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Research Article | Araştırma Makalesi

NEUROPEPTIDE W (NPW) ALLEVIATES COGNITIVE IMPAIRMENT AND ANXIETY-LIKE BEHAVIORS IN RATS WITH CHRONIC STRESS

NÖROPEPTİT W (NPW), KRONİK STRESLİ SIÇANLARDA BİLİŞSEL BOZUKLUĞU VE KAYGI BENZERİ DAVRANIŞLARI HAFİFLETİR

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ABSTRACT

Objective: Neuropeptide W (NPW) plays a regulatory role in the neuroendocrine response to stress and the activation of the Hypothalamic–pituitary–adrenal (HPA) axis. The current study aimed to investigate the effects of peripherally administered NPW on the cognitive functions and anxiety-like behavior of rats under chronic stress conditions.

Methods: Wistar albino (250-290 gr) male rats were randomly divided as control, chronic stress group, and chronic stress exposed NPW-treated (0.1 µg/kg, subcutaneously) group. Chronic stress was induced by exposing the animals to water avoidance stress (WAS) for 1 h/day for ten consecutive days. At the end of the experimental stress procedure, an object recognition test was used to evaluate cognitive functions, and a hole-board test was used to assess anxiety levels. After the experiments, blood samples were collected to measure corticosterone levels.

Results: WAS significant increases in the level of corticosterone when compared with control rats ($p<0.05$), showing the activation of HPA axis. Application of WAS also caused anxiety and diminished cognitive functions. In NPW-treated rats with WAS, the corticosterone level was not different from the control group. The number of rearing up and head-dips by WAS-induced rats increased after NPW application ($p<0.05$-0.01) and also decreased the immobilization time ($p<0.01$). Moreover, the difference score in the object recognition test was increased by the NPW application in WAS group ($p<0.05$).

Conclusion: Our results showed for the first time that NPW pretreatment exerted an anxiolytic effect and attenuated cognitive function in rats subjected to water avoidance stress.

Keywords: Neuropeptide W, stress, anxiety, cognitive function, memory.

ÖZ

Amaç: Nöropeptit W (NPW), NPW, strese karşı nöroendokrin yanıtta ve hipotalamik-hipofiz-adrenal (HPA) ekseninin aktivasyonunda düzenleyici bir rol oynar. Bu çalışmanın amacı, kronik stres koşullarında periferik olarak uygulanan NPW'nin sıçanların bilişsel işlevler ve kaygı benzeri davranışlar üzerindeki etkilerini araştırmaktır.

Yöntem: Wistar albino (250-290 gr) erkek sıçanlar kontrol, kronik stres grubu ve kronik strese maruz kalan ve NPW ile tedavi edilen (0.1 µg/kg, subkutan) grup olarak rastgele ayrıldı. Kronik stres indüksiyonu hayvanlara art arda on gün boyunca günde 1 saat sudan kaçınma stresi (WAS) uygulanarak yapıldı. Deneysel stres işleminin sonunda bilişsel işlevleri değerlendirmek için obje tanıma testi, kaygı düzeylerini değerlendirmek için delikli tahta testi kullanıldı. Deneylerden sonra, kortikosteron seviyelerini ölçmek için kan örnekleri alındı.

Bulgular: Kontrol sıçanları ile karşılaştırıldığında WAS grubunda kortikosteron seviyesinde anlamlı artış görüldü ($p<0.05$), bu da HPA ekseninin aktivasyonunu göstermektedir. WAS uygulanması ayrıca kaygıya ve bilişsel işlevlerin azalmasına neden oldu. WAS uygulanıp NPW ile tedavi edilen sıçanlarda, kortikosteron seviyesi kontrol grubundan farklı değildi. NPW uygulaması, WAS ile indüklenen sıçanlarda şaha kalkma ve burun sokma sayılarını artırdı ($p<0.05$-0.01) ve immobilizasyon süresini azalttı ($p<0.01$). Ayrıca WAS grubunda NPW uygulaması ile obje tanıma testinde fark skoru arttı ($p< 0.05$).

Sonuç: Sonuçlarımız ilk kez NPW ön tedavisinin sudan kaçınma stresi uygulanan sıçanlarda anksiyolitik etki gösterdiğini ve bilişsel işlevi hafiflettiğini gösterdi.

Anahtar Kelimeler: Nöropeptit W, stres, anksiyete, bilişsel işlev, hafıza.

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Introduction

Stress is regulated by the autonomic nervous system and increases sympathetic modulation when a threat is detected.[1] The hypothalamic-pituitary-adrenal (HPA) axis is an intricate hormonal network that is activated by stress.[2] Physiological and psychological stresses cause corticotropin-releasing hormone (CRH) to be secreted from the hypothalamus. CRH causes pituitary corticotrope cells to secrete adrenocorticotropic hormone (ACTH), which in turn causes the adrenal cortex to secrete cortisol. Chronic stress (CS) disturbs HPA axis and increases glucocorticoid release, which concerns neural plasticity in the hippocampus and may lead to learning and memory deficits.[3] Experimental studies have shown that stress induction causes learning and memory dysfunction and increases anxiety-like behaviors.[4,5] Repeated unpredictable stress and social isolation elevate hypothalamic CRH and cause HPA axis dysfunction, which is associated with mental and physical illness.[6,7]

CS also contributes to the formation of oxidative stress, which is involved in developing neurological diseases such as depression and Alzheimer's disease.[8]

Neuropeptide W (NPW) is a newly defined neuropeptide with two endogenous molecular isoforms, 23 and 30 amino acids. Two distinct G-protein-coupled receptors called NPBWR1 (GPR7) and NPBWR2 (GPR8) mediate the effects of NPW. [9,10] NPW has regulatory functions in the activation of the HPA axis and the neuroendocrine response to stress.[11] Peripheral application of NPW has been reported to exhibit neuroprotective and antioxidant effects in hypoxic-ischemia-induced brain injury.[12] However, the role of NPW on chronic stress-induced cognitive impairment has not yet been clarified. The aim of the current study was to investigate the effects of peripherally administered NPW on the cognitive functions and anxiety-like behavior of rats under chronic stress conditions.

Methods

Animals

Wistar albino male rats weighing between 250 and 290 grams, provided by the Sakarya University Animal Center (SÜDETAM), were housed in an air-conditioned space with 12-hour day and dark cycles, where the temperature was maintained at 22°C and the relative humidity at between 67% and 70%. The animals were fed a standard pellet and had unrestricted access to food and water. The principles and criteria established by the New York Academy of Sciences were followed, and the tests were carried out in accordance with Turkish law regarding the use of animals in experiments. The experimental procedures were approved by the Sakarya University Animal Care and Use Committee (approval number: 35; date: 06/07/2022).

Experimental Procedures

Rats were randomly divided as control (n=7), a chronic stress group (n=7), and chronic stress-exposed NPW-treated group (n=7). NPW (Phoenix Pharmaceuticals, USA) was applied subcutaneously at dose of 0.1 µg/kg before water avoidance stress (WAS). Saline as NPW solvent was administrated similarly in chronic stress group. Dose of NPW was selected based on the previous reports.[13]

An object recognition test was utilized to assess cognitive abilities after the experimental stress technique, and a hole-board test was performed to assess anxiety levels. Hole-board test was repeated before and after the stress procedure. After the experiments, the cardiac puncture was made to obtain blood samples from rats under anesthesia (100 mg kg^{-1} ketamine and 10 mg kg^{-1} xylazine, intraperitoneally), and the experimental animals were killed.

Water Avoidance Stress-WAS

The animals were subjected to WAS for one hour every day for ten straight days in order to create chronic psychological stress in them. Before the study, the same researcher handled rats every day for two weeks before subjecting them to WAS. The plastic container with a 90 cm diameter and 50 cm height was filled with fresh, room-temperature water to a depth of 1 cm below the surface of the platform, and rats in the WAS group were placed on a platform (8x8 cm) mounted in the center of the container. All experimental procedures were performed between 02:00 and 04:00 pm hours to minimize the effect of a circadian rhythm.

Measurements of Corticosterone Levels

According to the manufacturer's instructions, serum corticosterone levels (E0828Ra, BT LAB) were determined using rat ELISA kits. Corticosterone level was expressed as ng/ml.

Evaluation of the Anxiety Level

All rats underwent the hole-board test using a wooden box (100x100x50 centimeter) with 16 evenly spaced holes (3.8 cm in diameter) at the bottom. Each rat was placed separately in one of the box's corners, and a video camera captured all of its motion for five minutes. The box was cleansed with alcohol after each test to eliminate the odor. The number of head dips into the holes and rearing up on two hind legs were then recorded from videotapes by a watcher who was blind to the experimental groups to assess the rats' level of anxiety based on their exploratory behavior in the box. Rats with less head dipping and rearing up showed less exploratory activity, which led to increased anxiety.[14]

Object Recognition Test

A test for object recognition was performed to evaluate the cognitive function of the rats. The rat was given a day before the object identification test to acclimate to the new environment by spending 10 minutes in the empty test box (50x50x30 cm). The rat was then placed in the

same box on the 10th day of the experiment to examine the two identical objects for ten minutes before being returned to its cage. A video camera captured the rat's interest in the new object for three minutes an hour later after one of the objects was replaced (new object) while keeping one familiar object. The box and the items were cleaned with alcohol (70%), following each test. By comparing the amount of time spent with the known and unfamiliar things, recordings were processed and the outcomes were assessed. The number of contacts defined as determined the contact of the rat's nose with the object was evaluated. The following formula was used to determine the time difference spent with the objects:

Difference score (sec): time spent with the novel object – time spent with the sample object.[15] An improvement in cognitive processes was indicated by a higher difference score.

Statistical Analysis

One-way ANOVA followed by the Bonferroni multiple comparisons test was used to define the level of statistically significant between experimental groups by using GraphPad Prism 9.3.0 (GraphPad Software, San Diego, CA, USA). Student-t test was applied to compare two groups. The mean values and standard errors for all the data were reported. Statistical significance was determined to be $p<0.05$.

Results

Serum corticosterone levels were measured to assess for HPA axis activation during stress response in the control or stress-applied groups. When compared with the control rats, WAS significantly increased in the level of corticosterone compared with control rats ($p<0.05$; Figure 1), showing the activation of HPA axis. In NPW-treated rats with WAS, the corticosterone level was not changed respected to the control group.

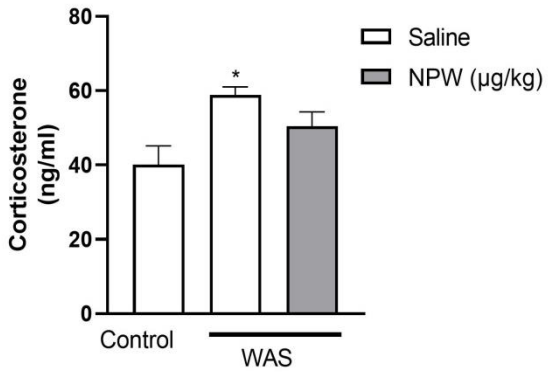

Figure 1. The corticosterone levels of control and water avoidance stress (WAS) groups were treated with either saline or NPW (0.1 µg/kg/day). *p<0.05, compared to the control group.

The hole-board test was performed to evaluate the degree of anxiety in the experimental groups. Elevation of the immobilization time and reduction in the rats' free exploratory behavior (head-dipping and rearing up) demonstrated increased anxiety. Application of WAS for ten days caused the increased immobilization time and reduced the numbers of head-dipping and rearing up compared to the control group ($p<0.05$-0.001; Figure 2), exhibiting increased anxiety. NPW treatment elevated the numbers of rearing up and head-dips by WAS-induced rats ($p<0.05$-0.01) and also decreased the immobilization time ($p<0.01$), indicating the anxiolytic effect of NPW.

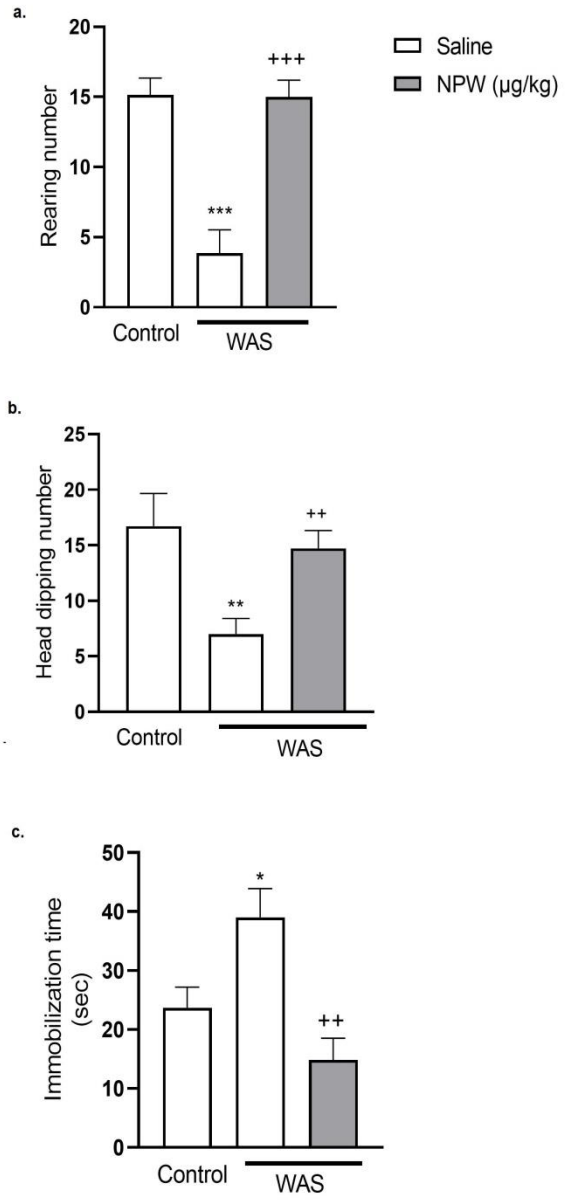

Figure 2. Numbers of rearing (a), head-dipping (b), and immobilization time (c) were recorded during the hole-board test of all experimental groups. *p<0.05, ** p<0.01, ***p<0.001, compared to control group; ++p<0.01, +++p<0.001 compared to saline-treated WAS group.

When performing an object recognition test to evaluate cognitive function, the difference of the time spent with novel and same objects has reduced in WAS group compared to the control group ($p<0.05$; Figure 3), demonstrating diminished cognitive functions. The difference score was increased by the NPW application in WAS group ($p< 0.05$).

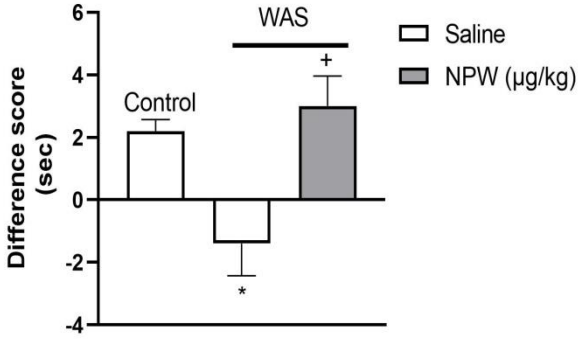

Figure 3. Object recognition test results of control or water avoidance stress (WAS) groups rats that were treated with either saline or NPW (0.1 µg/kg/day). Difference score (second): Time spent with the novel object − time spent with the sample object. *p<0.05, compared to control group; +p<0.05, compared to saline-treated WAS group.

Discussion

The results of the present study show that the application of WAS in rats created a cognitive impairment with increased activity of the HPA axis and anxiety-like behavior. Our results showed for the first time that NPW pretreatment exerted an anxiolytic effect and attenuated cognitive function in rats subjected to water avoidance stress.

The term "stress" refers to a situation in which the homodynamic balance is endangered by various stressors, which might be intrinsic or extrinsic, actual or imagined difficulties or stimuli.[16] Stress and the neurobiological alterations have been linked to depression, anxiety, and cognitive dysfunction in many studies.[17,18] The HPA axis has an important role in this research area because it is a modulator of pathophysiological alterations and a marker of the stress response.[19] It was shown that reduced dopaminergic transmission and dysregulation of the HPA axis, which is characterized by glucocorticoid negative feedback resistance, is frequently seen in stress-induced depression.[20] Resulting from a dysregulated HPA axis, increased corticosterone has been linked to behavioral and cognitive abnormalities.[21] The clinical results showed that hyperactivity of the HPA axis was strongly associated with depressive and anxiety disorders.[22] It was reported that chronic stress in rodents can lead to anomalies in the HPA axis, including elevation of corticosterone levels.[23,24] In the present study, we showed that serum corticosterone level was elevated in WAS-induced rats, demonstrating that the HPA axis was activated, which supports the previous study that demonstrated the elevation of corticosterone level due to WAS in mice.[25] Peripheral NPW application restored the secretion of corticosterone to normal levels. The production of corticosterone, which could be increased by the elevation of HPA axis activity, was inhibited by peripheral administration of NPW prior to stress ulcer formation, as we recently described.[13,26] Contrary to these findings, NPW encourages human adrenocortical cells to secrete cortisol [27] and has been found to increase plasma corticosterone levels in healthy rats.[28]

Water avoidance stress is widely used as a model of chronic psychological stress.[29] Chronic stress causes neuronal changes that lead to the development of anxiety, depression, cognitive decline and memory impairment.[30,31] Stressful situations cause the hypothalamic paraventricular nucleus to release corticotrophin-releasing factor (CRF), which then causes the pituitary to release ACTH, which in turn causes the adrenal cortex to secrete cortisol in humans and corticosterone in rodents.[32] Also, stressful events may result in blood-brain barrier malfunction, characterized by inflammation and leaking.[34] These all-important factors can contribute to the disturbance of cognitive functions and the development of anxiety and depression.[33,34] In this study, we found that the administration of NPW ameliorated WAS-induced anxiety-like behaviors observed in the hole board test. The hole board test is a method to evaluate anxiety, it can also be used in experimental studies for the anxiogenic-like effect of pharmacological agents, hormones, and drugs.[14] To our knowledge, there is no study on the anxiolytic effects of NPW in chronically stressed animals. The results of the object recognition test showed that water avoidance stress-induced cognitive impairments and NPW significantly prevented this memory dysfunction. Several studies demonstrated that synaptic plasticity was decreased, and cognitive impairment was brought on by stress-related impairment of the hippocampus' neuronal activity.[35] On the other hand, The BDNF/TrkB/CREB system controls the proliferation, differentiation, and migration of hippocampal neurons, which are crucial for cognitive function.[36] The increased expression of BDNF, TrkB, the pCREB/CREB ratio, and the inhibition of acetylcholinesterase activity were associated with cognitive improvements.[37] It was reported that about the interrelationships between BDNF and NPW, BDNF could modulate the expression of NPW in neuronal cells via the PI3K/Akt pathway and the application of exogenous BDNF to mice increased synthesis of NPW and also CRH levels in the hypothalamus.[38] NPW participates in the neuroendocrine regulation of pituitary hormone production and may have a significant role in the hypothalamic organization of the endocrine response to stress.[11,39] Central administration of NPW elevated ACTH levels via activating hypothalamic CRF in rats.[40] Additionally, pre-treatment with NPW did not affect serum levels of CRH or ACTH in rats with stress-induced stomach ulcers, but it did bring corticosterone back to normal ranges.[13] It indicates that NPW does not directly affect the activation of the HPA axis brought on by stress; instead, it may be inflammatory mediators released by the wounded stomach. Cognitive dysfunction may be significantly influenced by neuroinflammation.[41] According to Michelucci et al.[42] microglia and astrocyte activation, as well as the production of cytokines, chemokines, or growth factors, are the main causes of inflammation in the brain and often occur before cognitive failure.[43] Our results revealed that NPW alleviates cognitive

dysfunctions and anxiety-like behaviors, which may be related to its anti-inflammatory properties.

In conclusion, our results indicated that WAS caused the deterioration of cognitive functions and anxiety-like behaviors. Furthermore, our findings show for the first time that NPW alleviated cognitive function and anxiety-like behavior along with a reduction of corticosterone level in the water avoidance stress-induced rats.

Compliance with Ethical Standards

The Sakarya University Animal Care and Use Committee approved this research project (approval number: 35; date: 06/07/2022).

Conflict of Interest

There are no relevant conflicts of interest for the authors of this article.

Author Contribution

SAT: Design of work, completing the experiments and data collection, data interpretation and use of statistical analysis, manuscript drafting, critical revision of the manuscript, approval of the last version of the manuscript. EK: Completing the experiments and data collection, data interpretation and use of statistical analysis and approval of the last version of the manuscript

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References

1. Bustamante-Sánchez Á, Tornero-Aguilera JF, Fernández-Elías VE, Hormeño-Holgado AJ, Dalamitros AA, Clemente-Suárez VJ. Effect of Stress on Autonomic and Cardiovascular Systems in Military Population: A Systematic Review. *Cardiol Res Pract*. 2020;2020:7986249.
2. Karin O, Raz M, Tendler A, et al. A new model for the HPA axis explains dysregulation of stress hormones on the timescale of weeks. *Mol Syst Biol*. 2020;16(7):e9510.
3. Choobdar S, Vafaei AA, Sedaghat K. Protective role of vitamin-D on chronic stress induced-learning and memory deficits in rats. *Koomesh*. 2019;21(4):708-715.
4. Kasimay Cakir O, Ellek N, Salehin N, et al. Protective effect of low dose caffeine on psychological stress and cognitive function. *Physiol Behav*. 2017;168:1-10. doi:10.1016/j.physbeh.2016.10.010.
5. Zhang H, Wei M, Sun Q, et al. Lycopene ameliorates chronic stress-induced hippocampal injury and subsequent learning and memory dysfunction through inhibiting ROS/JNK signaling pathway in rats. *Food Chem Toxicol*. 2020;145:111688. doi:10.1016/j.fct.2020.111688
6. Algamal M, Pearson AJ, Hahn-Townsend C, et al. Repeated unpredictable stress and social isolation induce chronic HPA axis dysfunction and persistent abnormal fear memory. *Prog Neuropsychopharmacol Biol Psychiatry*. 2021;104:110035. doi:10.1016/j.pnpbp.2020.110035
7. Leistner C, Menke A. Hypothalamic-pituitary-adrenal axis and stress. *Handb Clin Neurol*. 2020;175:55-64. doi:10.1016/B978-0-444-64123-6.00004-7
8. Juszczyk G, Mikulska J, Kasperek K, Pietrzak D, Mrozek W, Herbet M. Chronic Stress and Oxidative Stress as Common Factors of the Pathogenesis of Depression and Alzheimer's Disease: The Role of Antioxidants in Prevention and Treatment. *Antioxidants (Basel)*. 2021;10(9):1439. Published 2021 Sep 9. doi:10.3390/antiox10091439
9. Shimomura Y, Harada M, Goto M, et al. Identification of neuropeptide W as the endogenous ligand for orphan G-protein-coupled receptors GPR7 and GPR8. *J Biol Chem*. 2002;277(39):35826-35832.doi:10.1074/jbc.M205337200
10. Fujii R, Yoshida H, Fukusumi S, et al. Identification of a neuropeptide modified with bromine as an endogenous ligand for GPR7. *J Biol Chem*. 2002;277(37):34010-34016. doi:10.1074/jbc.M205883200
11. Niimi M, Murao K. Neuropeptide W as a stress mediator in the hypothalamus. *Endocrine*. 2005;27(1):51-54. doi:10.1385/ENDO:27:1:051
12. Koyuncuoglu T, Ipek BE, Dertsiz EK, Akakin D, Yuksel M, Yegen BC. The neuroprotective effects of neuropeptide W in newborn rats with cerebral palsy. *In Acta Physiologica.* 2019;227:87-87.
13. Arabacı Tamer S, Akbulut S, Peker Eyüboğlu İ, et al. Peripheral administration of Neuropeptide-W protects against stress-induced gastric injury in rats. *Life Sci*. 2022;310:121087. doi:10.1016/j.lfs.2022.121087
14. Arabacı Tamer S, Üçem S, Büke B, et al. Regular moderate exercise alleviates gastric oxidative damage in rats via the contribution of oxytocin receptors. *J Physiol*. 2020;598(12):2355-2370. doi:10.1113/JP279577
15. Koyuncuoğlu T, Sevim H, Çetrez N, et al. High intensity interval training protects from Post Traumatic Stress Disorder induced cognitive impairment. *Behav Brain Res*. 2021;397:112923. doi:10.1016/j.bbr.2020.112923
16. Agorastos A, Chrousos GP. The neuroendocrinology of stress: the stress-related continuum of chronic disease development. *Mol Psychiatry*. 2022;27(1):502-513. doi:10.1038/s41380-021-01224-9
17. Chen P, Li X, Yu Y, et al. Administration Time and Dietary Patterns Modified the Effect of Inulin on CUMS-Induced Anxiety and Depression. *Mol Nutr Food Res*. 2023;67(8):e2200566. doi:10.1002/mnfr.202200566
18. Liu C, Shi R, Liu Y, et al. Low-frequency transcranial magnetic stimulation protects cognition in mice with chronic unpredictable mild stress through autophagy regulation. *Behav Brain Res*. 2023;444:114366. doi:10.1016/j.bbr.2023.114366
19. Mello AF, Mello MF, Carpenter LL, Price LH. Update on stress and depression: the role of the hypothalamic-pituitary-adrenal (HPA) axis. *Braz J Psychiatry*. 2003;25(4):231-238. doi:10.1590/s1516-44462003000400010
20. Mizoguchi K, Shoji H, Ikeda R, Tanaka Y, Tabira T. Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *Pharmacol Biochem Behav*. 2008;91(1):170-175. doi:10.1016/j.pbb.2008.07.002
21. Lim DW, Han D, Lee C. *Pedicularis resupinata* Extract Prevents Depressive-like Behavior in Repeated Corticosterone-Induced Depression in Mice: A Preliminary Study. *Molecules*. 2022;27(11):3434. Published 2022 May 26. doi:10.3390/molecules27113434

22. Vinkers CH, Kuzminskaite E, Lamers F, Giltay EJ, Penninx BWJH. An integrated approach to understand biological stress system dysregulation across depressive and anxiety disorders. *J Affect Disord*. 2021;283:139-146. doi:10.1016/j.jad.2021.01.051
23. Eliwa H, Brizard B, Le Guisquet AM, Hen R, Belzung C, Surget A. Adult neurogenesis augmentation attenuates anhedonia and HPA axis dysregulation in a mouse model of chronic stress and depression. *Psychoneuroendocrinology*. 2021;124:10507. doi:10.1016/j.psyneuen.2020.105097
24. Gao K, Farzi A, Ke X, et al. Oral administration of *Lactococcus lactis* WHH2078 alleviates depressive and anxiety symptoms in mice with induced chronic stress. *Food Funct*. 2022;13(2):957-969. doi:10.1039/d1fo03723d
25. 25.West EG, McDermott C, Chess-Williams R, Sellers DJ. Partial recovery of voiding function in female mice following repeated psychological stress exposure. *PLoS One*. 2022;17(4):e0266458. doi:10.1371/journal.pone.0266458
26. Atici AE, Arabacı Tamer S, Levent HN, et al. Neuropeptide W Attenuates Oxidative Multi-Organ Injury in Rats Induced with Intra-Abdominal Sepsis. *Inflammation*. 2022;45(1):279-296. doi:10.1007/s10753-021-01545-5
27. Mazzocchi G, Rebuffat P, Ziolkowska A, Rossi GP, Malendowicz LK, Nussdorfer GG. G protein receptors 7 and 8 are expressed in human adrenocortical cells, and their endogenous ligands neuropeptides B and w enhance cortisol secretion by activating adenylate cyclase- and phospholipase C-dependent signaling cascades. *J Clin Endocrinol Metab*. 2005;90(6):3466-3471. doi:10.1210/jc.2004-2132
28. Hochol A, Belloni AS, Rucinski M, et al. Expression of neuropeptides B and W and their receptors in endocrine glands of the rat. *Int J Mol Med*. 2006;18(6):1101-1106.
29. Zareie M, Johnson-Henry K, Jury J, et al. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut*. 2006;55(11):1553-1560. doi:10.1136/gut.2005.080739
30. Bozorgi H, Rashidy-Pour A, Moradikor N, et al. Reversal of chronic restraint stress-induced memory impairment by Japanese sake yeast supplement in mice: Role of adenosine A_1 and A_{2A} receptors. *J Psychiatr Res*. 2023;161:123-131. doi:10.1016/j.jpsychires.2023.02.033
31. Ahmed Z, Tokhi A, Arif M, et al. Fraxetin attenuates disrupted behavioral and central neurochemical activity in a model of chronic unpredictable stress. *Front Pharmacol*. 2023;14:1135497. doi:10.3389/fphar.2023.1135497
32. Kageyama K, Iwasaki Y, Daimon M. Hypothalamic Regulation of Corticotropin-Releasing Factor under Stress and Stress Resilience. *Int J Mol Sci*. 2021;22(22):12242. doi:10.3390/ijms222212242
33. Welcome MO. Cellular mechanisms and molecular signaling pathways in stress-induced anxiety, depression, and blood-brain barrier inflammation and leakage. *Inflammopharmacology*. 2020;28(3):643-665. doi:10.1007/s10787-020-00712-8.
34. Qin D, Wang J, Le A, Wang TJ, Chen X, Wang J. Traumatic Brain Injury: Ultrastructural Features in Neuronal Ferroptosis, Glial Cell Activation and Polarization, and Blood-Brain Barrier Breakdown. *Cells*. 2021;10(5):1009. Published 2021 Apr 24. doi:10.3390/cells10051009
35. Yan Y, Xu X, Chen R, et al. Down-regulation of MST1 in hippocampus protects against stress-induced depression-like behaviours and synaptic plasticity impairments. *Brain Behav Immun*. 2021;94:196-209. doi:10.1016/j.bbi.2021.02.007
36. Lei H, Zhang Y, Huang L, et al. L-3-n-Butylphthalide Regulates Proliferation, Migration, and Differentiation of Neural Stem Cell In Vitro and Promotes Neurogenesis in APP/PS1 Mouse Model by Regulating BDNF/TrkB/CREB/Akt Pathway. *Neurotox Res*. 2018;34(3):477-488. doi:10.1007/s12640-018-9905-3
37. Choi GY, Kim HB, Cho JM, et al. Umbelliferone Ameliorates Memory Impairment and Enhances Hippocampal Synaptic Plasticity in Scopolamine-Induced Rat Model. *Nutrients*. 2023;15(10):2351. doi:10.3390/nu15102351
38. Wang R, Yan F, Liao R, Wan P, Little PJ, Zheng W. Role of brain-derived neurotrophic factor and nerve growth factor in the regulation of Neuropeptide W in vitro and in vivo. *Mol Cell Endocrinol*. 2017;447:71-78. doi:10.1016/j.mce.2017.02.040
39. Taylor MM, Yuill EA, Baker JR, et al. Actions of neuropeptide W in paraventricular hypothalamus: implications for the control of stress hormone secretion. *Am J Physiol Regul Integr Comp Physiol.* 2005;288(1):R270-R275. doi:10.1152/ajpregu.00396.2004
40. Yogo K, Oki Y, Iino K, et al. Neuropeptide W stimulates adrenocorticotrophic hormone release via corticotrophin-releasing factor but not via arginine vasopressin. *Endocr J*. 2012;59(7):547-554. doi:10.1507/endocrj.ej11-0221
41. Hu Y, Li H, Zhang J, et al. Periodontitis Induced by *P. gingivalis*-LPS Is Associated With Neuroinflammation and Learning and Memory Impairment in Sprague-Dawley Rats. *Front Neurosci*. 2020;14:658. doi:10.3389/fnins.2020.00658
42. Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid-beta. *J Neuroimmunol*. 2009;210(1-2):3-12. doi:10.1016/j.jneuroim.2009.02.003