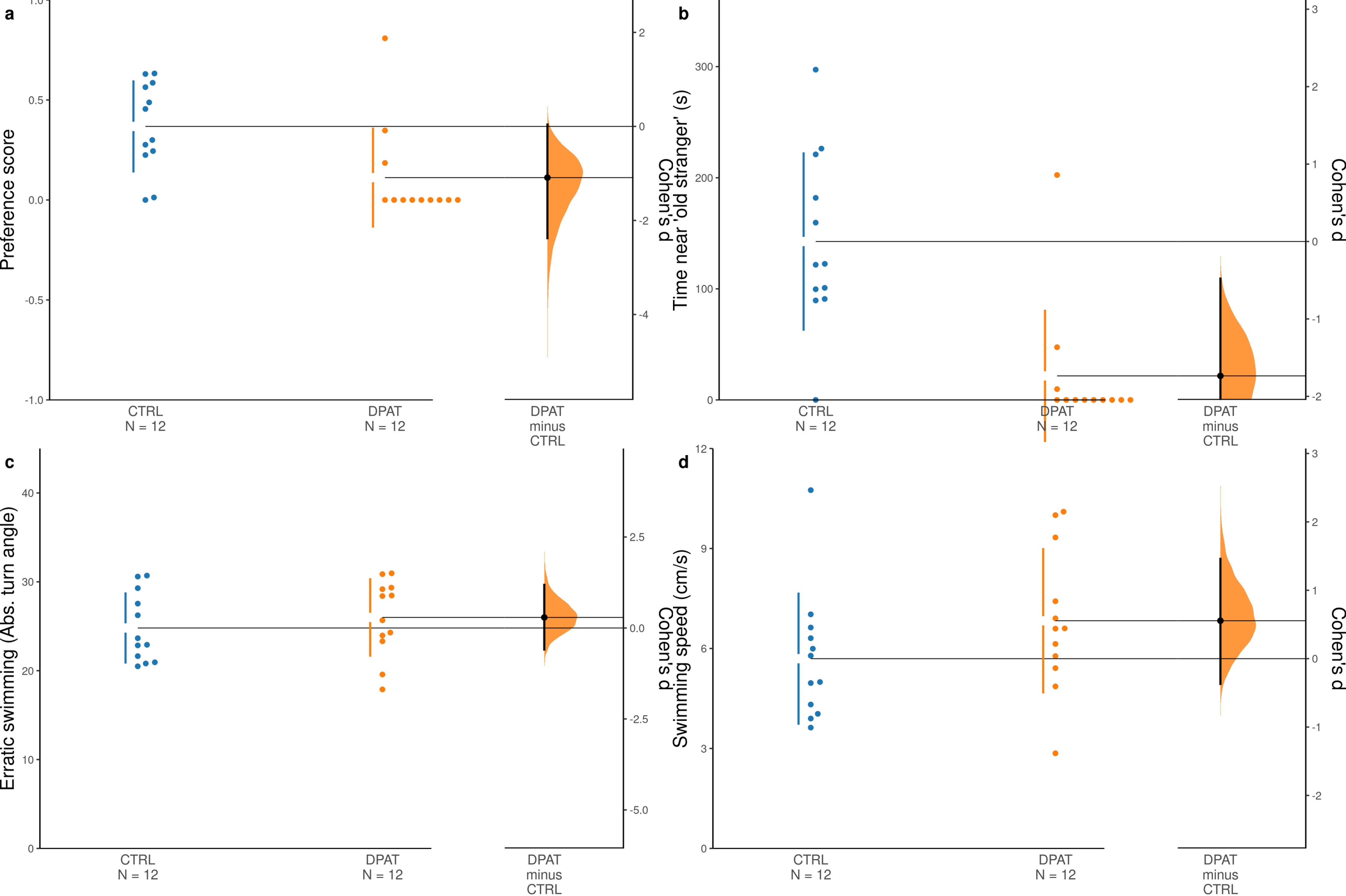
bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.

Figure 4 – 8-OH-DPAT decreases social preference in the social novelty test. (A) Preference score. (B) Social avoidance. (C) Erratic swimming. (D) Swimming speed. For a description of variables, refer to Table 1. Blue dots represent animals treated with vehicle (CTRL), and orange dots represent animals treated with 8-OH-DPAT (DPAT). The Cohen's *d* between CTRL and DPAT is shown in Gardner-Altman estimation plot. Both groups are plotted on the left axes; the effect size (Cohen's *d*) is plotted on floating axes on the right as a bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% confidence interval is indicated by the ends of the vertical error bar. 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated.


a
Time on white (s)
Cohen's d
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
b
Risk assessment (N)
Cohen's d
c
Erratic swimming (Abs. turn angle)
Cohen's d
d
Freezing (s)
e
Mean difference
Thigmotaxis (%)
f
Swimming speed (cm/s)


a
Time on bottom third of the tank (s)
Cohen's d
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
b
Time on top third of the tank (s)
Cohen's d
c
Erratic swimming (Abs. turn angle)
Cohen's d
d
Freezing (s)
Cohen's d
e
Swimming speed (cm/s)
Cohen's d


a
Preference score
1.0
0.5
0.0
-0.5
-1.0
0.0
-2.5
-5.0
-7.5
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
b
Time near empty tank (s)
Cohen's d
300
200
100
0
10.0
7.5
5.0
2.5
0.0
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
c
Erratic swimming (Abs. turn angle)
40
30
20
10
0
3
0
-3
-6
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
d
Swimming speed (cm/s)
Cohen's d
12
9
6
3
0
2
0
-2
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL


a
Preference score
1.0
0.5
0.0
-0.5
-1.0
2
0
-2
-4
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
b
Time near 'old stranger' (s)
300
200
100
0
Cohen's d
3
2
1
0
-1
-2
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
c
Erratic swimming (Abs. turn angle)
40
30
20
10
0
2.5
0.0
-2.5
-5.0
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL
d
Swimming speed (cm/s)
12
9
6
3
0
Cohen's d
3
2
1
0
-1
-2
CTRL
N = 12
DPAT
N = 12
DPAT
minus
CTRL