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Original Article

Forced swim test-induced serotonin alterations in white laboratory rats

ARBNORE IBRAHIMAJ GASHI[1], VUJICA ZIVKOVIC[2], ICKO GJORGOVSKI[3], SERYOZHA GONTAREV[4], ARJETA AZEMI[5], EQREM GARA[6]
[1,5,6]Physiotherapy department, Universityof Prishtina "Hasan Prishtina" Faculty of Medicine, KOSOVO
[2,4]Faculty of Physical Education, Sport and Health, University "Ss. Cyril and Methodius", NORTH MACEDONIA
[3]Faculty of Natural Sciences and Mathematics, Institute of Biology, University "Ss. Cyril and Methodius", NORTH MACEDONIA

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Abstract
Introduction: Stress induced by continuous swimming activates serotonergic activity in the brainstem and hippocampus. In several animal models of depression, exposure to stressful conditions results in observable behavioral changes, including a shift from proactive to reactive emotional responses, which are believed to reflect signs of depression. The primary aim of this experimental study was to evaluate serotonin levels in Wistar rats subjected to the forced swim test. **Material and Methods:** This experimental study spanned 22 days and involved twenty Wistar rats, divided into two groups of ten. The rats underwent the forced swim test for 21 days, swimming for 60 to 90 minutes each day. Serotonin levels were measured on days one, seven, fourteen, and twenty-one using an ELISA kit protocol. **Results:** The study found no discernible variation in serotonin levels in the control group ($p > 0.20$). After seven days of swimming, serotonin levels in the experimental group measured 0.095, which was lower than in the control group but not statistically significant. Following 14 days of swimming, the experimental rats showed no significant changes in serotonin production or release, with serotonin levels at 0.139—slightly higher than in the control group. After 21 days, serotonin levels increased to 0.249, higher than levels recorded on days seven and fourteen, yet still not significantly different from the control group ($p > 0.20$). **Conclusion:** These findings suggest that prolonged swimming does not significantly increase serotonin release or synthesis. Serotonin levels may instead be influenced by other factors, such as physiological adaptation, duration of exposure, behavioral immobility, or acute stress induced by the forced swim test.
Keywords: serotonin, swimming, aerobic activity, physical stress, rats

Introduction

Serotonin is a neurotransmitter that regulates communication between neurons and functions as a growth factor in certain cell types, indicating its role in wound healing. It performs a wide range of physiological functions, including enhancing motor activity, regulating cerebral blood flow, modulating body temperature, producing analgesic effects, and suppressing appetite Kanova and Kohout, 2021; O'Mahony et al., 2015).

Reduced serotonin production in the nervous system has been linked to mood disorders such as depression and anxiety (Wu et al., 2016). Stress-induced depression is considered a precursor to clinical depression and may be influenced by genetic predisposition. When combined with external stressors, this predisposition can lead to the development of depressive symptoms (Tafet et al., 2016).

In relation to the forced swim test (FST) and the use of drug supplements for depression, one study suggests that taurine may offer a potential treatment for depression in individuals with diabetes (Caletti, Olguins, Pedrollo, Barros, & Gomez, 2012). Furthermore, evidence indicates that prolonged restraint stress may accelerate aging and induce apoptosis (Seo, Park, Choi, Kim, Jeon, & Ryu, 2016). Changes in cortisol levels due to long-term stress exposure have also been associated with an increased risk of various metabolic disorders (Lee, et al., 2007), including obesity, insulin resistance, bone demineralization, and cognitive decline (Hewagalamulage, Lee, Clarke, & Henry, 2016). A recent investigation evaluated the impact of voluntary versus forced exercise on central nervous system functions in rats, concluding that exercise can induce molecular adaptations in neuronal activity (Morgan, Corrigan, & Baune, 2015). Findings from another study suggest that physical exercise during developmental stages may protect against aversive memory impairment and brain oxidative damage caused by chronic stress later in life (Marcon dos Santosa, et al., 2016). According to current evidence, comparisons of various acute stressors in ratssuch as forced swimming, immobilization, noise exposure, and overcrowding over a 7- to 15-day period—indicate that forced swimming is the most stressful activity, while overcrowding is the most harmful (Smitha & Mukkadan, 2014). These results may be attributed to the strenuous nature of forced swimming, which induces both mental and physical stress. The Rat Forced Swimming Test, developed by Porsolt and colleagues (Porsolt, Deniel, & Jalfre, 1978), is a widely used animal model for assessing antidepressant

Corresponding Author: EQREM GARA, E-mail: eqrem.gara@uni-pr.edu

behavior in rats (Slattery & Cryan, 2012). The test involves placing rats in a tank from which they cannot escape and evaluating their active or passive behaviors. Researchers have modified the test for use with mice, offering a shorter, cost-effective, and less training-intensive alternative. Both versions feature unique experimental setups, incorporating a 10–15-minute pretest to emphasize behavioral changes following drug treatment (Cryan, Valentino, & Lucki , 2005).

Swimming, which involves the coordinated movement of the human body in water through the combined action of the upper and lower limbs, is a widely practiced physical activity enjoyed by both healthy and unhealthy individuals. The exercise and flexibility involved in swimming can help alleviate tension, thereby reducing anxiety and stress. Consequently, a growing number of researchers and psychologists advocate for its effectiveness (Greer & Hamer, 2012). Exercise is known to increase serotonin levels in the animal brain, and the serotonin system is believed to play a significant role in the neuronal effects induced by physical activity (Kondo & Shimada, 2015). Swimming, in particular, can promote "hippocampal neurogenesis"—the growth of new brain cells in a region that typically atrophies under chronic stress. Controlled studies have shown that stress from continuous swimming activates serotonergic activity in the brainstem and hippocampus within the first 15 minutes of exposure, under regulated conditions of time and water temperature (Jiang et al., 2014).

According to the literature, aerobic exercise demonstrates therapeutic effects comparable to those of pharmacological treatment or psychological therapy in patients with depressive disorders. The influence of non-pharmacological interventions on brain serotonin, and the broader implications of elevated serotonin for mood and behavior, should be more widely recognized and applied (Chen, Nakagawa, An, Ito, Kitaichi, & Kusumi, 2017).

When comparing different types of acute stressors in rats—such as forced swimming, immobilization, noise, and overcrowding over a period of 7 to 15 days some researchers concluded that forced swimming was the most stressful activity, while overcrowding was the most harmful (Smitha & Mukkadan, 2014). These results may stem from the strenuous nature of forced swimming, which induces both mental and physical stress. The primary objective of this experimental study was to observe the effect of the forced swim test on serotonin levels in female Wistar rats.

Materials and Methods

Ethical Statement

The experimental procedures and protocols were approved in accordance with the *Manual for Care and Use of Laboratory Animals* and were authorized by the Macedonian Center for Bioethics and the Ethics Committee for Animals at the University "St. Kiril and Metodij – Skopje." The study followed recommendations for biomedical research involving animals as outlined by the Council of International Organizations for Medical Sciences. Anesthetics were administered in compliance with the guidelines of EU Directive 86/609/EEC.

Experimental Procedures

This experimental study lasted 22 days and involved twenty Wistar rats, divided into two groups of ten. The first group served as the control, while the second group underwent the forced swim test for 21 days. During the first week, the rats swam for 60 to 90 minutes per day over six consecutive days. In the following weeks, the duration gradually increased from 50 to 90 minutes per day, with sessions conducted between 08:30 AM and 12:00 PM. This intensity was maintained until the end of the training program. The rats swam in a cylindrical tank filled with tepid water (±34˚C) with a depth that exceeded the rats' body length and width. The swimming sessions were recorded using video and photo cameras (Figure 1).

Figure 1. The rats exposed to Forced Swimming test

Blood samples were collected from the tail of each rat on the first, seventh, and fourteenth days of the experiment to assess serotonin levels. During the final seven days, five rats from the experimental group continued the swimming protocol. On day twenty-one, all rats were euthanized, and blood was collected from the abdominal aorta. The samples were centrifuged at 3000 rpm for 15 minutes at 4°C. The resulting serum was analyzed for serotonin concentration using the ELISA technique.

This research was conducted at the Faculty of Natural Science and Mathematics, Institute of Biology in Skopje. Throughout the study, the subjects were provided with a standard diet and water *ad libitum* and were housed in a controlled environment with a consistent 12-hour light/dark cycle (light from 06:00 to 18:00) at a thermoneutral temperature of 26°C. This study represents the third phase of a broader experiment; in this paper, the results related to serotonin levels are analyzed and presented, while findings on adrenaline and cortisol have been reported in separate publications (Gashi et al., 2020; 2020a; 2021).

Collection and Preparation of Blood Serum and Plasma

Blood collection was consistently performed in the morning, prior to subjecting the rats to the swimming protocol. Samples were obtained from the tail vein on days 1, 7, and 14 while the rats were awake, eliminating the need for anesthesia. A small incision, approximately 2–3 mm from the tip of the tail, was made over the lateral tail vein using a sterile scalpel blade. Blood was collected by allowing it to drip into a 1.5 ml collection tube and was left to clot for 30 minutes at room temperature.

The serum was isolated by centrifuging at 3000 rpm for 15 minutes. Following centrifugation, 0.75 ml of serum was collected and stored at -20˚C. Hormone levels were measured using the serotonin ELISA kit protocol (ABNOVA, KA1894). On the final day of the experiment (day 21), all experimental rats were euthanized, and blood was drawn from the abdominal artery. Whole blood was collected into centrifuge tubes containing potassium EDTA as an anticoagulant and was immediately centrifuged at 3000 rpm for 15 minutes. After centrifugation, 5–6 ml of plasma was collected and stored at -20˚C. The analysis was conducted using the same ELISA kit technique.

Statistical Analyses

From the existing methods of data processing, those commonly used in previous research and suitable for the specific characteristics of the obtained data were selected. For all variables measured on an interval scale, the following basic statistical parameters were calculated: arithmetic mean (X), standard deviation (SD), coefficient of variability (V), minimum (MIN), maximum (MAX), asymmetry (skewness), and flatness (kurtosis) of the distribution. The Kolmogorov-Smirnov (KS) test was used to assess the normality of the data distribution. A multivariate analysis was performed to evaluate the effect of time on the dependent variables, using Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root. Differences between variables were analyzed using one-way repeated-measures ANOVA.

Results

As presented in Table 1, the control group had a mean serotonin level of 0.47 with a standard deviation (SD) of 0.11, indicating moderate variability. The coefficient of variation (CV) was 23.26%, suggesting relatively high variability. The distribution showed a negative skewness of -1.37, indicating a slight leftward skew, while kurtosis was also -1.37, suggesting a flatter-than-normal distribution (platykurtic). The mean value on the seventh day of swimming was 0.43, slightly lower than the control group, with an SD of 0.06, which is lower than the control, suggesting less variability. A slight positive skewness (0.22) indicates that the distribution has a longer tail to the right, while kurtosis (0.82) shows it to be higher than normal, indicating a more peaked distribution (leptokurtic).

By the fourteenth day of swimming, the mean serotonin level further decreased to 0.37, with an SD of 0.06. The group that rested starting on day fourteen exhibited a similar mean value (0.37) with a slightly lower SD of 0.04, indicating low variability. On the twenty-first day, the swimming group recorded the lowest mean serotonin level (0.36) across all groups, with an SD of 0.05, reflecting low variability (Table 1).

Table 1: Statistical data of Serotonin levels among groups

SEROTONIN	Mean	Min	Max	SD	CV	s.e.	Skew	Kurtos
CONTROL	0,47	0,33	0,65	0,11	23,26	0,03	0,02	-1,37
7 DAYS SWIM	0,43	0,32	0,57	0,06	13,79	0,01	0,22	0,82
14 DAYS SWIM	0,37	0,29	0,48	0,06	15,18	0,01	0,15	-0,73
REST FROM DAY 14	0,37	0,28	0,41	0,04	11,84	0,01	-1,01	-0,24
21 DAY SWIM	0,36	0,30	0,49	0,05	14,67	0,01	1,68	2,59

Serotonin levels in the control group were 0.153, with a p-value greater than 0.20, suggesting no significant variation from baseline levels. The lack of statistical significance indicates that there were no discernible changes in serotonin levels in the control animals throughout the trial. After seven days of swimming, serotonin levels decreased to 0.095, which is lower than the control group. However, this reduction in serotonin levels was not statistically significant, as the p-value remained greater than 0.20. This suggests that, unlike the control group, seven days of swimming did not induce a noticeable change in serotonin levels.

Following a 14-day swimming period, the experimental subjects showed no significant changes in serotonin production or release. Serotonin levels increased to 0.277 after a rest period beginning on Day 14. The p-value in this case was lower than 0.15, indicating a potential trend toward statistical significance, although it did not reach the conventional threshold of $p < 0.05$.

Following 14 days of swimming, serotonin levels increased slightly to 0.139, showing a modest rise compared to day seven of the swimming group. However, these levels remained lower than those of the control group. Once again, the p-value exceeded 0.20, indicating that the increase in serotonin levels after 14 days of swimming was not statistically significant. On day 21 of swimming, serotonin levels were measured at 0.249, higher than the levels recorded on days seven and fourteen but not significantly different from the control group ($p > 0.20$) (Table 2).

Table 2: The results of Kolmogorov Smirnov's procedure for Serotonin

SEROTONIN	max D	K-S
CONTROL	0,153	p > .20
7 DAYS SWIM	0,095	p > .20
14 DAYS SWIM	0,139	p > .20
REST FROM DAY 14	0,277	p < ,15
21 DAY SWIM	0,249	p > .20

A multivariate analysis was performed to evaluate the effect of time on the dependent variables. The results from multiple statistical tests (Pillai's Trace, Wilks' Lambda, Hotelling's Trace, and Roy's Largest Root) consistently indicated a statistically significant effect of time ($p = 0.001$). The partial eta squared value of 0.76 suggests that time accounted for 76% of the variance in the outcome variables, indicating a large effect size (Table 3).

Table 3. The results of one-factor analysis of the variance of repeated measurements for Serotonin

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Time	Pillai's Trace	0,76	10,51	4,00	13,00	0,001	0,76
	Wilks' Lambda	0,24	10,51	4,00	13,00	0,001	0,76
	Hotelling's Trace	3,24	10,51	4,00	13,00	0,001	0,76
	Roy's Largest Root	3,24	10,51	4,00	13,00	0,001	0,76

Post-hoc analyses (Table 4) revealed significant differences, primarily between Conditions 1 and 5. Condition 1 consistently exhibited lower values compared to the other conditions, while Condition 5 demonstrated significant differences from the other conditions, with higher mean values in several comparisons. Condition 3 showed fewer significant differences, particularly when compared to Conditions 4 and 5.

Table 4. The comparison of Serotonin serum among groups

(I) factor1		Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
1	2	,041	,034	,245	-,031	,113
	3	,104*	,030	,003	,040	,169
	4	,101*	,024	,001	,050	,152
	5	,110*	,031	,002	,046	,175
2	1	-,041	,034	,245	-,113	,031
	3	,063*	,021	,009	,018	,108
	4	,060*	,019	,007	,019	,101
	5	,069*	,013	,000	,042	,097
3	1	-,104)*	,030	,003	-,169	-,040
	2	-,063)*	,021	,009	-,108	-,018
	4	-,004	,016	,827	-,037	,030
	5	,006	,019	,754	-,034	,046
4	1	-,101)*	,024	,001	-,152	-,050
	2	-,060)*	,019	,007	-,101	-,019
	3	,004	,016	,827	-,030	,037
	5	,010	,014	,516	-,021	,040
5	1	-,110)*	,031	,002	-,175	-,046
	2	-,069)*	,013	,000	-,097	-,042
	3	-,006	,019	,754	-,046	,034
	4	-,010	,014	,516	-,040	,021

These findings emphasize the influence of the experimental conditions on the measured outcomes, with Conditions 1 and 5 showing the most pronounced differences across the groups.

Discussion

The primary objective of this experimental study was to observe the effect of the forced swim test on serotonin levels in Wistar rats, which were divided into two groups of ten and forced to swim for 21 days in a cylindrical swim tank. The results showed changes in serotonin levels across different groups and time points. The control group had a mean serotonin level of 0.47, with a high coefficient of variation. The negative skew and platykurtic distribution suggest a flatter distribution compared to a normal curve. At the seven-day mark, serotonin levels in the experimental group were slightly lower than those of the control group. The study demonstrated a significant decrease in serotonin levels after the swimming protocol, reaching a low point on Day 21. In contrast, the control group showed no significant changes in serotonin levels. These results support the idea that intense aerobic activity, such as forced swimming, can induce stress and temporarily reduce serotonin levels as the brain prioritizes exercise demands. This aligns with previous studies suggesting that exercise-induced stress can increase the synthesis of stress hormones, which may interfere with serotonin production during prolonged physical exertion (Arnold et al., 2020).

The mean serotonin levels in the rest group were consistent with those in the seven-day swimming group, and the rest period showed similar levels to the 14-day swimming group, but with lower variability. The increase in serotonin levels after 14 days of swimming was not significant, suggesting that serotonin production or release did not undergo significant changes after two weeks of swimming. These findings imply that serotonin levels may have stabilized following the prolonged swimming protocol. A recent study observed the effects of both short- and long-term exercise on serotonin levels and confirmed that while physical activity, such as swimming, can temporarily influence serotonin activity, major shifts in serotonin levels tend to stabilize after a few weeks as the body adapts to the new exercise routine (Gomez-Pinilla & Hillman, 2013).

According to the results, after a rest period beginning on Day 14, serotonin levels increased significantly to 0.277, indicating a potential trend toward statistical significance. This suggests that the rest period allowed for recovery or adaptation, which may have influenced serotonin levels. However, further investigation is required to determine whether this increase is attributable to the rest period itself or to an underlying biological response to the stress of prior swimming. After 21 days of swimming, serotonin levels were higher than those observed in the earlier phases of the experiment but did not significantly differ from control group levels. This suggests that prolonged swimming does not have a significant cumulative effect on serotonin release or synthesis. Despite this, there is positive evidence supporting the effect of the forced swim test on serotonin levels, demonstrating an increase in serotonin in proportion to the duration of the swimming protocol. Aerobic exercises, such as swimming, cycling, or running, are generally believed to increase serotonin levels, especially when performed regularly over extended periods. However, short-term fluctuations in serotonin levels and, in some cases, a decrease in serotonin activity can result from acute aerobic exercise (Arnold et al., 2020). Previous findings from this in vivo experiment confirm that aerobic physical activity positively affects cortisol and adrenaline levels. Therefore, the forced swim test can serve as a non-pharmacological strategy to enhance the stress hormone response (Gashi et al., 2020; 2020a; 2021). Regular aerobic exercise may promote serotonin production and receptor sensitivity over time, potentially leading to long-term improvements in mood (Zhou et al., 2022). This explains why consistent aerobic exercise over weeks or months is often associated with enhanced mood and mental health, even though the short-term effects may not be as pronounced. The Forced Swim Test conducted with Wistar rats may have significant clinical relevance, particularly regarding serotonin levels and their alterations during physical stress. These findings could influence the development of new treatment approaches for mood disorders and depression, offering alternative options beyond traditional drug therapies.

Conclusion

The results suggest that prolonged swimming does not significantly enhance the release or synthesis of serotonin over 21 days. Statistical analysis revealed no significant changes in serotonin production across the experimental groups, indicating that serotonin levels may be influenced by other factors, such as adaptation, prolonged duration, immobility, or acute stress induced by the forced swim test. Further research involving larger groups of Wistar rats, extended time frames, or alternative methodologies may be necessary to gain a more comprehensive understanding of the impact of the forced swim test on serotonin fluctuations.

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Conflict of interest: None declared.

References

Arnold, M. R., Greenwood, B. N., McArthur, J. A., Clark, P. J., Fleshner, M., & Lowry, C. A. (2020). Effects of repeated voluntary or forced exercise on brainstem serotonergic systems in rats. *Behavioural brain research*, *378*, 112237. https://doi.org/10.1016/j.bbr.2019.112237

Caletti, G., Olguins, D. B., Pedrollo, E. F., Barros, H. M., & Gomez, R. (2012). Antidepressant effect of taurine in diabetic rats. *Amino Acids, 43*(4), 1525-33.

Chen, C., Nakagawa, S., An, Y., Ito, K., Kitaichi, Y., & Kusumi, I. (2017). The exercise-glicocoticoid paradox: How exercise is beneficial for cognition, mood, and the brain while increasin glucocorticoid levels. *Frontiers in Neuroendocrinology, 44*, 83-102.

Cryan, J. F., Valentino, R. J., & Lucki , I. (2005). Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *neurosciennce and Biobehavioral reviews, 29*,

Dos Santos, T. M., Kolling, J., Siebert, C., Biasibetti, H., Bertó, C. G., Grun, L. K., Dalmaz, C., Barbé-Tuana, F. M., & Wyse, A. T. (2017). Effects of previous physical exercise to chronic stress on long-term aversive memory and oxidative stress in amygdala and hippocampus of rats. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*, *56*, 58–67. https://doi.org/10.1016/j.ijdevneu.2016.12.003

Gomez-Pinilla, F., & Hillman, C. (2013). The influence of exercise on cognitive abilities. *Comprehensive Physiology*, *3*(1), 403–428. https://doi.org/10.1002/cphy.c110063

Greer, T. L., & Hamer, R. M. (2012). The role of exercise in reducing depression and anxiety in adolescents: a review of the literature. *Psychology of Sport and Exercise*, 13(6), 688-696. HYPERLINK "https://doi.org/10.1016/j.psychsport.2012.05.001" https://doi.org/10.1016/j.psychsport.2012.05.001

Gashi, A. I., Gontarev, S., Zivkovic, V., Gjorgovski, I., & Azemi, A. (2020). The Effect of Aerobic Physical Activity in Adrenaline Level in White Laboratory Rats. *Medical archives (Sarajevo, Bosnia and Herzegovina)*, *74*(2), 84–89. https://doi.org/10.5455/medarh.2020.74.84-89

Gashi, A. I., Zivkovic, V., Gjorgovski, I., Gontarev, S., & Azemi, A. (2020a). Regular physical activity may influence stress hormone cortisol in Wistar rats. Journal of Physical Education and Sport, *20*(1),138–141. DOI:10.7752/jpes.2020.01018

Gashi, A. I., Azemi, A. Zivkovic, V., Gontarev, S., & Gjorgovski, I., (2021). HYPERLINK "https://scholar.google.com/citations?view_op=view_citation&hl=en&user=cmUQRPUAAAAJ&citation_for_view=cmUQRPUAAAAJ:eQOLeE2rZwMC" The effect of regular aerobic activity on cortisol levels in female Wistar rats . Biomedical Research and Therapy, *8*(2), 4219-4227.DOI :10.15419/bmrat.v8i2.660

Hewagalamulage, S. D., Lee, T. K., Clarke, I. J., & Henry, B. A. (2016). Stress, cortisol, and obesity: a role for cortisol responsiveness in identifying individuals prone to obesity. *Domestic Animal Endocrinology, 56*, S112-S120.

Jiang, P., Dang, R. L., Li, H. D., Zhang, L. H., Zhu, W. Y., Xue, Y., & Tang, M. M. (2014). The impacts of swimming exercise on hippocampal expression of neurotrophic factors in rats exposed to chronic unpredictable mild stress. *Evidence-based complementary and alternative medicine : eCAM*, *2014*, 729827. https://doi.org/10.1155/2014/729827

Kondo, M., & Shimada, S. (2015). Serotonin and exercise-induced brain plasticity. *Neurotransmitter, 2*, e793.

Kanova, M., & Kohout, P. (2021). Serotonin-Its Synthesis and Roles in Healthy and the Critically Ill. *International journal of molecular sciences*, *22*(9), 4837. HYPERLINK "https://doi.org/10.3390/ijms22094837" https://doi.org/10.3390/ijms22094837

Lee, B. K., Glass, T. A., Mcatee, M. J., Wand, G. S., Banden-Roche, K., Bolla, K. I., & Schwartz, B. S. (2007). Associations of salivary cortisol with cognitive function in the Baltimore memory study. *Arch Gen Psychiatry, 64*(7), 810-818.

Morgan, J. A., Corrigan, F., & Baune, B. T. (2015). Effects of physical exercise on central nervous system functions: a review of brain region specific adaptations. *Journal of molecular psychiatry*, *3*(1), 3. https://doi.org/10.1186/s40303-015-0010-8

O'Mahony, S. M., Clarke, G., Borre, Y. E., Dinan, T. G., & Cryan, J. F. (2015). Serotonin, tryptophan metabolism and the brain-gut-microbiome axis. *Behavioural brain research*, *277*, 32–48. HYPERLINK "https://doi.org/10.1016/j.bbr.2014.07.027" https://doi.org/10.1016/j.bbr.2014.07.027

Porsolt, R. D., Deniel, M., & Jalfre, M. (1978). Behavioural despair in rats and mice : strain differences and the effects of imipramine. *European Journal of Pharmacology, 51*(3), 291-294.

Seo, H., Park, C.-H., Choi, S., Kim, W., Jeon, B.-D., & Ryu, S. (2016). Effects of voluntary exercise on apoptosis and cortisol after chronic restraint stress in mice. *J exerc Nutrition biochem, 20*(3), 016-023.

Slatteryl, D. A., & Cryan, J. F. (2012). Using the rat forced swim test to assess antidepressant-like activity in rodents. *Nature Protocols, 7*(6), 1009-1014.

Smitha, K. K., & Mukkadan, J. K. (2014). Effect of different forms of acute stress in the generation of reactive oxygen species in albino wistar rats. *Indian J Physiol Pharmacol, 58*(3), 228-231.

Wu, T., et al. (2016). The role of serotonin in depression and the relationship between serotonin and stress. *Frontiers in Psychology*, 7, 1003. https://doi.org/10.3389/fpsyg.2016.01003

Zhou, Y. S., Meng, F. C., Cui, Y., Xiong, Y. L., Li, X. Y., Meng, F. B., Niu, Z. X., Zheng, J. X., Quan, Y. Q., Wu, S. X., Han, Y., & Xu, H. (2022). Regular Aerobic Exercise Attenuates Pain and Anxiety in Mice by Restoring Serotonin-Modulated Synaptic Plasticity in the Anterior Cingulate Cortex. *Medicine and science in sports and exercise*, *54*(4), 566–581. https://doi.org/10.1249/MSS.0000000000002841