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Light of different wavelengths influence stress response in the monoamines of zebrafish brain

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ABSTRACT

Environment plays a major role in conditioning the physiological functions of all organisms. Any slight changes in the perceived surroundings bring about behavioural, neurological and occasionally physical changes in the organism. In this study, the main focus was to quantify the neurotransmitter levels in the total brain of zebrafish exposed to different wavelengths of light 300 - 700 nm (daylight), 650 – 700 nm (red) and 450 – 490 nm (blue) within the tanks. HPLC with the PDA detection method was administered to quantify the levels in each group. Serotonin (5-HT), norepinephrine (NE) and epinephrine (E) were detected in all three groups with 1hr exposure. Dopamine (DA) was less than the detectable level in all groups. Levels of E and NE increased in both red and blue groups compared to the daylight group indicating an acute stress response, while 5-HT levels decreased. Light affects the rhythms and functioning of the brain, this study takes a closer look at monoamine regulation as a response to light stimuli in female zebrafish.

Key words : *Brain, Colour, Light, HPLC, Neurotransmitters, Stress*

Introduction

Every organism comes across various external stimuli that hinder its internal balance. Anxiety is caused when an unpredictable stressor or stimuli interrupts the homeostasis of an organism. A diurnal being like zebrafish has a fixed light/dark circadian rhythm. Zebrafish can differentiate between UV and various wavelengths of the daylight spectrum, they have tetrachromatic visibility (Siregar *et al.*, 2020). They are also a very popular model organism in neurobiology research. Like in most organisms, light and colour play a crucial role in the circadian rhythm, mating choices, and other social behaviour in zebrafish (Bault *et al.*, 2015; de Abreu *et al.*, 2021). It is also known that zebrafish change their body colour into a lighter or darker shade depending on their mood, health and even the background colours of the tank (Singh and Nüsslein-Volhard, 2015; de Abreu *et al.*, 2021). For humans, the colour blue is considered to be calming as it is in the cooler shade of the spectrum while the warm-toned red is associated with alarm and danger (Palmer and Schloss, 2010). Colour perception and its effect on zebrafish physiology is a rising topic in animal welfare and neurobiology research, given the popularity of the model organism.

Serotonin (5-HT), Norepinephrine (NE), Epinephrine (E) and Dopamine (DA) are chemical messengers that play an important role in communication, behavioural modulation and stress response. Catecholamines synthesis and secretion is a primary stress response followed by increased oxygen intake, cardiac output and other physiological actions (Bonga, 1997). Levels of neurotransmitters along with cortisol indicate the primary stress response

and it differs in acute, combined and chronic stress (Mommsen *et al.*, 1999; Pavlidis *et al.*, 2015; Sreelekshmi *et al.*, 2022).

The impact of light and colour on behaviour and neural regulations is examined along with finding a correlation between the synthesis of neurotransmitters and anxiety-like behaviour. Though colour preference tests have been done previously, the main objective of this study was to observe and elucidate the stress/ease responses of female zebrafish brains when exposed to various wavelengths of light in their housing tanks. Also to see if non-preference induces any anxiety-like behaviour. The present study seeks further evidence on the role of monoamines in regulating the stress response.

Materials and Methods

Animals

Zebrafish (*Danio rerio*) belonging to the family *Cyprinidae* were used as model organisms for this experiment. The fishes were of approximately the same age and size. 30 wild adult female shortfin zebrafish were divided equally into three experimental groups. They were acclimatised to the laboratory conditions before the inception of the experiment. 14L/10D photoperiod and commercial feed (Taiyo®Aini fish feed) were provided ad libitum.

Chemicals and Instruments

Epinephrine (E), norepinephrine (NE), dopamine (DA) and serotonin (5-HT) standards from SIGMA. Acetonitrile, ethanol, ethylene glycol monophenyl ether, disodium hydrogen phosphate, trifluoroacetic acid (TFA), and millipore filtered water (AR grade) are used in the experiment.

Instruments used are Hamilton syringe injector (25µl), HPLC (Shimadzu: SPD – 10A VP, Shimadzu, Kyoto, Japan). Reverse-phase Primesep column with dimensions of 150mm length, 4.6mm internal diameter (particle size 5µm) (SIELC, IL). 20 µl sample loop capacity. Ultrasonic homogeniser and REMI cooling centrifuge were used for the tissue preparation.

Experimental Setup and Sampling

The fish were divided into three groups based on the wavelength of the light. The first group were kept in a transparent tank with exposure to natural daylight (300 to 700 nm). The next group of fish were introduced to a blue tank with exposure to shorter wavelength light (450 - 490 nm). The final group of fish were kept in a red tank with exposure to longer wavelengths of 650 - 700 nm. All tanks were covered with respective coloured tops. Tanks were ensured to be well aerated with aquarium aerators. All fish were sacrificed after an hour of exposure and brains were collected for analysis.

Neurotransmitter Analysis

Standard neurotransmitters 5-HT, E, NE and DA were analysed prior and the retention time was validated. Freshly homogenised brains (n=10) were centrifuged, supernatant filtered and injected immediately to prevent exposure to light. HPLC with UV-vis and PDA detection was carried out to calculate the concentration of neurotransmitters in the pooled brain samples of each group. The mobile phase used was Na_2HPO_4: ACN: TFA (90:10:0.3) at 1ml/min for 30mins. Retention times were noted and concentration was calculated by determining the peak areas of standard and sample chromatograms.

Statistical analysis

Calibration curves were constructed by plotting standard neurotransmitter concentrations vs peak area. The mean and standard error of the mean (SEM) of sample concentrations were calculated. A one-way ANOVA followed by Tukey's multiple comparison test was carried out to find the significance using Graphpad PRISM software. Values were considered significant when $P < 0.05$.

Results

Retention time for each standard was noted down to seconds and the same was identified in the experimental sample chromatograms. The peaks were identified with the help of the component-specific spectrum and λ_{max}. The standard monoamines were identified in the order 5-HT, NE, E and DA at retention times 2.01 ± 0.05 mins, 4.68 ± 0.043 mins, 6.31 ± 0.054 mins and 7.5±0.58 mins, respectively at 254 nm. The λmax observed for 5-HT, NE, E and DA in the PDA detector showed 253, 250, 249 and 249, respectively.

The neurotransmitters were identified and concentration was calculated from peak areas of the chromatograms. E, NE and 5-HT were identified and concentrations were determined in all samples

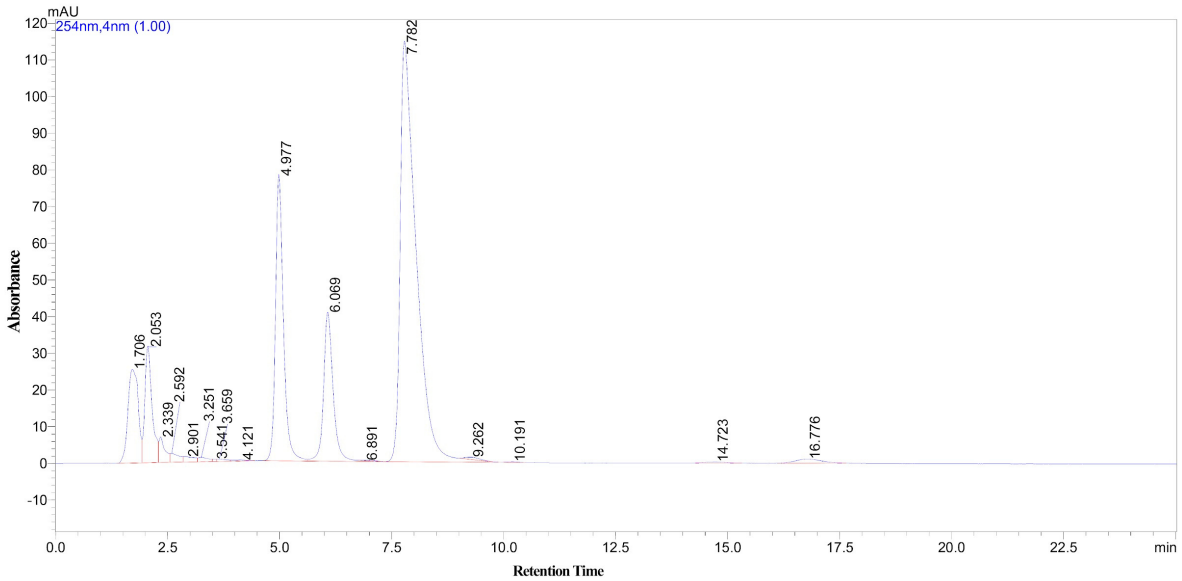

Fig 1. Chromatogram of the monoamines with their retention time (min) and maximum absorbance at 254 nm.

(Fig. 1.). DA was below the range of detection. There was an increase in the levels of E in fish exposed to the blue and red wavelengths compared to the daylight, meanwhile levels of 5-HT decreased (Fig. 2 and 3). Levels of NE were similar in fish brains exposed to daylight and blue wavelengths while increased in the red light (Fig. 3). To understand the significance of the variation of concentration levels between the groups, statistical tools - 2 way ANOVA followed by Tukey's test were employed. The results for each neurotransmitter are compiled in Table 1.

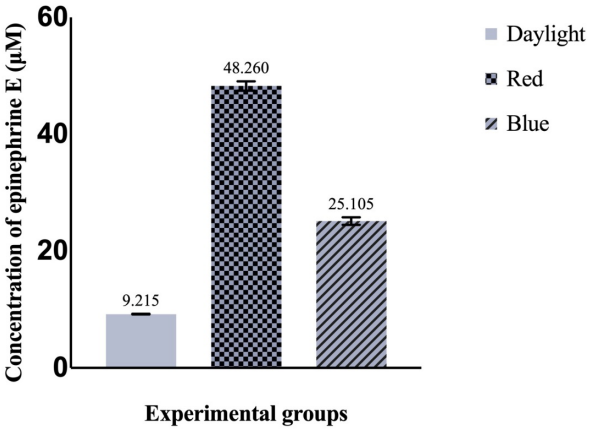

Fig. 2. Histograms depict the level of monoamine, epinephrine (E) in the brain of zebrafish housed in different colour tanks subjecting them to various wavelengths of light. n=10

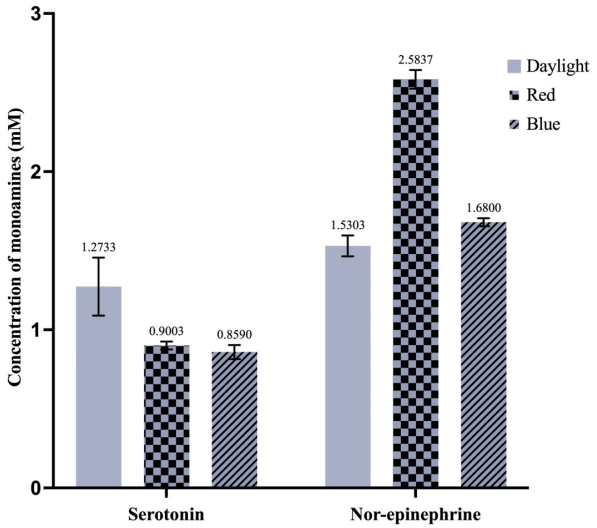

Fig. 3. Histograms depict the level of serotonin (5-HT) and nor-epinephrine (NE) in the brain of zebrafish housed in different colour tanks subjecting them to various wavelengths of light. n=10

The E and NE monoamine levels showed a significant decrease in the blue group compared to the red. Table 1 shows that there was no significance statistically between the levels of 5-HT in the red and blue groups, but it decreased when compared with the daylight group.

Discussion

Light is an easily controllable stimulus that directly

Table 1. Tukey's multiple comparisons test for concentration of Epinephrine, Norepinephrine and Serotonin

P values	Daylight vs Red tank		Daylight vs Blue tank		Red tank vs Blue tank	
Epinephrine (E)	****	<0.0001	***	0.007	***	0.002
Norepinephrine (NE)	****	<0.0001	ns	>0.05	****	<0.0001
Serotonin (5-HT)	*	0.025	*	0.013	ns	>0.05

ns - non-significant

affects the metabolism of zebrafish. Given the wide spectrum of wavelengths in the daylight, it was compelling to identify if and how the wavelengths at the extreme ends necessitate a stress response. Zebrafish have the ability to regain their spectral sensitivity after a long exposure or deficit of light (Fleisch and Neuhauss, 2006). Does preference for one colour necessarily means the other colour is a stressor? Though there was a clear preference for blue light, levels of E increased in red and blue groups. This could account for a sudden shift in their natural environment and as the first physiological response along with an increase in body cortisol levels.

There are various factors that affect the stress response like temperature, net handling, crowding, individual preferences and social interactions (Bonga, 1997) which were effectively kept at a minimum and consistent throughout the experiment. Prior to conducting the experiment, it was also ensured that all fish received 11 hours of natural light to maintain their circadian rhythm.

A previous study shows that in larval zebrafish the serotonergic signalling was affected by light-activated 5-HT (Rea *et al.*, 2013). Many inconsistencies remain pertinent in the results of multiple experiments arising from the differences in various factors like temperature, the intensity of light, age of organisms and other factors (Facciol *et al.*, 2017). Another study also showed that zebrafish larvae did not prefer red colour environments and it decreased their feeding and social behaviour, reducing the chances of survival (Villamizar *et al.*, 2014).

In this experiment, before standardisation, there was a high mortality rate in the red group compared to the other two. Many studies showed zebrafish prefered blue/black environments rather than white, yellow, red or green (Peeters *et al.*, 2016; Jia *et al.*, 2017). A darker environment closely resembles the natural habitats of a riverbank near a forest or a turbid pond (Engeszer *et al.*, 2007). This accounts for the decreased levels of E and NE in the blue group when compared to the red.

5-HT is an analgesic that mediates pain in the central nervous system (Rea *et al.*, 2013). A reduced level of 5-HT at the onset of stress in the two experimental groups suggests that 5-HT metabolism is involved in the stress response. A clearer pathway needs to be established linking the catecholamines and glucocorticoids to the stress and HPA axis. DA release is known to be controlled by the biological clock and day length can also alter the brain's dopaminergic activity (Badruzzaman *et al.*, 2021). Zebrafish are asynchronous breeders and their breeding depends highly on light as they spawn at daybreak (Nasiadka and Clark, 2012). The zebrafish in this study were in the post-vitellogenic stage with fully mature oocytes. Further studies can also look into the relation between colour preference, stress response and its effect on the HPG axis and reproduction.

In conclusion, neurotransmitters play a role in regulating the stress response in zebrafish. The changing levels of neurotransmitters are the first-line response to stress and indicate the onset of ease response. Colour preferences exist and it directly alters the behaviour and response. The blue (450 nm) and daylight (300-700 nm) spectrum are highly preferred over the red colour (650 nm).

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References

Badruzzaman, M., Goswami, C. and Sayed, M. A. 2021. Photoperiodic light pulse induces ovarian development in the catfish, *Mystuscavasius*: Possible roles of dopamine and melatonin in the brain. *Ecotoxicology and Environmental Safety*. 227: 112941. https://doi.org/10.1016/j.ecoenv.2021.112941

Bault, Z. A., Peterson, S. M. and Freeman, J. L. 2015. Directional and color preference in adult zebrafish: Implications in behavioral and learning assays in

neurotoxicology studies. *Journal of Applied Toxicology*. 35: 1502–1510. https://doi.org/10.1002/jat.3169

Bonga, S. E. W. 1997. The stress response in fish. *Physiological Reviews*. 77(3): 591-625. https://doi.org/10.1152/physrev.1997.77.3.591

de Abreu, M. S., Giacomini, A. C. V. V., Genario, R., dos Santos, B., Marcon, L., Demin, K. and Kalueff, A. 2020. The impact of housing environment color on zebrafish anxiety-like behavioral and physiological (cortisol) responses. *General and Comparative Endocrinology*. 294: 113499. https://doi.org/10.1016/j.ygcen.2020.113499

de Abreu, M. S., Giacomini, A., Genario, R., Dos Santos, B. E., Marcon, L., Demin, K. A., Galstyan, D. S., Strekalova, T., Amstislavskaya, T. G. and Kalueff, A. V. 2021. Color as an important biological variable in zebrafish models: Implications for translational neurobehavioral research. *Neuroscience and Biobehavioral Reviews*. 124: 1–15. https://doi.org/10.1016/j.neubiorev.2020.12.014

Engeszer, R. E., Patterson, L. B., Rao, A. A. and Parichy, D. M. 2007. Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish.* 4: 21–40. https://doi.org/10.1089/zeb.2006.9997

Facciol, A., Tran, S. and Gerlai, R. 2017. Re-examining the factors affecting choice in the light-dark preference test in zebrafish. *Behavioural Brain Research.* 327: 21–28. https://doi.org/10.1016/j.bbr.2017.03.040

Fleisch V. C. and Neuhauss S. C. 2006. Visual behavior in zebrafish. *Zebrafish*. 3(2): 191-201. https://doi.org/10.1089/zeb.2006.3.191.

Jia, L., Raghupathy, R. K., Albalawi, A., Zhao, Z., Reilly, J., Xiao, Q. and Shu, X. 2017. A colour preference technique to evaluate acrylamide-induced toxicity in zebrafish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*. 199: 11–19. https://doi.org/10.1016/j.cbpc.2017.01.004

Mommsen, T. P., Vijayan, M. M. and Moon, T. W. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. *Reviews in Fish Biology and Fisheries*. 9: 211-268. https://doi.org/10.1023/A:1008924418720

Nasiadka, A. and Clark, M. D. 2012. Zebrafish breeding in the laboratory environment. *ILAR Journal*. 53(2): 161–168. https://doi.org/10.1093/ilar.53.2.161

Palmer, S. E. and Schloss, K. B. 2010. An ecological valence theory of human color preference. *The Proceedings of the National Academy of Sciences U.S.A*. 107: 8877–8882. https://doi.org/10.1073/pnas.0906172107

Pavlidis, M., Theodoridi, A. and Tsalafouta, A. 2015. Neuroendocrine regulation of the stress response in adult zebrafish, *Danio rerio*. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 60: 121–131. https://doi.org/10.1016/j.pnpbp.2015.02.014.

Peeters, B. W., Moeskops, M. and Veenvliet, A. R. J. Z. 2016. Color preference in *Danio rerio*: effects of age and anxiolytic treatments. *Zebrafish.* 13: 330–334. https://doi.org/10.1089/zeb.2015.1150

Rea, A. C., Vandenberg, L. N., Ball, R. E., Snouffer, A. A., Hudson, A. G., Zhu, Y., McLain, D. E., Johnston, L. L., Lauderdale, J. D., Levin, M. and Dore, T. M. 2013. Light-activated serotonin for exploring its action in biological systems. *Chemistry & Biology*. 20(12): 1536–1546. https://doi.org/10.1016/j.chembiol.2013.11.005

Singh, A. P. and Nüsslein-Volhard, C. 2015. Zebrafish stripes as a model for vertebrate colour pattern formation. *Current Biology*. 25(2): R81–R92. https://doi.org/10.1016/j.cub.2014.11.013

Siregar, P., Juniardi, S., Audira, G., Lai, Y. H., Huang, J. C., Chen, K. H., Chen, J. R. and Hsiao, C. D. 2020. Method Standardization for Conducting Innate Color Preference Studies in Different Zebrafish Strains. *Biomedicines.* 8(8). https://doi.org/10.3390/biomedicines8080271

Sreelekshmi, S., Manish, K., Peter, M. C. S. and Inbaraj, R. M. 2022. Analysis of neuroendocrine factors in response to conditional stress in zebrafish Danio rerio (Cypriniformes: Cyprinidae). *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology.* 252: 109242. https://doi.org/10.1016/j.cbpc.2021.109242.

Villamizar, N., Vera, L. M., Foulkes, N. S. and Sa ìnchez-Va ìzquez, F. J. 2014. Effect of lighting conditions on zebrafish growth and development. *Zebrafish.* 11: 173–181. https://doi.org/10.1089/zeb.2013.0926