

1 Bold zebrafish (*danio rerio*) learn faster in an associative learning task

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10 **Abstract**

11 Animals differ in their ability to learn. One potential factor contributing to learning  
12 differences is personality types. We investigated the relationship between learning and the bold-  
13 shy continuum by comparing performance of bold and shy zebrafish in conditioned place  
14 preference (CPP) and 2 choice tasks. Bold fish learned significantly faster than the shy fish but  
15 there were no differences in their final performance. When tested in the 2 choice task, we found  
16 no clear evidence of learning, however bold fish made more initial choices than shy fish. Overall,  
17 our study suggests that bold fish tend to be faster learners when compared to shy fish. The lack  
18 of differences in the final change in behavior suggests that the learning difference is due to  
19 neophobic tendencies and resulting initial interactions with the learning stimulus.

20 **Keywords:** Personality, cognition, bold, shy, operant, classical, associative learning

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27 **Introduction**

28 As animals interact with their environment, how quickly they learn and recall these  
29 interactions can vary between individuals (Boogert et al., 2018, Cauchoix et al., 2018). It has  
30 been hypothesized that variation in learning between individuals can be explained in part by  
31 differing personality types (Dingemanse & Wolf, 2010, Sih & Del Guidice, 2012, Sih et al.,  
32 2004). Across many animal taxa, studies demonstrate that one common dimension of personality  
33 is the bold-shy continuum (Réale et al., 2007). Bold individuals are characterized by displaying  
34 lower neophobic and stress-related behaviors and have higher exploratory activity. In contrast  
35 shy individuals tend to have opposing traits (Wilson et al., 1994, Sih et al., 2004, Baker et al.,  
36 2018).

37 However, studies across taxa find a conflicting relationship between personality and  
38 learning. Many studies showed that bold individuals learn faster than shy, in animals such as  
39 mammals, birds, to teleost fish (Mazza et al., 2018, Guenther et al., 2014, Dugatkin & Alfieri,  
40 2002, DePasquale et al., 2014, Bensky et al., 2017, Daniel & Bhat, 2020, Kareklaas, Elwood &  
41 Holland, 2017). Fewer studies either found the opposite (e.g. shy learn faster than bold) or no  
42 relationship between personality and learning speed (Lermite, Peneaux & Griffin, 2016, Ferron  
43 et al., 2015, Sommer-Trembo & Plath, 2018, Baker & Wong, 2019). Inconsistencies across  
44 studies suggest that other factors likely influence learning performance beyond personality type.  
45 Aspects of the learning assay like the type of task (e.g. operant or classical conditioning) or the

46 context that the animal is tested in could affect the relationship between personality and learning  
47 (Poirer et al., 2020, Dingemanse & Wolf, 2010).

48 Individuals of varying personality types likely vary in their interactions with different  
49 learning tasks or stimuli, which may influence learning performance (Sih & Guidice, 2012). For  
50 example, different training paradigms require that the animal engage with the stimulus in  
51 different ways. Some studies found that learning is not correlated across training paradigms  
52 (Guillette et al., 2015, Ducatez et al., 2014, Kassai et al., 2022, Poirer et al., 2020) and one found  
53 that changing the difficulty of the learning task changed the relationship between personality and  
54 learning speed (Chang et al., 2018). Similarly, a meta-analysis in non-human animals found a  
55 low correlation between learning ability across cognitive tasks (Poirer et al., 2020). This  
56 potential variation across tasks suggests a need for measurements in multiple learning tasks  
57 (Griffin et al., 2015). Neophobia, associated with a shy personality, has been seen to affect  
58 operant learning of a food reward due to higher latencies to approach (Stöwe et al., 2006). Thus,  
59 comparing a passive (classical) task that does not require the animal to approach a novel object  
60 to an active (operant) task that does require approach may produce different results.

61 In this study, we investigated the effect of personality type on learning performance  
62 across two associative learning paradigms in zebrafish (*Danio rerio*). Using a within-subjects  
63 and counter-balanced design we individually trained bold and shy zebrafish to associate a visual  
64 stimulus with a food reward in both conditioned place preference and 2 choice tasks. We tested  
65 the prediction that bold individuals will be faster learners compared to shy fish because of their  
66 decreased neophobia. We also evaluated the prediction that there will be an interaction effect of  
67 personality and training paradigm on learning speed. Given that the operant task requires fish to

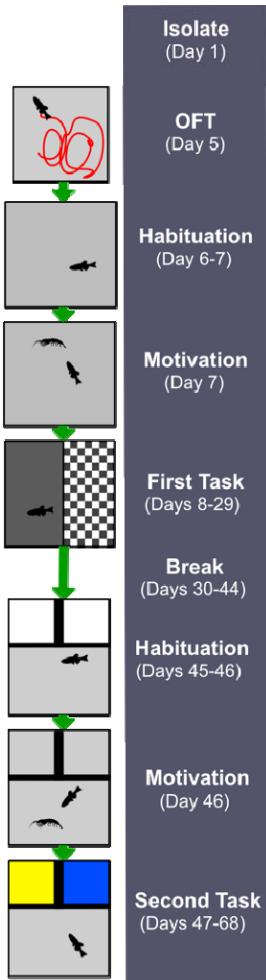
68 actively make a choice, we expected that bold fish would learn faster in this task than shy fish  
69 due to their decreased neophobia.

70

71 **Methods**

72 *Animals*

73 We used zebrafish from selectively bred lines that exhibit shy (high stationary behavior,  
74 HSB) or bold (low stationary behavior, LSB) personality traits (n = 48 per line). Across six  
75 different stress and anxiety-like behavioral assays, the HSB line exhibits a greater amount of  
76 behaviors consistent with a shy personality type (e.g., freezing, less exploratory, higher cortisol  
77 levels) than the LSB line (Wong et al., 2012, Baker & Wong, 2019). Additionally, the  
78 exploratory behavior of the lines in an open field test is repeatable and reliable (Baker & Wong,  
79 2019). The HSB line also shows faster release of cortisol under stress compared to the LSB line  
80 (Wong et al., 2019). For simplicity, we will refer to the HSB and LSB lines as shy and bold  
81 personality types, respectively. The fish used in this study were selectively bred for 13  
82 generations from wild caught zebrafish. Before testing, we housed the fish together in 40L tanks  
83 and fish were fed twice a day with Tetramin Tropical Flakes (Tetra, USA). One week prior to  
84 testing we physically isolated fish into 3-liter tanks on a recirculating water system (Pentair  
85 Aquatic Eco-Systems or Aquaneering) using UV and solid filtration on a 14:10 L/D cycle at a  
86 temperature of 27 °C. Fish had visual and olfactory access to each other. Starting three days  
87 before testing we withheld food from the fish to reduce the possibility of satiation while training.



**Figure 1.** Overview of Experiment Timeline. Fish of all groups started with isolation on the first day then went through an open field test (OFT), habituation, motivation test, and training on the first task (conditioned place preference task in this illustration). After a break fish went through the same for the second task (2 choice discrimination task in this illustration). Our study design was counterbalanced and half of the fish began with the CPP task while the other half began with the 2 choice discrimination task.

88

89

90 *Behavioral assays overview*

91 We conducted four behavioral assays on each fish: an open field test (OFT), a test for  
92 food motivation, a 2 choice discrimination task (operant conditioning), and a conditioned place  
93 preference (CPP, classical conditioning) task. The OFT and food motivation test were performed  
94 prior to training. Using a within-subjects design, we tested each fish in both associative learning  
95 paradigms and counterbalanced the starting paradigm (Figure 1). We used frozen adult brine  
96 shrimp (*Artemia* spp., San Francisco Bay Brand, USA) administered in liquid form as the food  
97 reward. Half of the fish received distilled water instead of brine shrimp to serve as controls. We

98 started with four groups of 24 fish (bold control, shy control, bold treatment, and shy treatment).  
99 All behavioral assays were performed between 3-8 hours after light onset. After 4 days of  
100 isolation, we tested each fish in the open field test to validate behavioral phenotype. We then  
101 habituated each fish for two consecutive days in the conditioning tank. We assessed biases in  
102 food motivation for the brine shrimp before starting baseline trials of the associative learning  
103 assays. Each fish had a 14 day inter-assay testing interval to minimize the influence of the tasks  
104 on each other.

105 *Open field test*

106 We individually tested fish in an OFT in a tank that was 31.75cm x 31.75cm x 10cm  
107 containing 4L of water. Immediately after placing fish in the tank we video-recorded the  
108 individual's behaviors for 5 minutes. We used Ethovision XT 17 (Noldus, Netherlands) to  
109 quantify the amount of time that each individual spent frozen during the trial.

110 *Motivation test*

111 This test was performed in the AD and LT models of the Zantiks semi-automated  
112 behavioral units (Zantiks, Cambridge, UK). After 30 seconds for acclimation, the food reward  
113 was administered 3 times at 30 second intervals. We quantified the time spent in a 9x12 cm  
114 rectangle centered around the food administration tube. The time that was being measured started  
115 immediately after the first brine shrimp administration until the end of the test to measure the  
116 motivation of the fish for the food reward. We performed the test in both Zantiks models but due  
117 to the size and height of the tank in the larger LT unit, the food drifted outside the fish tracking  
118 zone. Thus, we only used the data from the AD unit to assess motivation.

119

120 *Conditioned place preference*

121 We used a modified conditioned place preference protocol (Lau et al., 2006) in the  
122 Zantiks LT unit. The testing tank (36 cm x 27 cm x 30 cm) was filled with 5.8 L of water. We  
123 tested each fish in the CPP task for three weeks that consisted of 2 days of habituation, 1 day of  
124 baseline testing, 11 days of conditioning, and 3 days of probe trials (Figure S1a). To habituate  
125 each fish to the assay we placed the fish in the tank for 10 minutes with no training stimulus  
126 lights. After habituation we determined the baseline preference for the light stimuli (gray or  
127 checkered pattern) for each fish. Fish swam freely for 10 minutes in the tank where one half was  
128 illuminated from the bottom with a gray screen and the other half a checkered screen. We  
129 determined the conditioned and non-conditioned stimuli as the stimulus where the fish spent the  
130 least and most amount of time, respectively. During conditioning days, we sequentially presented  
131 each stimulus for 5 minutes to each fish. The non-conditioned stimulus was presented for the  
132 first five minutes followed by the conditioned stimulus. One hundred microliters of brine shrimp  
133 or distilled water was administered every minute during presentation of the conditioning and  
134 non-conditioning stimulus, respectively. Food reward consisted of 11.4 grams of frozen brine  
135 shrimp in 30 mL of distilled water. We fed control fish an equivalent amount of brine shrimp  
136 after each conditioning trial. Probe trials were administered the day after a conditioning trial.  
137 Probe trials were conducted after 3 days, 7 days, and 11 days after the first day of conditioning  
138 with a total of 3 probe trials. Probe trial methods were the same as those used in the baseline  
139 preference step where we quantified the time spent in each stimulus for each fish. The order of  
140 stimulus presentation was consistent within a fish but random across fish for probe and baseline  
141 trials.

142

143 *2 choice discrimination task*

144 We used a modified 2 choice discrimination task from an established protocol (Bilotta et  
145 al., 2006). We used the AD model Zantiks unit (Zantiks, Cambridge, UK) with a 14cm x 20cm x  
146 15cm tank filled with 2.5 L of water (Figure S1b). We habituated each fish for 20 minutes a day  
147 for two consecutive days with white lights on in the wells as shown in Figure S1b. We tested  
148 each fish every other day for a total of 10 testing days. Fish were fasted on non-testing days. In  
149 this task the fish were presented with two 6.5 cm x 5.1 cm light stimuli (blue and yellow) from  
150 below at one end of the tank. Prior studies show that with appetitive learning in zebrafish there is  
151 a bias towards red compared to other colors such as blue and yellow (Spence & Smith, 2008,  
152 Kim et al., 2017). For each fish, a color was randomly chosen at the start of testing to be the  
153 reinforced stimulus where a food reward (brine shrimp) was administered at the other end of the  
154 tank when the fish swam into the designated reinforced color. The food reward consisted of 5.7  
155 grams of frozen brine shrimp suspended in 30 mL of distilled water. Each trial began with an  
156 acclimation period of two minutes with white lights in the two wells. After two minutes blue and  
157 yellow lights were presented for 30 seconds. Swimming into the designated correct choice  
158 resulted in the correct colored light staying on for an additional 30 seconds and we  
159 simultaneously administered 25  $\mu$ l of the food reward. An incorrect choice resulted in all lights  
160 turning off for 30 seconds. This sequence ran for a total of 20 trials each day for each fish (i.e.,  
161 one session consists of 20 trials). The position of the yellow and blue lights (e.g., left or right)  
162 was randomly set for each trial. There was an intertrial interval of 10 seconds. Control fish  
163 underwent the same protocol with distilled water administered instead of brine shrimp and were  
164 fed brine shrimp after each testing day. We compared the number of correct choices and the total  
165 number of choices across sessions to assess learning.

166

167

168 *Statistical analysis*

169 We performed all statistical tests using R statistical software(R 4.2.2 GUI 1.79 Big Sur  
170 ARM build) and Rstudio version 2022.12.0+353 (R Core Team, 2021). Due to fish mortality  
171 during the experiment, the sample sizes for statistical analyses between the conditioned place  
172 preference (bold control (n = 20), shy control (n = 20), bold treatment (n = 19), and shy treatment  
173 (n = 19)) and 2 choice (bold control (n = 20), shy control (n = 17), bold treatment (n = 19), and  
174 shy treatment (n = 20)) tasks differed. We conducted post-hoc tests using the emmeans (Lenth et  
175 al., 2022) package and normality and assumptions were checked using base R. The lme4 package  
176 (Bates et al., 2022) was used to test negative binomial linear mixed effect models. We obtained  
177 simple statistics for all measures using the psych package (Revelle, 2022) (Table 1). Sex was  
178 included in all models but was not significant and therefore removed. Model assumptions,  
179 including normality were inspected in R.

180

181 *Open field test and motivation*

182 We tested for differences between the bold and shy groups in the OFT and motivation  
183 test using a Welch two-sample t-test. This test was used due to unequal variances between bold  
184 and shy groups. We compared the duration of time frozen in the OFT between the bold and shy  
185 personality types. To investigate difference in food motivation, we compared the duration of  
186 time spent around the food administration tube between the bold and shy personality types using  
187 the same test.

188

189 *Conditioned place preference*

190 We modeled the duration of time spent in the conditioned stimulus for the last half (5  
191 minutes) of the baseline and probe trials to test for a change in preference for the conditioned  
192 stimulus across the task within the different groups. We did not include the first half (5 minutes)  
193 in the analysis to minimize the influence of handling on fish behavior. We performed a repeated  
194 measures ANOVA to investigate the effects of treatment, personality type, and conditioning day  
195 on the time spent in the conditioned stimulus with a linear mixed effects model with individual  
196 as the random effect. We included all interactions in the model and used type II sums of squares.  
197 We used Tukey post-hoc tests to evaluate differences in the response variables across trials for  
198 each group and within trials between groups.

199

200 *2 choice discrimination task*

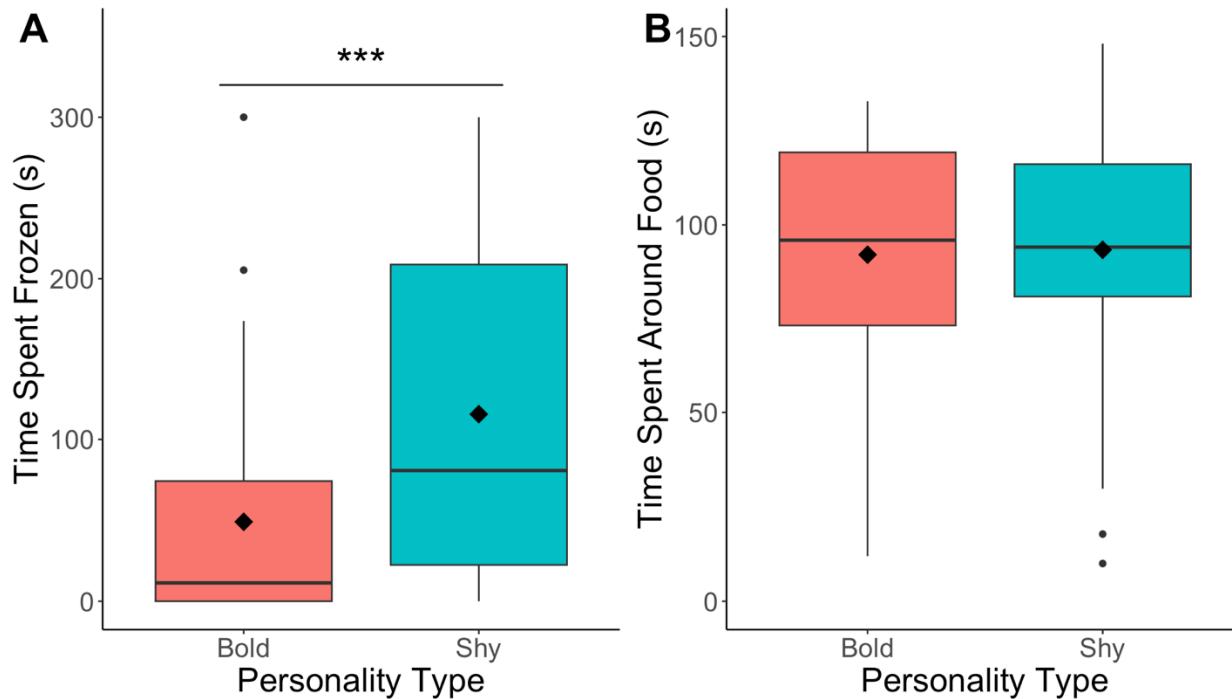
201 We modeled the number of correct choices over the conditioning days to examine  
202 changes in correct choices over time within groups. We performed a negative binomial mixed  
203 effect regression on the number of correct choices with treatment, personality type and session as  
204 the fixed effects and ID as the random effect. Simple slopes were obtained to test for increases in  
205 correct choices within each group using the interactions package in R and plotted using the same  
206 package. Additionally, we performed a negative binomial mixed effect regression on the total  
207 number of choices with treatment, personality type and session as the fixed effects and ID as the  
208 random effect. We also obtained simple slopes for this model.

209

210 **Results**

211 *Shy fish freeze more but had equal motivation to eat*

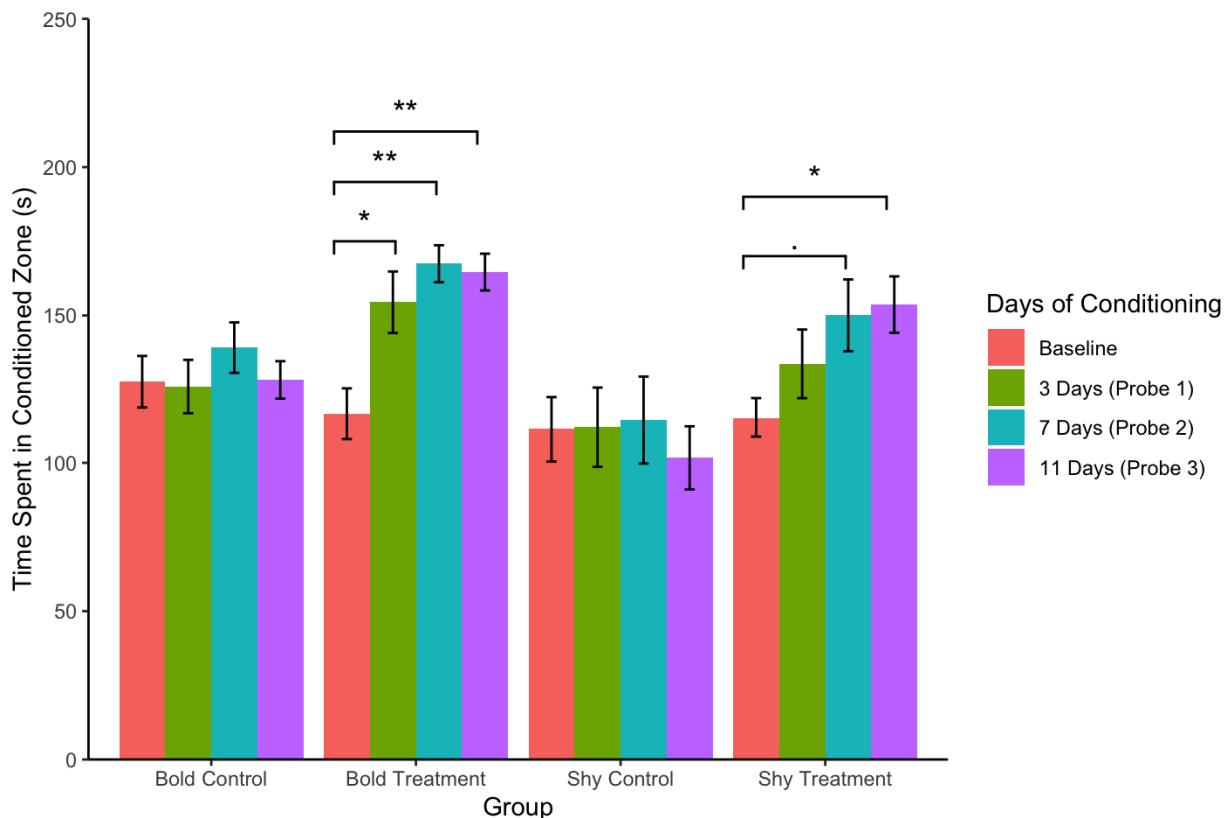
212 There was a significant effect of personality type on freezing time in the open field test  
213 (Figure 2a). Shy fish spent significantly more time frozen than bold fish ( $t = -3.55$ ,  $df = 90$ ,  $p =$   
214  $6.4 \times 10^{-4}$ ). There were no significant differences between personality types ( $t = -0.19$ ,  $df = 82$ ,  $p =$   
215 .85) in the amount of time spent around the food in the motivation task (Figure 2b).



216  
217 **Figure 2.** A. Boxplot of time spent frozen in the open field test and B. boxplot of time spent around the food in the  
218 motivation task. Bold fish are in red and shy fish are in teal. The diamond indicates the mean and the line is at the  
219 median.\* $p < .05$ , \*\* $p < .01$  \*\*\* $p < .001$

220  
221 *Bold fish change their behavior before shy fish*  
222 Treatment fish increased time spent in the conditioned stimulus in the CPP task, with  
223 bold fish increasing time spent in the conditioned stimulus earlier in the task than shy fish  
224 (Figure 3). In the full model (Table S1) the interaction effect between treatment and probe trial  
225 was approaching significance ( $F(3, 292) = 4.09$ ,  $p = .09$ ). A Tukey post hoc test (Table S2)  
226 revealed that there were no significant differences in the duration of time in the conditioned

227 stimulus between trials for the control groups for either personality type ( $p > .05$ ). In the bold  
228 treatment group there was a significant difference between baseline and probe 1 (i.e., after 3 days  
229 of conditioning;  $t = -2.64$ ,  $df = 296$ ,  $p = .03$ ), probe 2 (i.e., after 7 days of conditioning;  $t = -3.55$ ,  
230  $df = 296$ ,  $p = 2.8*10^{-3}$ ), and probe 3 (i.e., after 11 days of conditioning;  $t = -3.35$ ,  $df = 296$ ,  $p =$   
231  $4.7*10^{-3}$ ). In the shy treatment group there was no significant difference in time spent in the  
232 conditioned stimulus between baseline and probe 1 ( $t = -1.27$ ,  $df = 296$ ,  $p = .45$ ) but there was a  
233 trend for a difference between baseline and probe 2 ( $t = -2.42$ ,  $df = 296$ ,  $p = .07$ ) and at probe 3  
234 shy treatment group spent significantly more time in the CS compared to baseline ( $t = -2.67$ ,  $df =$   
235  $296$ ,  $p = .04$ ). No significant differences in duration of time in the CS between probe 1, 2, or 3  
236 were detected in any of the groups ( $p > .05$ ). There were no differences in time spent in the  
237 conditioned zone at any of the time points between personality types ( $p > .05$ ). Additionally,  
238 there was no significant correlation between learning speed (change in CS time from baseline  
239 after 3 days of conditioning) and final time spent in the conditioned stimulus in the CPP task for  
240 the bold fish ( $\rho = .19$ ,  $p = .44$ ) or for the shy fish ( $\rho = .22$ ,  $p = .35$ ).



241

242 **Fig. 3** Time spent in the conditioned zone by group and day of conditioning in the CPP. Pink bars are at baseline,  
243 green bars are after 3 days of conditioning (Probe 1), blue bars are after 7 days (Probe 2) and purple bars are after 11  
244 days of conditioning (Probe 3). Error bars indicate standard error.  $p < .1$ ,  $*p < .05$ ,  $**p < .01$ .

245

246 *No evidence of learning in 2 choice discrimination task with correct choices*

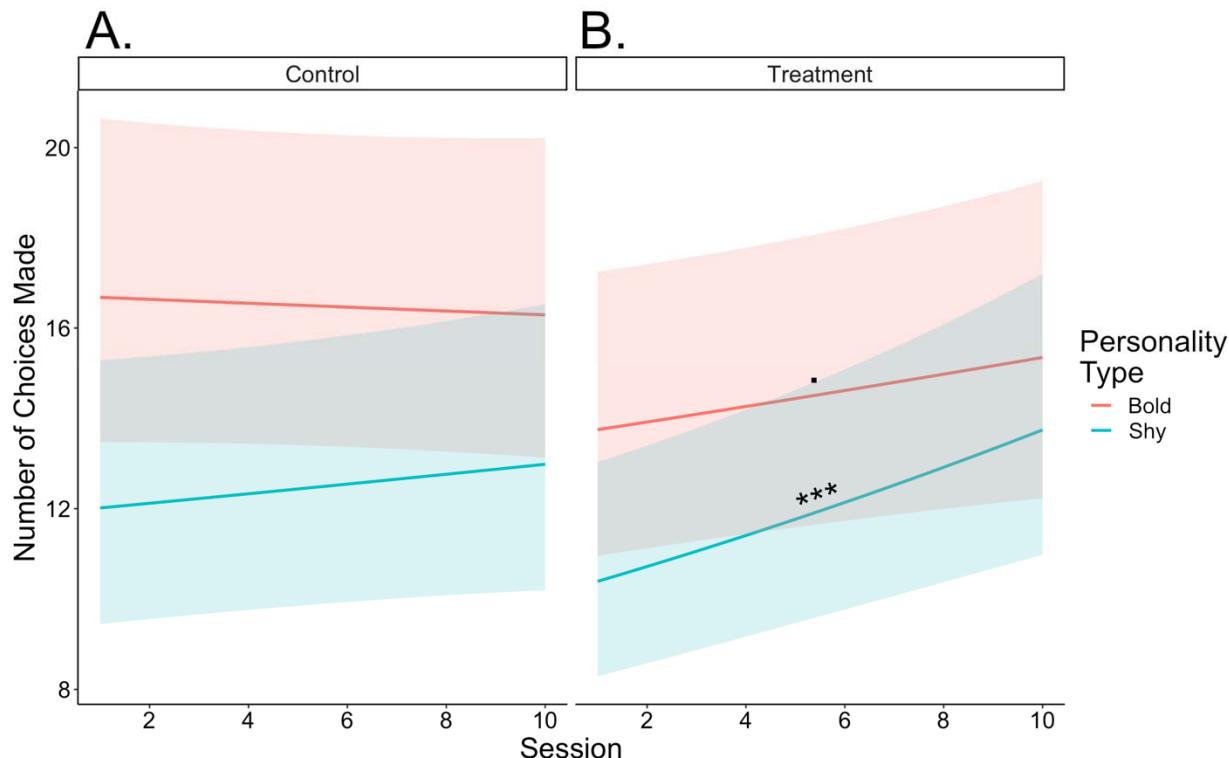
247 In the 2 choice discrimination task there was no significant difference in number of  
248 correct choices between control and treatment fish (Table S3). There was only a significant main  
249 effect of personality type such that bold fish made more correct choice compared to shy fish ( $b =$   
250  $-.49$ ,  $t = -2.84$ ,  $p = .01$ ) and a significant interaction between personality type and session ( $b =$   
251  $0.03$ ,  $t = 2.601$ ,  $p = .01$ ). Testing for the simple slopes (Table S4, Figure S2), both shy control ( $m$   
252  $= 0.03$ ,  $t = 3.15$ ,  $p = 2.2*10^{-5}$ ) and shy treatment ( $m = 0.04$ ,  $t = 4.24$ ,  $p = 4.4*10^{-6}$ ) groups had a

253 significant positive slope while bold control ( $m = 0, t = -0.26, p = .79$ ) and bold treatment ( $m =$   
254  $0.01, t = 0.98, p = .33$ ) have no significant relationship.

255

256 *Difference across treatment and control only in total number of choices in 2 choice*  
257 *discrimination task*

258 For the total number of choices, there was a significant difference between control and  
259 treatment fish (Figure 4, Table S5). There was a main effect of personality type on total number  
260 of choices ( $b = -.34, t = -2.07, p = .04$ ) where bold fish had higher total number of choices than  
261 shy. The interaction between session and treatment is approaching significance ( $b = 0.15, t =$   
262  $1.69, p = .09$ ). Testing for the simple slopes, shy control ( $m = 0.01, t = 1.22, p = .22$ ), and bold  
263 control ( $m = 0, t = -0.44, p = .66$ ) did not have a significant relationship (Figure 4a). Only shy  
264 treatment ( $m = 0.03, t = 4.59, p = .4.4*10-6$ ) had a significant positive slope (Figure 4b, Table  
265 S6). In contrast, bold treatment had a slope approaching significance ( $m = 0.01, t = 1.90, p = .06$ )  
266 (Figure 4b).



267 **Figure 4.** Regression lines of the number of choices made by personality type and treatment. 4A. shows the  
268 regression lines for control fish and 4B. shows regression lines for treatment fish. The bold group is in red and the  
269 shy group is in blue. Shaded regions indicate a 95% confidence interval. The simple slopes significance is indicated.  
270  $p < .1$ ,  $*p < .05$ ,  $**p < .01$ ,  $***p < .001$ .

## 271 **Discussion**

272 Variation in learning performance can be due to complex interactions between intrinsic  
273 (e.g., personality type) and extrinsic (e.g. learning task) factors (Sih & Guidice, 2012). We  
274 investigated the effects of personality type and learning task by testing zebrafish of differing  
275 personalities across two different associative learning assays. Overall, we found that learning  
276 performance in one of the tasks was influenced by an animal's personality type.

277 Bold fish increased time spent in the conditioned stimulus earlier than shy fish in the  
278 conditioned place preference task, which suggests that bold fish learned faster in this task. The  
279 bold fish showed a significant increase in time spent in the conditioned stimulus after just 3

280 conditioning days whereas shy fish took an additional 8 days of conditioning to show a  
281 significant change from baseline (Figure 3). These results are consistent with other studies  
282 demonstrating that individuals with bold personality types learn faster than shy individuals  
283 (Mazza et al., 2018, Guenther et al., 2014, Dugatkin & Alfieri, 2002, DePasquale et al., 2014,  
284 Bensky et al., 2017, Daniel & Bhat, 2020, Kareklaas, Elwood & Holland, 2017). Differences in  
285 learning speed between personality types in this task may be due to differences in behavior such  
286 as stress reactivity, exploration, and neophobia (Sih & Guidice, 2012, Sommer-Trembo & Plath,  
287 2018). Our observed differences in learning speeds between personality types cannot be  
288 explained by differences in motivation for the food reward (Figure 2). Interestingly, there were  
289 no differences in the amount of time spent in the conditioned stimulus between the personality  
290 types after 11 days of conditioning, suggesting that individuals approach an asymptotic level of  
291 performance. This suggests that both personality types are capable of changing their behavior  
292 (e.g. learn) to similar extents and therefore differences in cognitive ability between personality  
293 types is an unlikely explanation for differences in learning speed.

294 When testing the same fish in the 2 choice discrimination assay, there was no significant  
295 difference in the number of correct choices between treatment and control groups, which  
296 suggests the fish did not learn the stimulus-reward association in this task (Figure 4). However,  
297 there were differences across personality types in which both shy treatment and control increased  
298 the number of correct choices while the bold groups did not. The positive slope for the shy  
299 groups is likely due to an overall increase in total choices with repeated exposure. When looking  
300 at the total number of choices made over sessions, the control groups did not change over time  
301 while the treatment groups increased the total number of choices made over sessions. This  
302 suggests that the treatment fish did not learn the color association but may instead have learned

303 to go into the wells. Animals can attend to several cues in discrimination learning and sometimes  
304 attend to unintentional or general cues (Mackintosh, N., J., 1965). We also cannot rule out that  
305 rewarding the fish in a different location than the stimuli may have decreased the strength of  
306 pairing between action and reward (Murphy & Miller, 1958). While in the 2 choice  
307 discrimination task fish did not learn the color association, the bold fish made more choices than  
308 shy fish in the first session. This is likely due to decreased neophobia and increased exploration  
309 in the bold fish as demonstrated in the open-field test (Sih et al., 2004, Wong et al., 2012).

310 Differences in neophobia (e.g. latency to approach novel objects) classically distinguish  
311 bold and shy personality types (Carter et al., 2012, Sih et al., 2004, Wilson et al., 1994). In the  
312 current study one potential explanation for bold fish learning quicker in the conditioned place  
313 preference and making more initial choices in the 2 choice discrimination task relative to shy fish  
314 are differences in neophobia between the personality types. The shy fish could have found the  
315 colored lights in the 2 choice discrimination task initially aversive and increased their choices as  
316 they habituated to the novel stimuli. Shy individuals tend to have increased neophobia and  
317 habituate slower, which would result in the shy fish taking longer to make active choices (Carter  
318 et al., 2012). The two days of habituation in the 2 choice discrimination task only allowed the  
319 fish to experience the tank and lighted wells but at start of conditioning they were naïve to the  
320 color of the lights and the changing stimuli. A similar effect was seen in *Gallus gallus* where  
321 individuals that were less exploratory (i.e., shy) habituated slower to a loud sound than those that  
322 were more exploratory (Dissegna et al., 2022). Neophobia may also explain shy fish learning  
323 slower in the CPP task, as shy fish could have experienced more stress than the bold fish at the  
324 start of the task even after habituation and so learned the positive association slower. Mollies  
325 (*Poecilia mexicana*) that were desensitized to the lights and sounds used in the task showed no

326 differences in learning related to personality type (Sommer-Trembo & Plath, 2018). Increasing  
327 familiarity with the task environment and stimuli could explain why shy fish were slower to  
328 increase their preference for the conditioned stimulus but ultimately reached a level of  
329 performance similar to bold fish after 11 days of conditioning. Bold individuals tend to make  
330 associations faster likely because they are less neophobic and in a simple conditioned place  
331 preference task, this leads to them learning faster but does not change the plateau of performance  
332 (Dugatkin & Alfieri, 2002, DePasquale et al., 2014, Daniel & Bhat, 2020).

333 The relationship between more rapid learning and bold personality type is not consistent  
334 across all studies (Ferron et al., 2015, Lermite, Peneaux & Griffin, 2016). Potential explanations  
335 are that the relationship between speed of learning and personality can depend on aspects of the  
336 task such as learning stimulus valence or task complexity. Shy zebrafish trained in a contextual  
337 fear learning paradigm showed faster learning than bold zebrafish (Baker et al., 2019). As shy  
338 zebrafish have a faster glucocorticoid response to a novelty stressor than bold fish, this may  
339 facilitate quicker learning of aversive stimuli (Wong et al., 2019, Rau et al., 2005, Riggenbach et  
340 al., 2019) but inhibit learning of appetitive stimuli seen in current study. For task complexity, a  
341 study looking at learning accuracy found that aggressive spiders (e.g. bold personality type) were  
342 more accurate in a simple task but not in a more complex task (Chang et al., 2018). Future work  
343 may consider testing whether the same trend holds in a more complex classical conditioning  
344 task. In a more complex task, bold fish may make incorrect associations and not learn as quickly  
345 as shy fish.

346 Overall, we found support for differences between bold and shy individuals in how they  
347 interact with two different learning tasks. These differences in performance could be explained  
348 by varying neophobia between bold and shy individuals. In a 2 choice task requiring an active

349 behavioral response, we found differences in initial number of choices made between personality  
350 types, suggesting that the personality types naively interacted with the stimulus differently. In the  
351 conditioned place preference task, the bold fish learned faster than the shy fish. Differences in  
352 performance between bold and shy individuals in both tasks could be explained by variation in  
353 neophobia related to personality type. Additionally, the bold and shy fish reached a similar level  
354 of performance. We encourage future studies to test the performance of bold and shy individuals  
355 across different tasks to compare their behavior both within and across tasks. Future work should  
356 also consider explicitly measuring how individuals interact with the task environment, perhaps  
357 measuring neophobia and motivation for the task.

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