

Research



Cite this article: Lucon-Xiccato T, Montalbano G, Dadda M, Bertolucci C. 2020 Lateralization correlates with individual differences in inhibitory control in zebrafish. *Biol. Lett.* **16**: 20200296.
<http://dx.doi.org/10.1098/rsbl.2020.0296>

Received: 28 April 2020

Accepted: 13 July 2020

Subject Areas:

behaviour, cognition

Keywords:

cognitive abilities, executive functions, fish cognition, laterality

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Electronic supplementary material is available online at <https://doi.org/10.6084/m9.figshare.c.5069686>.

Lateralization correlates with individual differences in inhibitory control in zebrafish

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Individual fitness often depends on the ability to inhibit behaviours not adapted to a given situation. However, inhibitory control can vary greatly between individuals of the same species. We investigated a mechanism that might maintain this variability in zebrafish (*Danio rerio*). We demonstrate that inhibitory control correlates with cerebral lateralization, the tendency to process information with one brain hemisphere or the other. Individuals that preferentially observed a social stimulus with the right eye and thus processed social information with the left brain hemisphere, inhibited foraging behaviour more efficiently. Therefore, selective pressures that maintain lateralization variability in populations might provide indirect selection for variability in inhibitory control. Our study suggests that individual cognitive differences may result from complex multi-trait selection mechanisms.

1. Introduction

Fitness often depends on an individual's ability to fine-tune behaviour to a given situation; this includes inhibiting non-adaptive behaviour (hereafter, inhibitory control; [1]). For example, animals may benefit from inhibiting foraging when predators are present [2] or when providing parental care [3]. Evidence from diverse vertebrate taxa suggests that the individuals of the same species can differ in performance in inhibitory control tasks (mammals: [4,5]; birds: [6,7]; teleost fishes: [3,8,9]). One remarkable example comes from a fish, *Poecilia reticulata*, tested in a laboratory task in which an unreachable prey was presented enclosed in a transparent tube. Some individuals inhibited the tendency to attack the prey after a couple of attempts, whereas others required as many as 300 attempts [8]. Despite the potential impact of inhibitory control variability on fitness, its maintenance and psychological mechanisms remain unclear.

Functional magnetic resonance imaging in humans has revealed that neural circuit activation during inhibition tasks is lateralized to the right brain hemisphere [10,11]. Most humans exhibit the same functional organization between the two brain hemispheres, whereas other species exhibit large individual variation in lateralization [12]. The same cognitive task can be processed by the right hemisphere in some individuals and by the left hemisphere in others. Moreover, individuals may differ regarding how strongly they rely on the preferred hemisphere for a certain cognitive function.

If inhibitory control is also lateralized in an animal species, but individuals vary regarding the direction and strength of lateralization, this may contribute to individual differences in inhibitory control. We tested this hypothesis in the zebrafish, *Danio rerio*, a teleost fish in which variability in inhibitory control and lateralization were reported previously [13,14].

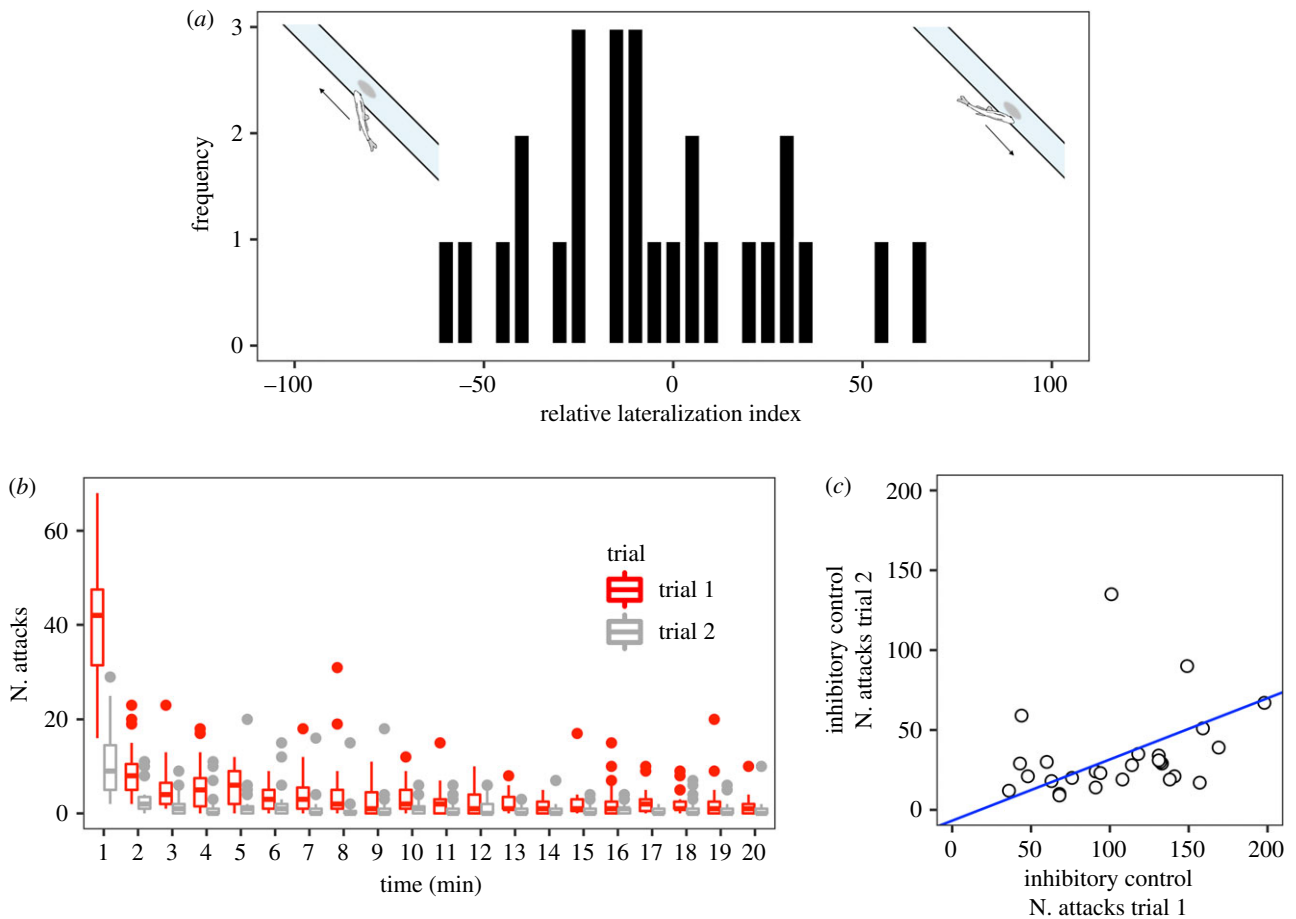


Figure 1. (a) Frequency distribution of relative lateralization. (b) Boxplots show number of attacks in the inhibitory control test per minute and trial; internal lines and box edges represent median and interquartile range (IQR), respectively, whiskers represent $1.5 \times$ IQR and points represent outliers. (c) Scatterplot of the number of attacks in the first versus the second trial of the inhibitory control test.

2. Material and methods

Using 28 adult zebrafish (15 males and 13 females), we studied the potential correlation between lateralization, which we measured using with a well-established mirror test [14], and inhibitory control, which we assessed using a foraging task [13].

(a) Lateralization test

Lateralization could not be measured during inhibition in our study species. Therefore, we measured it using a social task. Because lateralization of different cognitive functions is correlated in individuals [15,16], our social lateralization measure provided a proxy for overall individual differences in lateralization, including those of inhibitory control functions. Zebrafish were observed for 20 min in an octagonal apparatus with mirror walls (electronic supplementary material, figure S1). Subjects exhibited strong social attraction towards their mirror images, which they misperceived as conspecific [14,16]. Lateralized processing of social information caused subjects to look at the mirror images with either the left eye (corresponding to right-hemisphere processing) or the right eye (left-hemisphere processing). We analysed this behaviour using video recordings, by measuring time spent swimming in proximity (2 body lengths) to the mirror in clockwise (left eye, right hemisphere use) and anticlockwise (right eye, left hemisphere use; [16]) directions. We used this variable to calculate two lateralization indices [17]. The relative lateralization index ranged between -100 and 100 , indicating subjects that used exclusively the right or the left eye, respectively. The absolute lateralization index described lateralization strength, but not its direction. This index ranged

from 0 (subjects using both eyes equally) to 100 (subjects using exclusively one eye).

(b) Inhibitory control test

The inhibitory test was successfully used in this species [13] and was not detectably affected by motivation and habituation [18]. Each individual fish was housed for 4 days in a 4 L plastic tank (electronic supplementary material, figure S2). After a three-day habituation (electronic supplementary material), we presented a transparent glass tube containing live prey (*Artemia salina*) for 20 min and counted the number of attacks. We expected individuals with higher inhibitory control to inhibit the tendency to attack the unreachable prey sooner, resulting in a low number of attacks. To investigate individual differences, as well as the role of learning and memory, we repeated the prey exposure 2 h later. One zebrafish did not approach the tube, so we dropped it from the dataset (final $N=27$).

3. Results

(a) Lateralization

Zebrafish spent most of their time ($85.64 \pm 11.38\%$, mean \pm s.d.) in proximity to the mirror. Relative lateralization was -5.35 ± 32.02 and did not differ from 0 (one-sample Wilcoxon signed-rank test: $N=27$, $V=150$, $p=0.361$), suggesting its symmetrical distribution in our subjects' sample (figure 1a). Absolute lateralization was 42.65 ± 14.21 . The time spent in proximity to the mirror did not correlate with relative

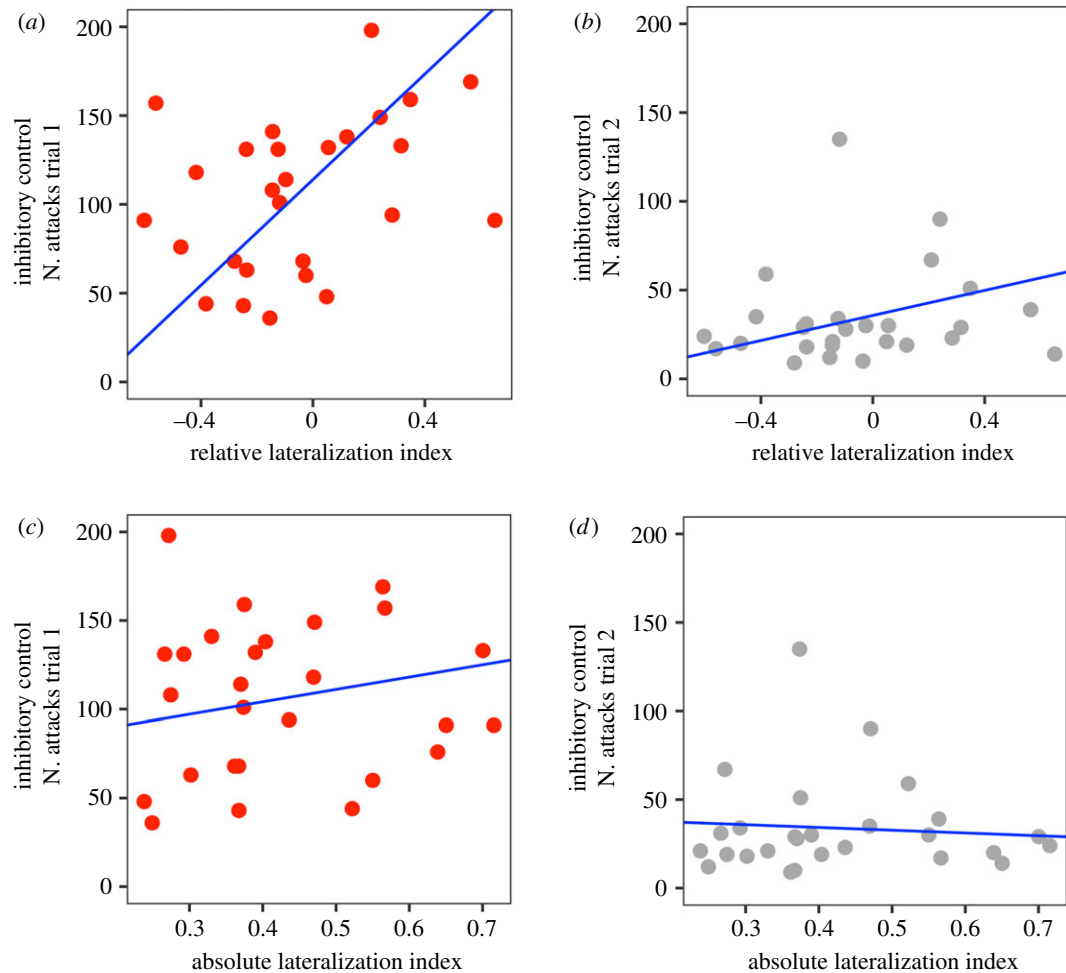


Figure 2. Scatterplots of relative lateralization versus the (a) first and the (b) second trial of the inhibitory control test and scatterplots of absolute lateralization versus the (c) first and the (d) second trial of the inhibitory control test. Lines represent predicted values from reduced major axis regression.

(Spearman's rank correlation: $N = 27$, $\rho = -0.241$, $p = 0.226$) or absolute lateralization ($N = 27$, $\rho = 0.056$, $p = 0.781$).

(b) Inhibitory control

Overall, fish attempted to attack the prey 139.81 ± 57.55 times. The number of attacks was significantly higher in the first trial (105.96 ± 43.53) compared to the second trial (33.85 ± 27.26 ; Wilcoxon signed-rank test: $N = 27$, $V = 370$, $p < 0.001$; figure 1b). Within trials, the number of attacks decreased over time (Friedman test: trial 1: $\chi^2_{19} = 214.900$, $p < 0.001$; trial 2: $\chi^2_{19} = 126.130$, $p < 0.001$; figure 1b), indicating that zebrafish progressively inhibited attack behaviour. The number of attacks was positively correlated between the first and the second trial (Spearman's rank correlation: $N = 27$, $\rho = 0.404$, $p = 0.037$), indicating individual differences in inhibitory control (figure 1c). We repeated the analysis without one outlier subject that showed high number of attacks in trial 2, finding a similar result ($N = 26$, $\rho = 0.431$, $p = 0.028$).

(c) Correlation between lateralization and inhibitory control

Relative lateralization correlated positively with the number of attacks in the first inhibitory control trial (Spearman's rank correlation: $N = 27$, $\rho = 0.403$, $p = 0.037$; figure 2a), but not in the second trial (Spearman's rank correlation: $N = 27$, $\rho = 0.226$, $p = 0.257$; figure 2b). Absolute lateralization did

not correlate with the number of attacks in the first ($N = 27$, $\rho = 0.154$, $p = 0.443$; figure 2c) or the second inhibitory control trial ($N = 27$, $\rho = 0.110$, $p = 0.584$; figure 2d).

The relative decrease in the number of attacks between inhibitory control trials 1 and 2 did not correlate with relative or absolute lateralization (Spearman's rank correlation: $N = 27$, $\rho = -0.061$, $p = 0.762$; and $N = 27$, $\rho = -0.097$, $p = 0.629$, respectively). The time spent in proximity to the mirror image did not correlate with the number of attacks during the inhibitory control test (first trial: $N = 27$, $\rho = 0.024$, $p = 0.905$; second trial: $N = 27$, $\rho = -0.093$, $p = 0.643$).

4. Discussion

Our experiment revealed a significant correlation between individual differences in the directionality of lateralization and inhibitory control in zebrafish. Individuals that preferentially observed a social stimulus with the right eye, and thus processed social information with the left brain hemisphere [19], inhibited the fruitless attacking behaviour more efficiently.

Lateralization has pervasive effects on cognitive tasks in a range of species (birds: [20]; amphibians: [17]; fish: [21,22]). The novelty of our study derives from the type of cognitive ability we investigated. Inhibitory control is one of the executive functions, i.e. cognitive functions necessary to control

behaviour; executive functions are often combined together and with more specific functions to accomplish complex cognitive processes [1]. Part of the covariation between lateralization and cognitive performance observed in prior investigations could be caused by inhibitory control. For instance, Bibost and Brown [21] found that rainbowfish, *Melanotaenia duboulayi*, with left-eye preference for viewing their mirror images, acquired a food–light association more quickly than right-lateralized individuals did. The cause of this correlation could be inhibitory control, which might have enabled the fish to focus on the light stimulus as a food release signal. Indeed, in our study, performance in the second trial of the inhibitory control test, which was also affected by learning and memory [23], did not significantly correlate with lateralization. Nevertheless, this does not exclude the possibility that lateralization covaries with other cognitive functions, as observed in humans [24].

The relationship was not significant when considering lateralization strength, irrespective of directionality (absolute lateralization). Therefore, one hemisphere appeared more efficient than the other in inhibiting behaviour. Interspecies similarities in hemisphere use [19] suggest that the base architecture of the brain, including lateralization, is conserved across vertebrates owing to inheritance from a common ancestor [12]. Thus, one might ask whether the right hemisphere is responsible for inhibition in fish, as it is in humans [10,11]. However, hemispheric use is task dependent [24], and we measured social lateralization [14,16]. This is expected to correlate with inhibitory control lateralization, but it does not necessarily predict the hemisphere involved [15]. Patterns of neural activity, which can be measured in larvae, are necessary to answer this question.

Precise behavioural modulation should confer benefits, causing directional selection that disrupts individual differences in inhibitory control. However, lateralization variability is maintained by a balance of costs and benefits, resulting in an evolutionary stable strategy [12]. If lateralization determines at least partly individuals' inhibitory control, then selection that maintains variability in lateralization might be indirectly responsible for inhibitory control variability. However, the reverse might also occur. Although it

remains undemonstrated, enhanced inhibitory control might bear costs that, along with its benefits, might determine selective pressures that directly maintain inhibitory control variability and indirectly affect lateralization. The high occurrence of individuals with right-hemisphere social processing (the lateralization phenotype with higher inhibitory control) in teleost species seems to align with this hypothesis [18].

Notably, lateralization is not the only factor affecting inhibitory control variability. Various studies have reported covariation between inhibitory control and personality [6], including in zebrafish [13]. In our experiment, a measure of personality (sociability as time spent close to the mirror image) did not correlate with inhibitory control. However, the simultaneous effect of multiple cognitive traits remains possible and provides an explanation, along with non-cognitive factors [25], for the moderate covariation in our study. Nonetheless, the correlation between lateralization and inhibitory control has likely high biological relevance because its effect size mirrors those of inhibitory control repeatability in our study and correlations between other cognitive traits in prior fish experiments [26]. Overall, it is becoming clear that a complex multi-trait selection mechanism involving lateralization and personality maintains variability in executive and non-executive functions.

Ethics. This study was conducted in accordance with national law (D.L. 4 Marzo 2014, n. 26) and was approved by Ferrara University Ethical Committee (TLX n. 2/2018).

Data accessibility. The datasets supporting this article have been uploaded in Dryad: <https://doi.org/10.5061/dryad.vq83bk3qc> [27].

Authors' contributions. T.L.-X. conceived the study, carried out the statistical analyses and drafted the manuscript; G.M. collected data and critically revised the manuscript; M.D. and C.B. designed the study and helped draft the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

Competing interests. We declare we have no competing interests.

Funding. This work was supported by FAR2019 and FIR2018 grant from University of Ferrara to T.L.-X.

Acknowledgements. We thank Andrea Margutti and Francesco Moda for help in building the apparatuses and scoring the recordings.

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