Divergent effect of fluoxetine on the response to physical or chemical stressors in zebrafish

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Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that increases serotonin concentration in the central nervous system and modulates various systems, including the control of sympathetic outflow and the hypothalamus–pituitary–adrenal. However, it is not yet established whether fluoxetine can modulate the responses to stressors stimulants (physical or chemical) that trigger cortisol response in zebrafish . We demonstrate that fluoxetine blunts the response to physical stress, but not to chemical stress.

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Abstract

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) that increases serotonin concentration in the central nervous system and modulates various systems, including the control of sympathetic outflow and the hypothalamus–pituitary–adrenal. However, it is not yet established whether fluoxetine can modulate the responses to stressors stimulants (physical or chemical) that trigger cortisol response in zebrafish. We demonstrate that fluoxetine blunts the response to physical stress, but not to chemical stress.

1. Introduction

Fluoxetine (FLU), a selective serotonin reuptake inhibitor (SSRI), increases serotonin concentration in the central nervous system (Wong et al., 1995). Serotonin is one of the major neurotransmitters in the central nervous system and modulates various systems, including the control of sympathetic outflow and the hypothalamus–pituitary–adrenal axis (HPA), via serotonergic fibers that innervate structures such as the hippocampus, prefrontal cortex, amygdala and hypothalamus (Lowry, 2002). SSRIs and cognitive-behavioral therapy are both effective treatments for generalized anxiety disorder, and are know to reduce the peak of cortisol in older adults (Rosnick et al., 2016). Fluoxetine has been shown to blunt the cortisol response (Abreu et al., 2014) and, as a consequence, prevent stress-related osmoregulation changes in zebrafish (Abreu et al., 2015). In addition, fluoxetine reverses the anxiogenic effects of acute (Giacomini et al., 2016) and chronic (Marcon et al., 2016) stress in this species.

Stress depends on a stressor stimulus to occur, and in mammals it triggers a stimulatory process in the hippocampus and amygdala (LeDoux, 2000; 2007). In the hypothalamus, stress stimulates the release of corticotropin-releasing factor (CRF), which is the key neurotransmitter regulating the release of adrenocorticotropic hormone (ACTH) from the pituitary, which in turn induces the release of glucocorticoids (cortisol) from the adrenal. In teleost fish like in zebrafish, the hypothalamic–pituitary–interrenal axis (HPI axis) is the HPA axis homolog (Wendelaar Bonga, 1997).

Stress stimuli can be varied (e.g., social, physical, chemical), such as exposures to neighborhood-level violence, which can influence physiological and cellular markers of stress, even in children (Theall et al., 2017). In addition, physical stimuli elicit robust stress responses in fish (Perry et al., 1996). Physical stressors such as chasing have been used as standardized

64 stressors (Abreu et al., 2014; Giacomini et al., 2015, 2016), and spatial restriction is used as a
65 stress model for behavioral assessment in zebrafish (Piato et al., 2011; Ghisleni et al., 2012).
66 Stressor stimulus can also be chemical, such as alarm substances, originally described in the
67 minnow (*Phoxinus phoxinus*) (Frisch, K 1941), which are produced and stored in epidermal
68 'club' cells (Barbosa Jr et al., 2012) and are released into the water after skin injuries as those
69 provoked by predator attack (Chivers & Smith, 1998; Korpi & Wisenden, 2001). Alarm
70 substance is known to induce fear responses in a range of fish species (Pfeiffer W, 1977).
71 Moreover, blood (Barreto et al., 2013) and diamines (putrescine and cadaverine) (Hussain et al.,
72 2013) have also been documented as potential chemical stressors. However, it is not yet
73 established whether FLU can modulate the responses to different modalities of stressors
74 stimulants (physical or chemical) that trigger cortisol response in zebrafish.
75

76 **2. Materials and Methods**

77 **2.1. Experimental animals**

78 A stock population of 200 mixed-sex (50/50) 180-day-old wild-type zebrafish (*D. rerio*),
79 weighing 0.45 ± 0.05 g, short-fin (SF) strain, was maintained in two tanks equipped with
80 biological filters, under constant aeration, and with a natural photoperiod (approximately 14 h
81 light: 10 h dark). Water temperature was maintained at 26 ± 1 °C; pH at 7.0 ± 0.2; dissolved
82 oxygen at 6.1 ± 0.2 mg/L; total ammonia at < 0.01 mg/L; total hardness at 6 mg/L; and alkalinity
83 at 22 mg/L $CaCO_3$. This study was approved by the Ethics Commission for Animal Use (CEUA)
84 of Universidade de Passo Fundo, UPF, Passo Fundo, RS, Brazil (Protocol #29/2014-CEUA) and
85 met the guidelines of Conselho Nacional de Controle de Experimentação Animal (CONCEA).
86

87 **2.2. Experimental protocol**

88 Our aim was to verify whether FLU modulates cortisol changes induced by physical and
89 chemical stressors in zebrafish. After a 15-day period for acclimation to laboratory conditions,
90 fish were randomly distributed into two groups, i.e., untreated fish (control group) and fish
91 exposed to FLU. The latter group was exposed to FLU (Daforin®, EMS, Brazil) at a
92 concentration of 50 μg/L for 15 min. before the stressor stimuli (Figure 1); this concentration and
93 duration of exposure were previously shown to elicit behavioral responses (Giacomini et al.,
94 2016) and decrease cortisol response in acute chasing stress (Abreu et al., 2014).

95

96 **2.2.1 Physical stimuli on stress response**

97 To evaluate the physical stress response, we then subdivided control and treated fish into
98 groups of 10 animals (duplicate) that were submitted or not to the following types of physical
99 stress: chasing with a net (duration 2 min., and waiting to complete 15 min to sampling); spatial
100 restriction in a microtube (duration 15 min) (Fig. 1A). After the 15 min of exposure to each
101 stressor, fish were captured, euthanized by medulla sectioning and immediately frozen in liquid
102 nitrogen for storage at −80 °C until cortisol extraction (Figure 1). This time interval was based
103 on previous studies showing that cortisol levels peak 15 min following presentation of a stressor
104 stimulus (Abreu et al., 2014; Idalencio et al., 2014; Ramsay et al., 2009).

105

106 **2.2.2. Chemical stimuli on stress response**

107 To evaluate the chemical stress response, we then subdivided control and treated fish into
108 groups of 10 animals (duplicate) that were submitted or not to the following types of chemical
109 stress: exposure to conspecific blood (duration 15 min); and exposure to alarm substance of

conspecifics (duration 15 min). Exposure to blood (5 mL, extracted from zebrafish and jundia (*Rhamdia quelen*) – the use of jundia blood was due to the low yield of zebrafish blood extraction) was in a 10-L aquarium (Barreto et al., 2013); and exposure to alarm substance of conspecifics (Speedie & Gerlai, 2008) (1 mL, zebrafish) was in a 10-L aquarium (Barreto et al., 2010). After 15 min of exposure to each stressor, fish were captured, euthanized and stored as described above (Fig. 1B). For collection of fish blood (zebrafish and jundia), fish were anesthetized by eugenol (400 mg/L), then the caudal peduncle was sectioned for the collection of blood. For extraction of alarm substance, fish were quickly killed by medulla sectioning, then shallow cuts were made on each side of fish and the cuts were washed with distilled water; at the end of the process a total of 100 mL of alarm substance in solution were collected (Speedie & Gerlai, 2008).

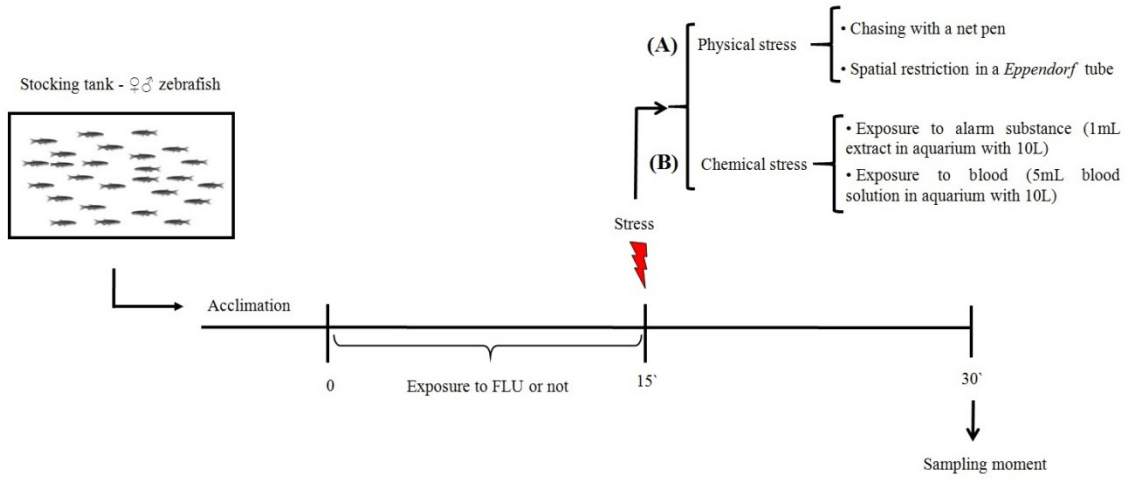


Figure 1. Schematic representation of the experimental design.

2.3. Cortisol analysis

Whole-body cortisol levels were determined using the method described by Sink et al., 2007. Fish were weighed, minced and homogenized with phosphate buffered saline (PBS, pH 7.3). Samples were transferred into tubes with ether, vortexed, centrifuged, and then immediately frozen in liquid nitrogen (three times this last process). The unfrozen portion (ethyl ether containing cortisol) was decanted and transferred to a new tube and completely evaporated, yielding a lipid extract containing the cortisol. The samples were then placed on the plate of enzyme-linked immunosorbent assay kit. The accuracy was tested by calculating the recoveries from samples spiked with known amounts of cortisol (50, 25 and 12.5 ng/mL), the mean detection of spiked samples was 94.3%. All cortisol values were adjusted for recovery with the following equation: cortisol value = measured value × 1.0604. Whole-body cortisol levels were measured in duplicate for each extraction using the commercially available enzyme-linked immunosorbent assay kit (EIAgen CORTISOL test, BioChem Immunosystems). Reading was carried out in microplate reader equipment (ASYS UVM 340, ASYS, England).

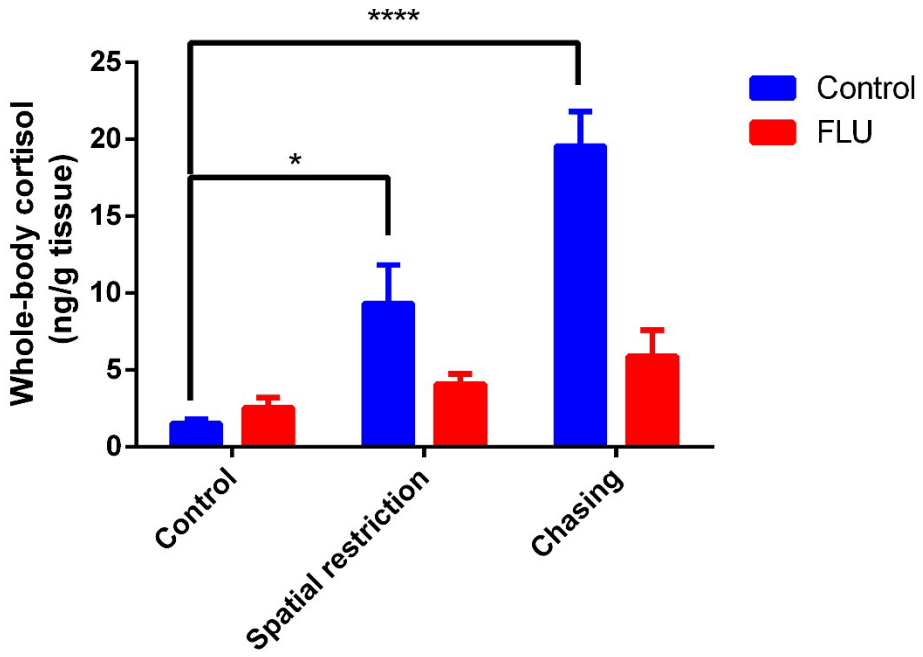
2.4. Statistical analysis

After testing the homogeneity of variance and normality of data (Hartley and Kolmogorov–Smirnov tests, respectively), we compared the whole-body cortisol levels using two-way analysis of variance (ANOVA) followed by Dunnett´s post hoc test. Differences were considered statistically significant at $p<0.05$. The data are expressed as mean + standard error of mean (SEM).

3. Results

3.1. Physical stimuli on stress response

149 Fish exposed to physical stressors (spatial restriction or chasing) displayed an increase in
150 cortisol levels, and FLU blunted the increase in cortisol levels in fish subjected to physical
151 stressors (Fig. 2). Two-way ANOVA revealed significant interaction between the factors ($F_{2, 45}$ =
152 6.080, p = 0.0046), main effects of drug ($F_{1, 45}$ = 13.89, p = 0.0005) and stress ($F_{2, 45}$ = 12.93, p <
153 0.0001).



154

155 Figure 2. Effects of physical acute stressors (spatial restriction or chasing) on cortisol levels in
156 whole-body zebrafish. Data were expressed as mean + SEM. Two-way ANOVA followed by
157 Dunnett´s post hoc test. FLU (fluoxetine). * p < 0.05 and **** p < 0.0001.
158

159

160 **3.2. Chemical stimuli on stress response**

161 Fish exposed to chemical stressors (alarm substance or blood) displayed an increase in
162 cortisol levels, but FLU did not blunt the increase in cortisol levels in fish subjected to chemical

163 stressors (Figure 3). Two-way ANOVA revealed a significant main effect of stress ($F_{2, 48}$ =
164 5.623, p = 0.0064), but not interaction effect between the factors ($F_{2, 48}$ = 0.7045, p = 0.4994) or a
165 main effect of drug ($F_{1, 48}$ = 0.01718, p = 0.8963).

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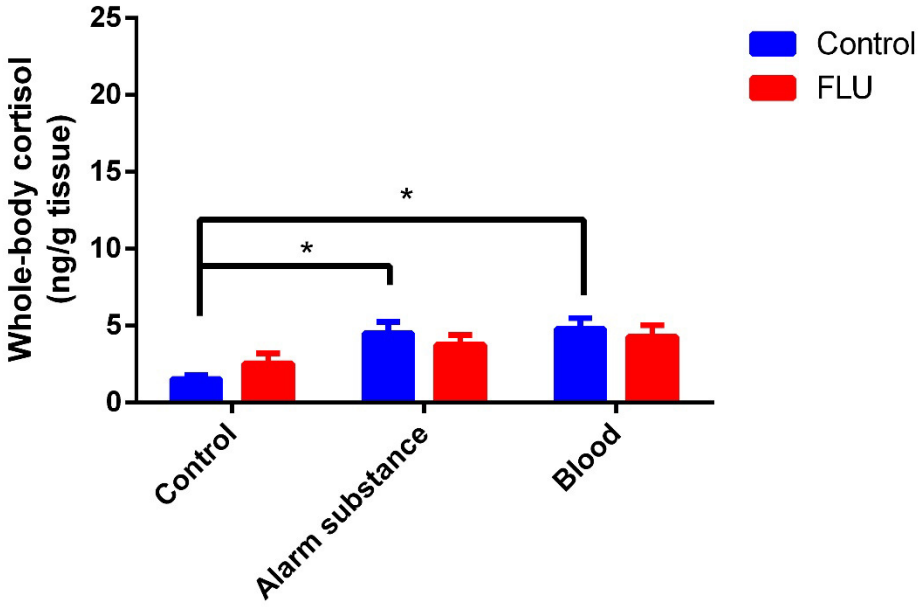

167

168 Figure 3. Effects of chemical acute stressors (alarm substance or blood) on cortisol levels in
169 whole-body zebrafish. Data were expressed as mean + SEM. Two-way ANOVA followed by
170 Dunnett´s post hoc test. FLU (fluoxetine). * $p < 0.05$.

171

172

173 **4. Discussion**

174 Here we show that fluoxetine blunts the response to physical, but not chemical, stress.
175 Even if physical (Ramsay et al., 2009) or chemical (Teles et al., 2017) stress increase cortisol
176 levels in zebrafish.

177 The greater magnitude of response to a physical stressor could be related to its high
178 impact can cause a clear aversive response in fish (Abreu et al., 2016). Besides, confinement

179 stress also resulted in elevated cortisol for being ´´high-impact stress`` (Silva et al., 2015),
180 perhaps physical stressors act in dorsolateral and dorsomedial regions of the pallium that have
181 been characterized as functional homologues to the mammalian amygdala and hippocampus
182 (Goodson & Kingsbury, 2013; O'Connell & Hofmann, 2011; Vargas et al., 2009), with
183 consequent action under the hypothalamus. On the other hand, chemical stress does not trigger a
184 response of such magnitude (Silva et al., 2015). Our hypothesis is that the chemical stressor
185 stimulus depends on more than one sensory pathway (e.g., smell, tactile) for the perception of the
186 stimulus, which would result in a suppression of the stimulation force of the hypothalamic
187 system, with consequent pituitary and later adrenergic stimulation.

188 We demonstrated that fluoxetine prevents the increase of cortisol in fish in response to
189 physical stressor stimulus. Previously, we showed that fluoxetine blocked cortisol response to
190 acute chasing stress in a dose-dependent manner (Abreu et al., 2014) as well as in fish subjected
191 to different forms of housing (Giacomini et al., 2016). Fluoxetine also blocked the stress
192 response following chronic exposure in zebrafish (Egan et al., 2009), besides stress increases
193 serotoninergic activity in the telencephalon in fish (e.g. Øverli et al., 2004; Winberg et al., 1992).
194 In fact, the levels of serotonin in the brain regions considered homologous to the mammalian
195 hippocampus and amygdala are altered in fish subjected to spatial restriction (Silva et al., 2015).
196 This effect reinforces the participation of these regions in response to physical stress, as well as
197 the involvement of serotonin in these pathways.

198 Still, we have shown that fluoxetine did not block the increase of cortisol in fish in
199 response to chemical stressor stimulus. Alarm substance induced stress responses in *Nile tilapia*
200 (*Oreochromis niloticus*), increasing ventilation rate and cortisol level (Sanches et al., 2015) as
201 well as increasing erratic movements in zebrafish (Speedie & Gerlai 2008). The exposure to

202 blood has also been shown to induce antipredator behavior in the fish species *Nile*
203 *tilapia* (Barreto et al., 2013). The exposure to alarm substance also increased anxiety-like
204 behavior in the light/dark test in zebrafish and decreased nocifensive behavior, however
205 pretreatment with fluoxetine blocked the anxiogenic effects of alarm substance on the light/dark
206 test and also increased extracellular brain 5-HT (Maximino et al., 2014), the same behavioral
207 relationship between alarm substance and serotoninergic system was not observed in the
208 relationship between neuroendocrine and serotoninergic system. Serotonin receptors (5-HT_{1A}
209 and 5-HT_4) expressed in steroidogenic cells in the interrenal glands mediate the effects of
210 serotonin on cortisol response, and this direct mechanism may underlie the effects of fluoxetine
211 observed in physical stress response, namely the inhibition of cortisol release.

212

213 **References**

214 Abreu MS, Giacomini AC, Koakoski, G, Oliveira TA, Gusso D, Baldisserotto B, Barcellos LJ.
215 2015. Effects of waterborne fluoxetine on stress response and osmoregulation in zebrafish.
216 *Environmental Toxicology and Pharmacology* **40**: 704-707. DOI 10.1016/j.etap.2015.09.001.

217 Abreu MS, Giacomini ACV, Gusso D, Koakoski G, Oliveira TA, Marqueze A, Barreto
218 RE, Barcellos LJ. 2016. Behavioral responses of zebrafish depend on the type of threatening
219 chemical cues. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **202**: 895-901. DOI
220 10.1007/s00359-016-1129-5

221 Abreu MS, Koakoski G, Ferreira D, Oliveira TA, Rosa JS, Gusso D, Giacomini AC, Piato
222 AL, Barcellos LJ. 2014. Diazepam and fluoxetine decrease the stress response in zebrafish. *PLoS*
223 *ONE* **9 (7)**: e103232. DOI 10.1371/journal.pone.0103232

224 Barbosa Jr A, Alves FL, Pereira AS, Ide LM, Hoffmann A. 2012. Behavioral characterization
225 of the alarm reaction and anxiolytic-like effect of acute treatment with fluoxetine in piauçu fish.
226 *Physiology & Behavior* **105**: 784–790. DOI 10.1016/j.physbeh.2011.10.007

227 Barreto RE, Barbosa A, Giassi ACC, Hoffmann A. 2010. The'club' cell and behavioural and
228 physiological responses to chemical alarm cues in the Nile tilapia. *Mar Fresh Behav Physiol* **43**:
229 75–81. DOI 10.1080/10236241003654139.

Barreto RE, Miyai CA, Sanches FHC, Giaquinto PC, Delicio HC, Volpato GL. 2013. Blood cues induce antipredator behavior in Nile tilapia conspecifics. *Plos One* **8**: 54642. DOI 10.1371/journal.pone.0054642.

Chivers DP, Smith JF. 1998. Chemical alarm signalling in aquatic predator– prey systems: a review and prospectus. *Ecoscience* **5**: 338–352.

Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF, Elkhayat SI, Bartels BK, Tien AK, Tien DH, Mohnot S, Beeson E, Glasgow E, Amri H, Zukowska Z, Kalueff AV. 2009. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behav Brain Res* **205**: 38–44. DOI 10.1016/j.bbr.2009.06.022.

Frisch K. 1941. Uber einen schreckstoff der fischhaut und seine biologische Bedeutung. *Z vergl Physiol* **29**: 46–145.

Ghisleni G, Capiotti KM, Da Silva RS, Oses JP, Piato AL, Soares V, Bogo MR, Bonan CD. 2012. The role of CRH in behavioral responses to acute restraint stress in zebrafish. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **36:** 176–182. DOI 10.1016/j.pnpbp.2011.08.016.

Giacomini AC, de Abreu MS, Koakoski G, Idalêncio R, Kalichak F, Oliveira TA, da Rosa JG, Gusso D, Piato AL, Barcellos LJ. 2015. My stress, our stress: Blunted cortisol response to stress in isolated housed zebrafish. *Physiology & Behavior* **139**: 182-187. DOI 1 0.1016/j.physbeh.2014.11.035.

Giacomini ACVV, Abreu MS, Giacomini LV, Siebel AM, Zimerman FF, Rambo CL, Mocelin R, Bonan CD, Piato AL, Barcellos LJ. 2016. Fluoxetine and diazepam acutely modulate stress induced-behavior. *Behavioural Brain Research* **296**: 301–310. DOI 10.1016/j.bbr.2015.09.027.

Goodson JL, Kingsbury MA. 2013. What's in a name? Considerations of homologies and nomenclature for vertebrate social behavior networks. *Horm Behav* **64**: 103-112. DOI : 10.1016/j.yhbeh.2013.05.006.

Herculano AM, Maximino C. 2014. Serotonergic modulation of zebrafish behavior: Towards a paradox. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **55**: 50–66. DOI 10.1016/j.pnpbp.2014.03.008.

Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, McLaren S, Sealy I, Caccamo M, Churcher C, Scott C, Barrett JC, Koch R, Rauch GJ, White S, Chow W, Kilian B, Quintais LT, Guerra-Assunção JA, Zhou Y, Gu Y, Yen J, Vogel JH, Eyre T, Redmond S, Banerjee R, Chi J, Fu B, Langley E, Maguire SF, Laird GK, Lloyd D, Kenyon E, Donaldson S, Sehra H, Almeida-King J, Loveland J, Trevanion S, Jones M, Quail M, Willey D, Hunt A, Burton J, Sims S, McLay K, Plumb B, Davis J, Clee C, Oliver K, Clark R, Riddle C, Elliot D, Threadgold G, Harden G, Ware

D, Begum S, Mortimore B, Kerry G, Heath P, Phillimore B, Tracey A, Corby N, Dunn M, Johnson C, Wood J, Clark S, Pelan S, Griffiths G, Smith M, Glithero R, Howden P, Barker N, Lloyd C, Stevens C, Harley J, Holt K, Panagiotidis G, Lovell J, Beasley H, Henderson C, Gordon D, Auger K, Wright D, Collins J, Raisen C, Dyer L, Leung K, Robertson L, Ambridge K, Leongamornlert D, McGuire S, Gilderthorp R, Griffiths C, Manthravadi D, Nichol S, Barker G, Whitehead S, Kay M, Brown J, Murnane C, Gray E, Humphries M, Sycamore N, Barker D, Saunders D, Wallis J, Babbage A, Hammond S, Mashreghi-Mohammadi M, Barr L, Martin S, Wray P, Ellington A, Matthews N, Ellwood M, Woodmansey R, Clark G, Cooper J, Tromans A, Grafham D, Skuce C, Pandian R, Andrews R, Harrison E, Kimberley A, Garnett J, Fosker N, Hall R, Garner P, Kelly D, Bird C, Palmer S, Gehring I, Berger A, Dooley CM, Ersan-Ürün Z, Eser C, Geiger H, Geisler M, Karotki L, Kirn A, Konantz J, Konantz M, Oberländer M, Rudolph-Geiger S, Teucke M, Lanz C, Raddatz G, Osoegawa K, Zhu B, Rapp A, Widaa S, Langford C, Yang F, Schuster SC, Carter NP, Harrow J, Ning Z, Herrero J, Searle SM, Enright A, Geisler R, Plasterk RH, Lee C, Westerfield M, de Jong PJ, Zon LI, Postlethwait JH, Nüsslein-Volhard C, Hubbard TJ, Roest Crollius H, Rogers J, Stemple DL.. 2013. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* **496 (7446):** 498-503. DOI 10.1038/nature12111.

Hussain A, Saraiva LR, Ferrero DM, Ahuja G, Krishna VS, Liberles SD, Korsching SI. 2013. High-affinity olfactory receptor for the death-associated odor cadaverine. *Proc. Natl. Acad. Sci. U. S. A*. **110**: 19579–19584. DOI 10.1073/pnas.1318596110.

Idalencio R, Kalichak F, Rosa JGS, Oliveira TA, Koakoski G, Gusso D, Abreu MS, Giacomini AC, Barcellos HH, Piato AL, Barcellos LJ. 2015. Waterborne Risperidone Decreases Stress Response in Zebrafish. *Plos One* **10**: e0140800. DOI 10.1371/journal.pone.0140800.

Kalueff AV, Echevarria DJ, Stewart AM. 2014. Gaining translational momentum: more zebrafish models for neuroscience research. *Progress in neuro-psychopharmacology & biological psychiatry* **55**: 1-6. DOI 10.1016/j.pnpbp.2014.01.022.

Korpi NL, Wisenden BD. 2001. Learned recognition of novel predator odour by zebra danio, Danio rerio, following time-shifted presentation of alarm cue and predator odour. *Environmental Biology of Fishes* **61:** 205-211.

LeDoux J. 2007. The amygdala. *Curr. Biol.* **17**: 868-874. DOI 10.1016/j.cub.2007.08.005

LeDoux JE. 2000. Emotion circuits in the brain. *Annu. Rev. Neurosci.* **23**: 155-184.

Lowry CA. 2002. Functional subsets of serotonergic neurones: implications for control of the hypothalamic–pituitary–adrenal axis. *J Neuroendocrinol* **14**: 911–23.

Marcon M, Herrmann AP, Mocelin R, Rambo CL, Koakoski G, Abreu MS, Conterato GM, Kist LW, Bogo MR, Zanatta L, Barcellos LJ, Piato AL. 2016. Prevention of unpredictable chronic

stress-related phenomena in zebrafish exposed to bromazepam, fluoxetine and nortriptyline. *Psychopharmacol*. **233**: 3815-3824.

Maximino C, Lima MG, Costa CC, Guedes IML, Herculano AM. 2014. Fluoxetine and WAY 100.635 dissociate increases in scototaxis and analgesia induced by conspecific alarm substance in zebrafish (*Danio rerio* Hamilton 1822). *Pharmacol. Biochem. Behav.* **124**: 425–433. DOI 1 0.1016/j.pbb.2014.07.003.

O'Connell LA, Hofmann HA. 2011. The Vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *J Comp Neurol* **519**: 3599-3639. DOI 10.1002/cne.22735.

Øverli Ø, Korzan WJ, Larson ET, Winberg S, Lepage O, Pottinger TG, Renner KJ, Summers CH. 2004. Behavioural and neuroendocrine correlates of displaced aggression in trout. *Horm. Behav.* **45**: 324–329. DOI 10.1016/j.yhbeh.2004.01.001

Perry S, Reid S, Salama A. 1996. The effects of repeated physical stress on the β-adrenergic response of the rainbow trout red blood cell. *Journal of experimental biology* **199**: 549 -562.

Pfeiffer W. 1977. The distribution of fright reaction and alarm substance cells in fishes. *Copeia* **4**: 653–65.

Piato AL, Rosemberg DB, Capiotti KM, Siebel AM, Herrmann AP, Ghisleni G, Vianna MR, Bogo MR, Lara DR, Bonan CD. 2011. Acute restraint stress in zebrafish: behavioral parameters and purinergic signaling. Neurochem. Res. **36**: 1876-86.

Ramsay JM, Feist GW, Varga ZM, Westerfield M, Kent ML, Schreck CB. 2009. Whole-body cortisol response of zebrafish to acute net handling stress. *Aquaculture* **297**: 157–162.

Rosnick CB, Wetherell JL, White KS, Andreescu C, Dixon D, Lenze EJ. 2016. Cognitive-behavioral therapy augmentation of SSRI reduces cortisol levels in older adults with generalized anxiety disorder: A randomized clinical trial. *J Consult Clin Psychol.* **84(4)**: 345-52. DOI 10.1037/a0040113.

Sanches FHC, Miyai CA, Pinho-Neto CF, Barreto RE. 2015. Stress responses to chemical alarm cues in Nile tilapia. *Physiology & Behavior* **149**: 8–13. DOI 10.1016/j.physbeh.2015.05.010.

Silva PIM, Martins CI, Khan UW, Gjøen HM, Øverli Ø., Höglund E. 2015. Stress and fear responses in the teleost pallium. *Physiology & Behavior* **141**: 17–22.DOI 10.1016/j.physbeh.2014.12.020.

Sink TD, Kumaran S, Lochmann RT. 2007. Development of a whole-body cortisol extraction procedure for determination of stress in golden shiners, Notemigonus crysoleucas. *Fish Physiol. Biochem.* **33**: 189–193. DOI 10.1007/s10695-007-9130-0.

Speedie N, Gerlai R. 2008. Alarm substance induced behavioral responses in zebrafish (*Danio rerio*). *Behav. Brain Res.* **188**: 168-177. DOI 10.1016/j.bbr.2007.10.031.

Teles M, Soares AM, Tort L, Guimarães L, Oliveira M. 2017. Linking cortisol response with gene expression in fish exposed to gold nanoparticles. *Sci Total Environ.* **17**: 30163-8. DOI 10.1016/j.scitotenv.2017.01.153.

Theall KP, Shirtcliff EA, Dismukes AR, Wallace M, Drury SS. 2017. Association Between Neighborhood Violence and Biological Stress in Children. *JAMA Pediatr.* **171(1)**: 53-60. DOI 10.1001/jamapediatrics.2016.2321.

Vargas JP, López JC, Portavella M. 2009. What are the functions of fish brain pallium? *Brain Res Bull* **79**: 436-440. DOI 10.1016/j.brainresbull.2009.05.008.

Wendelaar Bonga SE. 1997. The stress response in fish. *Am. Physiol. Soc. Rev.* **77**: 591-625.

Winberg S, Nilsson GE, Olsen KH. 1992. The effect of stress and starvation on brainserotonin utilization in Arctic charr (Salvelinus alpinus). *J. Exp. Biol.* **165**: 229–239.

Wong DT, Bymaster FP, Engleman EA. 1995. Prozac (Fluoxetine, Lilly 110140), the first selective serotonin uptake inhibitor and an antidepressant drug: twenty years since its first publication. *Life Sci.* **57**: 411–441.