The role of the stress axis in mediating behavioural flexibility in a social cichlid, *Neolamprologus pulcher*

Stefan Fischer[1,2], Zala Ferlinc[2], Katharina Hirschenhauser[3], Barbara Taborsky[4], Leonida Fusani[1,2] & Sabine Tebbich[2]

[1]Konrad Lorenz Institute for Ethology, Department of Interdisciplinary Life Sciences, University of Veterinary Medicine Vienna, Savoyenstrasse 1, 1160 Vienna, Austria

[2]Department of Behavioral and Cognitive Biology, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria

[3]University College for Education of Upper Austria (PH OÖ), Kaplanhofstraße 40, 4020 Linz, Austria

[4]Division of Behavioural Ecology, University of Bern, Wohlenstrasse 50a, CH-3032 Hinterkappelen, Switzerland

Correspondence and requests for materials should be addressed to S.F. (email:Stefan.fischer@vetmeduni.ac.at)

ORCIDs:

0000-0001-8811-7518 (Stefan Fischer)

0000-0001-8710-9022 (Katharina Hirschenhauser)

0000-0003-1690-8155 (Barbara Taborsky)

0000-0001-8900-796X (Leonida Fusani)

0000-0003-3971-2139 (Sabine Tebbich)

Key words: cortisol; glucocorticoid receptor; mifepristone; RU 484; Detour task; predator; Lepidiolamprologus elongatus; learning;

Abstract

Behavioural flexibility plays a major role in the way animals cope with novel situations, and physiological stress responses are adaptive and highly efficient mechanisms to cope with unpredictable events. Previous studies investigating the role of stress responses in mediating behavioural flexibility were mostly done in laboratory rodents using stressors and cognitive challenges unrelated to the ecology of the species. To better understand how stress mediates behavioural flexibility in a natural context, direct manipulation of the stress response and cognitive tests in ecologically relevant contexts are needed. To this aim, we pharmacologically blocked glucocorticoid receptors (GR) in adult cichlid fish using a minimally invasive application of a GR antagonist. GRs blockade prevents the termination of the stress response, which we predicted to impair behavioural flexibility. After the application of the GR antagonist, we repeatedly exposed fish to a predator and tested their behavioural flexibility using a detour task, i.e. fish had to find a new, longer route to the shelter when the shortest route was blocked. While the latencies to find the shelter were not different between treatments, GR blocked fish showed more failed attempts during the detour tasks than control fish. Furthermore, reduced performance was accompanied by an increase of fear related behaviours. This suggests that blocking GRs plays an important role in determining perceived fear and resulted in an impaired behavioural flexibility. We confirm a causal role of terminating the stress response in mediating behavioural flexibility with potential consequences for an effective and adaptive coping with changing environments.

50 **Introduction**

51 Stress is a ubiquitous feature of life and all organisms have evolved internal mechanisms to cope
52 with external stressors (Taborsky et al., 2021). Stressors can be abiotic or biotic, ranging from
53 extreme temperatures to predator attacks or social stressors such as aggressive conflicts. An
54 adequate response to a stressor, the so-called stress response, has major fitness consequences
55 because it is of utmost importance for the survival of the individual (Lemonnier et al., 2022; McEwen
56 and Wingfield, 2003; Romero et al., 2009; Sopinka et al., 2015).

57 When a stressor is experienced the adaptive stress response in vertebrates is generally characterised
58 by a physiological increase of circulating glucocorticoids (GCs) (Sapolsky et al., 2000). Cortisol is the
59 major GC and a main actor of the stress response in fish and most mammals (Sopinka et al., 2015).
60 Cortisol can cross the blood-brain barrier where it binds to high affinity mineralcorticoid receptors
61 and lower affinity glucocorticoid receptors (GRs) that are expressed across the brain (Joels et al.,
62 2008; Roozendaal et al., 2009). Cortisol acts on GRs to induce a negative feedback that reduces the
63 further release of cortisol, which slowly returns to baseline levels (Joels et al., 2008). This swift
64 increase and slow decrease of cortisol is an adaptive stress response leading to beneficial changes in
65 physiology, behaviour and cognition to successfully cope with the stressor. On the contrary, if
66 cortisol concentrations do not return to baseline levels after the stressor ends, prolonged elevation
67 of cortisol has adverse effects on health and behaviour particularly on cognitive abilities (Sandi and
68 Pinelo-Nava, 2007; Schwabe et al., 2012).

69 Behavioural flexibility is an important mechanism to cope with environmental change and requires
70 different cognitive abilities including learning and the seeking of alternative solutions (Tebbich et al.,
71 2010). GR activity has been shown to play an important role in mediating cognition and particularly
72 in regulating learning performances (for reviews see: de Kloet et al., 2005; Roozendaal et al., 2009;
73 Sandi and Pinelo-Nava, 2007). For example, laboratory rats receiving an application of a specific GR
74 antagonist (mifepristone) performed worse in a spatial learning task than rats receiving a control

75 manipulation (Oitzl and Dekloet, 1992). Conversely, an early-life treatment of the same GR
76 antagonist in a social cichlid (*Neolamprologus pulcher*), marginally enhanced learning but had no
77 effects on reversal learning, a commonly used proxy for behavioural flexibility (Reyes-Contreras and
78 Taborsky, 2022). Thus, more research is needed to better understand the causal role of GRs in
79 mediating cognition and in particular behavioural flexibility. Furthermore, it is not known whether a
80 short-term application of a GR antagonist immediately before a cognitive challenge is sufficient to
81 induce changes in behavioural flexibility.

82 Previous studies that tried to link mediators of the stress axis to behavioural flexibility in adult
83 individuals manipulated mostly type or duration of stressor (e.g. Bryce and Howland, 2015; Butts et
84 al., 2013; Calandreau et al., 2011; George et al., 2015; Graybeal et al., 2014; Graybeal et al., 2011;
85 Thai et al., 2013). However, we do not know whether the responsiveness of the stress axis to the
86 same stressor impairs or facilitates behavioural flexibility. Furthermore, most studies have been
87 conducted on laboratory rodents using stressors and cognitive tasks with limited ecological
88 relevance. Such studies are limited in their ability to reveal how adaptive stress responses mediate
89 behavioural flexibility in a changing environment. A more appropriate approach to study whether
90 adaptive stress responses determine behavioural flexibility is to (i) pharmacologically block GR
91 activity to verify the causal role of the stress response in mediating behavioural flexibility and (ii) use
92 ecologically relevant stressors and cognitive challenges.

93 In this work, we administered, minimally invasive, a GR antagonist (mifepristone) to adult *N. pulcher*
94 and tested their behavioural flexibility in a spatial task after being repeatedly exposed to a predator.
95 In this spatial task, individuals had to navigate through a maze to hide in a shelter after encountering
96 a predator. After displaying flight responses using the shortest route to the shelter, we tested each
97 individual`s ability to find a new route to the shelter when the shortest route was blocked. Our study
98 species *N. pulcher* is a highly social cichlid and in nature individuals live in large groups consisting of
99 dominant and subordinate individuals that are structured in a size-based, linear hierarchy (Wong and

Balshine, 2011). Individuals that live in more complex social environments often have enhanced cognitive skills, including an enhanced behavioural flexibility (e.g. Amici et al., 2008; Ashton et al., 2018; Bond et al., 2007; Johnson-Ulrich and Holekamp, 2020).

Based on the role that GRs play in the negative feedback mechanism to terminate stress responses we formulated two predictions: (1) GR antagonist-treated fish show impaired behavioural flexibility, particularly after repeated exposures to a predator. (2) Effects that are causally mediated by GRs are specific to behavioural flexibility and the ability to cope with stress and do not affect other behaviours such as the motivation of the fish to solve the task.

Methods

Subjects and housing

The experiment was conducted at the Konrad Lorenz Institute of Ethology, University of Veterinary Medicine, Vienna, Austria, under the licence (ETK-101/06/2019). All experimental tanks (45L, 30 x 50 x 30 cm) were equipped with a 2 cm layer of sand, a biological filter, a heater and one-half clay flowerpot that served as a shelter. The water temperature was kept at 27 ± 1°C with a light-dark regime of 13:11 h and a dimmed-light phase of 10 min. During the experiment all fish were fed commercial flake food 6 days a week.

Our study species was the cooperatively breeding cichlid *Neolamprologus pulcher* because stress responses are well researched (Antunes et al., 2021a; Mileva et al., 2009) in this species, and it is a model to study the evolution of vertebrate sociality highlighting their social and non-social behavioural flexibility (Taborsky, 2016; Wong and Balshine, 2011). These fish are endemic to Lake Tanganyika and groups always consist of a dominant breeding pair and related or unrelated helpers (Balshine et al., 2001; Heg et al., 2005; Taborsky, 1984, 1985). Helpers assist the breeding pair in raising the offspring by cleaning and fanning the eggs, cleaning the breeding shelters, and defending

124 the common territory (Grantner and Taborsky, 1998; Taborsky, 1984, 1985). Larger territories are
125 divided into sub-territories where each group member occupies their own shelter. As an ecologically
126 relevant stress stimulus, we used the piscivorous cichlid *Lepidiolamprologus elongatus* which occurs
127 in sympatry with *N. pulcher* (Ochi and Yanagisawa, 1998). *L. elongatus* preys on all sizes of *N.*
128 *pulcher*, except eggs, and is one of its most important predators (Groenewoud et al., 2016). Previous
129 research indicates that *N. pulcher* can innately recognise this predator based on visual or olfactory
130 cues (Fischer et al., 2014; Zöttl et al., 2013). In nature, groups of *N. pulcher* face a high predation
131 pressure and individuals either attack or hide when predators intrude their territories, particularly
132 when faced with a large predator such as *L. elongatus*. Sexually mature *N. pulcher* feed on
133 zooplankton in the water column that requires them to stay far away from their shelters (Taborsky,
134 2016). Thus, flexibility in the way how individuals return to their shelter in case of danger is of
135 utmost importance for their survival.

136 Our test fish were laboratory-reared first- and second-generation offspring of wild-caught fish. We
137 randomly selected 35 male and 35 female adult fish (N=70, mean standard length [SL; from the tip of
138 the snout to the end of the caudal peduncle]: 7.2 cm, [range: 5.6-8.2 cm]) from the laboratory stock,
139 where they had been kept before in tanks separated by sex. The experiment was conducted in seven
140 blocks over seven weeks with 10 test fish per block. Test fish were permanently marked using
141 subcutaneous injections of Visible Implant Elastomer tags (VIE; Northwest 191 Marine Technology),
142 at least three days before each block started, except for one block where test fish had been marked
143 one day before the experiment started. To avoid any confounding effects, we excluded all test fish
144 from this block from the behavioural analysis because some individuals had not fully recovered from
145 the marking procedures which reduced the final sample size to N=60.

146 We also randomly chose adult *L. elongatus* fish (N = 10, mean SL: 12.68 cm [range: 10.3-14.7 cm])
147 from the laboratory stock population, where they had been housed in pairs or small groups. To avoid
148 exposing predators to repeated stress from catching and transporting them to the presentation

tanks (see below), which could influence their behaviours, we used five individuals for the first three blocks and another five individuals for the last four blocks. During the experiment, each predator was kept in a separate 45L tank. These tanks were set-up close to the test fish but with a visual and olfactory separation. Body sizes of predators were obtained after the last block of the experiment was completed. All fish were kept under the same housing conditions and food regimes before and during the experiment.

Experimental set-up

During the trials, we visually separated the experimental tanks using opaque dividers. The mazes used to carry out the detour trials remained in the experimental tanks throughout all the experimental phases (see below). The tanks were separated from the observer and the rest of the room using a dark curtain to minimize any disturbances from the surrounding area. All trials were recorded from above and from the front using two web cameras (Creative Live Cam Sync HD webcam) placed inside the curtain and connected to a computer outside of the curtain. Prior to each trial, a randomly chosen predator was transported to a small presentation tank (25 x 30 cm) filled with water, that was placed on a small movable rack. The front of the presentation tank – the part facing the experimental tanks – was covered with an opaque divider which could be moved with a string by the observer from outside the curtain. The divider was lifted before each trial for 60s to visually expose the test fish to the predator.

For all the trials, we used a transparent, U-shaped maze that prevented fish from using the maze as a shelter. The walls of the maze were covered with a mesh-like patterning that was fine enough to clearly see though. Halved clay flowerpots were used as shelters. The shelter could be reached using two routes: either via the direct route where fish only entered the arm of the maze that contains the shelter (‘shelter arm’, see Figure 1), or via a detour route where fish entered first the arm furthest away from the shelter (‘non-shelter arm’, see Figure 1)and had to cross to the other side in the back

of the maze to enter the shelter arm. The middle arm of the maze was a dead end (Figure 1). The area in front of the maze (30 x 10 cm) that faced towards the predator was used as a starting area that could be separated from the rest of the tank with a removable opaque divider. Again, using a string the observer could remotely move the divider with minimal disturbance to the fish from outside the curtain. Biological filters and heaters were removed during the trials to ensure fish could only seek shelter inside the flowerpot.

Experimental phases

Each block consisted of five distinctive experimental phases: a habituation phase (day 1, day 2), a pre-treatment direct phase (day 3), a treatment immersion phase (day 4, day 5), a post-treatment direct phase (day 6), and a detour phase (day 6).

Habituation. After fish were measured (SL) to the closest mm, each fish was individually transported into one of the experimental tanks. Each tank included a maze, and a shelter was placed in the shelter arm. Fish were allowed to habituate to the new environment for 48h. During the habituation phase we removed the opaque dividers between the experimental tanks to allow fish to visually interact with their neighbours.

Pre-treatment direct. After the habituation phase (day 3, 10a.m.), we tested whether the fish used the shortest route to the shelter after being exposed to a brief predator presentation. First, the observer gently guided the fish to the starting area using a small handheld net. Second, the observer lowered the divider to separate the start area from the rest of the tank. Third, the observer added an olfactory predator cue (15 ml of water from a predator tank) to the starting area and lifted the divider inside the predator tank to visually expose the fish to the predator for 60s. Fourth, directly afterwards, the observer lifted the divider separating the start area from the rest of the tank, and the fish could enter the maze and seek shelter. In this phase, the entrance of the shelter faced towards the starting area. Fish successfully completed this phase if they used the direct route to

199 reach the shelter within 60s without any detours. Fish that did not use the direct route (N=8) were
200 excluded from the experiment.

201 Immersion treatment. The subjects that successfully completed the previous phase (N=52),
202 underwent a 48h period of either a mifepristone (N=26, females=14) or a control immersion
203 treatment (N=26, females=13) starting on the next day (day 4, 8a.m.). Half of the females and half of
204 the males were randomly assigned to one of the two treatments, in a way that ensured that the
205 observer was blind to the treatments of the fish within each block. For this a second person (SF)
206 applied the treatments to the experimental tanks without the observer (ZF) present. The treatments
207 were applied in the form of water baths following Nyman et al. (2018). In short, we dissolved
208 mifepristone (RU 484) in dimethylsulfoxide at 50 mg ml-1, then serially diluted it in 0.1 M acetic acid
209 (1:10), phosphate-buffered saline (1:100), and finally, diluted it in distilled water for an immersion
210 concentration of 400 ng l^{-1}. Controls were prepared with diluents without mifepristone. Both
211 mifepristone and control solutions were prepared and stored in a -20C freezer one week prior to the
212 experiment. The fish were immersed in 2L of water inside 3L glass containers that were place inside
213 the experimental tank.

214 Post-treatment direct. After the immersion treatments (day 6, 9a.m.) we repeated the previous
215 direct phase to test whether (i) the fish memorised the shortest route to the shelter, and (ii) whether
216 the immersion treatments influenced the motivation or memory of fish. Seven out of 52 fish did not
217 take the direct route in this phase. The immersion treatments did not influence whether fish took
218 the short or the longer route (control: 4/26; mifepristone: 3/26). This time all fish proceeded to the
219 detour phase because they already used the direct route in the pre-treatment direct phase. This
220 ensured that (i) all fish that proceeded to the detour phase had used at least once the direct route
221 and (ii) the immersion treatments had no effect on the motivation of the fish to seek shelter.

222 Detour. For the detour trials (day 6, 10:30a.m.) we blocked the direct route to the shelter arm (Fig.
223 1) with a rectangular barrier (5 x 25 cm), made from the same material as the maze. The shelter

entrance was turned to face away from the starting area. This ensured that fish approached the shelter from the same side irrespective of whether they entered the shelter arm via the direct or via the longer route (Figure 1). We used the same procedures as described for the pre-treatment direct phase. Each fish experienced 4 detour trials with a 2h recovery period between each trial.

Video analysis

Video recordings were analysed using BORIS (Friard and Gamba, 2016). One trial (block 3, detour 1) was excluded from the analysis due to problems with the video quality. For each trial the following information was recorded: (i) the type of the test (post-treatment direct/detour 1/detour 2/detour 3/detour 4), (ii) the latency to cross the shelter arm line, (iii) the latency to enter the shelter, (iv) whether the fish entered the shelter or not and (v) the number of failed attempts. In the post-treatment direct trials, we additionally recorded whether the fish used the shortest route to the shelter or not. A failed attempt in the post-treatment direct trials was defined as either crossing the middle arm line or the non-shelter arm line. A failed attempt in the detour trials was defined as crossing the middle arm line, attempting, and trying to cross the blocked shelter arm line, or crossing the non-shelter arm line but then returning back to the starting area without entering the shelter arm. A fish was classified as having crossed into a new arm when both pectoral fins crossed the virtual line separating the two arms. We started measuring the latencies immediately after the predator exposure – i.e. immediately after the divider in the presentation tank was closed and the divider in the experimental tank was lifted. If fish did not cross any arm line or entered the shelter before the end of the experiment, we assigned maximum latencies (600s) in the respective trial.

Statistical analysis

247 Data were analysed using R 4.1.1. (R Core Team, 2021) with the packages “lme4” (Bates et al., 2015)
248 and “afex” (Singmann et al., 2021). We analysed treatment effects using Linear Models (LMs),
249 Generalized Linear Models (GLMs), Linear Mixed Models (LMMs) and Generalized Linear Mixed
250 Models (GLMMs). Residuals and Q/Q-plots of all LMs and LMMs were visually inspected, and the
251 distributions of residuals were compared to a normal distribution using Kolmogorov-Smirnov and
252 Shapiro tests. If residuals were not normally distributed, a log transformation was applied, and
253 residuals again checked. All GLMs and GLMMs were checked for over-dispersion, but none required
254 a correction. To obtain p-values, we used either the mixed() function in the package ‘afex’ with a
255 Satterthwaite's approximation for degrees of freedom, or the drop1() function to perform either a
256 type II anova (for LM and LMM) or a χ^2-test (for GLM and GLMM). Interactions, and control variables
257 (sex and body size of test fish) were stepwise removed if non-significant (Engqvist, 2005) and
258 reported p-values refer to the final model without non-significant interactions, and control variables.
259 An overview of all full models can be found in Supplementary Table S1.

260 Except for the analyses of the probability to enter the shelter with increasing numbers of predator
261 exposures (see below) we always included ‘Treatment’ (GR antagonist or control treatment), the
262 body size and sex of test fish in the models. When analysing the detour trials, we additionally
263 included test fish identity as a random effect to control for multiple observations on the same
264 individual and the number of the detour trial as a fixed effect. If the interaction ‘Nr. of detour trial x
265 Treatment’ was significant we compared the marginal means of both treatments within each detour
266 trial separately (four comparisons) with a Tukey correction for multiple testing. To analyse the
267 probability to enter the shelter with increasing numbers of predator presentations we used all trials
268 that were done after the fish received their respective treatments (post-treatment direct, detour 1,
269 detour 2, detour 3, detour 4). Fish identity was included as a random effect and the number of daily
270 predator presentations as a continuous covariate. Initially we constructed two models, one that
271 included a linear interaction between the number of daily predator presentations and treatment and
272 one that included a non-linear, polynomial relationship with a degree of 2. We compared both

273 models using a χ^2- goodness of fit test. To foster comparability, we kept the structure of the models
274 similar and did not include the body size or sex of fish in this analysis. To further investigate the
275 significant non-linear interaction, we constructed two additional models separately for GR blocked
276 and control fish. Both models included the identity of the fish as a random factor and the number of
277 daily predator presentations as a linear predictor or as a non-linear polynomial predictor with a
278 degree of 2.

279

280 **Results**

281

282 *Does the application of a GR antagonist influence memory or motivation to seek shelter in the post-*
283 *treatment learning trials?*

284 As expected, we found no behavioural differences between the GR blocked and control fish in the
285 post-treatment direct trials. There were no significant differences between the two immersion
286 treatments in the latency to cross the shelter line ($F_{1,50}$=0.08, p=0.78; see factor: 'Treatment' in Table
287 1a), the latency to enter the shelter ($F_{1,50}$=0.1, p=0.77; see factor 'Treatment' in Table 1b) or the
288 probability to show a failed attempt (χ^2=0.12, p=0.73, N=52; see factor 'Treatment' in Table 2).

289

290 *Does the application of a GR antagonist influence behavioural flexibility in the detour trials during*
291 *repeated exposures to a stressor?*

292 GR blocked fish showed less behavioural flexibility during the detour trials than control fish. That is,
293 the application of a GR antagonist influenced the number of errors that fish made over time with
294 repeated exposures to predators (χ^2=23.96, p<0.01, N=52; see interaction 'Nr. of detour trial x
295 Treatment' in Table 3, Figure 2). Post-hoc analysis revealed that GR blocked fish made significantly
296 more errors in the third detour trial compared to control fish (see Table 4 for pairwise comparisons).

297 In contrast, GR blocked fish did not show a longer latency to enter the shelter arm ($F_{1,50.11}$=0.0,
298 p=0.97, see factor 'Treatment' in Table 5a) or a longer latency to enter the shelter ($F_{1,48.98}$=0.42,
299 p=0.52, see factor 'Treatment' in Table 5b) than the control fish. Overall, all fish reduced the latency
300 to enter the shelter arm with increasing predator exposures ($F_{1,152.25}$=3.78, p=0.01; see factor 'Nr. of
301 detour trial' in Table 5a), whereas this was not the case for the latency to enter the shelter
302 ($F_{1,152.18}$=0.6, p=0.61; see factor 'Nr. of detour trial' in Table 5b). Irrespective of the treatments, we
303 found a positive relationship between body size and the latency to enter the shelter ($F_{1,48.9}$=5.1,
304 p=0.03; see factor 'Body size' in Table 5b) indicating that larger fish took longer to enter the shelter.

305

306 *Does the application of a GR antagonist determine how fish behaviourally cope with repeated*
307 *exposures to a stressor?*

308 When analysing the probability of fish to enter the shelter with increasing numbers of daily predator
309 exposures, we found that a model containing a non-linear interaction between the number of daily
310 predator exposures and treatment explained the data significantly better than a linear interaction
311 term ($\chi2$=15.37, p<0.01). GR blocked fish showed a linear reduction in the probability to enter the
312 shelter with increasing numbers of daily predator exposures (z=-2.17, p=0.03, N=62; see factor 'Nr.
313 of predator exposure' in Table 6a). In contrast, control fish showed a non-linear, U-shaped
314 relationship between the probability to enter the shelter and the number of daily predator
315 exposures [z=3.26, p<0.01, N=26; see factor 'poly (Nr. of predator exposure, 2)' in Table 6b]. Here,
316 control fish entered the shelter more frequently during the initial and the last predator exposures
317 than during any of the intermediate predator exposures (Figure 3).

318

319 **Discussion**

320 In the present experiment we applied, minimally invasive, a GR antagonist or a control immersion to
321 groups of fish and subsequently tested their behavioural flexibility after repeated exposures to an
322 ecologically relevant stressor. Fish treated with a GR antagonist (1) made more errors and (2) had a
323 higher probability to enter the shelter during the intermediate compared to the initial and last
324 predator exposures. Furthermore, the application of a GR antagonist did not influence the individual
325 motivation to participate in the task or their spatial memory.

326 Our results show that GR play an important role for behavioural flexibility in a spatial task when
327 repeatedly exposed to a mild naturalistic stressor. Previous studies using laboratory rodents have
328 shown that GR play a causal role in determining memory formation (Oitzl and Dekloet, 1992;
329 Roozendaal and McGaugh, 1997), fear conditioning (Donley et al., 2005), and reversal learning
330 (Bryce and Howland, 2015). Taken together with the current study, these results indicate that GR
331 activity affects specific cognitive abilities across a diverse set of cognitive tasks. In contrast to
332 previous studies, we used an ecologically relevant task where fish had to find a new route to avoid a
333 predator. This revealed that the stress response and particularly the activity of GRs play an
334 important role in determining the way individuals cope with environmental change.

335 A slower retention of previously formed associations during an increasingly stressful situation might
336 be the underlying mechanism determining the observed behavioural differences between GR
337 blocked and control fish. Although GRs are distributed across the entire brain, GR activity in the
338 basolateral amygdala (BLA) and the ventral hippocampus (VH) have been specifically linked to the
339 retention of fear memory (Donley et al., 2005). Similarly, we found that GR blocked fish showed a
340 higher probability to enter the shelter during the intermediate predator presentations compared to
341 control fish. This might be an indicator of a higher perceived fear in GR blocked fish. We assume that
342 the higher number of fear related behaviours was associated with increased concentrations of
343 circulating GCs, in our case cortisol because previous studies showed that GCs are essential for fear
344 related behaviours (for review see Schulkin et al., 2005). Cortisol might have accumulated more in

345 GR blocked fish due to their reduced ability to terminate the stress response before encountering
346 the next stressful predator exposure. Interestingly, this increase in fear related behaviours and thus
347 cortisol was accompanied by a reduction in behavioural flexibility as shown by a higher number of
348 errors of GR blocked fish while searching for the new, longer route to the shelter.

349 Generally, the effects of GC concentrations on cognition are diverse and context dependent (for
350 reviews see de Kloet et al., 2005; Roozendaal et al., 2009; Sandi and Pinelo-Nava, 2007). In *N.*
351 *pulcher* experimentally elevating GCs during early life reduced social competence and behavioural
352 flexibility in a reversal learning task (Reyes-Contreras et al., 2019; Reyes-Contreras and Taborsky,
353 2022). In contrast, a study using the same GR antagonist in rainbow trout found that GR blocked fish
354 had significantly reduced GC concentrations after experiencing a single stressor compared to control
355 fish (Alderman et al., 2012). The current study employed adult fish and repeated predator exposures
356 and we cannot conclude whether GC concentrations have increased or decreased after repeated
357 exposures to a predator. Further investigations are needed to disentangle the complex interplay
358 between GCs, GRs and cognition particularly when repeatedly experiencing a stressor during
359 development or later in life.

360 Behavioural flexibility generally refers to adaptive changes in behaviour, in response to
361 environmental changes (Brown and Tait, 2014). The flexible adjustment of behaviour is thought to
362 involve several cognitively demanding components such as learning and memory (Tebbich et al.,
363 2010), and flexibility has also been used as evidence of complex cognition (Mikhalevich et al., 2017).
364 On an evolutionary perspective, complex cognition covaries with the increased demands of more
365 complex or unpredictable social ('social brain hypothesis' Shultz and Dunbar, 2007) or ecological
366 environments ('cognitive buffer hypothesis' Sol, 2009). For example, between species comparisons
367 of varying sociality, as well as within species comparisons of individuals that experience different
368 social environments showed that the demands of a more complex social life are important
369 evolutionary drivers of behavioural flexibility (Ashton et al., 2018; Bond et al., 2007). Similarly to the

370 social environment, a more complex ecological environment, for example exposure to predation
371 cues has been linked to a stronger learnt antipredator response and longer memory retention
372 (Ferrari, 2014; Ferrari et al., 2005; Mitchell et al., 2016).

373 In *N. pulcher*, social and ecological experiences during the first two month of life are of particular
374 relevance for the development of social and anti-predator behaviours, as well as for non-social
375 behavioural flexibility (Bannier et al., 2017; Fischer et al., 2017). These organisational effects are
376 most likely mediated by a life-long reprogramming of the physiological stress axis (Antunes et al.,
377 2021a; Antunes et al., 2021b; Nyman et al., 2018) although an early-life manipulation of the stress
378 axis using the same GR antagonist did not determine behavioural flexibility when adult individuals
379 were tested in a reversal learning paradigm (Reyes-Contreras and Taborsky, 2022). In contrast, our
380 study shows an activational effect on behavioural flexibility mediated by differences in the
381 immediate stress response to a predator. Future studies focusing on both the organizational and the
382 activational effects of the stress response in determining cognition and in particular behavioural
383 flexibility are needed to better understand the way how animals respond to naturalistic
384 environmental challenges.

385 In conclusion, our study provides novel insights into the way how animals adapt to environmental
386 change. In this study we used an ecologically relevant stressor and cognitive task to highlight the role
387 of GRs in mediating behavioural flexibility. The results of the current study may stimulate further
388 research to better understand how stress responses shape cognitive abilities and their associated
389 behaviours in a natural context.

390 **Data availability**

391 Data for this study are available at Figshare public repository:
392 https://doi.org/10.6084/m9.figshare.24083952

393

References

Alderman, S.L., McGuire, A., Bernier, N.J., Vijayan, M.M., 2012. Central and peripheral glucocorticoid receptors are involved cortisol response to an acute stressor in rainbow trout. Gen. Comp. Endocrinol. 176, 79-85. https://doi.org/10.1016/j.ygcen.2011.12.031

Amici, F., Aureli, F., Call, J., 2008. Fission-fusion dynamics, behavioral flexibility, and inhibitory control in primates. Curr. Biol. 18, 1415-1419. https://doi.org/10.1016/j.cub.2008.08.020

Antunes, D.F., Reyes-Contreras, M., Glauser, G., Taborsky, B., 2021a. Early social experience has life-long effects on baseline but not stress-induced cortisol levels in a cooperatively breeding fish. Horm. Behav. 128, 104910. https://doi.org/10.1016/j.yhbeh.2020.104910

Antunes, D.F., Teles, M.C., Zuelling, M., Friesen, C.N., Oliveira, R.F., Aubin-Horth, N., Taborsky, B., 2021b. Early social deprivation shapes neuronal programming of the social decision-making network in a cooperatively breeding fish. Mol. Ecol. 30, 4132. https://doi.org/10.1111/mec.16019

Ashton, B.J., Ridley, A.R., Edwards, E.K., Thornton, A., 2018. Cognitive performance is linked to group size and affects fitness in Australian magpies. Nature 554, 364–367. https://doi.org/10.1038/nature25503

Balshine, S., Leach, B., Neat, F., Reid, H., Taborsky, M., Werner, N., 2001. Correlates of group size in a cooperatively breeding cichlid fish (*Neolamprologus pulcher*). Behav. Ecol. Sociobiol. 50, 134-140. https://doi.org/10.1007/s002650100343

Bannier, F., Tebbich, S., Taborsky, B., 2017. Early experience affects learning performance and neophobia in a cooperatively breeding cichlid. Ethology 123, 712-723. https://doi.org/https://doi.org/10.1111/eth.12646

Bates, D., Maechler, M., Bolker, B.M., Walker, S., 2015. Fitting linear mixed-effects models using {lme4}. J. Stat. Softw. 67, 1-48. https://doi.org/ https://doi.org/10.18637/jss.v067.i01

Bond, A.B., Kamil, A.C., Balda, R.P., 2007. Serial reversal learning and the evolution of behavioral flexibility in three species of North American corvids (Gymnorhinus cyanocephalus, Nucifraga columbiana, Aphelocoma californica). J. Comp. Psycho. 121, 372-379. https://doi.org/10.1037/0735-7036.121.4.372

Brown, V.J., Tait, D.S., 2014. Behavioral Flexibility: Attentional Shifting, Rule Switching, and Response Reversal, in: Stolerman, I., Price, L. (Eds.), Encyclopedia of Psychopharmacology. Springer, Berlin, Heidelberg.

Bryce, C.A., Howland, J.G., 2015. Stress facilitates late reversal learning using a touchscreen-based visual discrimination procedure in male Long Evans rats. Behav. Brain Res. 278, 21-28. https://doi.org/10.1016/j.bbr.2014.09.027

Butts, K.A., Floresco, S.B., Phillips, A.G., 2013. Acute stress impairs set-shifting but not reversal learning. Behav. Brain Res. 252, 222-229. https://doi.org/10.1016/j.bbr.2013.06.007

Calandreau, L., Bertin, A., Boissy, A., Arnould, C., Constantin, P., Desmedt, A., Guemene, D., Nowak, R., Leterrier, C., 2011. Effect of one week of stress on emotional reactivity and learning and

memory performances in Japanese quail. Behav. Brain Res. 217, 104-110. https://doi.org/10.1016/j.bbr.2010.10.004

de Kloet, E.R., Joels, M., Holsboer, F., 2005. Stress and the brain: from adaptation to disease. Nat. Rev. Neurosci. 6, 463-475. https://doi.org/10.1038/nrn1683

Donley, M.P., Schulkin, J., Rosen, J.B., 2005. Glucocorticoid receptor antagonism in the basolateral amygdala and ventral hippocampus interferes with long-term memory of contextual fear. Behav. Brain Res. 164, 197-205. https://doi.org/10.1016/j.bbr.2005.06.020

Engqvist, L., 2005. The mistreatment of covariate interaction terms in linear model analyses of behavioural and evolutionary ecology studies. Anim. Behav. 70, 967-971. https://doi.org/10.1016/j.anbehav.2005.01.016

Ferrari, M.C.O., 2014. Short-term environmental variation in predation risk leads to differential performance in predation-related cognitive function. Anim. Behav. 95, 9-14. https://doi.org/10.1016/j.anbehav.2014.06.001

Ferrari, M.C.O., Trowell, J.J., Brown, G.E., Chivers, D.P., 2005. The role of learning in the development of threat-sensitive predator avoidance by fathead minnows. Anim. Behav. 70, 777-784. https://doi.org/10.1016/j.anbehav.2005.01.009

Fischer, S., Bohn, L., Oberhummer, E., Nyman, C., Taborsky, B., 2017. Divergence of developmental trajectories is triggered interactively by early social and ecological experience in a cooperative breeder. Proc. Natl. Acad. Sci. U.S.A. 114, E9300-E9307. https://doi.org/10.1073/pnas.1705934114

Fischer, S., Taborsky, B., Burlaud, R., Fernandez, A.A., Hess, S., Oberhummer, E., Frommen, J.G., 2014. Animated images as a tool to study visual communication: a case study in a cooperatively breeding cichlid. Behaviour 151, 1921-1942. https://doi.org/10.1163/1568539x-00003223

Friard, O., Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. Methods Ecol. Evol. 7, 1325-1330. https://doi.org/10.1111/2041-210x.12584

George, S.A., Rodriguez-Santiago, M., Riley, J., Abelson, J.L., Floresco, S.B., Liberzon, I., 2015. Alterations in cognitive flexibility in a rat model of post-traumatic stress disorder. Behav. Brain Res. 286, 256-264. https://doi.org/10.1016/j.bbr.2015.02.051

Grantner, A., Taborsky, M., 1998. The metabolic rates associated with resting, and with the performance of agonistic, submissive and digging behaviours in the cichlid fish *Neolamprologus pulcher* (Pisces: Cichlidae). J. Comp. Physiol. 168, 427-433. https://doi.org/10.1007/s003600050162

Graybeal, C., Bachu, M., Mozhui, K., Saksida, L.M., Bussey, T.J., Sagalyn, E., Williams, R.W., Holmes, A., 2014. Strains and stressors: an analysis of touchscreen learning in genetically diverse mouse strains. Plos ONE 9, e87745. https://doi.org/10.1371/journal.pone.0087745

Graybeal, C., Feyder, M., Schulman, E., Saksida, L.M., Bussey, T.J., Brigman, J.L., Holmes, A., 2011. Paradoxical reversal learning enhancement by stress or prefrontal cortical damage: rescue with BDNF. Nat. Neurosci. 14, 1507-1509. https://doi.org/10.1038/nn.2954

Groenewoud, F., Frommen, J.G., Josi, D., Tanaka, H., Jungwirth, A., Taborsky, M., 2016. Predation risk drives social complexity in cooperative breeders. Proc. Natl. Acad. Sci. U.S.A., 4104–4109. https://doi.org/10.1073/pnas.1524178113

Heg, D., Brouwer, L., Bachar, Z., Taborsky, M., 2005. Large group size yields group stability in the cooperatively breeding cichlid *Neolamprologus pulcher*. Behaviour 142, 1615-1641. https://doi.org/10.1163/156853905774831891

Joels, M., Karst, H., DeRijk, R., de Kloet, E.R., 2008. The coming out of the brain mineralocorticoid receptor. Trends Neurosci. 31, 1-7. https://doi.org/10.1016/j.tins.2007.10.005

Johnson-Ulrich, L., Holekamp, K.E., 2020. Group size and social rank predict inhibitory control in spotted hyaenas. Anim. Behav. 160, 157-168. https://doi.org/10.1016/j.anbehav.2019.11.020

Lemonnier, C., Bize, P., Boonstra, R., Dobson, F.S., Criscuolo, F., Viblanc, V.A., 2022. Effects of the social environment on vertebrate fitness and health in nature: Moving beyond the stress axis. Horm. Behav. 145, 105232. https://doi.org/10.1016/j.yhbeh.2022.105232

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Horm. Behav. 43, 2-15. https://doi.org/10.1016/s0018-506x(02)00024-7

Mikhalevich, I., Powell, R., Logan, C., 2017. Is behavioural flexibility evidence of cognitive complexity? How evolution can inform comparative cognition. Interface Focus 7, 20160121. https://doi.org/10.1098/rsfs.2016.0121

Mileva, V.R., Fitzpatrick, J.L., Marsh-Rollo, S., Gilmour, K.M., Wood, C.M., Balshine, S., 2009. The stress response of the highly social African cichlid *Neolamprologus pulcher*. Physiol. Biochem. Zool. 82, 720-729. https://doi.org/10.1086/605937

Mitchell, M.D., Chivers, D.P., Brown, G.E., Ferrari, M.C.O., 2016. Living on the edge: how does environmental risk affect the behavioural and cognitive ecology of prey? Anim. Behav. 115, 185-192. https://doi.org/10.1016/j.anbehav.2016.03.018

Nyman, C., Fischer, S., Aubin-Horth, N., Taborsky, B., 2018. Evolutionary conserved neural signature of early life stress affects animal social competence. Proc. R. Soc. B-Biol. Sci. 285, 9. https://doi.org/10.1098/rspb.2017.2344

Ochi, H., Yanagisawa, Y., 1998. Commensalism between cichlid fishes through differential tolerance of guarding parents toward intruders. J. Fish Biol. 52, 985-996. https://doi.org/10.1111/j.1095-8649.1998.tb00598.x

Oitzl, M.S., Dekloet, E.R., 1992. Selective Corticosteroid antagonists modulate specific aspects of spatial orientation learning. Behav. Neurosci. 106, 62-71. https://doi.org/10.1037/0735-7044.106.1.62

R Core Team, 2021. R: a language and environment for statistical computing.

Reyes-Contreras, M., Glauser, G., Rennison, D.J., Taborsky, B., 2019. Early-life manipulation of cortisol and its receptor alters stress axis programming and social competence. Philos. Trans. R. Soc. B-Biol. Sci. 374. https://doi.org/10.1098/rstb.2018.0119

Reyes-Contreras, M., Taborsky, B., 2022. Stress axis programming generates long-term effects on cognitive abilities in a cooperative breeder. Proc. R. Soc. B-Biol. Sci. 289, 20220117. https://doi.org/10.1098/rspb.2022.0117

Romero, L.M., Dickens, M.J., Cyr, N.E., 2009. The reactive scope model - a new model integrating homeostasis, allostasis, and stress. Horm. Behav. 55, 375-389. https://doi.org/10.1016/j.yhbeh.2008.12.009

Roozendaal, B., McEwen, B.S., Chattarji, S., 2009. Stress, memory and the amygdala. Nat. Rev. Neurosci. 10, 423-433. https://doi.org/10.1038/nrn2651

Roozendaal, B., McGaugh, J.L., 1997. Glucocorticoid receptor agonist and antagonist administration into the basolateral but not central amygdala modulates memory storage. Neurobiol. Learn. Mem. 67, 176-179. https://doi.org/10.1006/nlme.1996.3765

Sandi, C., Pinelo-Nava, M.T., 2007. Stress and memory: behavioral effects and neurobiological mechanisms. Neural Plasticity 2007, 2007: 78970. https://doi.org/10.1155/2007/78970

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55-89. https://doi.org/10.1210/er.21.1.55

Schulkin, J., Morgan, M.A., Rosen, J.B., 2005. A neuroendocrine mechanism for sustaining fear. Trends Neurosci. 28, 629-635. https://doi.org/10.1016/j.tins.2005.09.009

Schwabe, L., Joels, M., Roozendaal, B., Wolf, O.T., Oitzl, M.S., 2012. Stress effects on memory: an update and integration. Neurosci. Biobehav. Rev. 36, 1740-1749. https://doi.org/10.1016/j.neubiorev.2011.07.002

Shultz, S., Dunbar, R.I.M., 2007. The evolution of the social brain: anthropoid primates contrast with other vertebrates. Proc. R. Soc. B-Biol. Sci. 274, 2429-2436. https://doi.org/10.1098/rspb.2007.0693

Singmann, H., Bolker, B., Westfall, J., Aust, F., Ben-Shachar, M.S., 2021. afex: analysis of factorial experiments, R package version 1.0-1 ed.

Sol, D., 2009. Revisiting the cognitive buffer hypothesis for the evolution of large brains. Biol. Lett. 5, 130-133. https://doi.org/10.1098/rsbl.2008.0621

Sopinka, N.M., Patterson, L.D., Redfern, J.C., Pleizier, N.K., Belanger, C.B., Midwood, J.D., Crossin, G.T., Cooke, S.J., 2015. Manipulating glucocorticoids in wild animals: basic and applied perspectives. Conserv. Physiol. 3, cov031. https://doi.org/10.1093/conphys/cov031

Taborsky, B., English, S., Fawcett, T.W., Kuijper, B., Leimar, O., McNamara, J.M., Ruuskanen, S., Sandi, C., 2021. Towards an Evolutionary Theory of Stress Responses. Trends Ecol. Evol. 36, 39-48. https://doi.org/10.1016/j.tree.2020.09.003

Taborsky, M., 1984. Broodcare helpers in the cichlid fish *Lamprologus brichardi*: their costs and benefits. Anim. Behav. 32, 1236-1252. https://doi.org/10.1016/s0003-3472(84)80241-9

Taborsky, M., 1985. Breeder-helper conflict in a cichlid fish with broodcare helpers: an experimental analysis. Behaviour 95, 45-75. https://doi.org/10.1163/156853985x00046

Taborsky, M., 2016. Cichlid fishes: A model for the integrative study of social behavior, in: Koenig, W., Dickinson, J. (Eds.), Cooperative Breeding in Vertebrates: Studies of Ecology, Evolution, and Behavior. Cambridge University Press, Cambridge.

Tebbich, S., Sterelny, K., Teschke, I., 2010. The tale of the finch: adaptive radiation and behavioural flexibility. Philos. Trans. R. Soc. B-Biol. Sci. 365, 1099-1109. https://doi.org/10.1098/rstb.2009.0291

Thai, C.A., Zhang, Y., Howland, J.G., 2013. Effects of acute restraint stress on set-shifting and reversal learning in male rats. Cogn. Affect. Behav. Neurosci. 13, 164-173. https://doi.org/10.3758/s13415-012-0124-8

Wong, M., Balshine, S., 2011. The evolution of cooperative breeding in the African cichlid fish, *Neolamprologus pulcher*. Biol. Rev. 86, 511-530. https://doi.org/https://doi.org/10.1111/j.1469-185X.2010.00158.x

Zöttl, M., Frommen, J.G., Taborsky, M., 2013. Group size adjustment to ecological demand in a cooperative breeder. Proc. R. Soc. B-Biol. Sci. 280, 20122772. https://doi.org/10.1098/rspb.2012.2772

Factors	Estimate ± SE	Num. D.F.	Den. D.F.	F-value	*P*-value
(a) Latency to enter the shelter arm					
Intercept	2.92 ± 0.31	-	-	-	-
Treatment	0.12 ± 0.44	1	50	0.08	0.78
(b) Latency to enter the shelter					
Intercept	3.29 ± 0.3	-	-	-	-
Treatment	0.12 ± 0.42	1	50	0.1	0.77

Table 1: Latencies recorded during the post-treatment direct trials. (a) The latency to enter the shelter arm, and (b) the latency to enter the shelter were not significantly different in glucocorticoid receptor antagonist treated or control treated fish. The dependent variables were log transformed to obtain normally distributed residuals. Estimates are shown on a log scale and as differences to the reference level 'control' for factor 'Treatment'. Body size and sex of test fish (N=52) did not predict any of the latencies and were subsequently removed from the models. F-values and p-values refer to comparisons of models with or without the factor of interest.

637

638

Factors	Estimate ± SE	X^2-value	*P*-value
Intercept	1.2 ± 0.47	-	-
Treatment	0.23 ± 0.68	0.12	0.73

639

640 **Table 2**: Probability of a failed attempt during the post-treatment direct trials. The probability of a
641 failed attempt was not significantly different in glucocorticoid receptor antagonist treated or control
642 treated fish. Estimates are shown on a logit scale and as differences to the reference level 'control'
643 for factor 'Treatment'. Body size and sex of test fish (N=52) did not predict the probability of a failed
644 attempt and were subsequently removed from the model. X^2 and p-values refer to comparisons of
645 models with or without the factor of interest.

646

647

648

Factors	Estimate ± SE	X^2-value	*P*-value
Intercept	1.44 ± 0.18	-	-
Treatment	-0.26 ± 0.26	0.65	0.42
Nr. of detour trial	-*	288.56	**<0.01**
Nr. of detour trial x Treatment	-*	23.96	**<0.01**

*Estimates are shown in Table 4

Table 3: Number of failed attempts during the four detour trials. Fish treated with a glucocorticoid receptor (GR) antagonist showed a significantly different learning curve during the detour trials compared to control treated fish. (see significant interaction 'Nr. of detour trials x Treatment'). Post-hoc tests revealed that GR blocked fish showed significantly more errors during detour trial 3 than control fish (see Table 4 for the pairwise comparisons). Estimates are shown on a log scale and as differences to the reference level 'control' for factor 'Treatment'. Estimates for the factor 'Nr. of detour trials' and for the interaction 'Nr. of detour trials x Treatment' are presented as post-hoc comparisons in Table 4. Body size and sex of test fish did not predict the number of a failed attempts and were subsequently removed from the model. X^2 and p-values refer to comparisons of models with or without the factor of interest. N=52 (207 observations in total). P-values < 0.05 are highlighted in bold.

667

668

Contrast	Estimate ± SE	z-value	*P*-value
Detour 1			
Control vs Mifepristone	0.26 ± 0.26	1	0.31
Detour 2			
Control vs. Mifepristone	-0.62 ± 0.37	-1.7	0.09
Detour 3			
Control vs. Mifepristone	-1.15 ± 0.42	-2.72	**0.01**
Detour 4			
Control vs. Mifepristone	0.64 ± 0.41	1.57	0.12

669

670 **Table 4:** Posthoc comparisons with a Tukey correction of the significant interaction term in Table 3.
671 Fish treated with a glucocorticoid receptor antagonist made significantly more errors during detour
672 trail 3 leading to a significantly different error pattern during the detour trials (see Figure 2).
673 Estimates are shown on a log scale, the direction of comparison within a contrast is left to right and
674 the estimate value refers to the treatment at the right. N=52 (207 observations in total). P-values <
675 0.05 are highlighted in bold.

676

677

678

679

Factors	Estimate ± SE	Num. D.F.	Den. D.F.	F-value	*P*-value
(a) Latency to cross the shelter arm					
Intercept	4.92 ± 0.19	-	-	-	-
Treatment	0.01 ± 0.23	1	50.11	0.0	0.97
Nr. of detour trial	-	3	152.25	3.78	**0.01**
Detour 2	-0.49 ± 0.16	-	-	-	-
Detour 3	-0.39 ± 0.16	-	-	-	-
Detour 4	-0.36 ± 0.16	-	-	-	-
(b) Latency to enter the shelter					
Intercept	1.71 ± 1.6	-	-	-	-
Treatment	-0.14 ± 0.22	1	48.98	0.42	0.52
Body size	0.5 ± 0.22	3	48.9	5.1	**0.03**
Nr. of detour trial	-		152.18	0.6	0.61
Detour 2	-0.2 ± 0.2	-	-	-	-
Detour 3	-0.19 ± 0.17	-	-	-	-
Detour 4	-0.18 ± 0.17	-	-	-	-

680

681 **Table 5:** Latencies recorded during the detour trials. (a) The latency to enter the shelter arm, and (b)
682 the latency to enter the shelter was not significantly different in glucocorticoid receptor antagonist
683 treated or control treated fish. The dependent variables were log transformed to obtain normally
684 distributed residuals. Estimates are shown on a log scale and as differences to the reference levels
685 'control' for factor 'Treatment' and 'Detour 1' for factor 'Nr. of detour trial'. Sex of test fish did not
686 predict the latencies and was removed from the models, whereas body size was only dropped in (a).
687 F-values and p-values refer to comparisons of models with or without the factor of interest. N=52
688 (207 observation in total). P-values < 0.05 are highlighted in bold

689

690

691

692

Factors	Estimate ± SE	z-value	*P*-value
(a) GR blocked fish			
Intercept	2.07 ± 0.47	-	-
Nr. of predator exposure	-7.69 ± 3.54	-2.17	**0.03**
poly(Nr. of predator exposure, 2)	3.42 ± 3.18	1.08	0.28
(b) Control fish			
Intercept	1.8 ± 0.51		
Nr. of predator exposure	-4.23 ± 3.5	-1.21	0.23
poly(Nr. of predator exposure, 2)	11.87 ± 3.64	3.26	**<0.01**

693

694 **Table 6:** The probability to enter the shelter with increasing numbers of daily predator
695 presentations. Fish treated with a glucocorticoid receptor (GR) antagonist showed a significantly
696 different pattern over the course of the experiment than control treated test fish. Separate models
697 were performed only after confirming that a non-linear interaction term between the number of
698 daily predator presentations and treatment explained the data better than a linear relationship (see
699 Results section). (a) GR blocked fish show a linear decrease in the probability to enter the shelter
700 with increasing daily predator presentations, whereas (b) control fish show a significant U-shape
701 relationship (see also Figure 3). Estimates are shown on a logit scale. The factor 'Nr. of predator
702 exposure' was included as a continues variable either as a linear predictor or as a polynomial
703 predictor (poly) of degree 2. N=26 (a: 129 observations; b: 130 observations in total).

704

705

706

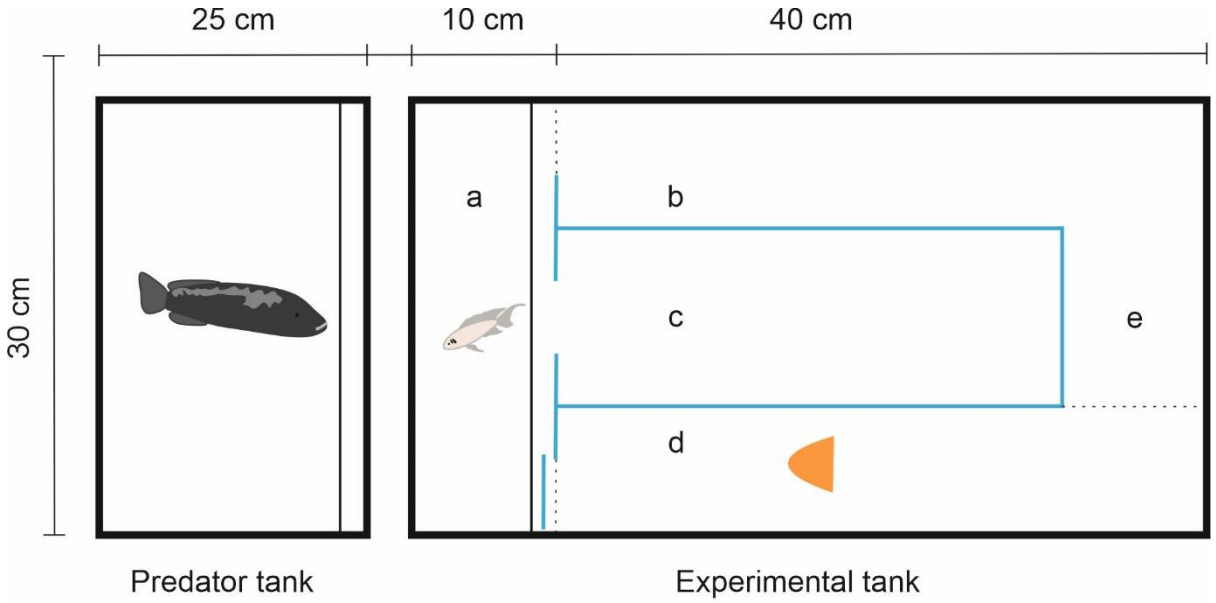
Figure legends

Figure 1: Experimental set-up consisting of one predator tank and a 45L experimental tank. The predator tank contained a removable opaque divider that could be remotely lifted during the predator presentations. The experimental tank was divided into five areas that are indicated with letters (a)-(e): (a) The start area which could be separated by a removable opaque divider from the rest of the experimental tank. (b) The non-shelter arm which served as the correct entrance during the detour trials. (c) The middle arm leading to a dead end. (d) The shelter arm that always contained a half clay flowerpot with the opening either facing towards the start area during the learning trials or with the opening facing away from the start area during the detour trials (as depicted here). (e) The back side arm which test fish had to use to enter the shelter arm in the detour trials. The U-shaped maze consisted of transparent Perspex material. A mesh was glued on top to make the maze clearly recognizable. Dashed lines indicate virtual lines that were used to record latencies and failed attempts from the video recordings (see Methods section for more details). The shortest route towards the shelter was either directly accessible (direct trials) or blocked (detour trials) with a barrier made from the same material as the maze.

Figure 2: The number of failed attempts in the detour trials. Glucocorticoid receptor (GR) antagonist treated fish showed a different pattern of failed attempts during the detour trials than control treated fish. GR blocked fish made more errors particularly during detour trial 3. Individual datapoints (smaller dots in the background) and predicted values (larger dots in the front) are shown with 95% confidence intervals for control (red dots) and GR blocked (blue dots) fish.

Figure 3: Probability to enter the shelter with increasing numbers of daily predator presentations for glucocorticoid receptor (GR) antagonist treated (red dots and line) and control treated (blue dots and line) fish. GR blocked fish showed a constant decrease in the probability to enter the shelter with successive predator presentations whereas control fish showed a nonlinear U-shape relationship. Dots represent the raw data and lines the model prediction with standard errors depicted as shaded areas.

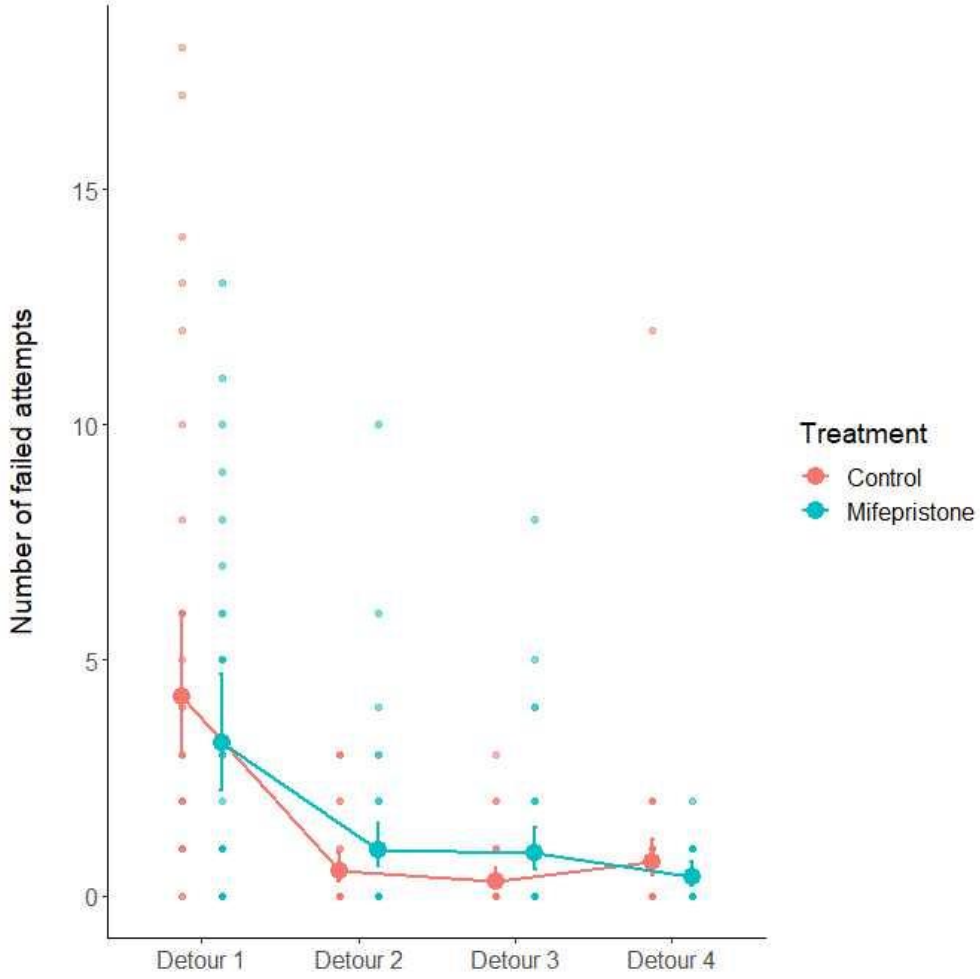
Figure 1



743

744

745 Figure 2



746

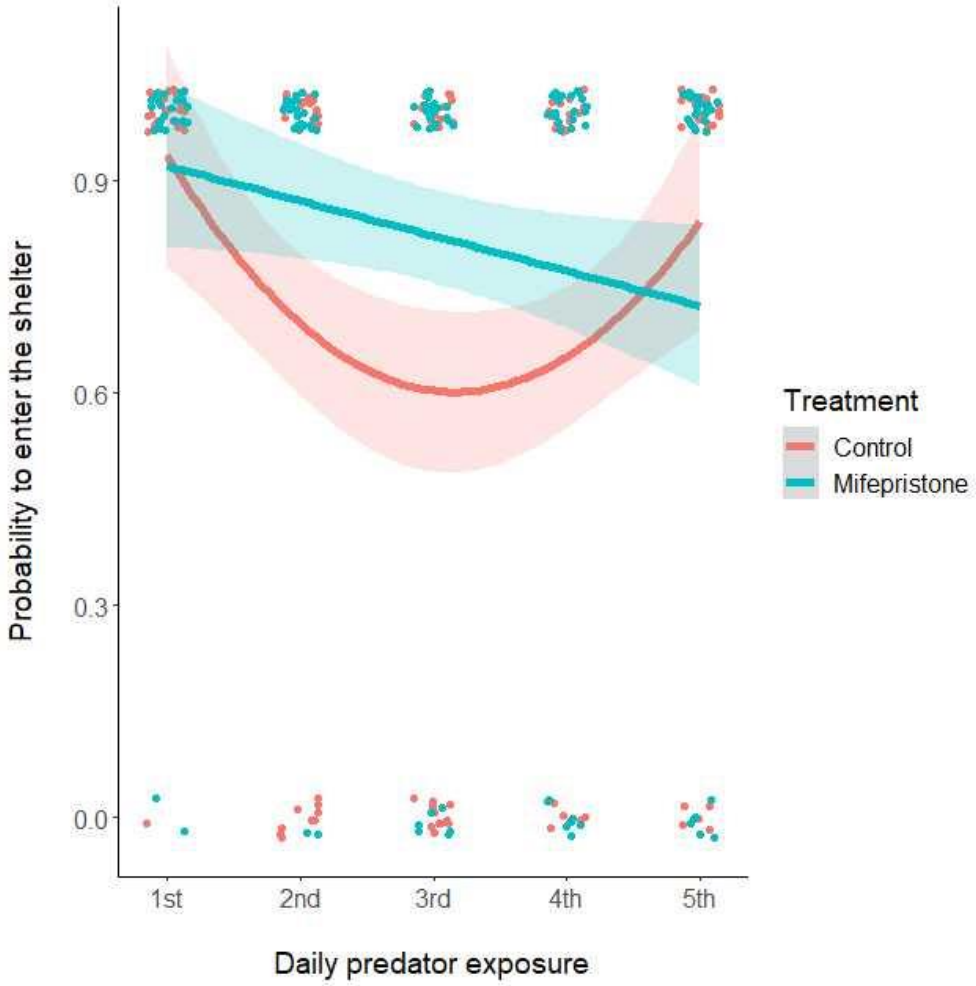
747

748

749

750

751 Figure 3



752

753

754

755

756

757