Physiology and Pharmacology 27 (2023) 171-181
Original Article

Venlafaxine and synbiotic attenuated learned fear-like behavior and recognition memory impairment in immobilized-stressed rats

Sarawut Lapmanee[1*], Sakkarin Bhubhanil[1], Siriwan Sriwong[2], Mattaka Khongkow[3], Katawut Namdee[3], Prapimpun Wongchitrat[4], Pawin Pongkorpsakol[5]

1. Department of Basic Medical Sciences, Faculty of Medicine, Siam University, Bangkok, Thailand
2. Laboratory Animal Center, Thammasat University, Patumthani, Thailand
3. National Nanotechnology Centre (NANOTEC), National Science and Technology Development Agency, Pathumthani, Thailand
4. Center for Research and Innovation, Faculty of Medical Technology, Mahidol University, Nakon Pathom, Thailand
5. Princess Srisavangavadhana College of Medicine, Chulabhorn Royal Academy, Bangkok, Thailand

ABSTRACT

Introduction: Stress disturbs the gut-brain axis and contributes to the development of mood disorders and memory impairment. Recent findings on the anti-stress effects of monoamine modulators have shown that the serotonin and norepinephrine reuptake inhibitor (SNRI) venlafaxine (Vlx) is more effective in stressed rodents. However, the effects of Vlx on microbiota and memory impairment- and stress-related behaviors are still unknown. Synbiotics (Syn), a mixture of probiotics and prebiotics, can modify the gut microbiome; however, the effects of anti-anxiety and anti-memory deficits are still not well understood. Therefore, this study proposed to compare the effectiveness of Vlx and Syn in the reduction of learned fear- and memory impairment-like behaviors in an animal model of stress.

Methods: Forty male adult Wistar rats were subjected to stress by immobilization in a restrainer for 2h per day and were administered 10mg/kg Vlx and/or 2g of Syn containing 1.0×10^{10} CFU probiotic strains and prebiotic oligosaccharides daily for 14 days. Learned fear, recognition memory and locomotor activity were evaluated by the elevated-T maze, novel objective recognition and open field tests. Blood samples and adrenal glands were collected to measure the circulating corticosterone levels and relative adrenal weights, which were used as markers of stress responses.

Results: The immobilized-stressed rats showed hyperactivity in stress responses, as demonstrated by increased relative adrenal weights and serum corticosterone levels. Both Vlx and Syn reduced the increase in serum corticosterone levels in stressed rats. Furthermore, Vlx- and/or Syn-treated stressed rats had fewer learned fear-like behaviors and a higher discrimination index without any locomotor activity changes than vehicle-treated stressed rats.

Conclusion: Syn supplementation had comparable effects to SNRIs in alleviating the risk of developing anxiety disorders and memory impairment in stressed individuals or psychiatric patients.

Keywords:
Fear
Immobilized stress
Memory
Venlafaxine
Synbiotics

* Corresponding author: Sarawut Lapmanee, sarawut.lap@siam.edu
Received 23 January 2022; Revised from 4 May 2022; Accepted 2 July 2022

Citation: Lapmanee S, Bhubhanil S, Sriwong S, Khongkow M, Namdee K, Wongchitrat P, Pongkorpsakol P. Venlafaxine and synbiotic attenuated learned fear-like behavior and recognition memory impairment in immobilized-stressed rats. Physiology and Pharmacology 2023; 27: 171-181. http://dx.doi.org/10.61186/phypha.27.2.171

Introduction

Rapid changes in economic and social conditions are leading to the world's population experiencing more stress, resulting in psychological changes and reduced quality of life. Continuous exposure to stress results in hypothalamic-pituitary adrenal (HPA) axis dysfunction and an increase in circulating glucocorticoids that disturb the functions of several of the body's organs. Indeed, stress has been shown to affect both the nervous and digestive systems, leading to anxiety, depression, peptic ulcers and irritable bowel symptoms (McEwen et al., 2016). Moreover, stress reduces the amounts of microorganisms in the digestive organs and lessens the communication between the central and enteric nervous systems, which are contributing factors to the development of mood disorders (Foster et al., 2017; Heijtz et al., 2011; Maslanik et al., 2012). However, no conclusive evidence exists on the relationship between the gut-brain axis and memory impairment in individuals with stress and psychiatric disorders.

Currently, antidepressants are prescribed to treat stressed and psychiatric patients. Previous studies found that the serotonin and norepinephrine reuptake inhibitor (SNRI) venlafaxine (Vlx) appeared to be more effective than the first-line drugs (i.e., diazepam and fluoxetine) and showed anxiolytic- and antidepressant-like actions in stressed rats (Lapmanee et al., 2013; Lapmanee et al., 2012). In addition to SNRIs, some antidepressants and anti-anxiety agents, such as selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants, can reduce the bacterial content of the gut (Ayaz et al., 2015; Macedo et al., 2017; Ait Chait et al., 2020). The changes in the quantity and the compositions of gut bacteria have been shown to down-regulate genes in brain plasticity and induce depressive behavior in anti-stress agent-treated mice (Lukić et al., 2019). Moreover, there is a lack of evidence of the effects of Vlx on microbiota and memory impairment- and stress-related behaviors. Therefore, it is necessary to search for antistress agents that relieve stress without the depletion of gut microbiota in the treatment of digestive and mental health.

Dietary supplements for the maintenance of microbial homeostasis to alleviate pathological changes in the digestive organs can be obtained commercially. Synbiotics (Syn) are food products that contain probiotics and prebiotics. Probiotics are living microorganisms in the digestive tract that can maintain a balanced number of good bacteria, facilitate the communication of the digestive and immune systems and help to reduce inflammation. Prebiotic supplements, which are indigestible portions of food (i.e., xylooligosaccharides and fructooligosaccharides), stimulate probiotic growth in the digestive tract (Pandey et al., 2015). Interestingly, Syn have beneficial effects on lowering fatty liver accumulation and blood sugar levels in patients with nonalcoholic liver disease and diabetes (Mofidi et al., 2017; Ebrahimi et al., 2017). However, there is a lack of studies on the effect of synbiotic supplements on the treatment of complications of the disorders brain regions that control behavior and memory formation when exposed to stressful situations.

In addition, the effect of SNRIs in combination with Syn supplements on neurobehavioral changes in learned fear and memory remains elusive. Therefore, the present study proposed to compare the effectiveness of Vlx and Syn supplements in the reduction of learned fear and memory impairment in an animal model of stress to develop treatments and prevent stress-induced neurological complications with anxiety and recognition memory deficits.

Material and methods

Animals

Forty adult male Wistar rats (8 weeks old, weighing 180-210g) were obtained from Nomura Siam International Company Limited (Bangkok, Thailand). The rats were housed, 2 rats/cage, (n=8/group) in a room with controlled conditions, at 25±2°C and 55±5% humidity, with a 12h light/dark cycle (average illuminance 250 lux). The rats were under this condition for at least 7 days before the start of the experiments. All animals received standard rat pellets (CP Company Limited, Bangkok, Thailand) and water *ad libitum*. The experimental procedures were certified and approved by the Thammasat University Animal Care and Use Committee, Pathum Thani, Thailand (Animal Ethics No. 003/2020).

Experimental procedure

The rats were randomly assigned to 5 equal groups: (*i*) control+ vehicle group (received 5ml/kg body weight sterile saline; Con), (*ii*) stress+ vehicle group (received 5ml/kg body weight sterile saline; Veh), (*iii*) stress+ Vlx group (received 10mg/kg/body weight), (*iv*) stress+ Syn group (received Syn containing 1.0×10^{10} CFU probiotic

strains and oligosaccharides), and (*v*) stress+ Vlx/Syn group (received Vlx and Syn), as shown in Figure 1A. After completing stress induction and anti-stress agent administration, all animals were evaluated for learned fear (day 15), recognition memory impairment (days 15–16) and locomotor activities (day 17) by using neurobehavioral assessment tools, i.e., elevated T-maze (ETM), novel object recognition (NOR) and open field test (OFT, Figure 1A). To minimize the impact of behavioral tests and determine the long-lasting effects of the interventions on animals, all rats were euthanized after the last behavioral tests (day 18) between 9.00-12.00 hours (Miller et al., 2011; Davies et al., 2016; Ifergane et al., 2018; Noronha et al., 2019; Wang et al., 2019; Fan et al., 2022).Blood samples and adrenal glands were collected to measure circulating corticosterone levels and relative adrenal weights, which were used as markers of stress responses (Lapmanee et al., 2017).

Immobilized stress protocol

The stress induction followed the methods of Yang et al., 2014 and Tian et al., 2018. The stressed rats were immobilized daily in a 23×6cm transparent restrainer fixed with plastic tape for 2h/day for 14 days. There was a ~0.5cm hole at the end of the restrainer to allow for breathing. The immobilization stress was conducted from 9:00 to 11:00 AM.

Antistress agent administration

Based on our recent findings, SNRIs could modulate central monoamine neurotransmitters and reduce anxiety- and depression-like behaviors in stressed male rats. Therefore, the treatment protocols followed previous studies by (Alves et al., 2017; Lapmanee et al., 2012). The antistress agents were Vlx (10mg/kg/day) and 2g of Syn containing 1.0×10^{10} CFU probiotic strains, including *Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium longum, Lactobacillus acidophilus Lactobacillus casei* and *Lactococcus lactis,* and prebiotic oligosaccharide (produced by Inter Pharma Public Co., Ltd., Bangkok, Thailand). Sterile saline (0.9%) was used as the vehicle (5ml/kg/day). Vlx and Syn were freshly prepared and administered orally daily between 15:00 and 16:00 PM for 14 days.

Behavioral tests

Elevated T-maze test (ETM)

The ETM is a validated model of anxiety and is used to evaluate the simultaneous anxiolytic- and amnesic-like effects in preclinical studies (Asth et al., 2012). The ETM apparatus was made from a black plastic material and was elevated 50cm from the ground. The T-shaped model used for the ETM for this study consisted of 3 arms (50cm long × 10cm wide). The closed arm with a 40cm wall was placed at the perpendicular right angle. The ETM test followed the method of (Graeff et al., 1993). The ETM test evaluated latency in three inhibitory avoidance trials (i.e., baseline, avoidance 1 and avoidance 2) at 30s intervals and a one-way escape trial. A rat was gently placed at the end of the closed arm in the inhibitory avoidance task. It was then placed at the end of the right side of the open arm in the escape task. The time taken for the rat to leave this arm with all paws was recorded. Learned fear (general anxiety disorder) was represented by inhibitory avoidance, whereas innate fear (panic disorder) was represented by one-way escape.

Open field test (OFT)

The OFT apparatus was made of a black plastic material (76cm long × 57cm wide × 35cm high) with a floor painted with white gridlines. The apparatus was under 60 lux lighting, similar to a dimly lit room. The rat was gently placed in one of the four corner squares facing the arena center and was allowed to explore for 5min. The changes in the number of lines crossed indicated locomotor activity and exploration. These were recorded using an infrared video camera (Lapmanee et al., 2017).

Novel object recognition (NOR)

The NOR test is considered a less stressful test and is widely used to evaluate learning and short-term memory, which represent the process of recognition in the rodent hippocampus (Antunes and Biala, 2012). The NOR apparatus was made of a black plastic material (63cm long × 63cm wide × 45cm high) under 350 lux lighting, equivalent to bright room lighting. On the habituation or familiarization day, the rat was allowed to explore the empty arena for 10min/session for 2 sessions. After 24h, on the acquisition day, the rat was exposed to congruent objects (two pepper bottles) for 3min. Thereafter, the rat was transferred to its cage and allowed to stay there for 1h. In the testing session, the rat was allowed to explore the unfamiliar object (glass paperweight) for 3min. The

behavior profiles, i.e., sniffing, licking or touching, were recorded by a video camera. The reduction in exploration of the novel object or the discrimination index (DI) indicated a loss of recognition memory (Lapmanee et al., 2017; Redrobe et al., 2010), which was calculated as followed:

$$DI = \frac{\text{Exploring novel object time} - \text{Exploring familiar object time}}{\text{Total exploration time}}$$

Measurement of body and adrenal weights

The rats were weighed daily (in grams). When the experiments were completed, the adrenal glands were removed by laparotomy and cleaned of the surrounding fat tissues. The adrenal glands were then washed and dried in an incubator at a constant temperature of 80°C for 3 days. The dry weight of the adrenal glands was measured, and the relative weight of the adrenal glands

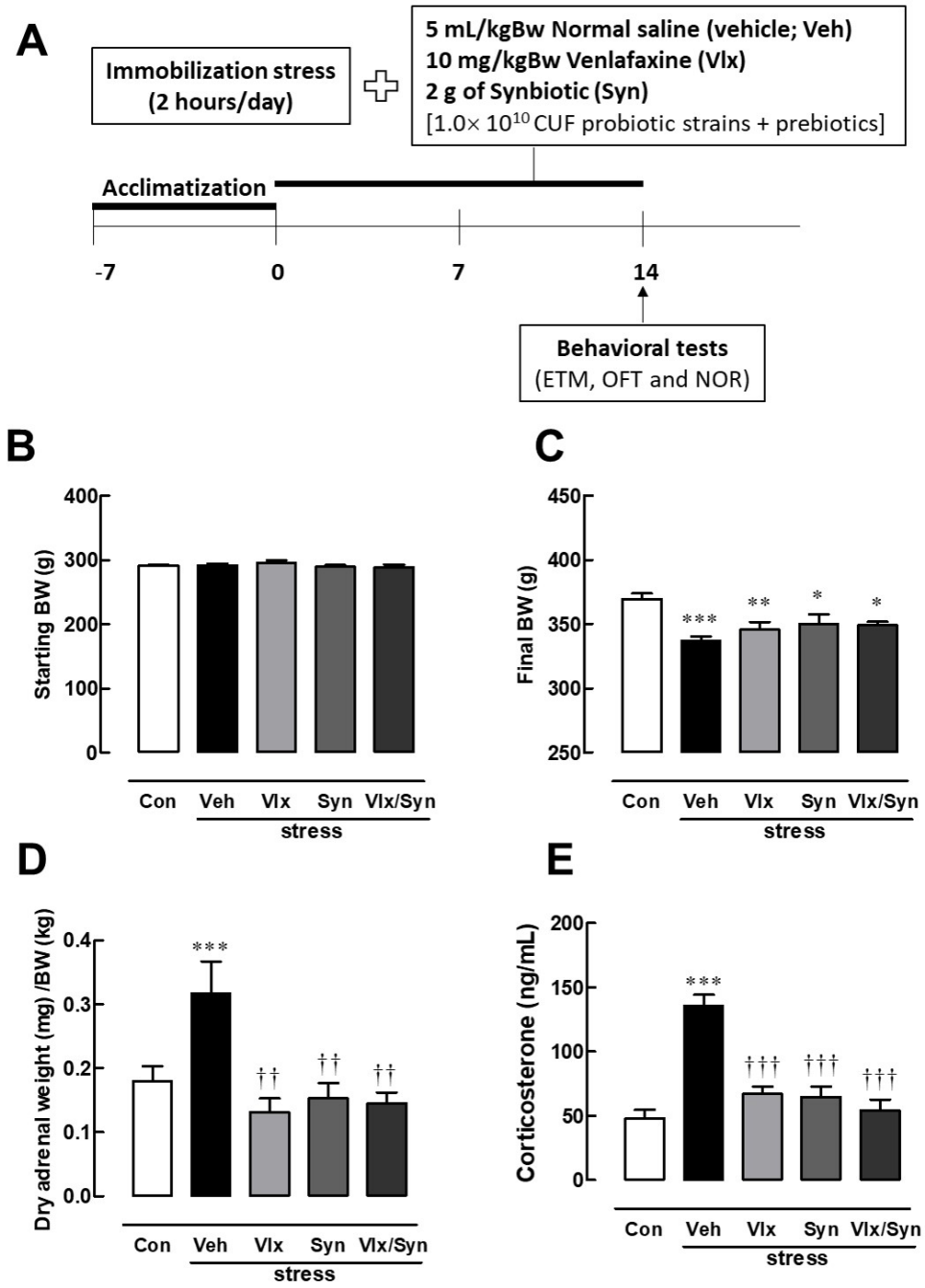

FIGURE 1. Time course experiments (A) and physiological alteration in stress responses. (B) The starting body weight, (C) the final body weight, (D) relative dry adrenal gland weight and (E) serum corticosterone levels. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ compared to vehicle-treated non-stress control group. $^{\dagger\dagger}P<0.01$ and $^{\dagger\dagger\dagger}P<0.001$ compared to vehicle-treated stressed group. Con: control; Veh: vehicle; Vlx: venlafaxine; Syn: synbiotic.

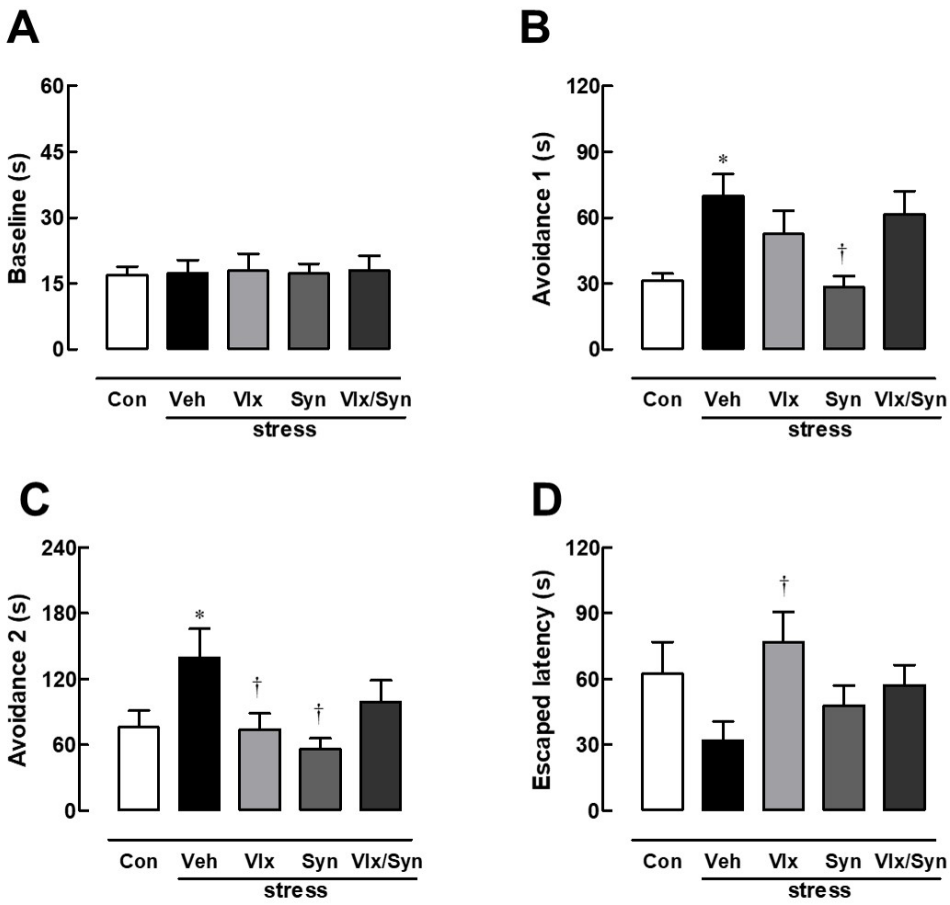

FIGURE 2. Learned fear alteration as determined by ETM. Inhibitory avoidance trails, (A) baseline latency, (B) avoidance 1 latency, (C) avoidance 2 latency and (D) latency of one-way escape trail. $^{*}P<0.05$ compared to vehicle-treated non-stress control group. $^{\dagger}P<0.05$ compared to vehicle-treated stressed group. Con: control; Veh: vehicle; Vlx: venlafaxine; Syn: synbiotic.

(adrenal weight/body weight) was calculated.

Measurement of corticosterone levels

Blood sample collection was performed through cardiac puncture under isoflurane anesthesia. The blood was allowed to clot at room temperature for 15min. The serum was centrifuged (1500g, 15min, 4°C), and the corticosterone levels were analyzed by a commercial ELISA kit (Immunodiagnostic Systems Ltd, Tyne and Wear, UK).

Statistical analysis

The results are presented as the mean±SEM. GraphPad Prism 7.0 software (San Diego, CA) was used to perform the statistical analysis. Multiple sets of data were analyzed by one-way analysis of variance (ANOVA) with Dunnett's post-hoc test. An alpha level of 0.05 ($P<0.05$) was set as statistically significant.

Results

Body weight, adrenal weight and serum corticosterone levels

All rats had the same starting body weight and gained weight throughout the study period (Figures 1B and C). After completing the 14-day experiments, the body weights of stressed rats were significantly lower than the weights of the rats in the control groups. Both Vlx and Syn reduced body weight in stressed rats ($P=0.0026$, Figure 1C). Veh-treated stressed rats had higher relative dry adrenal gland weights ($P=0.0009$) and serum corticosterone levels ($P<0.001$) than the control groups, while Vlx- and/or Syn-treated stressed rats had significantly lower adrenal dry weights ($P=0.0011$) and serum corticosterone levels ($P<0.0001$) than Veh-treated stressed rats, as shown in Figures 1D-E.

Learned fear- and anxiety-like behaviors

In inhibitory avoidance trials, there was no signifi-

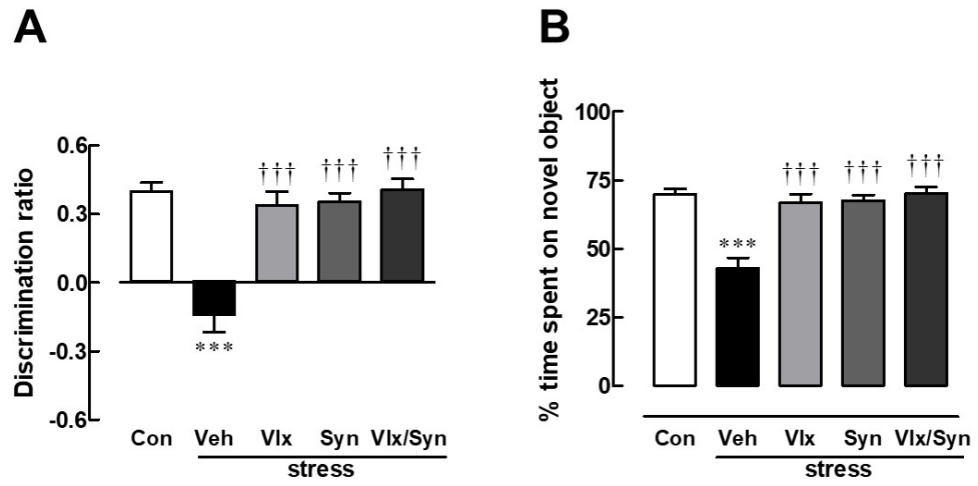

FIGURE 3. Recognition memory alteration as determined by NOR. (A) discrimination index and (B) percent of time spent on novel objective. ***P<0.001 compared to vehicle-treated non-stress control group. †††P<0.001 compared to vehicle-treated stressed group. Con: control; Veh: vehicle; Vlx: venlafaxine; Syn: synbiotic.

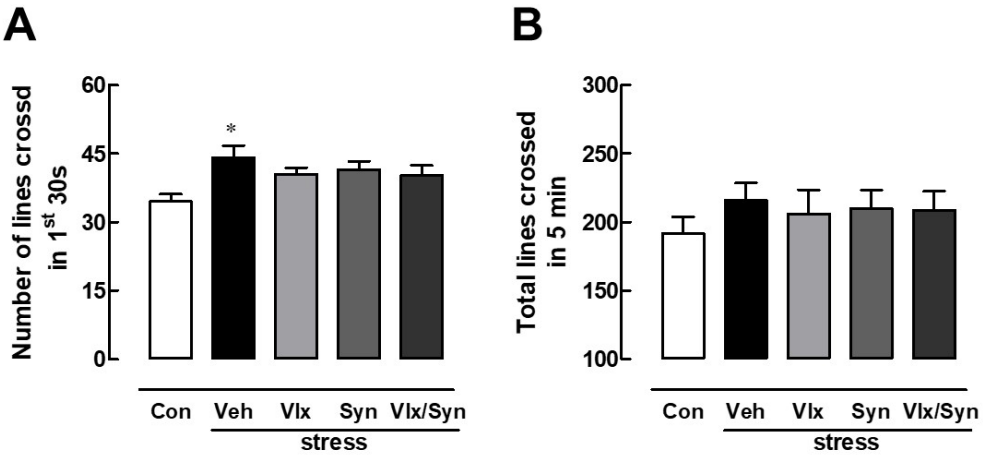

FIGURE 4. Locomotor alteration as determined by OFT. (A) the number of lines crossed in the first 30 s, and (B) the total number of lines crossed in 5min. *P<0.05 compared to vehicle-treated non-stress control group. Con: control; Veh: vehicle; Vlx: venlafaxine; Syn: synbiotic.

cant difference in baseline latency among the groups. Veh-treated stressed rats displayed learned fear-like behavior, as indicated by increased latencies of avoidance 1 (P=0.048) and avoidance 2 (P=0.0247, Figures 2A and B). Furthermore, it was observed that exposure of Veh-treated stressed rats to an unfamiliar area induced an increase in the number of lines crossed in the first 30s (Figure 4A). As expected, Vlx treatment produced anxiolytic-like action in stressed male rats, as it significantly decreased avoidance 2 (P=0.0248) and increased escape latencies (P=0.0377, Figures 2C and D). Interestingly, Syn-treated stressed rats had less avoidance 1 (P=0.0262) and avoidance 2 (P=0.0248) than Veh-treated stressed rats (Figures 2B and C).

Novel-objective recognition

As shown in Figures 3A-B, the discrimination index of the Veh-treated stressed rats significantly decreased, and less time was spent on the novel object compared to the control group (P<0.0001), suggesting impaired recognition memory in stressed rats. Vlx and/or Syn treatment alleviated stress-induced memory impairment in rats, as determined by an increased discrimination index in the NOR test (P<0.0001).

Locomotor activity

Although increased exploratory locomotor activity in the first 30s was observed in Veh-treated stressed rats (Figure 4A), there were no significant effects on the locomotor activity of either the Veh-, Vlx- or Syn-treated

stressed rats, as indicated in the total lines crossed under the OFT (Figure 4B).

Discussion

Following restraint stress induction (2h/day for 14 consecutive days), rats had a decrease in weight and increases in serum corticosterone levels as well as dry adrenal weight. In behavioral responses, the stressed rats displayed learned fear in the ETM, impaired recognition memory in the NOR test and hyperarousal reaction to an unfamiliar open area without changes in total locomotor activity in the OFT. These findings indicated physical and biochemical changes in response to exposure to stressors, which were consistent with previous studies (Lapmanee et al., 2017; Tian et al., 2018). Chronic stress exposure results in increases in adrenal stress hormone production and glucocorticoids via hyperactivation of the HPA axis (Franco et al., 2016), which interferes with the production of ghrelin, pro-opiomelanocortin and leptin within the neural circuit of food intake and body weight regulation (Herman et al., 2016; Jeong et al., 2013). The final body weights were significantly lower in both the untreated stressed rats and the Vlx/Syn-treated stressed rats compared with those in the control groups. Stress induced increased inflammation and disruption in the rhythmic release of hypothalamic and adrenal hormones and was the possible mechanism responsible for weight reduction in rats exposed to stress (Lucassen and Cizza, 2012; Stefanaki et al., 2018). Furthermore, Vlx modulates dopamine and serotonin neurotransmission and induces weight loss in sleep-deprived rats (De Oliveira et al., 2004), while Syn restores gut microbial homeostasis and reduces body weight gain in obese mice (Ke et al., 2019). These reasons could explain the weight loss in stressed rats, and none of the treatments were able to reverse the body weight.

Moreover, stress reduces the production of brain-derived neurotrophic factor (BDNF) and neurogenesis in the hippocampus associated with mood disorders and cognitive impairment (Lapmanee et al., 2017; Mizoguchi et al., 2020; Schoenfeld et al., 2017). In addition, the disruption of microbiological homeostasis in the gastrointestinal tract has been reported as chronic stress exposure (Farzi et al., 2018; Misiak et al., 2020) from the decrease in microbial counts, including *Lactabacilli* and *Provetella*, (Bailey and Coe 1999; Maslanik et al., 2012). Stress-induced dysbiosis causes high levels of cytokines and inflammatory markers, imbalances in monoamines, malabsorption of essential nutrients and minerals and metabolism of lipids in the brain, resulting in anxiety, depression and memory impairment (Bassett et al., 2019; Caspani et al., 2019; Li et al., 2019; Madison and Kiecolt-Glaser 2019; van de Wouw et al., 2018; Zafar 2020).

It is known that antidepressants are used to alleviate mood disorders, i.e., anxiety and depression. Although SSRIs (i.e., citalopram, fluoxetine, paroxetine and sertraline) are first-line antidepressant therapies, they may lack long-lasting effects and provide adverse side effects in long-term use (Li et al., 2019; Sanchez et al., 2014). In the present study, Vlx-treated stressed rats had significant avoidance latency in the ETM and an increased discrimination index in the NOR, which indicated that Vlx improved learned fear and memory impairment in stressed rats. Consistently, our previous findings on SNRIs showed that Vlx was effective in reducing anxiety-, depression- and cognitive impairment-like behaviors in rats exposed to chronic restraint stress by reducing the level of corticosterone and restoring the reduction in hippocampal BDNF protein expression (Lapmanee et al., 2017; McEwen et al., 2016). Additionally, long-term treatment with Vlx or duloxetine reduced the number of bacterial taxa, including *Ruminococcus flavefaciens OTU 228330, Ruminococcus flavefaciens, Adlercreutzia OTU 245324* and *Adlercreutzia equolifaciens* (Lukić et al., 2019).

The microbiome in the gastrointestinal tract is currently being widely studied, especially the correlation between the microbiota-gut-brain axis and neurobehavioral disorders. In particular, supplementation with psychobiotics, including prebiotics, probiotics and synbiotics can modify microbial function and enhance communication between the nervous system and the gastrointestinal tract (Tremblay et al., 2021). Generally, microorganisms are found within the gastrointestinal tract, i.e., *Firmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria*. The changes in the quantity or equilibrium of these microbiota in the gastrointestinal tract result in abnormal behavioral responses (Cryan and Dinan 2012). The results from both animal and human studies have shown that psychobiotic supplements can relieve symptoms of anxiety and depression (Vaghef-Mehrabany et al., 2020). However, there is no conclusive evidence on the efficacy of psychobiotic supplementation on mem-

ory impairment. The results of this study revealed that synbiotics can have beneficial effects on recognition memory impairment as determined by an increase in the discrimination index of NOR tests in stressed rats. In contrast, antibiotic treatment results in disturbance in the intestinal microbiota in rodents, which exhibit anxiety and depressive behaviors, but their learning behavior and memory are unaffected (O'Mahony et al., 2014).

The underlying mechanisms in the use of Syn supplements against the development of stress-induced learned fear and memory impairment are discussed further here; *Lactobacillus* supplementation can have protective effects on the integrity and inflammation of the intestinal mucosa (Blackwood et al., 2017). In addition, an increase in *Bifidobacteria* can increase microglial function and synapse density in the cerebellum, cortex and hippocampus (Erny et al., 2015; Luck et al., 2020). Moreover, *Lactobacillus helveticus R0052* and *Bifidobacterium longum R0175* supplements attenuate hippocampal apoptosis and memory impairment in rats with lipopolysaccharide-induced memory loss (Mohammadi et al., 2019). Taken together, the supplementation of psychobiotics (i.e., Syn) with Vlx further reduced stress-induced learned fear, anxiety and memory impairment by modulating serotonin and norepinephrine neurotransmission and balancing microorganisms in the brain-gut-microbiome axis.

Conclusion

The stress induction performed in this study was a successful test protocol, as displayed by the physical and behavioral consequences in the stress responses of male rats. Stress is one of the causes of disturbances in the balance of intestinal microflora, and it affects brain function by regulating learned fear, anxiety and memory formation processes. Syn can be an alternative supplement taken in combination with SNRIs to prevent disturbances in intestinal microbiota abnormalities and alleviate the risk of developing anxiety disorders and memory impairment in stressed individuals. The effects of Syn supplementation on the changes in gene or protein expression promoting hippocampal neurogenesis and neuronal growth can be demonstrated to explain the underlying mechanisms for reducing anxiety and restoring impaired memory in an animal model of stress.

Acknowledgements

The authors thank Assistant Professor Dr. Jantarima Charoenphandhu and Laboratory Animal Center, Thammasat University for behavioral testing facility. We would like to thank Conrado Heruela for his assistance in proofreading of the manuscript. This work was supported by research grants from the Faculty of Medicine, Siam University (001/2563 to S. Lapmanee) and the target development group from the National Science and Technology Development Agency (NSTDA) (to M. Khongkow and K. Namdee).

Conflicts of interest

None.

References

Ait Chait Y, Mottawea W, Tompkins TA, Hammami R. Unravelling the antimicrobial action of antidepressants on gut commensal microbes. Sci Rep 2020; 10:17878. https://doi.org/10.1038/s41598-020-74934-9

Alves CC, Waitzberg DL, de Andrade LS, dos Santos Aguiar L, Reis MB, Guanabara CC, et al. Prebiotic and synbiotic modifications of beta oxidation and lipogenic gene expression after experimental hypercholesterolemia in rat liver. Front Microbiol 2017; 8: 2010. https://doi.org/10.3389/fmicb.2017.02010

Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process. 2012;13(2):93-110. https://doi.org/10.1007/s10339-011-0430-z

Asth L, Lobão-Soares B, André E, Soares Vde P, Gavioli EC. The elevated T-maze task as an animal model to simultaneously investigate the effects of drugs on long-term memory and anxiety in mice. Brain Res Bull. 2012;87(6):526-33. https://doi.org/10.1016/j.brainresbull.2012.02.008

Ayaz M, Subhan F, Ahmed J, Khan AU, Ullah F, Ullah I, et al. Sertraline enhances the activity of antimicrobial agents against pathogens of clinical relevance. J Biol Res (Thessalon) 2015; 22: 4. https://doi.org/10.1186/s40709-015-0028-1

Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Dev Psychobiol 1999; 35: 146-55. https://doi.org/10.1002/(SICI)1098-2302(199909)35:2<146::AID-DEV7>3.0.CO;2-G

Bassett SA, Young W, Fraser K, Dalziel JE, Webster J, Ryan L, et al. Metabolome and microbiome profiling of a

stress-sensitive rat model of gut-brain axis dysfunction. Sci Rep 2019; 9: 14026. https://doi.org/10.1038/s41598-019-50593-3

Blackwood BP, Yuan CY, Wood DR, Nicolas JD, Grothaus JS, Hunter CJ. Probiotic Lactobacillus species strengthen intestinal barrier function and tight junction integrity in experimental necrotizing enterocolitis. J Probiotics Health 2017; 5:159. https://doi.org/10.4172/2329-8901.1000159

Caspani G, Kennedy S, Foster JA, Swann J. Gut microbial metabolites in depression: understanding the biochemical mechanisms. Microb Cell 2019; 6: 454-81. https://doi.org/10.15698/mic2019.10.693

Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 2012; 13: 701-12. https://doi.org/10.1038/nrn3346

Davies DR, Olson D, Meyer DL, Scholl JL, Watt MJ, Manzerra P, Renner KJ, Forster GL. Mild traumatic brain injury with social defeat stress alters anxiety, contextual fear extinction, and limbic monoamines in adult rats. Frontiers in behavioral neuroscience. 2016 Apr 19;10:71. https://doi.org/10.3389/fnbeh.2016.00071

de Oliveira RA, Cunha GM, Borges KD, de Bruin GS, dos Santos-Filho EA, Viana GS, de Bruin VM. The effect of venlafaxine on behaviour, body weight and striatal monoamine levels on sleep-deprived female rats. Pharmacol Biochem Behav. 2004 Nov;79(3):499-506. https://doi.org/10.1016/j.pbb.2004.09.001

Ebrahimi SZ, Nasli-Esfahani E, Nadjarzade A, Mozaffari-Khosravi H. Effect of symbiotic supplementation on glycemic control, lipid profiles and microalbuminuria in patients with non-obese type 2 diabetes: a randomized, double-blind, clinical trial. J Diabetes Metab Disord 2017;16:23. https://doi.org/10.1186/s40200-017-0304-8

Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 2015; 18: 965-77. https://doi.org/10.1038/nn.4030

Fan Y, Zhang L, Kong X, Liu K, Wu H. Different exercise time on 5-HT and anxiety-like behavior in the rat with vascular dementia. American Journal of Alzheimer's Disease & Other Dementias®. 2022;37:15333175221082743. https://doi.org/10.1177/15333175221082

Farzi A, Fröhlich EE, Holzer P. Gut microbiota and the neuroendocrine system. Neurotherapeutics 2018; 15: 5-22. https://doi.org/10.1007/s13311-017-0600-5

Foster JA, Rinaman L, Cryan JF. Stress & the gut-brain axis: regulation by the microbiome. Neurobiol Stress. 2017;7:124-36. https://doi.org/10.1016/j.ynstr.2017.03.001

Franco AJ, Chen C, Scullen T, Zsombok A, Salahudeen AA, Di S, et al. Sensitization of the hypothalamic-pituitary-adrenal axis in a male rat chronic stress model. Endocrinology 2016; 157: 2346-55. https://doi.org/10.1210/en.2015-1641

Graeff F, Viana M, Tomaz C. The elevated T maze, a new experimental model of anxiety and memory: effect of diazepam. Braz J Med Biol Res 1993; 26: 67-70.

Heijtz RD, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci U S A 2011;108:3047-52. https://doi.org/10.1073/pnas.1010529108

Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, et al. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Compr Physiol 2016; 6: 603-21. https://doi.org/10.1002/cphy.c150015

Ifergane G, Boyko M, Frank D, Shiyntum HN, Grinshpun J, Kuts R, Geva AB, Kaplan Z, Zeldetz V, Cohen H. Biological and behavioral patterns of post-stroke depression in rats. Canadian Journal of Neurological Sciences. 2018;45(4):451-61. https://doi.org/10.1017/cjn.2017.302

Jeong JY, Lee DH, Kang SS. Effects of chronic restraint stress on body weight, food intake, and hypothalamic gene expressions in mice. Endocrinol Metab (Seoul) 2013; 28: 288-96. https://doi.org/10.3803/EnM.2013.28.4.288

Ke X, Walker A, Haange SB, Lagkouvardos I, Liu Y, Schmitt-Kopplin P, von Bergen M, Jehmlich N, He X, Clavel T, Cheung PCK. Synbiotic-driven improvement of metabolic disturbances is associated with changes in the gut microbiome in diet-induced obese mice. Mol Metab. 2019;22:96-109. https://doi.org/10.1016/j.molmet.2019.01.012

Lapmanee S, Charoenphandhu J, Charoenphandhu N. Beneficial effects of fluoxetine, reboxetine, venlafaxine, and voluntary running exercise in stressed male rats with anxiety-and depression-like behaviors. Behav Brain Res 2013; 250: 316-25. https://doi.org/10.1016/j.bbr.2013.05.018

Lapmanee S, Charoenphandhu N, Krishnamra N, Charoenphandhu J. Anxiolytic-like actions of reboxetine, venlafaxine and endurance swimming in stressed male rats. Behav Brain Res 2012; 231: 20-8. https://doi.org/10.1016/j.bbr.2012.02.037

Lee SP, Sung IK, Kim JH, Lee SY, Park HS, Shim CS. The effect of emotional stress and depression on the prevalence of digestive diseases. J Neurogastroenterol Motil 2015; 21:

273-82. https://doi.org/10.5056/jnm14116

Li H, Wang P, Huang L, Li P, Zhang D. Effects of regulating gut microbiota on the serotonin metabolism in the chronic unpredictable mild stress rat model. Neurogastroenterol Motil 2019; 31: e13677. https://doi.org/10.1111/nmo.13677

Lucassen EA, Rother KI, Cizza G. Interacting epidemics? Sleep curtailment, insulin resistance, and obesity. Ann N Y Acad Sci. 2012;1264(1):110-34. https://doi.org/10.1111/j.1749-6632.2012.06655.x

Luck B, Engevik MA, Ganesh BP, Lackey EP, Lin T, Balderas M, et al. Bifidobacteria shape host neural circuits during postnatal development by promoting synapse formation and microglial function. Sci Rep 2020; 10: 7737. https://doi.org/10.1038/s41598-020-64173-3

Lukić I, Getselter D, Ziv O, Oron O, Reuveni E, Koren O, et al. Antidepressants affect gut microbiota and Ruminococcus flavefaciens is able to abolish their effects on depressive-like behavior. Transl Psychiatry 2019; 9:133. https://doi.org/10.1038/s41398-019-0466-x

Macedo D, Filho AJMC, de Sousa CNS, Quevedo J, Barichello T, Júnior HVN, et al. Antidepressants, antimicrobials or both? Gut microbiota dysbiosis in depression and possible implications of the antimicrobial effects of antidepressant drugs for antidepressant effectiveness. J Affect Disord 2017; 208:22-32. https://doi.org/10.1016/j.jad.2016.09.012

Madison A, Kiecolt-Glaser JK. Stress, depression, diet, and the gut microbiota: human-bacteria interactions at the core of psychoneuroimmunology and nutrition. Curr Opin Behav Sci 2019; 28: 105-10. https://doi.org/10.1016/j.cobeha.2019.01.011

Maslanik T, Bernstein-Hanley I, Helwig B, Fleshner M. The impact of acute-stressor exposure on splenic innate immunity: a gene expression analysis. Brain Behav Immun 2012; 26:142-9. https://doi.org/10.1016/j.bbi.2011.08.006

McEwen BS, Nasca C, Gray JD. Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. Neuropsychopharmacology 2016; 41: 3-23. https://doi.org/10.1038/npp.2015.171

Miller SM, Piasecki CC, Lonstein JS. Use of the light-dark box to compare the anxiety-related behavior of virgin and postpartum female rats. Pharmacol Biochem Behav. 2011;100(1):130-7. https://doi.org/10.1016/j.pbb.2011.08.002

Misiak B, Łoniewski I, Marlicz W, Frydecka D, Szulc A, Rudzki L, et al. The HPA axis dysregulation in severe mental illness: Can we shift the blame to gut microbiota? Prog Neuropsychopharmacol Biol Psychiatry 2020; 102: 109951. https://doi.org/10.1016/j.pnpbp.2020.109951

Mizoguchi Y, Yao H, Imamura Y, Hashimoto M, Monji A. Lower brain-derived neurotrophic factor levels are associated with age-related memory impairment in community-dwelling older adults: the Sefuri study. Sci Rep 2020; 10: 16442. https://doi.org/10.1038/s41598-020-73576-1

Mofidi F, Poustchi H, Yari Z, Nourinayyer B, Merat S, Sharafkhah M, et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial Br J Nutr. 2017;117:662-68. https://doi.org/10.1017/S0007114517000204

Mohammadi G, Dargahi L, Naserpour T, Mirzanejad Y, Alizadeh SA, Peymani A, et al. Probiotic mixture of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 attenuates hippocampal apoptosis induced by lipopolysaccharide in rats. Int Microbiol 2019; 22: 317-23. https://doi.org/10.1007/s10123-018-00051-3

Noronha SSR, Lima PM, Campos GSV, Chírico MTT, Abreu AR, Figueiredo AB, Silva FCS, Chianca DA Jr, Lowry CA, De Menezes RCA. Association of high-fat diet with neuroinflammation, anxiety-like defensive behavioral responses, and altered thermoregulatory responses in male rats. Brain Behav Immun. 2019;80:500-511. https://doi.org/10.1016/j.bbi.2019.04.030

O'Mahony SM, Felice VD, Nally K, Savignac HM, Claesson MJ, Scully P, Woznicki J, Hyland NP, Shanahan F, Quigley EM, Marchesi JR, O'Toole PW, Dinan TG, Cryan JF. Disturbance of the gut microbiota in early-life selectively affects visceral pain in adulthood without impacting cognitive or anxiety-related behaviors in male rats. Neuroscience. 2014;277:885-901. https://doi.org/10.1016/j.neuroscience.2014.07.054

Pandey KR, Naik SR, Vakil BV. Probiotics, prebiotics and synbiotics-a review. J Food Sci Technol 2015; 52:7577-87. https://doi.org/10.1007/s13197-015-1921-1

Redrobe JP, Bull S, Plath N. Translational aspects of the novel object recognition task in rats abstinent following sub-chronic treatment with phencyclidine (PCP): effects of modafinil and relevance to cognitive deficits in schizophrenia. Front Psychiatry 2010; 1:146. https://doi.org/10.3389/fpsyt.2010.00146

Sanchez C, Reines EH, Montgomery SA. A comparative review of escitalopram, paroxetine, and sertraline: are they all alike? Int Clin Psychopharmacol 2014; 29: 185-96. https://doi.org/10.1097/YIC.0000000000000023

Schoenfeld TJ, McCausland HC, Morris HD, Padmanaban V,

Cameron HA. Stress and loss of adult neurogenesis differentially reduce hippocampal volume. Biol Psychiatry 2017; 82: 914-23. https://doi.org/10.1016/j.biopsych.2017.05.013

Stefanaki C, Pervanidou P, Boschiero D, Chrousos GP. Chronic stress and body composition disorders: implications for health and disease. Hormones. 2018;17:33-43. https://doi.org/10.1007/s42000-018-0023-7

Tian W, Akanda MR, Islam A, Yang H-D, Lee S-C, Lee J-H, et al. The anti-stress effect of Mentha arvensis in immobilized rats. Int J Mol Sci 2018; 19: 355. https://doi.org/10.3390/ijms19020355

Tremblay A, Lingrand L, Maillard M, Feuz B, Tompkins TA. The effects of psychobiotics on the microbiota-gut-brain axis in early-life stress and neuropsychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 2021; 105: 110142. https://doi.org/10.1016/j.pnpbp.2020.110142

Vaghef-Mehrabany E, Maleki V, Behrooz M, Ranjbar F, Ebrahimi-Mameghani M. Can psychobiotics "mood" ify gut? An update systematic review of randomized controlled trials in healthy and clinical subjects, on anti-depressant effects of probiotics, prebiotics, and synbiotics. Clin Nutr 2020; 39: 1395-410. https://doi.org/10.1016/j.clnu.2019.06.004

Van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O'Sullivan O, et al. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J Physiol 2018; 596: 4923-44. https://doi.org/10.1113/JP276431

Wang XM, Zhang GF, Jia M, Xie ZM, Yang JJ, Shen JC, Zhou ZQ. Environmental enrichment improves pain sensitivity, depression-like phenotype, and memory deficit in mice with neuropathic pain: role of NPAS4. Psychopharmacology (Berl). 2019;236(7):1999-2014. https://doi.org/10.1007/s00213-019-5187-6

Yang HJ, Kim KY, Kang P, Lee HS, Seol GH. Effects of Salvia sclarea on chronic immobilization stress induced endothelial dysfunction in rats. BMC complementary and alternative medicine. 2014;14:1-5.https://doi.org/10.1186/1472-6882-14-396

Zafar T. Potential biomarkers of emotional stress induced neurodegeneration. eNeurologicalsci 2020; 21: 100292. https://doi.org/10.1016/j.ensci.2020.100292