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IMPACT OF CHRONIC UNPREDICTABLE STRESS ON THE EXPRESSION OF APOPTOTIC GENES IN ZEBRAFISH BRAIN

Dheepthi Jayamurali and Sathya Narayanan Govindarajulu*

Department of Physiology, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai - 600113, Tamil Nadu, India.

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Correspondence to Author:
Dr. G. Sathya Narayanan

Assistant Professor,
Department of Physiology,
Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai - 600113. Tamil Nadu, India.

E-mail: drgsathyanarayanan@gmail.com

ABSTRACT: Stress plays a major role in various pathophysiological processes related with neurodegenerative diseases and mental disorders. Zebrafish has emerged as a promising model organism in inducing Chronic Unpredictable Stress (CUS). In the present study, we have attempted to understand the molecular basis of neuronal changes underlying CUS in zebrafish model. An evidence for a connection between CUS and apoptosis in the brain of zebrafish was analyzed. For this purpose, zebrafish were subjected to CUS protocol twice a day for a period of 15 days. The impact of CUS on the brain was evaluated in relation to the expression of the stress marker genes: corticotropin releasing factor (*crf*) and glucocorticoid receptor (*gr*). The effects of CUS were also estimated from the expression of *p53* mediated apoptotic genes: *p53*, *noxa*, *bcl2* and *casp3*. CUS protocol increased the gene expression of *crf* ($p<0.05$) and decreased the expression of *gr* ($p<0.05$). The impact of CUS on the apoptotic pathway showed increased expression of *p53* ($p< 0.05$) and *noxa* ($p<0.05$) with a decrease in expression of mitochondrial *bcl2* ($p<0.05$). As a hallmark of apoptosis, CUS increased the expression of *casp3* ($p<0.05$). In principle, CUS has the potency to exert their detrimental effects on the neuronal cells of the brain and has confirmed p53 induced apoptosis in the brain of zebrafish.

INTRODUCTION: Neurodegenerative diseases are a heterogeneous group of disorders characterized by progressive, selective loss of anatomically or physiologically related neuronal systems [1]. Many lines of evidence suggest that the greatest risk factor for neurodegenerative diseases is ageing, and mitochondria have been thought to contribute to ageing through the accumulation of mitochondrial DNA mutations and net production of reactive oxygen species (ROS) [2].

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Despite ageing, Stress can also bring about structural and functional changes in the brain, including neural damage through the production of ROS [3] and mitochondrial dysfunction [4]. There are only limited printings on stress induced neurodegenerative diseases on mitochondrial dysfunction induced apoptotic pathway.

World Health Organization (WHO) has reported that stress is the central lifestyle risk factor, the leading cause of the global burden of disease. Within bounds, stress has positive effects such as improved memory performance [5], increased alertness, focus, energy and also it can help people to manage unfavorable situations. However chronic stress takes a toll on physiology affecting productivity and quality of life and ultimately leading to many affective disorders [6, 7].

Chronic Unpredictable Stress (CUS), one of the most clinically relevant stress paradigms in rodents, mimics a number of characteristics observed in patients with mental disorders [8, 9]. Numerous experimental studies of rodent models appear to be costly affairs for screening of thousands of novel, potential candidate small molecules. Recently, Zebrafish *(Danio rerio*) have emerged as a promising novel organism to study neuroscience [10] and other related areas. This species has relatively 84 percent genetic homology to humans [11] and provides many advantages when compared to other vertebrates such as low cost, easy handling and faster reproduction [12]. Moreover, zebrafish brain is neuroanatomically and functionally comparable to mammals [13] and it is the target organ of stress [14].

In zebrafish, stress system is characterized by the hypothalamus – pituitary – interrenal axis (HPI) [15, 16]. Similarly to the mammalian hypothalamus – pituitary – adrenal axis (HPA), the zebrafish HPI axis controls the levels of circulating cortisol. Hippocampus of the brain provides negative control over the HPA axis. Chronic Stress suppresses cell proliferation in the hippocampus [17] and leads to the activation of the HPA axis [18]. A stressful signal stimulates secretion of Corticotropin Releasing Factor (CRF) [19]. In response to CRF, the pituitary releases Adrenocorticotropic Hormone (ACTH) which reaches the head kidney of fish. Cortisol is secreted and binds to the Glucocorticoid Receptor (GR), a ligand activated nuclear transcription factor. GR regulates transcription of target genes related to glucose metabolism, immune and other physiological functions [20, 21].

There are two key aspects of stress response. Firstly, the body responds to many stressors by releasing chemical mediators. Secondly, the chronic elevation of these mediators can cause allostatic load leading to pathophysiology. The allostatic load model of chronic stress focuses on glucocorticoid dysregulation particularly glucose imbalance. Mitochondrial allostatic load defines the deleterious structural and functional changes that mitochondria undergo in response to glucose imbalance and stress related pathophysiology. Damaged mitochondria has been proved to generate toxic products and reactive oxygen species that can promote inflammation and alter gene expression [22, 23]. Increased reactive oxygen species has been shown to cause DNA fragmentation [24]. The tumor suppressor protein *p53* play an important part in the regulation of cellular response to DNA damage. It has been revealed to have a role in sensing oxidative DNA damage [25] and perhaps this can trigger *p53* dependent apoptosis in zebrafish brain.

Although there is evidence that zebrafish may be suitable to study neurobiology of human pathologies, the assessment of CUS induced apoptotic pathway in this model is scarcely characterized [26]. Therefore the purpose of the study is to establish a CUS zebrafish model. We evaluated the effects of chronic stress from the gene expression of *crf* and *gr* (stress marker genes). The impact of chronic stress on zebrafish brain was also assessed from the gene expression of *p53*, noxa, *bcl2* and *casp3* which forms the death cascade.

MATERIALS AND METHODS:

Animals and housing: 300 Adult wild type zebrafishes of both sexes were obtained from a commercial fish supplier. The fishes were acclimatized to the laboratory conditions by maintaining them at 28 ± 2ºC, 14/10 h light/dark cycle. They were fed two times a day with commercial flakes and live shrimp provided with constant aeration [27].The fishes were accommodated in groups of 20 fishes/ 15 l of water. The fishes were segregated into two groups – control and stress induced group with 150 fishes in each. All the protocols were approved by the Institutional Animal Ethical Committee.

Chronic Unpredictable Stress Protocol (CUS): Succeeding a two – week adaptation period, the fishes were subjected to a range of chronic stressors such as restrain stress, predator stress, low water level stress (dorsal body exposure), over - crowding stress, chasing stress, cold stress and heating stress. The fishes were exposed to one of the above mentioned stressors twice a day for a period of 15 days (**Table 1**).

Method of administration of stress: Restraint stress – each fish was restrained in a 2 ml micro centrifuge tube with perforations at both the ends to allow free flow of water (Duration 90 min).

Predator stress: The fishes were alarmed by a predator fish *(Archocentrus nigrofasciatus)* in close vicinity but avoiding direct contact (Duration 50 min). Low water level stress: The water was drained in the housing tanks to expose the animals' dorsal body surface (Duration 2 min). Over – crowding stress: 250 ml beaker crowded with 10 fishes/150 ml of water (Duration 50 min). Chasing stress: racing the animals using a net (Duration 8 min). Cold Stress: Exposing the animals to 23º C (Duration 30 min). Heating Stress: Heating the tank for 33º C (Duration 30 min) [19].

Optimum conditions were controlled during each stressor presentation. Time and sequence of stressors were altered on daily basis to prevent habituation and to promote unpredictability. A control group was also retained in the same room provided with ideal conditions for a period of 15 days. Two distinguished groups: control and stress induced group were used to evaluate the gene expression. Despite stressful conditions, no extreme harm was caused to the animals nor abnormal number of deaths witnessed.

mRNA extraction and cDNA synthesis: Zebrafishes were cryoanaesthetized and euthanized 24 hours after the CUS protocol [28]. By means of the established protocol, the brains were dissected and removed [29]. RNA Extraction: Total RNA was extracted from pooled adult zebrafish brain using Trizol Reagent (Sigma) in accordance with the kit's manual (Invitrogen). The purity of the RNA was spectrophotometrically quantified. cDNA synthesis - cDNA was synthesized from the isolated RNA by reverse transcription (iScript cDNA Synthesis Kit, BIO – RAD). Primers – The primers were designed in primer blast of NCBI. β actin was used as a control. The set of primers used are mentioned in **Table 2**.

Gene Expression analysis by qRT-PCR: Quantitative Real time (qRT) PCR analysis were done for the genes that code for *crf and gr*, molecular markers of stress related disorders. qRT – PCR was also performed for the apoptotic genes *p53, noxa , bcl2* and *casp3*. All qRT – PCR reactions were executed in CFX96 BIORAD Real - Time PCR using SYBR green master mix plus for SYBR assay. qRT – PCR was achieved in triplicate using gene specific primers as listed in **Table 2**, designed and produced as per the requirements to route the reactions in qRT – PCR. Thermal profiles for *βact, crf, p53, bcl2, casp3* in qRT – PCR: initial denaturation at 95ºC for 5 min, 40 cycles of 95ºC for 10 sec for denaturing, 1 min annealing step at 60ºC and a final 30 sec extension at 72 ºC. Similarly, thermal profiles for *gr and noxa*: initial denaturation at 95 ºC for 5 min, 40 cycles of 94 ºC for 1 min for denaturing, 1 min annealing step at 62ºC and a final 1 min extension at 72ºC. The results were expressed as relative expression levels. The relative abundance of gene expression were quantified by normalization to *βact* levels. The data was computed by the $2^{-\Delta\Delta C}{}_{T}$ method [30].

RESULTS:

Effect of CUS on *crf* and *gr* expression, molecular markers of stress: As a confirmation of chronic stress the gene expression of *crf* and *gr* were detected and perhaps it revealed significant alterations. The gene expression level of *crf* was significantly increased and the gene expression level of *gr* was considerably decreased in the stress induced group when compared to the control group.

Effect of CUS on the gene expression of apoptotic genes: As a confirmation of apoptosis induced by *p53*, the gene expression levels of *p53*, *noxa*, *bcl2* and *casp3* were detected in both control and stress induced group. Substantial alterations were seen in the gene expression of apoptotic genes. The gene expression levels of *p53* and *noxa* presented a significant increase ($p<0.001$) in stress induced group compared with control group. A significant decrease ($p<0.001$) in *bcl2* was observed in the stressed group compared with control. The expression levels of *casp3* exhibited a significant increase ($p<0.05$) in stress exposed group when compared to control group.

Statistical analysis: Student's t test was performed for each experiment using SPSS 20.0. Data were expressed as Mean ± SD. p value < 0.05 was considered significant.

DISCUSSION:

Dynamic Expression of *crf* and *gr* in zebrafish brain: Recent studies [31] revealed that the 15 day CUS paradigm was successful at inducing an anxiety and related mood disorders. Our aim was to determine whether the CUS established any

changes in the molecular markers of stress in zebrafish brain. Neurons in the hypothalamus in response to an environmental stressor secrete CRF. CRF is a neurotransmitter that regulates the release of ACTH which binds to the interrenal cells of the head kidney and excites the production and release of cortisol [32]. In the course of HPI activation, glucocorticoid and mineralocorticoid receptors are the mediators of transcriptional effects of cortisol on the peripheral tissues. On exposure to CUS, *crf* and *gr* exhibited remarkable variations. Centrally, chronic stress differentially modulated the gene expression of *crf* and *gr*, where the expression of *crf* was increased and the expression of *gr* was decreased.

In the present study, CUS could have activated the HPI axis and exhibited a sharp increase in the gene expression of *crf*. The changes observed in the expression of *crf* gene on exposure to CUS was similar to those reported in previous study in which zebrafish on exposure to unpredictable long term stress increased brain *crf* [33]. CUS has also shown to induce changes in the gene expression of *crf* in zebrafish brain [19]. This result also goes in parallel with previous results of stress protocols in which stressed fish of different species showed increased *crf* expression [34, 35].

A stressful signal stimulates the secretion of glucocorticoids from interrenal cells in response to ACTH which in turn is regulated by CRF as described earlier. Glucocorticoids serve a homeostatic function in acute stress responses by providing negative feedback to brain stress circuits. Activated GR bind to DNA and regulate the transcription of genes and terminate the stress response. But repeated stress may induce long – term neural adaptations, including downregulation of *gr* expression [36]. In the present study as predicted, zebrafish brain presented a decrease in the gene expression of *gr* when exposed to CUS. CUS could have downregulated the gene expression of gr by long term neural adaptations or by suppressing the gene by epigenetic modification [37, 38].

Dynamic Expression of apoptotic genes in zebrafish brain: Previous studies have revealed that chronic stress has increased mitochondrial allostatic load and it was effective in the net production of reactive oxygen species as described earlier. Reactive oxygen species functions as important physiological regulators of intracellular signaling pathway [39]. DNA damage occurs as a result of the production of reactive oxygen species [40]. Recent studies on zebrafish embryo have exposed the fact that *p53* signalling is activated on DNA damage and results in cell death [41].

The tumor suppressor *p53* induces apoptosis primarily via induction of pro – apoptotic protein phorbol – 12 – myristate – 13 – acetate – induced protein 1 (*pmaip1*; also known as *noxa*).The increase in levels of *p53* protein leads to accumulation of *noxa*. *noxa* binds to *bcl2* and apoptosis occurs when an apoptotic threshold is reached owing to the inhibition of *bcl2*. Apoptosis is driven by a highly controllable proteolytic cascade. The role of *p53* function as described above is underlined from the findings of several former studies [42].

The present study was designed to evaluate whether CUS lead to *p53* induced apoptosis in zebrafish brain. The effects were studied from the expression of apoptotic genes like *p53, noxa, bcl2* and *caspase 3.* In the present study, the expression of *p53* was considerably higher in the CUS induced group. This upregulation of *p53* can be the result of DNA damage caused by CUS. Review studies on *p53* showed that the tumor suppressor protein is a transcription factor induced by stress, which can promote cell cycle arrest and apoptosis [43]. Another study has proved that rat astrocyte on exposure to severe oxidative stress caused upregulation of *p53* due to DNA damage [44]. These findings support the present results of *p53* upregulation in the brain of zebrafish on exposure to CUS.

Current study provides evidence for CUS induced upregulation of *noxa*. The upregulation of *noxa* may be due to the accumulation of *p53* and increased expression of *p53* in the CUS induced brain of zebrafish. This is underscored from previous conclusions which displayed that doxorubicin induced accumulation of *p53* in the nucleus of neuroblastoma cells caused upregulation of *noxa* in the mitochondria of neuroblastoma cells [45]. Also other review studies support the fact that increased *p53* has a positive effect on *noxa* and leads to the corresponding increase of *noxa* [46].

Recent analyses revealed that repeated unpredictable stress reduced *bcl2* levels in the central nucleus of amygdala of rats [47]. Also several studies have established the fact that *noxa* when ectopically expressed underwent *bh3* dependent localization to mitochondria and interacted with anti-apoptotic *bcl-2* family members and caused its inhibition [48]. In the current study as expected the expression of *bcl2* was greatly decreased. The inhibition of *bcl2* may be due to upregulation of *noxa* or activation of pro – apoptotic *bax* and *bad* [49], which has not been included in this study. Pro and anti-apoptotic members of the *bcl-2* family regulate mitochondrial participation in cell death [50]. Earlier models have proposed that the release of cytochrome c from damaged mitochondria triggers the activation of cascade of caspases [51].

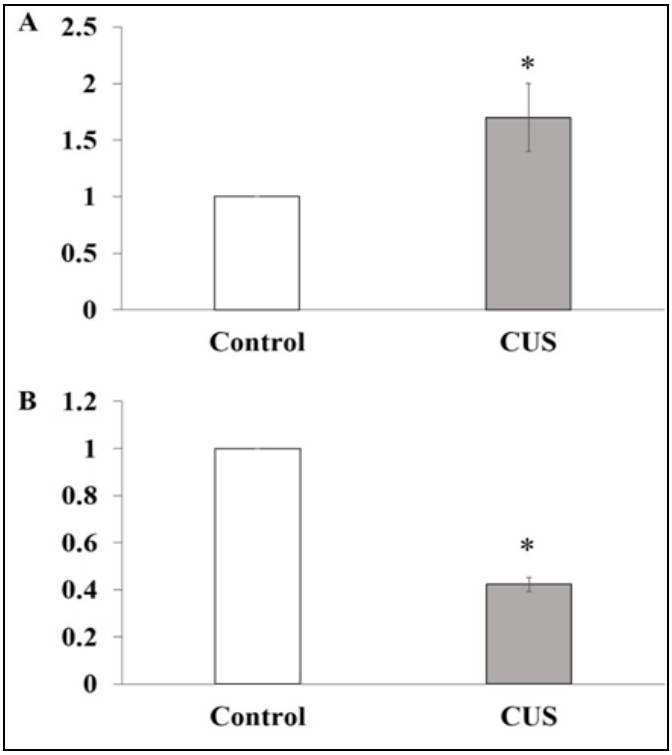

FIG. 1: EFFECT OF CUS ON THE GENE EXPRESSION OF STRESS MARKERS *crf* (A) AND *gr* (B) IN ZEBRAFISH BRAIN. The data are presented as the mean ± SD. * indicates significance compared with control (Student`s t test, *p<0.05)

In the present study as expected, the expression of *casp3* was increased in the CUS induced group when compared to the control group. c*asp3* activation may be due to *bcl2* downregulation contributed mitochondrial dysfunction, as seen in this study. Early reviews have also supported the fact that protein complexes activate the cascade of caspases in stress induced apoptosis [52]. Activation of the caspase-3 pathway is a hallmark of apoptosis and can be used to quantify activators and inhibitors of "death cascade" [53].

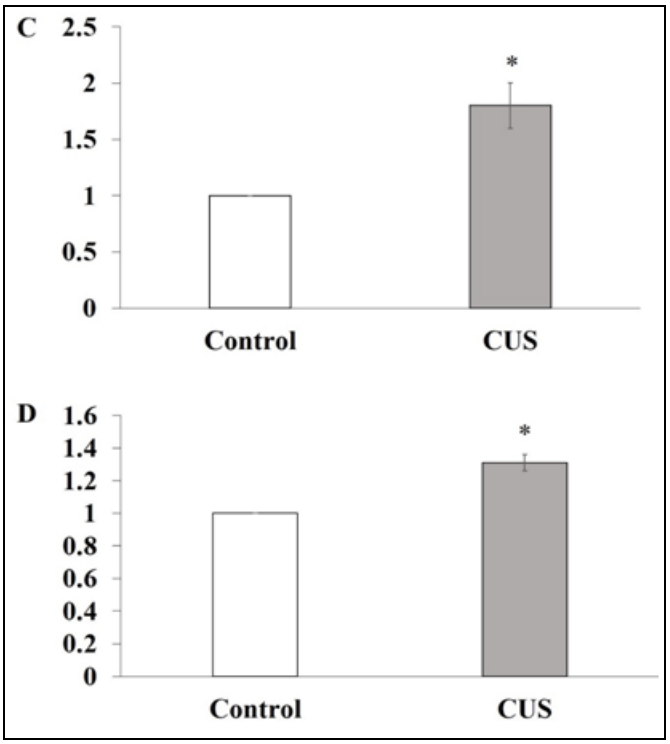

FIG. 2: EFFECT OF CUS ON THE GENE EXPRESSION OF APOPTOTIC GENES *p53* (C) AND NOXA (D) IN ZEBRAFISH BRAIN. The data are presented as the mean ± SD. * indicates significance compared with control (Student`s t test, *p<0.05)

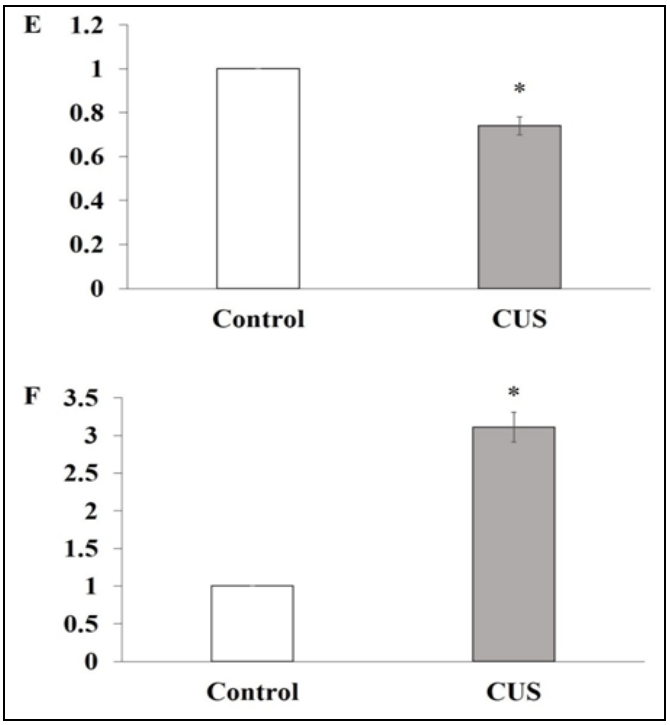

FIG. 3: EFFECT OF CUS ON THE GENE EXPRESSION OF APOPTOTIC GENES *bcl2* (E) AND *casp3* (F) IN ZEBRAFISH BRAIN. The data are presented as the mean ± SD. * indicates significance compared with control (Student`s t test, *p<0.05)

TABLE 1: CUS PROTOCOL IN ADULT ZEBRAFISH

Duration		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1	Morning	RS	CS	PS	HS	CS	RS	PS
	Evening	HS	OCS	C	LWLS	C	LWLS	HS
Week 2	Morning	LWLS	PS	OCS	HS	CS	RS	PS
	Evening	C	CS	RS	LWLS	C	OCS	HS

C – Chasing Stress, OCS – Over Crowding Stress, LWLS – Low Water Level Stress,
RS – Restrain Stress, PS – Predator Stress, CS – Cold Stress, HS – Heating Stress

TABLE 2: LIST OF PRIMERS USED IN QUANTITATIVE REAL-TIME PCR AMPLIFICATION

Gene		Primer Sequence
βact	Forward	5’ – CGA GCA GGA GAT GGG AAC C – 3’
	Reverse	5’ – CAA CGG AAA CGC TCA TTG C – 3’
crf	Forward	5’ – CCG CCG TAT GAA TGA TAG AGC – 3’
	Reverse	5’ – GAT GGA AAG TGA TGA CAG TG – 3’
gr	Forward	5’ – AAC ATG CTG TGT TTC GCT CC – 3’
	Reverse	5’ – CTG CAA GCA TTT CGG GAA AC – 3’
p53	Forward	5’ – GGG CAA TCA GCG AGC AAA – 3’
	Reverse	5’ – ACT GAC CTT CCT GAG TCT CCA – 3’
noxa	Forward	5’ – CGA ACC TGT GAC AGA AAC TTG – 3’
	Reverse	5’ – CTG CGC GCA CTC TAC TAC A – 3’
bcl2	Forward	5’ – AGG AAA ATG GAG GTT GGG ATG – 3’
	Reverse	5’ – TGT TAG GTA TGA AAA CGG GTG GA – 3’
casp3	Forward	5’ – CCG CTG CCC ATC ACT A– 3’
	Reverse	5’ – ATC CTT TCA CGA CCA TCT – 3’

CONCLUSION: In summary, we have presented evidence that exposure to CUS has well known roles in inducing apoptosis in the brain of zebrafish through p53 mediated pathway. The actual mechanism involving apoptosis in the present study might be due to DNA damage upon CUS exposure, which would have activated the death cascade. Further studies on zebrafish CUS model can enable a better understanding of stress induced neurological disorders via apoptosis.

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CONFLICT OF INTEREST: Authors declare they have no conflict of interest.

REFERENCES:

1. Chen, Y., Garcia, G. E., Huang, W., Constantini, S. The involvement of secondary neuronal damage in the development of neuropsychiatric disorders following brain insults. Frontiers in Neurology. 2014; 5, 22.
2. Cui, H., Kong, Y., Zhang, H. Oxidative stress, mitochondrial dysfunction, and aging. Journal of Signal Transduction. 2012; 646354, 1-13.
3. Guo, C., Sun, L., Chen, X., Zhang, D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regeneration Research. 2013; 8, 2003-2014.
4. Chan, F., Lax, N.Z., Davies, C.H., Turnbull, D.M., Cunningham, M.O. Neuronal oscillations: A physiological correlate for targeting mitochondrial dysfunction in neurodegenerative diseases?. Neuropharmacology. 2016; 102, 48-58.
5. McReynolds, J.R., Holloway-Erickson, C.M., Parmar, T.U., McIntyre, C.K. Corticosterone-induced enhancement of memory and synaptic Arc protein in the medial prefrontal cortex. Neurobiology of Learning and Memory. 2014; 112, 148-57.
6. Mahara, I., Rodriguez, B.F., Mechawar, N., José, N.N. Stress, serotonin, and hippocampal neurogenesis in relation to depression and antidepressant effects. Neuroscience & Biobehavioral Reviews. 2014; 38, 173-192.
7. Magalhaes, A.C., Holmes, K.D., Dale, L.B., Comps-Agrar, L., Lee, D., Yadav, P.N., Drysdale, L., Poulter, M.O., Roth, B.L., Pin, J.P., Anisman, H., Ferguson, S.S. CRF receptor 1 regulates anxiety behavior via sensitization of 5-HT2 receptor signaling. Nature Neuroscience. 2010; 13, 622-629.
8. Pittenger, C., Duman, R.S. Stress, Depression, and Neuroplasticity: A convergence of mechanisms. Neuropsychopharmacology. 2008: 33, 88-109.
9. LaPlant, Q., Chakravarty, S., Vialou, V., Mukherjee, S., Koo, J.W., Kalahasti, G., Bradbury, K.R., Taylor, S.V., Maze, I., Kumar, A., Graham, A., Birnbaum, S.G., Krishnan, V., Truong, H.T., Neve, R.L., Nestler, E.J., Russo, S.J. Role of nuclear factor kappaB in ovarian hormone-mediated stress hypersensitivity in female mice. Biological Psychiatry. 2009; 65, 874-880.
10. Becker, C.G., Becker, T. Adult zebrafish as a model for successful central nervous system regeneration. Restorative Neurology and Neuroscience. 2008; 26, 71-80.
11. Howe, K., Clark, M.D., Torroja, C.F., Torrance, J., Berthelot, C., Muffato, M., Stemple, D.L. The zebrafish reference genome sequence and its relationship to the human genome. Nature, 2013; 496, 498-503.

12. Egan, R.J., Bergner, C.L., Hart, P.C., Cachat, J.M., Canavello, P.R., Elegante, M.F., Elkhayat, S.I., Bartels, B.K., Tien, A.K., Tien, D.H., Mohnot, S., Beeson, E., Glasgow, E., Amri, H., Zukowska, Z., Kalueff, A.V. Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. Behavioural Brain Research. 2009; 205, 38-44.
13. Kalueff, A.V., Stewart, A.M., Gerlai, R. Zebrafish as an emerging model for studying complex brain disorders. Trends in Pharmacological Sciences. 2014; 35, 63–75.
14. McEwen, B.S. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiological Reviews. 2007; 87, 873-904.
15. Alderman, S.L., Bernier, N.J. Ontogeny of the corticotropin-releasing factor system in zebrafish. General and Compatative Endocrinology. 2009; 164, 61-69.
16. Alsop, D., Vijayan, M.M. Development of the corticosteroid stress axis and receptor expression in zebrafish. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2008; 294, R711-719.
17. Kim, E.J., Pellman, B., Kim, J.J. Stress effects on the hippocampus: a critical review. Learning & Memory. 2015; 22, 411-416.
18. Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann J, Myers, B. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Comprehensive Physiology. 2016; 6, 603-621.
19. Piato, Â.L., Capiotti, K.M., Tamborski, A.R., Oses, J.P., Barcellos, L.J., Bogo, M.R., Lara, D.R., Vianna, M.R., Bonan, C.D. Unpredictable chronic stress model in zebrafish (*Danio rerio*): behavioral and physiological responses. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2011; 35, 561-567.
20. Bury, N.R., Sturm, A. Evolution of the corticosteroid receptor signaling pathway in fish. General and Comparative Endocrinology. 2007; 153, 47-56.
21. Robert, H., Oakley, John Cidlowski, A. The Biology of the Glucocorticoid Receptor: New Signaling Mechanisms in Health and Disease. Journal of Allergy and Clinical Immunology. 2013; 132, 1033–1044.
22. Aschbacher, K., O'Donovan, A., Wolkowitz, O.M., Dhabhar, F.S., Su, Y., Epel, E. Good stress, bad stress and oxidative stress: insights from anticipatory cortisol reactivity. Psychoneuroendocrinology. 2013; 38, 1698-708.
23. Picard, M., Juster, R.P., McEwen, B.S. Mitochondrial allostatic load puts the 'gluc' back in glucocorticoids. Nature Reviews Endocrinology. 2014; 10, 303-310.
24. Zhu, L., Dong, X., Xie, H., Wang, J., Wang, J., Su, J., Yu, C. DNA damage and effects on glutathione-S-transferase activity induced by atrazine exposure in zebrafish (*Danio rerio*). Environmental Toxicology. 2011; 26, 480-488.
25. Zhao, M., Howard, E. W., Guo, Z., Parris, A.B., Yang, X. p53 pathway determines the cellular response to alcohol-induced DNA damage in MCF-7 breast cancer cells. PLoS ONE. 2017; 12, e0175121.
26. Champagne, D.L., Hoefnagels, C.C., de Kloet, R.E., Richardson, M.K. Translating rodent behavioral repertoire to zebra fish (*Danio rerio*): relevance for stress research. Behaviour Brain Research. 2010; 214, 332-342.
27. Westerfield, M. The zebrafish book, A guide for the laboratory use of zebrafish (*Danio rerio*). Fifth edition. University of Oregon Press, Eugene. 2007.
28. Wilson, J.M., Bunte, R.M., Carty, A.J. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). Journal of the American Association for Laboratory Animal Science. 2009; 48, 785-789.
29. Gupta, T., Mullins, M.C. Dissection of organs from the adult zebrafish. Journal of Visualized Experiments. 2010; 37, 1717.
30. Schmittgen, T.D., Livak, K.J. Analyzing real-time PCR data by the comparative CT method. Nature Protocols. 2008; 3, 1101-1108.
31. Chakravarty, S., Reddy, B.R., Sudhakar, S.R., Saxena, S., Das, T., Meghah, V., Swamy, C.V.B., Arvind Kumar, Idris, M.M. Chronic Unpredictable Stress (CUS) induced anxiety and related mood disorders in a zebrafish model: Altered brain proteome profile implicates mitochondrial dysfunction. PLoS One 2013; 8, e63302.
32. A. Tsalafouta, N. Papandroulakis, M. Gorissen, P. Katharios, G. Flik, and M. Pavlidis. Ontogenesis of the HPI axis and molecular regulation of the cortisol stress response during early development in Dicentrarchus labrax. Scientific Reports. 2014; 4: 5525.
33. Pavlidis, M., Theodoridi, A., Tsalafouta, A. Neuroendocrine regulation of the stress response in adult zebrafish, *Danio rerio*. Progress in Neuro-Psychopharmacology & Biological Psychiatry. 2015; 60, 121-131.
34. Bernier, N.J., Craig, P.M. CRF-related peptides contribute to stress response and regulation of appetite in hypoxic rainbow trout. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology. 2005; 289, R982-990.
35. Doyon, C., Trudeau, V.L., Moon, T.W. Stress elevates corticotropin-releasing factor (CRF) and CRF-binding protein mRNA levels in rainbow trout (*Oncorhynchus mykiss*). Journal of Endocrinology. 2005; 186, 123-130.
36. Howell, K.R., Kutiyanawalla, A., Pillai, A. Long-term continuous corticosterone treatment decreases VEGF receptor-2 expression in frontal cortex. PLoS One. 2011; 6, e20198.
37. Adrian, M., Stankiewicz, Artur Swiergiel, H., Lisowski, P. Epigenetics of stress adaptations in the brain. Brain Research Bulletin. 2013; 98, 76–92.
38. Chiba, S., Numakawa, T., Ninomiya, M., Richards, M.C., Wakabayashi, C., Kunugi, H.,. Chronic restraint stress causes anxiety- and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. Progress in Neuropsychopharmacology and Biological Psychiatry. 2013; 39, 112-9.
39. Schieber, M., Chandel, N.S. ROS Function in Redox Signaling and Oxidative Stress. Current biology. 2014; 24, R453-R462.
40. Laura, N., Eleonora, P., Mauro, R., Valeria, S., Alessio, C., Daniela, M., Eugenia, D. The Response to Oxidative DNA Damage in Neurons: Mechanisms and Disease. Neural Plasticity. 2016; 3619274, 1-14.
41. Zhou, R., Zhang, H., Wang, Z., Zhou, X., Si, J., Gan, L., Li, J., Liu, Y. The developmental toxicity and apoptosis in zebrafish eyes induced by carbon-ion irradiation. Life Sciences. 2015; 139, 114-122.
42. Storer, N.Y., Zon, L.I. Zebrafish models of p53 functions. Cold Spring Harbor Perspectives in Biology. 2010; 2, a001123.
43. Levine, A.J., Oren, M. The first 30 years of p53: growing ever more complex. Nature Reviews Cancer. 2009; 9, 749-758.

44. Bonini, P., Cicconi, S., Cardinale, A., Vitale, C., Serafino, A.L., Ciotti, M.T., Marlier, L.N. Oxidative stress induces p53-mediated apoptosis in glia: p53 transcription-independent way to die. Journal of Neuroscience Research. 2004; 75, 83-95.
45. Kurata, K., Yanagisawa, R., Ohira, M., Kitagawa, M., Nakagawara, A., Kamijo, T. Stress via p53 pathway causes apoptosis by mitochondrial noxa upregulation in doxorubicin-treated neuroblastoma cells. Oncogene. 2008; 27, 741-754.
46. Ploner, C., Kofler, R., Villunger, A. Noxa: at the tip of the balance between life and death. Oncogene. 2008; 27, S84-92.
47. Kosten, T.A., Galloway, M.P., Duman, R.S., Russell, D.S., D'Sa, C. Repeated unpredictable stress and antidepressants differentially regulate expression of the bcl-2 family of apoptotic genes in rat cortical, hippocampal, and limbic brain structures. Neuropsychopharmacology. 2008; 33, 1545-1558;
48. Delbridge, A.R.D., Strasser, A. The BCL-2 protein family, BH3-mimetics and cancer therapy. Cell Death and Differentiation. 2015; 22, 1071–1080.
49. Willis, S.N., Fletcher, J.I., Kaufmann, T., Van Delft, M.F., Chen, L., Czabotar, P.E., Ierino, H., Lee, E.F., Fairlie, W.D., Bouillet, P., Strasser, A., Kluck, R.M., Adams, J.M., Huang, D.C. Apoptosis initiated when bh3 ligands engage multiple bcl-2 homologs, not bax or bak. Science. 2007; 315, 856-859.
50. D'Orsi, B., Mateyka, J., Prehn, J.H. Control of mitochondrial physiology and cell death by the Bcl-2 family proteins Bax and Bok. Neurochemistry International. 2017; 17, S0197-0186.
51. Eleftheriadis, T., Pissas, G., Liakopoulos, V., Stefanidis, I. Cytochrome c as a Potentially Clinical Useful Marker of Mitochondrial and Cellular Damage. Frontiers in Immunology. 2016; 7, 279.
52. Lopez-Cruzan, M., Sharma, R., Tiwari, M., Karbach, S., Holstein, D., Martin, C.R., Lechleiter, J.D., Herman, B. Caspase-2 resides in the mitochondria and mediates apoptosis directly from the mitochondrial compartment. Cell Death Discovery. 2016; 2, 16005.
53. McIlwain, D.R., Berger, T., Mak, T.W. Caspase functions in cell death and disease. Cold Spring Harbor Perspectives in Biology. 2013; 5, a008656.

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