Zebrafish (*Danio rerio*) behavioral laterality predicts increased short-term avoidance memory but not stress-reactivity responses

Barbara D. Fontana[a*], Madeleine Cleal[a], James M. Clay[a] and Matthew O. Parker[a,b*]

[a]Brain and Behaviour Laboratory, School of Pharmacy and Biomedical Sciences, University of Portsmouth, UK.

[b]The International Zebrafish Neuroscience Research Consortium (ZNRC), 309 Palmer Court, Slidell, LA 70458, USA.

[*]Correspondence to:

Matthew O. Parker, PhD

School of Pharmacy and Biomedical Sciences, University of Portsmouth, Old St Michael's Building, White Swan Road, Portsmouth, PO1 2DT, UK. E-mail: matthew.parker@ac.uk

Barbara D. Fontana

School of Pharmacy and Biomedical Sciences, University of Portsmouth, Old St Michael's Building, White Swan Road, Portsmouth, PO1 2DT, UK. E-mail: barbara.fontana@port.ac.uk

Abstract

Once considered a uniquely human attribute, behavioral laterality has proven to be ubiquitous among non-human animals, being frequently associated with different neurophenotypes in rodents and fish species. Zebrafish (*Danio rerio*) are a **versatile vertebrate model system** that has been widely used in translational neuropsychiatric research due their highly conserved genetic homology, well characterized physiological and extensive behavioral repertoire. Although the spontaneous left- and right-bias responses and associated behavioral domains (e.g. stress reactivity, aggression and learning) have previously been observed in other teleost species, no information regarding **how spontaneous motor left-right bias responses of zebrafish predicts other behavioral domains** has been described. Thus, we aimed to investigate the existence and incidence of natural left-right bias of adult zebrafish in the Y-maze test and explore any relationship of biasedness on the performance of different behavioral domains. This included learning about threat-cues in the fear conditioning test and locomotion and anxiety-related behavior in the novel tank diving test. In conclusion, we showed for the first time that zebrafish exhibit a natural manifestation of **motor** behavioral lateralization which can influence aversive learning responses. **Although laterality did not change locomotion or anxiety-related behaviors, we found that biased animals had an altered exploration pattern in the Y-maze, making them easily discernable from their unbiased counterparts, and increased learning associated to fear cues.**

Keywords: Anxiety; Behavioral asymmetries; Left-bias; Pavlovian fear conditioning; Right-bias.

1. Introduction

Lateralization of brain and behavior is the apparent predisposition towards side bias, often manifested in terms of motor output, such as handedness. Laterality has been extensively studied in humans, **with functional laterality of different brain regions known to be an important aspect in language and cognition (Hull and Vaid 2007; Vikingstad et al. 2000). Research has revealed that motor functions may also be under the control of lateralized mechanisms which, in humans, may manifest as preference for one side over the other for handedness, footedness and eyedness** (Brown and Taylor 1988)**. Laterality** has been shown to be an evolutionarily conserved characteristic which is observed at the populational level and, when disrupted, has been associated with **cognitive and neuropsychiatric disorders, such as anxiety and major depressive disorder (Koster et al. 2010; Lichtenstein-Vidne et al. 2017).**

Lateralization is not a purely human characteristic, many studies now suggest that most vertebrate species, including monkeys (Fagot and Vauclair 1991; Hopkins 1994; McGrew and Marchant 1997), rodents (Robison 1981; Rodriguez and Afonso 1993; Rodriguez et al. 1992), birds (Bhagavatula et al. 2014; Franklin and Adams 2010; Gunturkun et al. 1998), fish (Bibost and Brown 2014; Bisazza and de Santi 2003; Dadda et al. 2010a; Dadda et al. 2010b) **and some invertebrate species (Anfora et al. 2010, 2011; Frasnelli, Vallortigara, and Rogers 2012) express brain functional asymmetries (MacNeilage, Rogers, and Vallortigara 2009). In rodents for example,** several behavioral tasks have been used to assess behavioral asymmetries such **as** turning rotometers, handedness, choice behavior, T-maze and Y-maze (Corballis 1986; Pisa and Szechtman 1986; Zimmerberg and Glick 1974). Variability in lateralization exerts a number of fitness benefits **at the individual level**. For example, lateralization has been associated with maximization of brain processes, enabling individuals to process two tasks simultaneously (Rogers 2000; Rogers 2002). **Studies have suggested that laterality evolved at the population level to maintain coordination among social groups once that lateralization in a same**

direction is considered as a potential benefit to group cohesion and a mechanism where individuals interact in predictable ways in social group (Rogers 2000).

Behavioral laterality is an evolutionarily conserved characteristic which is observed at populational level in humans and has been associated to different neurophenotypes (Corballis 2017; Frasnelli 2013). **Taxonomic and evolutionary aspects of brain laterality have been previously described in fish species, primarily focusing on CNS asymmetries, sensory organs and somatic lateralization, as well as the adaptive role of laterality in nature (de Perera and Braithwaite 2005; Lychakov 2013; Nepomnyashchikh and Izvekov 2006; Vallortigara and Rogers 2005). Behavioral** asymmetries have been related to high escape performance (Dadda et al. 2010b), social responses (Reddon and Balshine 2010) and even accelerated learning responses (Andrade et al. 2001) in **both fish and mammals. However, despite the clear** relevance of lateralization to several mammals and vertebrate species, we still have a limited understanding of the general origins of morphological and functional asymmetries in the brain and of their importance for behavior.

Zebrafish (*Danio rerio*) is a versatile vertebrate model system that has been widely used in translational neuropsychiatric research (Fontana et al. 2018; Stewart et al. 2015) and **to understand evolutionary aspects of animal cognition (Oliveira 2013; Reale et al. 2007). The last decade has seen an increase in the use of zebrafish to study the mechanisms underlying lateralization (Andersson et al. 2015; Ariyomo and Watt 2013; Barth et al. 2005; Dadda et al. 2010a; Sovrano and Andrew 2006). Larval zebrafish have an increased use of left-eye when interacting with their own reflection (Sovrano and Andrew 2006). Studies also showed that zebrafish initially use the right hemifield predominantly when interacting with novel object, but as the object becomes familiar, they switch to the left hemifield (Miklósi, Andrew, and Savage 1997). In a mutant zebrafish strain that shows strong laterality bias (*fsi*), asymmetry of diencephalic structures correlates with different behavior patterns such as boldness responses, with left-bias fish showing increased latency to interact with a novel object than right-bias fish (Barth et al. 2005). Despite a growing understanding of**

lateralization in zebrafish, very little is known about how spontaneously occurring motor asymmetry impacts on other behavioral domains. Therefore, in this study we aimed: (1) to investigate the existence and incidence of spontaneously occurring motor left-right bias of adult zebrafish in a continuous Y-maze test; and (2) to explore how spontaneously occurring motor left-right bias relates to performance on different behavioral domains, including learning about threat-cues in the fear conditioning test, and locomotion and anxiety-related behavior in the novel tank test.

2. Material and Methods

2.1. Animals

Adult zebrafish (AB wild-type; ~ 50:50 male:female ratio at 3-month of age) were bred in-house and reared in standard laboratory conditions on a re-circulating system (Aquaneering, USA). Animals were maintained on a 14/10-hour light/dark cycle (lights on at 9:00 a.m.), pH 8.4, at ~28.5 °C (±1 °C) in groups of 20 animals per 2.8 L. Fish were fed three times/day with a mixture of live brine shrimp and flake food, **except on the weekends when they were fed once a day**. **Animals were tested in the Y-maze apparatus and then pair-housed for 24 hours prior to analysis of shock-avoidance or tank diving,** to reduce stress from multiple handling in a single day (see **Fig. 1**). **During the pair-housing period, animals had shared water system and visual contact through transparent partition, the pair-housing system is used to separate animals for further identification and reduce the stress induced by social isolation (Parker et al. 2012).** After behavioral tests, all animals were euthanized using 2-phenoxyethanol from Aqua-Sed (Aqua-Sed™, Vetark,Winchester, UK). All experiments were carried out following approval from the University of Portsmouth Animal Welfare and Ethical Review Board, and under license from the UK Home Office (Animals (Scientific Procedures) Act, 1986) [PPL: P9D87106F].

2.2. Y-maze test

The Y-maze spontaneous alternation task is widely used for measuring the disposition of different animal models to explore new environments and to assess left- and right-biased responses (Barnard et al. 2016; Castellano et al. 1987; Frasnelli 2013; Rodriguez et al. 1992). **One-hundred and three** adult zebrafish were used for assessing Y-maze performance and right-left bias. Required sample size was calculated *a priori* following pilot tests (effect size (d) = 0.3, power = 0.8, alpha = 0.05). The apparatus consisted of a white Y-maze tank with three identical arms (5 cm length x 2 cm width; three identical arms at a 120º angle from each other) and a transparent base, filled with 3L of aquarium water (**Fig. 1**). Ambient light allowed some visibility in the maze, but no explicit intra-maze cues were added to the environment. **Behavioral tests were performed between 10 a.m. to 4 p.m. using the automated Zantiks (Zantiks Ltd., Cambridge, UK) AD system (Brock et al. 2017) to track animal behavior, and we carried out three independent replicate**s. **The Zantiks AD system was fully controlled via a web-enabled device during behavioral training. Fish behavior patterns were recorded for 1 hour** and was assessed according to overlapping series of four choices (tetragrams) and analyzed as a proportion of the total number of turns (Gross et al. 2011). **For assessing fish behavioral lateralization, the number of turns for the right or left arm according to the previous location in 10 minute time bins was used and the following formula was applied:** $\boldsymbol{bias\ percentage} = \frac{\textbf{number of turns L or R} * \textbf{100}}{\textbf{total number of turns}}$ **. Following this, the mean and coefficient of variation for right and left bias was calculated based on the bias percentage in 10-minute time bins to assess the consistency of data across the entire trial (1-hour test period). Behavioral lateralization was considered when the animal presented >60% of average preference for right or left turns in the Y-maze during the 1-hour test. To assess zebrafish search strategies we used the number of alternations (rlrl + lrlr) and repetitions (rrrr + llll) as proportion of total number of turns which are highly expressed through 1-hour and are commonly linked to animals' exploratory activity (Cleal and Parker 2018; Gross et al. 2011). Both alternations and repetitions were also previously linked to short-term spatial memory by reduction the number of alternations after developmental alcohol exposure (Cleal and Parker, 2018);**

however, more studies are need to clarify how these patterns are linked to learning and memory processes.

2.3. Pavlovian fear conditioning

The inhibitory avoidance paradigm is a valid method widely used to explore mechanisms underlying fear avoidance learning responses in zebrafish (Amorim et al. 2017; Manuel et al. 2014; Manuel et al. 2015; Ng et al. 2012). 24h after the completion of the Y-maze test, animals (*n*= 55) were tested on a Pavlovian fear conditioning procedure for 1 hour. The fear conditioning response was based on previous work (Cleal and Parker 2018; Valente et al. 2012) **where increased memory responses were linked to the decreased time spent in the fear conditioned area**. Fish were individually placed in one of four lanes of a tank (25 cm length x 15 cm, 1 L of water in each tank) **of the Zantiks (Zantiks Ltd., Cambridge, UK) AD system (Brock et al. 2017) (Fig. 1)**. Briefly, fish were habituated for 30 minutes in the test environment**, half of the arena had a check pattern on the base and the other half had a grey color on the base. Images were projected into the tank from a screen which was embedded in the base of the AD unit, on which the test tank sits. Every 5 minutes of the habituation the base patterns, check or grey, were switched to the opposite side of the tank. The baseline preference was ascertained over 30-mins, but only the last 10-mins baseline preference was used for assessing the area preference.** Following the baseline was the conditioning phase, which consisted of a conditioned stimulus (**CS+; full screen of "check" or "grey", half CS+ in "check" and CS+ in "grey"**) presented for 1.5-s and followed by a brief mild shock (9 V DC, 80ms; unconditioned stimulus (US). After this, an 8.5-s inter-trial interval (ITI) of the non-CS (CS−) exemplar was presented at the bottom of the tank. The CS+/US was **shown** nine times. Finally, avoidance of CS+ was assessed by presenting the baseline screen (CS+ and CS− simultaneously) for 1-min, and switching positions after 30-s.

2.4. Novel tank diving and thigmotaxis responses

The novel tank test is commonly used for analyzing locomotor and anxiety-like phenotypes in zebrafish presenting a high sensitivity to anxiolytic and anxiogenic drugs (Egan et al. 2009; Kalueff et al. 2013; Levin et al. 2007; Maximino et al. 2010; Mezzomo et al. 2016; Wong et al. 2010). 24 hours after the Y-maze test, animals (*n = 46*) were placed individually in a novel tank (30 cm length x 15 cm height x 12 cm width) containing 4 L of aquarium water. Behavioral activity was recorded using 2 webcams (front and top view, see **Fig. 1B**) for 5 min to analyze thigmotaxis and diving response (Egan et al. 2009; Parker et al. 2012; Rosemberg et al. 2012). Behaviors were **measured using an automated** video-tracking software (EthoVision, Noldus Information Technology Inc., Leesburg, VA - USA) at a rate of 60 frames/s. **Thigmotaxis (time spent in proximity to the edge/sides) was analyzed through the behavior tracking obtained by the top view camera. Meanwhile, for the detailed evaluation of vertical activity, the tank was separated in three virtual areas (bottom, middle and top) and the** following endpoints were measured: total distance traveled, time spent in each third of the tank, immobility and thigmotaxis.

2.5. Randomization and blinding

All behavioral testing was carried out in a fully randomized order, choosing fish at random from one of ten housing tanks for testing. Fish were screened for left-right bias in the Y-maze first, but analysis was not carried out prior to subsequent behavioral testing to avoid bias. Subsequent to **the** Y-maze screening, fish were pair housed and issued a subject ID, allowing all testing to be carried out in a fully blinded manner. **Once all data were collected and screened for extreme outliers (e.g., fish freezing and returning values of '0' for behavioral parameters indicating non-engagement), the bias was revealed, and data analyzed in full. Two animals were excluded in the Y-maze task and two in the novel tank due to poor engagement with the task (freezing and displaying no measurable behavioral patterns).**

*2.6. Data **processing** and statistical analysis*

Data from the Y-maze protocol was obtained as number of entries into each arm (1, 2, 3 and middle section 4) across a 1-hour trial. To analyze the data according to left and right turns in 10-minute time bins, raw data was processed using the Zantiks Y-maze Analysis Script created specifically for this purpose (available from: https:/ /github.com/thejamesclay/ZANTIKS_YMaze_Analysis_Script). Subsequently, data were analyzed in IBM SPSS® Statistics and the results were expressed as means ± standard error of the mean (S.E.M), to assess whether there were any effects of bias on total turns, alternations (lrlr + rlrl) **or** repetitions (rrrr + llll). **Alternations and repetitions were analyzed using generalized linear models (Poisson distribution, log link), with laterality (three levels – left bias, right bias, non-bias) and time (six levels - 10-min time bins across 1 hour) as the fixed factor, and ID as a random effect (to account for non-independence of replicates). To analyze novel tank responses, we used either one- or two-way ANOVAs, with bias and time spent in different tank zones fixed factors (tank diving) or bias only as a fixed factor for thigmotaxis, immobility and distance traveled.** Additionally, left-right bias effects on **the** shock avoidance test was assessed using two-way repeated measures ANOVA with 'bias' (left vs right vs neutral) and conditioning (pre-vs-post) as factors, and preference for conditioned stimulus as the dependent variable. Newman-Keuls test was used as post-hoc analysis, and results were considered significant when $p \leq 0.05$.

3. Results

2.1. Left-right bias profile in the Y-maze test

Zebrafish showed behavioral lateralization in the Y-maze (right-biased 27.18 %, left-biased 27.18% and non-biased 45.63%). To confirm if the behavioral laterality was **consistent across 1-hour (analyzed in 10-minute time bins)**, the coefficient of variation for the left and right-turn preferences were calculated for the non-biased (19.28 ± 2.52 and 21.05 ± 2.95), left-biased (30.40 ± 3.85 and 21.7 ± 3.61) and right-biased (27.23 ± 5.52 and 25.95 ± 2.31) groups. **Figure 2** displays the Y-maze data. A significant bias effect was observed for number of turns (F

$_{(2, 601)}$ = 13.115; p<0.0001), repetitions ($F_{(2, 601)}$ = 39.696; p<0.0001) and alternations ($F_{(2, 601)}$ = 45.437; p<0.0001). A time effect (data not shown) was also observed for number of turns ($F_{(5, 601)}$ = 9.769; p<0.0001), repetitions ($F_{(5, 601)}$ = 3.242; p=0.007) and alternations ($F_{(5, 601)}$ = 3.801; p=0.002). Additionally, a significant interaction effect (bias * time) was observed for repetitions ($F_{(10, 601)}$ = 2.504; p=0.006) and alternations ($F_{(10, 601)}$ = 2.390; p= 0.009). **Bias, to the left or the right, significantly increased the number of repetitions (p<0.0001 for right-bias and p<0.001 for left-bias) and decreased the percentage of alternations (p<0.0001 for right-bias and p<0.005 for left-bias) compared to non-biased animals. In addition, right-biased animals also decreased the number of turns (p<0.05) compared to non-biased animals. Biased differences were not just seen between biased vs. non-biased groups, but also between bias groups,** right-biased animals had a significant increase of repetitions (p<0.05) and decrease of alternations (p<0.05) compared to left-biased animals (**Fig. 2A**). The behavioral profile of biased and non-biased animals is displayed in **Fig. 2B** tetragrams where a high number of llll and rrrr configuration can be observed for left- and right-biased animals, respectively.

2.2. Short-term avoidance memory and novelty response of biased animals

Although no interaction effect bias *vs.* shock ($F_{(2, 98)}$ =1.259; p= 0.312) was observed for the Pavlovian responses, a significant effect for bias ($F_{(2, 98)}$ =3.128; p= 0.035) and **conditioning (probe *vs* baseline)** ($F_{(1, 98)}$ = 79.47; p<0001) effect was revealed. **ANOVA analysis are often underpowered and therefore unable to detect the significance of interaction terms** (Wahlsten 1990)**. Therefore post-hoc analysis was performed to specifically analyze the effects between groups. In general, all biased (p<0.0001 for right- and left-bias) and non-biased (p<0.0001) animals had a decreased time spent in the preference for conditioned stimulus (probe *vs* baseline)**. **However, both left- and right- biased animals (p<0.05) spent significantly less time in the conditioned area during the probe trial compared to non-biased group** (**Fig. 3), despite no significant differences in their baseline preferences.** No significant effect was observed for bias in all novel tank diving test-related parameters, including distance travelled ($F_{(2,}$

$_{43)}$ = 0.683; p = 0.510), immobility ($F_{(2, 43)}$ = 2.348; p = 0.107), time in tank zones time ($F_{(2, 129)}$ = 0.084; p = 0.918) (**Fig. 4**) and thigmotaxis ($F_{(2, 43)}$ = 1.289; p = 0.286) (**Fig. 5**).

4. Discussion

In this study we evaluated **spontaneously occurring motor left-right bias** in adult zebrafish using **an** unconditioned Y-maze **protocol and** evaluated the predictive validity of **spontaneous behavioral laterality** on both unconditioned and conditioned measures of fear **and anxiety**. We showed, for the first time, that the zebrafish presents natural behavioral laterality in the Y-maze test **when analyzed using 16 overlapping tetragrams to evaluate exploration strategy**, suggesting that the protocol may be useful for screening this species for behavioral asymmetry. Second, we found that behaviorally lateralized animals show **a decrease in the relative number of alternation** responses and increase **in relative number of repetitions, in the Y-maze**. **This suggests that search strategies used by spontaneously biased individuals differs from that used by non-biased fish and is quite likely directly due to the lateralization.** Third, we observed that behavioral asymmetry predicts increased learning in a Pavlovian fear conditioning protocol but did not predict measures of unconditioned anxiety (novel tank test, thigmotaxis). Collectively, these data suggest, contrary to theories that laterality is related to increased stress-reactivity, that increased behavioral laterality may be related to increased cue-reactivity, particularly in relation to aversive cues. This has connotations for translational models of human disorders of affective state, in which heightened attention to threat-related cues is observed (Lichtenstein-Vidne et al. 2017) and in which brain and behavioral laterality are thought to be risk factors (Bruder et al. 2016).

Left-right asymmetries in behavioral protocols including the T-maze and Y-maze have been widely utilized in rodents (Andrade et al. 2001; Nakagawa et al. 2004; Rodriguez et al. 1992). Here, for the first time, we observed that approximately a quarter of zebrafish present substantial natural left- (27.18 %) or right- (27.18%) locomotor lateralization, with the remaining 45.63% **of** animals showing stochastic patterns of left/right. These data are somewhat at odds from observed

bias in other models, in which there is a high number of right-biased animals (52.8%) and low numbers of left- (22.2%) and non-biased responses (25%) for rodents (Andrade et al. 2001). **A previous study (Chivers et al. 2016) showed that wild-caught fish, yellow-and-blueback fusiliers (*Caesio teres*), also present a natural increased bias for the right turn (65%) and decreased left bias (24%) and no biased (11%). However, they also observed that the strength of the behavioral lateralization was increased depending on predation pressure. Although the authors also did not find significant differences between the left- and right-biased groups, in general, the biased groups presented distinct patterns of behavior compared to non-biased animals. As mentioned earlier, larvae and adult zebrafish exhibit different patterns of left- and right eye use that correlate with boldness and response to novelty (Barth et al. 2005), aggression (Ariyomo and Watt 2013) and learning/memory (Andersson et al. 2015) behavior.**

Studies focusing on the evolutionary aspects of lateralization suggest that behavioral asymmetries exist at population level because individually asymmetrical organisms coordinate their behavior with other asymmetrical organisms (Frasnelli 2013). The differences between left- and right-bias in other fish species. For example, right-biased animals showed increased escape responses (Chivers et al. 2016) and increased right-eye use when attacking the mirror image aggression (Bisazza and de Santi 2003). In invertebrates, laterality has been also consistent with the vertebrate findings, where eusocial ants show motor left-bias when exploring unfamiliar nest sites (Hunt et al. 2014) and eusocial honeybees have olfactory asymmetries that predict learning and recall of memory (Frasnelli et al. 2010; Rigosi et al. 2011; Rogers and Vallortigara 2008).

In general, brain and behavior lateralization are more common in social rather than in solitary species (Bisazza et al. 2000; Frasnelli 2013). Although zebrafish is a social species (Saverino and Gerlai 2008), research on zebrafish almost only uses domesticated strains, that were removed from wild populations many generations ago (Spence et al. 2008), as we used here. Zebrafish lab strains present genetic divergence from wild populations (Whiteley

et al. 2011), and it is therefore important to consider that wild populations may have different lateralization patterns. Thus, behavioral patterns may change depending on stress-levels (Parker et al. 2012), predator pressure (Chivers et al. 2016), fish strain (Vignet et al. 2013) and animal origin (Spence et al. 2008), doing that more studies are needed to understand how behavioral laterality is influence by these variables in adult zebrafish.

We observed that behavioral laterality has an important role in Y-maze performance, where left-right biased animals presented an increase of repetition behavior and decrease of alternation, in particular in the left-biased animals. **Previous studies by** (Cleal and Parker 2018; Gross et al. 2011) **and continuing work in our laboratory (data not shown here) in conjunction with rodent T- and Y-maze data suggest that the normal search strategy employed in mazes is to use a high level of alternations.** Alternations have been directly associated with functionally distinct search patterns, where the seeking for change and novelty may have a role in their exploratory profiles (Kool et al. 2010). **Rodriguez et al. (1992) described decreases in pure alternations and increases in pure repetition behavior in lateralized animals, and demonstrated that both stress and over-training decrease the alternation/repetition ratio through the promotion of an increase of biased responses. Although the dominant strategy, alternations are broken up with other exploratory patterns, as indicated in Fig 2B showing the relative frequency of each of the 16 tetragrams over the duration of a 1-hour trial. This discontinuous, but dominant search strategy, leads us to believe that the exploration patterns in the Y-maze are possibly associated with short term memory rather than motor memory (McBride and Parker 2015). However, further work would have to be carried out to determine to what extent, if any, alternations and repetitions are implicated in short term memory in the Y-maze. Here, we confirmed that both alternations and repetitions remain as a highly reliable behavioral pattern that is conserved across species (Ghafouri et al. 2016; Lewis et al. 2017; Pickering et al. 2015).**

In agreement with previous studies (Andrade et al. 2001), we showed that locomotor lateralization is associated **with** increased learning in a **Pavlovian** fear conditioning protocol.

Studies using other fish species showed that lateralized animals have a better response in cognitive tasks such as spatial reorientation (Sovrano et al. 2005), and left-eye bias is related to faster learning in conditioning tasks (Bibost and Brown 2014). In general, the most predominant theory of how left-right bias affects learning and cognitive processing relates to a hypothesized increased stress-reactivity in lateralized animals (Carlson and Glick 1989; Neveu 1996; Westergaard et al. 2001). Interindividual differences in laterality have been shown to covary with, or predict, individual differences in stress-reactivity and susceptibility to stress-related pathology (Byrnes et al. 2016; Carlson and Glick 1989; Fride and Weinstock 1989; Ocklenburg et al. 2016). Here, we tested the hypothesis that left- and right-biased animals would differ in measures of stress-reactivity and anxiety-like phenotypes (Blaser and Rosemberg 2012; Egan et al. 2009; Parker et al. 2012). We found no significant differences in lateralized animals in our measures of anxiety, suggesting that the observed differences in behavioral phenotypes observed in the Y-maze and shock avoidance learning seems to not be related to stress-reactivity responses *per se*. Instead, our data may suggest that the lateralized fish are more reactive to stress-related cues. This would explain the increased performance **in** the Pavlovian fear conditioning, the fact that there were no differences in measures of anxiety, and the **altered search strategy in** the Y-maze test.

There are several theories regarding the mechanisms underlining behavior laterality in simple maze tasks. Diaz Palarea et al. (1987) were the first to report that left-right biased animals, as assessed via spatial asymmetry in a T-maze, had alterations in dopaminergic (DA) signaling. In addition, apomorphine (DA receptor agonist) and 6-hydroxydopamine lesions alters behavioral laterality of animals in the T-maze test (Castellano et al. 1987) and Y-maze (Nakagawa et al. 2004), confirming the involvement of DA system in behavioral asymmetry. DA receptors are strongly implicated in emotional learning and recall of emotionally relevant events in rats. For example, activation of D4-receptors in the medial pre-frontal cortex potentiates fear-associated memory formation but has no impact on recall (Lauzon et al. 2009; Laviolette et al. 2005), whereas activation of D1-like receptors blocks recalls of previously learned fear-associated

memories but has no impact on learning (Lauzon et al. 2009), suggesting a double dissociation of function. Interestingly, the serotonergic (5-HT) system has also been shown to have an important role in mediating individual differences in anxiety-like responses and locomotor activity in zebrafish and exerts a minor modulatory role of the DA system (Tran et al. 2016). Both behavioral laterality and aversive memory is mostly associated with **modulatory action of the DA system**, but the 5-HT system has a major role modulating zebrafish responses to novelty. The precise mechanisms of how behavioral laterality modulates neuropsychiatric conditions are yet to be firmly established, and further studies are required to better understand the mechanisms in which behavioral laterality modulates aversive memory in zebrafish.

5. Conclusion

Overall, we showed for the first time that zebrafish exhibit **spontaneously occurring motor lateralization which can influence aversive learning responses. We found that biased animals show an altered search exploratory pattern Y-maze and increased** in learning in a Pavlovian fear conditioning protocol. Coupled with a lack of differences between lateralized and non-lateralized animals in unconditioned tests of anxiety, our data suggest that lateralized zebrafish may **show heightened** reactivity to fear related cues. These results have important connotations for translational models of depression and anxiety, particularly in the light of well-established links between laterality and anxiety/depression in humans. Finally, because biased animals present different behavioral performances in the Y-maze and Pavlovian fear conditioning protocols, left- and right- preference should be considered when working with zebrafish behavior, particularly to control variability in performance on more complex tasks.

Compliance with Ethical Standards

Funding

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001 at the University of Portsmouth, UK. MOP receives funding from the Foundation for Liver Research and the British Academy. MC is supported by a Science Faculty Studentship from the University of Portsmouth.

Conflict of interest

The authors declare that no conflict of interest exists.

Ethical approval

All experiments were carried out following scrutiny by the University of Portsmouth Animal Welfare and Ethical Review Board, and under license from the UK Home Office (Animals (Scientific Procedures) Act, 1986) [PPL: P9D87106F].

References

Amorim RR, Silva PF, Luchiari AC (2017) Effects of Alcohol on Inhibitory Avoidance Learning in Zebrafish (Danio rerio) Zebrafish 14:430-437 doi:10.1089/zeb.2017.1438

Andersson MA, Ek F, Olsson R (2015) Using visual lateralization to model learning and memory in zebrafish larvae Scientific reports 5:8667 doi:10.1038/srep08667

Andrade C, Alwarshetty M, Sudha S, Suresh Chandra J (2001) Effect of innate direction bias on T-maze learning in rats: implications for research Journal of neuroscience methods 110:31-35

Ariyomo TO, Watt PJ (2013) Aggression and sex differences in lateralization in the zebrafish Animal Behaviour 86:617-622

Barnard S, Matthews L, Messori S, Podaliri-Vulpiani M, Ferri N (2016) Laterality as an indicator of emotional stress in ewes and lambs during a separation test Animal cognition 19:207-214 doi:10.1007/s10071-015-0928-3

Barth KA, Miklosi A, Watkins J, Bianco IH, Wilson SW, Andrew RJ (2005) fsi zebrafish show concordant reversal of laterality of viscera, neuroanatomy, and a subset of behavioral responses Current biology : CB 15:844-850 doi:10.1016/j.cub.2005.03.047

Bhagavatula PS, Claudianos C, Ibbotson MR, Srinivasan MV (2014) Behavioral lateralization and optimal route choice in flying budgerigars PLoS computational biology 10:e1003473 doi:10.1371/journal.pcbi.1003473

Bibost AL, Brown C (2014) Laterality influences cognitive performance in rainbowfish Melanotaenia duboulayi Animal cognition 17:1045-1051 doi:10.1007/s10071-014-0734-3

Bisazza A, Cantalupo C, Capocchiano M, Vallortigara G (2000) Population lateralisation and social behaviour: a study with 16 species of fish Laterality 5:269-284 doi:10.1080/713754381

Bisazza A, de Santi A (2003) Lateralization of aggression in fish Behavioural brain research 141:131-136

Blaser RE, Rosemberg DB (2012) Measures of anxiety in zebrafish (Danio rerio): dissociation of black/white preference and novel tank test PloS one 7:e36931 doi:10.1371/journal.pone.0036931

Brock AJ, Goody SMG, Mead AN, Sudwarts A, Parker MO, Brennan CH (2017) Assessing the Value of the Zebrafish Conditioned Place Preference Model for Predicting Human Abuse Potential The Journal of pharmacology and experimental therapeutics 363:66-79 doi:10.1124/jpet.117.242628

Bruder GE et al. (2016) Brain laterality, depression and anxiety disorders: New findings for emotional and verbal dichotic listening in individuals at risk for depression Laterality 21:525-548 doi:10.1080/1357650X.2015.1105247

Byrnes EE, Vila Pouca C, Brown C (2016) Laterality strength is linked to stress reactivity in Port Jackson sharks (Heterodontus portusjacksoni) Behavioural brain research 305:239-246 doi:10.1016/j.bbr.2016.02.033

Carlson JN, Glick SD (1989) Cerebral lateralization as a source of interindividual differences in behavior Experientia 45:788-798

Castellano MA, Diaz-Palarea MD, Rodriguez M, Barroso J (1987) Lateralization in male rats and dopaminergic system: evidence of right-side population bias Physiology & behavior 40:607-612

Cleal M, Parker MO (2018) Moderate developmental alcohol exposure reduces repetitive alternation in a zebrafish model of fetal alcohol spectrum disorders Neurotoxicology and teratology 70:1-9 doi:10.1016/j.ntt.2018.09.001

Corballis MC (1986) Brain asymmetries: cerebral lateralization in nonhuman species Science 231:1022-1023 doi:10.1126/science.231.4741.1022-a

Corballis MC (2017) The Evolution of Lateralized Brain Circuits Frontiers in psychology 8:1021 doi:10.3389/fpsyg.2017.01021

Dadda M, Domenichini A, Piffer L, Argenton F, Bisazza A (2010a) Early differences in epithalamic left-right asymmetry influence lateralization and personality of adult zebrafish Behav Brain Res 206:208-215 doi:10.1016/j.bbr.2009.09.019

Dadda M, Koolhaas WH, Domenici P (2010b) Behavioural asymmetry affects escape performance in a teleost fish Biology letters 6:414-417 doi:10.1098/rsbl.2009.0904

de Perera TB, Braithwaite VA (2005) Laterality in a non-visual sensory modality--the lateral line of fish Current biology : CB 15:R241-242 doi:10.1016/j.cub.2005.03.035

Diaz Palarea MD, Gonzalez MC, Rodriguez M (1987) Behavioral lateralization in the T-maze and monoaminergic brain asymmetries Physiology & behavior 40:785-789

Egan RJ et al. (2009) Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish Behavioural brain research 205:38-44 doi:10.1016/j.bbr.2009.06.022

Fagot J, Vauclair J (1991) Manual laterality in nonhuman primates: a distinction between handedness and manual specialization Psychological bulletin 109:76-89

Fontana BD, Mezzomo NJ, Kalueff AV, Rosemberg DB (2018) The developing utility of zebrafish models of neurological and neuropsychiatric disorders: A critical review Experimental neurology 299:157-171 doi:10.1016/j.expneurol.2017.10.004

Franklin RG, Jr., Adams RB, Jr. (2010) The two sides of beauty: laterality and the duality of facial attractiveness Brain and cognition 72:300-305 doi:10.1016/j.bandc.2009.10.002

Frasnelli E (2013) Brain and behavioral lateralization in invertebrates Frontiers in psychology 4:939 doi:10.3389/fpsyg.2013.00939

Frasnelli E, Vallortigara G, Rogers LJ (2010) Response competition associated with right-left antennal asymmetries of new and old olfactory memory traces in honeybees Behavioural brain research 209:36-41 doi:10.1016/j.bbr.2010.01.014

Fride E, Weinstock M (1989) Alterations in behavioral and striatal dopamine asymmetries induced by prenatal stress Pharmacology, biochemistry, and behavior 32:425-430

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

Gross AN, Engel AK, Richter SH, Garner JP, Wurbel H (2011) Cage-induced stereotypies in female ICR CD-1 mice do not correlate with recurrent perseveration Behav Brain Res 216:613-620 doi:10.1016/j.bbr.2010.09.003

Gunturkun O, Hellmann B, Melsbach G, Prior H (1998) Asymmetries of representation in the visual system of pigeons Neuroreport 9:4127-4130

Hopkins WD (1994) Hand preferences for bimanual feeding in 140 captive chimpanzees (Pan troglodytes): rearing and ontogenetic determinants Developmental psychobiology 27:395-407 doi:10.1002/dev.420270607

Hull R, Vaid J (2007) Bilingual language lateralization: a meta-analytic tale of two hemispheres Neuropsychologia 45:1987-2008 doi:10.1016/j.neuropsychologia.2007.03.002

Hunt ER, O'Shea-Wheller T, Albery GF, Bridger TH, Gumn M, Franks NR (2014) Ants show a leftward turning bias when exploring unknown nest sites Biology letters 10:20140945 doi:10.1098/rsbl.2014.0945

Kalueff AV et al. (2013) Towards a comprehensive catalog of zebrafish behavior 1.0 and beyond Zebrafish 10:70-86 doi:10.1089/zeb.2012.0861

Kool W, McGuire JT, Rosen ZB, Botvinick MM (2010) Decision making and the avoidance of cognitive demand Journal of experimental psychology General 139:665-682 doi:10.1037/a0020198

Koster EH, De Raedt R, Leyman L, De Lissnyder E (2010) Mood-congruent attention and memory bias in dysphoria: Exploring the coherence among information-processing biases Behaviour research and therapy 48:219-225 doi:10.1016/j.brat.2009.11.004

Lauzon NM, Bishop SF, Laviolette SR (2009) Dopamine D1 versus D4 receptors differentially modulate the encoding of salient versus nonsalient emotional information in the medial prefrontal cortex The Journal of neuroscience : the official journal of the Society for Neuroscience 29:4836-4845 doi:10.1523/JNEUROSCI.0178-09.2009

Laviolette SR, Lipski WJ, Grace AA (2005) A subpopulation of neurons in the medial prefrontal cortex encodes emotional learning with burst and frequency codes through a dopamine D4 receptor-dependent basolateral amygdala input The Journal of neuroscience : the official journal of the Society for Neuroscience 25:6066-6075 doi:10.1523/JNEUROSCI.1168-05.2005

Levin ED, Bencan Z, Cerutti DT (2007) Anxiolytic effects of nicotine in zebrafish Physiology & behavior 90:54-58 doi:10.1016/j.physbeh.2006.08.026

Lewis SA, Negelspach DC, Kaladchibachi S, Cowen SL, Fernandez F (2017) Spontaneous alternation: A potential gateway to spatial working memory in Drosophila Neurobiology of learning and memory 142:230-235 doi:10.1016/j.nlm.2017.05.013

Lichtenstein-Vidne L, Okon-Singer H, Cohen N, Todder D, Aue T, Nemets B, Henik A (2017) Attentional bias in clinical depression and anxiety: The impact of emotional and non-emotional distracting information Biological psychology 122:4-12 doi:10.1016/j.biopsycho.2016.07.012

Lychakov DV (2013) [Lateralization of behavioral reactions and otolith asymmetry] Zhurnal evoliutsionnoi biokhimii i fiziologii 49:299-312

Manuel R, Gorissen M, Roca CP, Zethof J, van de Vis H, Flik G, van den Bos R (2014) Inhibitory avoidance learning in zebrafish (Danio rerio): effects of shock intensity and unraveling differences in task performance Zebrafish 11:341-352 doi:10.1089/zeb.2013.0970

Manuel R, Zethof J, Flik G, van den Bos R (2015) Providing a food reward reduces inhibitory avoidance learning in zebrafish Behavioural processes 120:69-72 doi:10.1016/j.beproc.2015.08.013

Maximino C, de Brito TM, da Silva Batista AW, Herculano AM, Morato S, Gouveia A, Jr. (2010) Measuring anxiety in zebrafish: a critical review Behavioural brain research 214:157-171 doi:10.1016/j.bbr.2010.05.031

McGrew WC, Marchant LF (1997) On the other hand: Current issues in and meta□analysis of the behavioral laterality of hand function in nonhuman primates Physical Anthropology 104:201-232

Mezzomo NJ, Silveira A, Giuliani GS, Quadros VA, Rosemberg DB (2016) The role of taurine on anxiety-like behaviors in zebrafish: A comparative study using the novel tank and the light-dark tasks Neuroscience letters 613:19-24 doi:10.1016/j.neulet.2015.12.037

Nakagawa M, Ohgoh M, Nishizawa Y, Ogura H (2004) Dopaminergic agonists and muscarinic antagonists improve lateralization in hemiparkinsonian rats in a novel exploratory Y-maze The Journal of pharmacology and experimental therapeutics 309:737-744 doi:10.1124/jpet.103.059519

Nepomnyashchikh GI, Izvekov EI (2006) Laterality of behavioral responses in bony fishes: Inheritance, adaptive importance, and morphofunctional correlates Journal of Ichthyology 47:782-790

Neveu PJ (1996) Lateralization and stress responses in mice: Interindividual differences in the association of brain, neuroendocrine, and immune responses Behavior Genetics 26:373-377

Ng MC, Hsu CP, Wu YJ, Wu SY, Yang YL, Lu KT (2012) Effect of MK-801-induced impairment of inhibitory avoidance learning in zebrafish via inactivation of extracellular signal-regulated kinase (ERK) in telencephalon Fish physiology and biochemistry 38:1099-1106 doi:10.1007/s10695-011-9595-8

Ocklenburg S, Korte SM, Peterburs J, Wolf OT, Gunturkun O (2016) Stress and laterality - The comparative perspective Physiology & behavior 164:321-329 doi:10.1016/j.physbeh.2016.06.020

Oliveira RF (2013) Mind the fish: zebrafish as a model in cognitive social neuroscience Frontiers in neural circuits 7:131 doi:10.3389/fncir.2013.00131

Parker MO, Millington ME, Combe FJ, Brennan CH (2012) Housing conditions differentially affect physiological and behavioural stress responses of zebrafish, as well as the response to anxiolytics PloS one 7:e34992 doi:10.1371/journal.pone.0034992

Pickering C, Alsio J, Morud J, Ericson M, Robbins TW, Soderpalm B (2015) Ethanol impairment of spontaneous alternation behaviour and associated changes in medial prefrontal glutamatergic gene expression precede putative markers of dependence Pharmacology, biochemistry, and behavior 132:63-70 doi:10.1016/j.pbb.2015.02.021

Pisa M, Szechtman H (1986) Lateralized and compulsive exteroceptive orientation in rats treated with apomorphine Neuroscience letters 64:41-46

Reale D, Reader SM, Sol D, McDougall PT, Dingemanse NJ (2007) Integrating animal temperament within ecology and evolution Biological reviews of the Cambridge Philosophical Society 82:291-318 doi:10.1111/j.1469-185X.2007.00010.x

Reddon AR, Balshine S (2010) Lateralization in response to social stimuli in a cooperatively breeding cichlid fish Behavioural processes 85:68-71 doi:10.1016/j.beproc.2010.06.008

Rigosi E, Frasnelli E, Vinegoni C, Antolini R, Anfora G, Vallortigara G, Haase A (2011) Searching for anatomical correlates of olfactory lateralization in the honeybee antennal lobes: a morphological and behavioural study Behavioural brain research 221:290-294 doi:10.1016/j.bbr.2011.03.015

Robison DN (1981) Conceptual aspects of “laterality” syndromes Behavioral and Brain Sciences 4:33-34

Rodriguez M, Afonso D (1993) Ontogeny of T-maze behavioral lateralization in rats Physiology & behavior 54:91-94

Rodriguez M, Gomez C, Alonso J, Afonso D (1992) Laterality, alternation, and perseveration relationships on the T-maze test Behavioral Neuroscience 106:974-980

Rogers LJ (2000) Evolution of hemispheric specialization: advantages and disadvantages Brain and language 73:236-253 doi:10.1006/brln.2000.2305

Rogers LJ (2002) Lateralization in vertebrates: its early evolution, general pattern and development Advances in the study of behavioral and Brain Sciences 31:107 - 162

Rogers LJ, Vallortigara G (2008) From antenna to antenna: lateral shift of olfactory memory recall by honeybees PloS one 3:e2340 doi:10.1371/journal.pone.0002340

Rosemberg DB et al. (2012) Behavioral effects of taurine pretreatment in zebrafish acutely exposed to ethanol Neuropharmacology 63:613-623 doi:10.1016/j.neuropharm.2012.05.009

Saverino C, Gerlai R (2008) The social zebrafish: behavioral responses to conspecific, heterospecific, and computer animated fish Behavioural brain research 191:77-87 doi:10.1016/j.bbr.2008.03.013

Sovrano VA, Andrew RJ (2006) Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae Behavioural brain research 167:226-231 doi:10.1016/j.bbr.2005.09.021

Sovrano VA, Dadda M, Bisazza A (2005) Lateralized fish perform better than nonlateralized fish in spatial reorientation tasks Behavioural brain research 163:122-127 doi:10.1016/j.bbr.2005.04.012

Spence R, Gerlach G, Lawrence C, Smith C (2008) The behaviour and ecology of the zebrafish, Danio rerio Biological reviews of the Cambridge Philosophical Society 83:13-34 doi:10.1111/j.1469-185X.2007.00030.x

Stewart AM, Ullmann JF, Norton WH, Parker MO, Brennan CH, Gerlai R, Kalueff AV (2015) Molecular psychiatry of zebrafish Molecular psychiatry 20:2-17 doi:10.1038/mp.2014.128

Tran S, Nowicki M, Muraleetharan A, Chatterjee D, Gerlai R (2016) Neurochemical factors underlying individual differences in locomotor activity and anxiety-like behavioral responses in zebrafish Progress in neuro-psychopharmacology & biological psychiatry 65:25-33 doi:10.1016/j.pnpbp.2015.08.009

Valente A, Huang KH, Portugues R, Engert F (2012) Ontogeny of classical and operant learning behaviors in zebrafish Learning & memory 19:170-177 doi:10.1101/lm.025668.112

Vallortigara G, Rogers LJ (2005) Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization The Behavioral and brain sciences 28:575-589; discussion 589-633 doi:10.1017/S0140525X05000105

Vignet C, Begout ML, Pean S, Lyphout L, Leguay D, Cousin X (2013) Systematic screening of behavioral responses in two zebrafish strains Zebrafish 10:365-375 doi:10.1089/zeb.2013.0871

Vikingstad EM, George KP, Johnson AF, Cao Y (2000) Cortical language lateralization in right handed normal subjects using functional magnetic resonance imaging Journal of the neurological sciences 175:17-27

Wahlsten D (1990) Insensitivity of the analysis of variance to heredity-environment interaction Behavioral and Brain Sciences 13:109-120

Westergaard GC, Lussier ID, Suomi SJ, Higley JD (2001) Stress correlates of hand preference in rhesus macaques Developmental psychobiology 38:110-115

Whiteley AR et al. (2011) Population genomics of wild and laboratory zebrafish (Danio rerio) Molecular ecology 20:4259-4276 doi:10.1111/j.1365-294X.2011.05272.x

Wong K et al. (2010) Analyzing habituation responses to novelty in zebrafish (Danio rerio) Behavioural brain research 208:450-457 doi:10.1016/j.bbr.2009.12.023

Zimmerberg B, Glick SD (1974) Rotation and stereotypy during electrical stimulation of the caudate nucleus Research communications in chemical pathology and pharmacology 8:195

Figure Captions

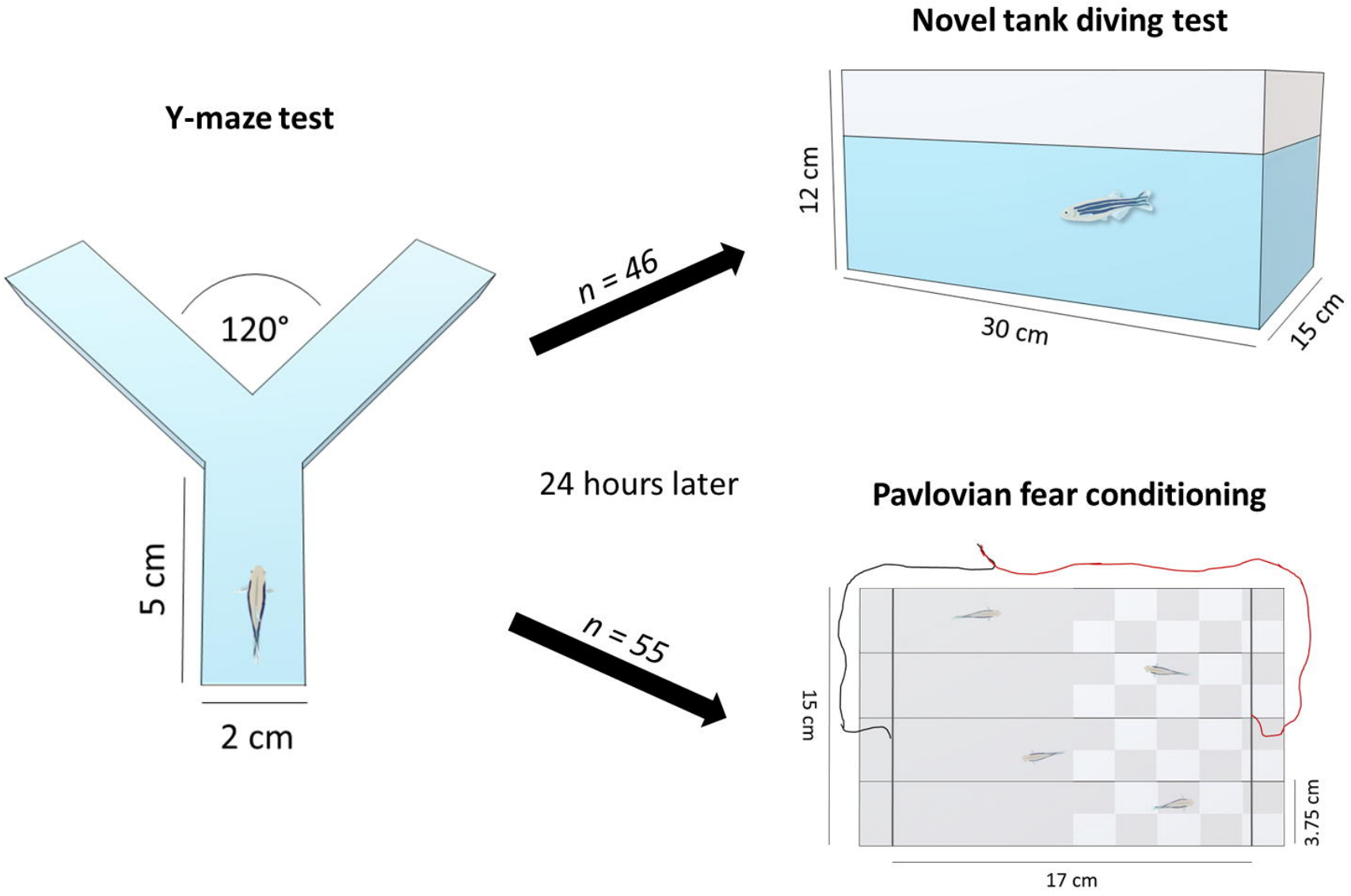
Figure 1. Schematic representation of the experimental design and the behavioral tasks.

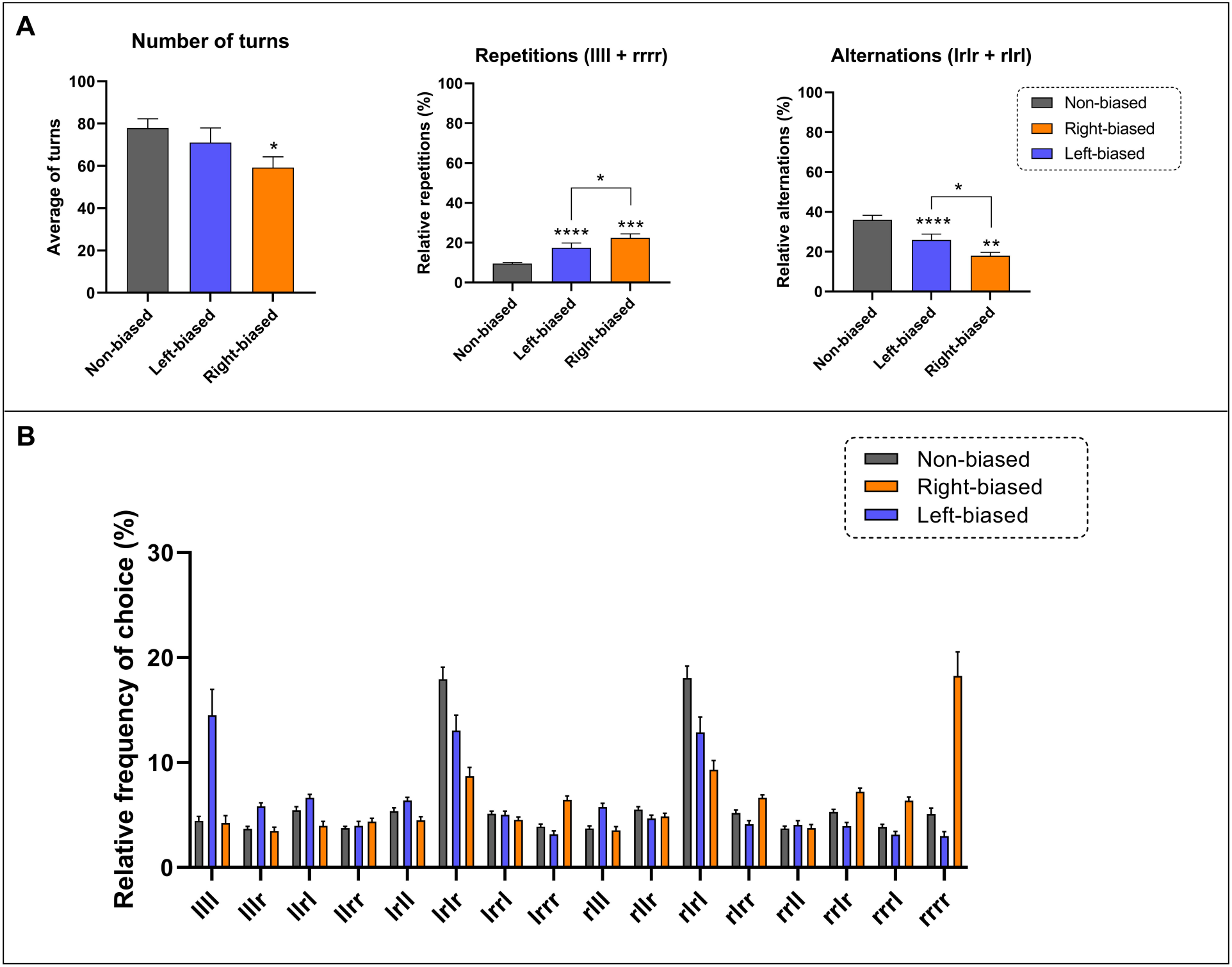
Figure 2. Effects of left- and right- bias in zebrafish on the Y-maze test. (A) Laterality affects total number of turns, repetitions and alternation of adult zebrafish. **(B) Y-maze tetragrams showing the behavioral phenotype of biased and non-biased animals considering the relative frequency of choice (tetragram frequency of choice*100/total number of turns).** Data were represented as mean ± S.E.M. and analyzed by linear mixed effects, followed by Tukey's multiple comparison test. Asterisks indicates statistical differences compared to non-biased group or between biased groups (*p < 0.05, **p <0.01, ***p <0.001 and ******p<0.0001**, n = 47 non-biased, n = 28 left-biased and n = 28 right-biased group).

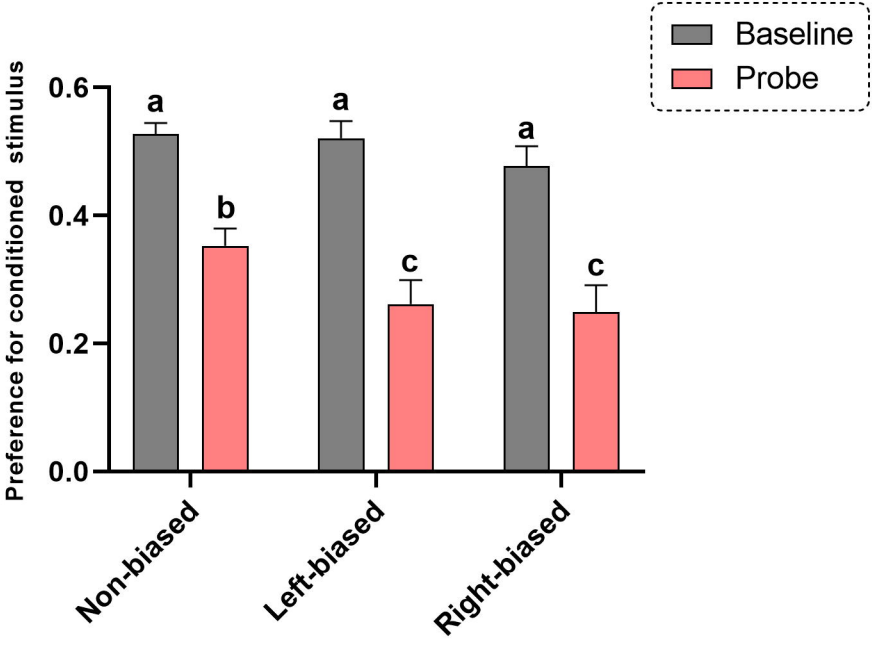
Figure 3. Left- and right- bias are related to fear avoidance learning responses in adult zebrafish. Data were represented as mean ± S.E.M. and analyzed by two-way RM ANOVA, followed by Tukey's multiple comparison test. **Different letters indicate significant differences between groups (*p < 0.05*;** *n* = 25 non-biased, *n* = 17 left-biased and *n* = 13 right-biased group).

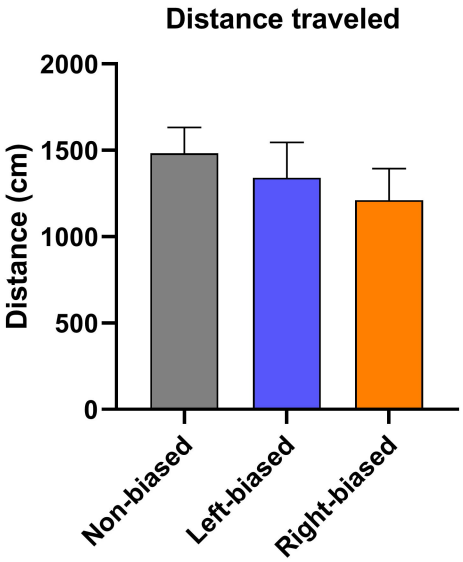
Figure 4. Behavioral laterality is not related to locomotor or anxiety-related phenotypes in adult zebrafish. Data were represented as mean ± S.E.M. and analyzed by **one- or two- way ANOVA** (n = 22 non-biased, n = 11 left-biased and n = 15 right-biased group).

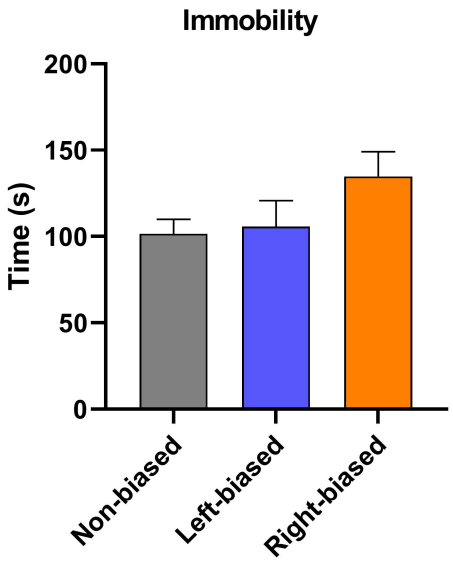
Figure 5. Left- and right- bias do not change thigmotaxis in adult zebrafish. Data were represented as mean ± S.E.M. and analyzed by **one-way ANOVA** (n = 20 non-biased, n = 11 left-biased and n = 15 right-biased group).

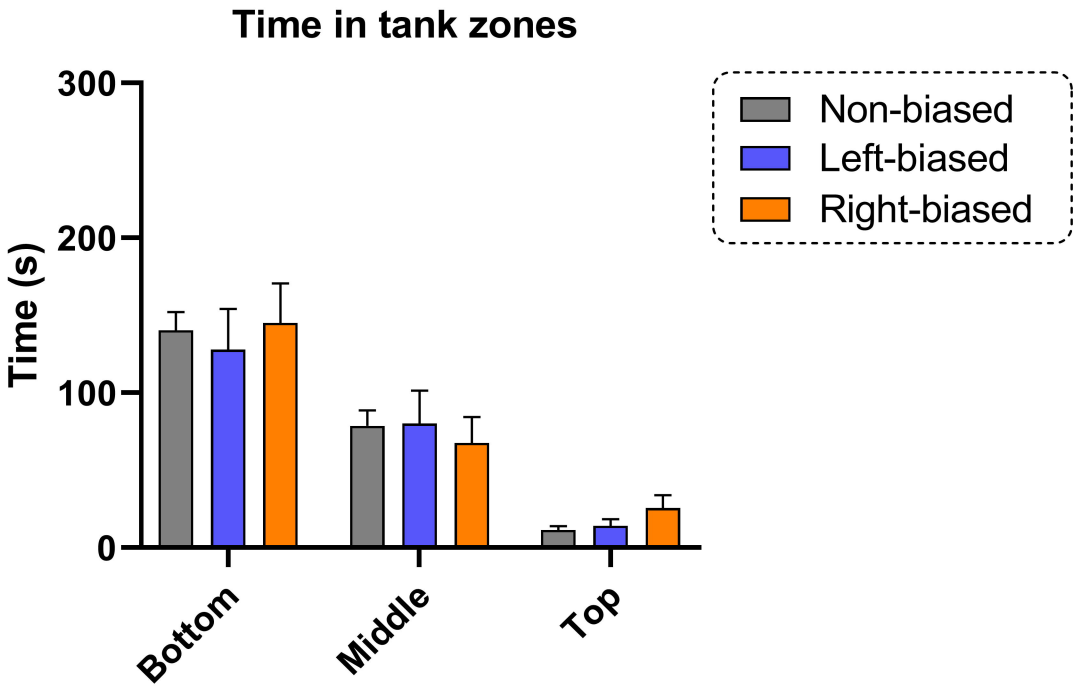

Y-maze test
120°
5 cm
2 cm
n = 46
24 hours later
n = 55
Novel tank diving test
12 cm
30 cm
15 cm
Pavlovian fear conditioning
15 cm
3.75 cm
17 cm

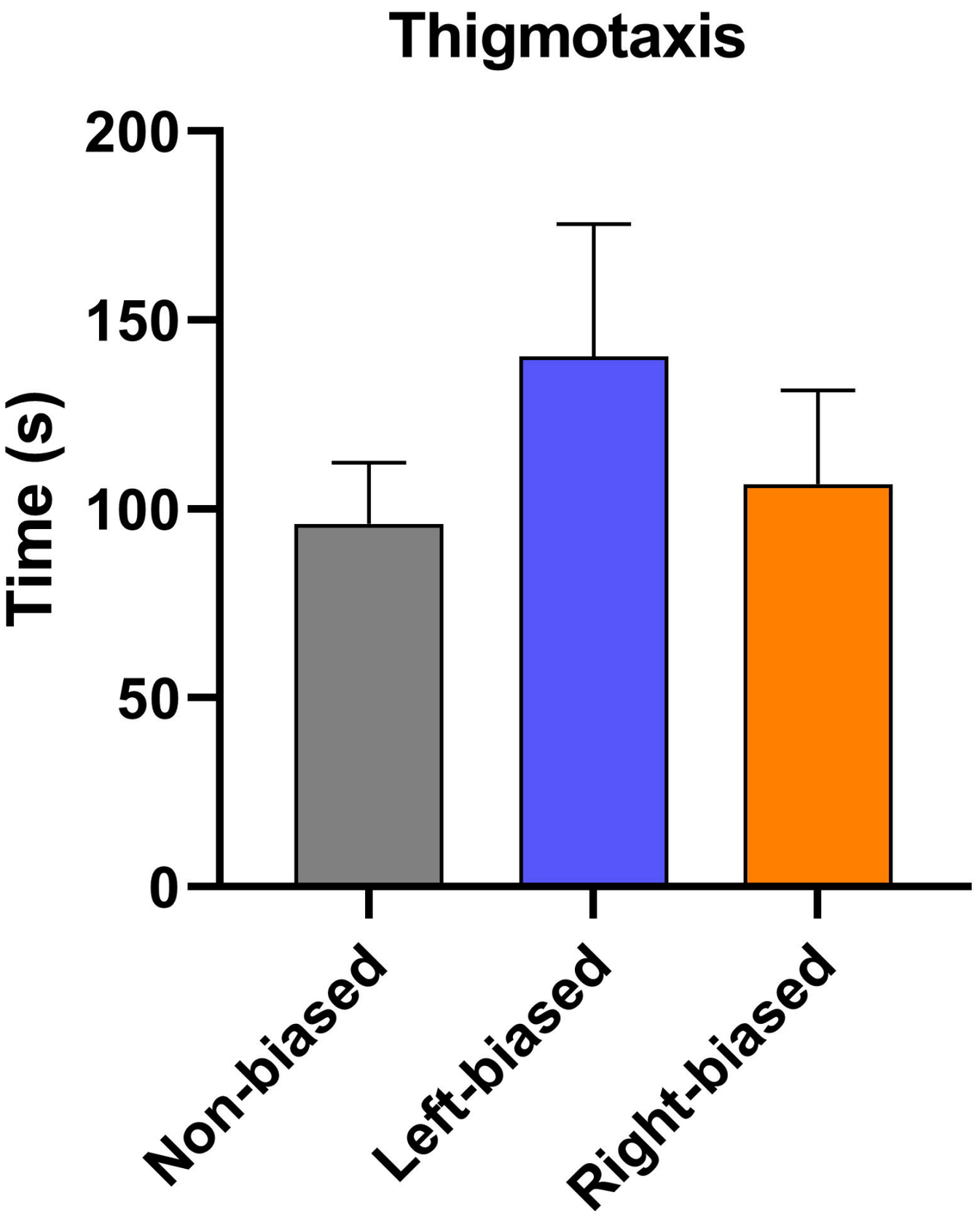

A
Number of turns
Average of turns
100
80
60
40
20
0
*
Non-biased
Left-biased
Right-biased
Repetitions (llll + rrrr)
Relative repetitions (%)
100
80
60
40
20
0
*
****
***
Non-biased
Left-biased
Right-biased
Alternations (lrlr + rlrl)
Relative alternations (%)
100
80
60
40
20
0
*
****
**
Non-biased
Left-biased
Right-biased
Non-biased
Right-biased
Left-biased
B
Non-biased
Right-biased
Left-biased
Relative frequency of choice (%)
30
20
10
0
llll
lllr
llrl
llrr
lrll
lrlr
lrrl
lrrr
rlll
rllr
rlrl
rlrr
rrll
rrlr
rrrl
rrrr


Baseline
Probe
Preference for conditioned stimulus
0.6
0.4
0.2
0.0
a
b
a
c
a
c
Non-biased
Left-biased
Right-biased


Distance traveled
Distance (cm)
2000
1500
1000
500
0
Non-biased
Left-biased
Right-biased


Immobility
Time (s)
200
150
100
50
0
Non-biased
Left-biased
Right-biased


Time in tank zones
Time (s)
300
200
100
0
Bottom
Middle
Top
Non-biased
Left-biased
Right-biased


Thigmotaxis
Time (s)
200
150
100
50
0
Non-biased
Left-biased
Right-biased