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Linalool Ameliorates Memory Loss and Behavioral Impairment Induced by REM-Sleep Deprivation through the Serotonergic Pathway

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Abstract

Rapid eye movement (REM) sleep has an essential role in the process of learning and memory in the hippocampus. It has been reported that linalool, a major component of *Lavandula angustifolia*, has antioxidant, anti-inflammatory, and neuroprotective effects, along with other effects. However, the effect of linalool on the cognitive impairment and behavioral alterations that are induced by REM-sleep deprivation has not yet been elucidated. Several studies have reported that REM-sleep deprivation-induced memory deficits provide a well-known model of behavioral alterations. In the present study, we examined whether linalool elicited an anti-stress effect, reversing the behavioral alterations observed following REM-sleep deprivation in mice. Furthermore, we investigated the underlying mechanism of the effect of linalool. Spatial memory and learning memory were assessed through Y maze and passive avoidance tests, respectively, and the forced swimming test was used to evaluate anti-stress activity. The mechanisms through which linalool improves memory loss and behavioral alterations in sleep-deprived mice appeared to be through an increase in the serotonin levels. Linalool significantly ameliorated the spatial and learning memory deficits, and stress activity observed in sleep-deprived animals. Moreover, linalool led to serotonin release, and cortisol level reduction. Our findings suggest that linalool has beneficial effects on the memory loss and behavioral alterations induced by REM-sleep deprivation through the regulation of serotonin levels.

Key Words: Linalool, REM-sleep deprivation, Memory, Stress, Behavior

INTRODUCTION

Insomnia is a common problem and results from a high work load, shift work and various other tasks imposed by modern society. In our society, insomnia has come to be known as a cause of many chronic illnesses that can significantly impair functioning and have a negative influence on ones quality of life (Huang *et al*., 2011). Sleep deprivation has a negative impact on endocrine, metabolic, cardiovascular, immune, stress, cognition, and neurological function (Dimitrov *et al*., 2004; Scheer *et al*., 2009). Sleep deprivation is known to be a stressor (Mabunga *et al*., 2015), and generally increases cortisol levels, which appear to play a key role in the molecular, neuronal, and behavioral consequences (Mirescu *et al*., 2006). Evidence indicates that sleep deprivation involves a temporary activation of the major neuroendocrine stress systems, the autonomic sympathetic system and the hypothalamic-pituitary-adrenal (HPA) axis (Andersen *et al*., 2005). Recent studies have demonstrated that sleep deprivation impairs memory (Hu *et al*., 2013) and that rapid eye movement (REM) sleep, especially, has an essential role in the process of learning and memory in the hippocampus. Many reports have demonstrated that REM-sleep deprivation in rodents results in memory deficits as demonstrated using several behavioral tests, such as the passive avoidance test and the Y maze test (Huang *et al*., 2011). However, the mechanism through which sleep deprivation produces these deficits has not been clearly demonstrated. The disruptive effect of REM-sleep deprivation may be related to an alteration in the levels of the essential signaling molecules involved in memory and synaptic plasticity.

Linalool, a monoterpene, is the major component of *Lavandula angustifolia*, *Melissa officinalis*, *Rosmarinus officinalis*,

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and *Cymbopogon citratus* (Linck *et al*., 2009). Linalool exhibits diverse pharmacological effects including antioxidant, anti-inflammatory, and cardiovascular effects in hypertensive rats (Anjos *et al*., 2013; Huo *et al*., 2013; Park *et al*., 2016). A recent study suggested that linalool has various effects in the CNS, such as the reduction of memory loss and behavioral impairments in a mouse model of Alzheimer's disease, alteration of hypothalamic gene expression in restrained mice, as well as a neuroprotective effect (Sabogal-Guaqueta *et al*., 2016). However, the effect of linalool on REM-sleep deprivation is not known. In the present study, we examined whether linalool can elicit an anti-stress effect and a reversal of the memory loss and behavioral alterations observed in REM-sleep-deprived mice. Furthermore, we investigated the underlying mechanism of linalool.

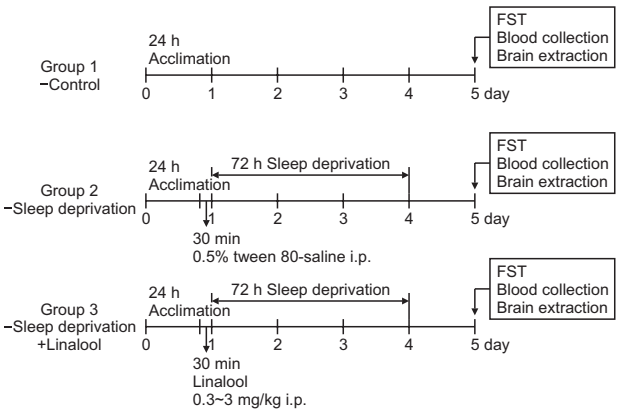

Fig. 1. Overview of experiments. Group 1 was a control group that was kept in their home cages. Group 2 was subjected to sleep deprivation for 72 h. Groups 3 was treated with linalool and subjected to sleep deprivation for 72 h.

MATERIALS AND METHODS

Animals

Four-week-old male ICR mice (weighing 18-22 g) were purchased from the OrientBio (Seongnam, Korea), and housed in a conventional animal facility with free access to food and water in a temperature and relative humidity monitored and controlled environment under artificial lighting (12 h of light per day). Animals were allowed to acclimatize for at least 7 days before the experiments were performed. All animal related study protocols were conducted in accordance with the guidelines of the National Institutes of Health guide for the care and use of Laboratory animals (NIH publication No. 8023, revised 1978), and were approved by the Committee on Animal Research at Ajou Medical Center, Ajou University.

REM-sleep deprivation model

In ICR mice, REM-sleep deprivation was induced using the modified multiple platform method, as described previously (Silva *et al*., 2004). The multiple platform method is the most widely used model to induce REM-sleep deprivation without affecting the non-REM-sleep period (van Hulzen and Coenen, 1981). Mice were placed in groups of four in water tanks (42×26×18 cm) containing 8 round platforms (3-cm-diameter) for 72 h. The tanks were filled with water to a level of 1 cm below the surface of the platforms. Each mouse was placed on the platform in water tanks without touching water. Mice were capable of moving within the tank and jumping up to the platforms. Previously, Suchecki *et al*. (2000) reported that the multiple platform method-induced animals exhibited an exclusive REM-sleep rebound, indicating significant suppression of REM-sleep. Food and water were provided ad libitum throughout the study and the water in the tanks was changed daily.

Experimental design

Animals were divided between a total of 10 groups, each comprising of 6-8 animals. Five groups were examined using the Y maze and forced swimming test (FST), the remaining five groups were examined using the passive avoidance test. Fig. 1 displays an overview of the experiments. Each group was either exposed to 72 h sleep deprivation using the multiple platform method or they remained in their home cages and acted as controls. After 30 min of 0.5% tween80-saline or linalool (0.3, 1 or 3 mg/kg, Sigma, St. Louis, MO, USA) administration, behavioral tests were performed on the day following the 72 h sleep deprivation. All behavioral tests were conducted between 10:00 and 17:00.

Y maze test

Spontaneous alternation behavior was examined using the Y maze test. This test is performed in a horizontal maze (30-cm long and 5-cm wide, with 12-cm high walls) with three arms (labeled A, B, and C). The maze floor and walls are constructed of black acrylic. Mice are initially placed within one arm, and the number of alternations (i.e., consecutive entry sequences of ABC, CAB, or BCA but not BAB) and the number of arm entries are manually recorded for each mouse over an 8-min period. In our experiment, 30 min before each test, the mice were given linalool (0.3, 1, 3 mg/kg, through intraperitoneal injection) or saline. The control group received 0.5% tween80-saline instead of linalool. The percentage alternation was calculated according to the following equation: Percentage alternation=[(Number of alternations)/(Total arm entries-2)]×100. The number of arm entries per trial was used as an indicator of locomotor activity. The Y maze arms were cleaned with 10% ethanol between tests to remove odors and residues.

Passive avoidance test

The passive avoidance test was performed essentially as has been described by Silva and colleagues (Silva *et al*., 2004). The apparatus employed was a two-way shuttle-box inter communicated by a guillotine door that was placed between the modular testing chambers. One chamber was clear, while the other chamber remained in the dark. In the training session, on the day before sleep deprivation, animals were given a foot shock (0.5 mA) whenever they entered the dark compartment. In the test sessions, the mice were again placed in the illuminated chamber, but no foot shock was applied; 30 min before the test, the mice were given linalool or saline. The latency to the chamber entrance was registered in each session.

Forced swim test (FST)

In the FST, mice were individually forced to swim in an open

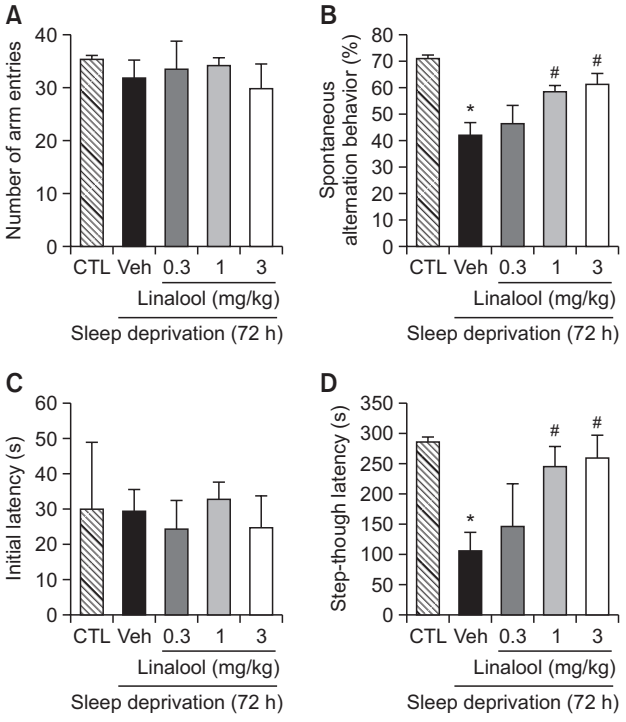

Fig. 2. Effect of linalool on the spontaneous alterative rates (%) in the Y maze test and the step-through latency in the passive avoidance test following sleep deprivation. Linalool (0.3, 1, 3, and 10 mg/kg, through intraperitoneal injection), or 0.5% tween80-saline (in vehicle (veh) group), were administered to 72 h sleep-deprived mice 30 min before the behavioral tests. (A, B) Y-maze test, (C, D) Passive avoidance test. Data are expressed as mean ± SEM (n=6-8). **p*<0.05 vs. control group (CTL), #*p*<0.05 vs. vehicle group.

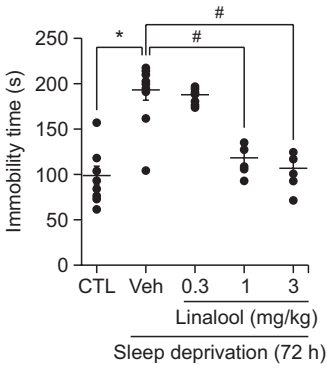

Fig. 3. Effect of linalool on the forced swimming test (FST) results following sleep deprivation. Linalool (0.3, 1, and 3 mg/kg, through intraperitoneal injection) or 0.5% tween80-saline (in vehicle (veh) group), were administered to 72 h sleep-deprived mice before the FST. Each dot represents the results from an individual animal. Data are expressed as mean ± SEM (n=6-8). **p*<0.05 vs. control group (CTL), #*p*<0.05 vs. vehicle group.

cylindrical container (10×25 cm), containing water at a temperature of 23 ± 1°C and a depth of 15 cm so that they could not escape or touch the bottom. Each mouse was gently placed in the cylinder and the total duration of floating was recorded during a 6-min period. The immobility time was measured during the last 4 min of the test. Each mouse was judged to be immobile when it ceased struggling and maintained motionless floating in the water, making only those movements necessary to keep its head above water.

Plasma hormones and serotonin levels

After performing the behavioral tests, mice were anaesthetized, and blood was collected from the abdominal vein using a 1 mL syringe, to include 60 μL 3.8% sodium citrate. Following this, plasma was clarified by centrifugation at 20°C for 10 min and stored at –80°C until experimentation. Plasma cortisol, adrenocorticotropic hormone (ACTH) and serotonin concentrations were analyzed using Mybiosource ELISA kits (San Diego, CA, USA). Absorbance was read using a Bio-Tek Synergy HT plate reader (Bio-Tek Instruments Inc., Winooski, VT, USA).

Hippocampal serotonin levels

Following linalool administration and linalool withdrawal, mice were sacrificed by decapitation immediately after the behavioral tests, and the hippocampi were dissected from the brain and stored in liquid nitrogen for the determination of serotonin levels. Serotonin levels were measured with Enzo Life Sciences ELISA kits (Farmingdale, NY, USA). The hippocampal samples were weighed and 300 μL lysis buffer was added. The samples were homogenized for 15 sec and centrifuged at 4°C for 20 min. The supernatant was stored at –20°C until analysis. Absorbance was read using a Bio-Tek Synergy HT plate reader (Bio-Tek Instruments Inc.).

Statistical analysis

Data are expressed as mean ± standard error of at least three separate determinations in each group. Numerical data were compare using Student's *t*-test or one-way ANOVA post-hoc test for the unpaired observations between the two groups. A *p*-value<0.05 was considered significant.

RESULTS

The effect of linalool on spatial and learning memory in REM-sleep-deprived mice

Spontaneous alteration behavior in the Y maze is used to measure spatial and working memory (Kwon *et al*., 2013). A significant difference was observed between the spontaneous alteration ratio performance in the REM-sleep-deprived and the control animals (Fig. 2A, 2B). The spontaneous alterative behavior of the REM-sleep-deprived group was markedly lower than the control group. Sleep-deprived mice treated with linalool (0.3, 1 or 3 mg/kg) displayed a higher performance rate in the Y maze test than the REM-sleep-deprived group (Fig. 2B). This result indicates that linalool may improve working and spatial memory. The spontaneous alteration ratio in the linalool 3 mg/kg treated group was similar to the spontaneous alteration ratio in the linalool 10 mg/kg treated group (data not shown). Therefore, linalool at 3 mg/kg was used as the maximum effect dose in all further experiments. To further assess the effect of linalool treatment on memory disorder in REM-sleep-deprived mice, the passive avoidance test was used to assess learning memory function in the rodents. In this test, a decline in the latency to enter into the dark chamber in the test

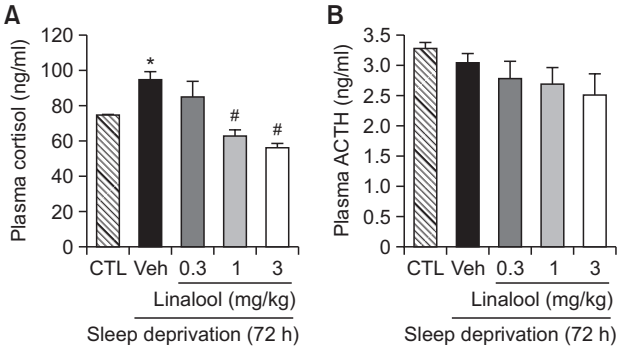

Fig. 4. Effect of linalool on the plasma hormone levels after sleep deprivation. (A) plasma cortisol levels, (B) plasma adrenocorticotropic hormone (ACTH) levels. Data are expressed as mean ± SEM (n=6-8). *p<0.05 vs. control group (CTL), $^{\#}p$<0.05 vs. vehicle group.

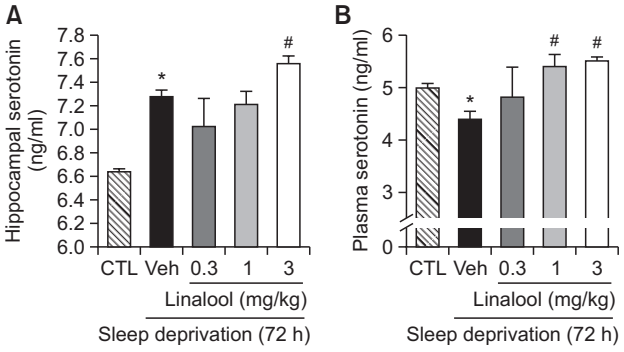

Fig. 5. Effect of linalool on hippocampal (A) and plasma (B) serotonin levels following sleep deprivation. Linalool (0.3, 1, and 3 mg/kg, through intraperitoneal injection) or 0.5% tween80-saline (in vehicle (veh) group), were administered to 72 h sleep-deprived mice. Data are expressed as mean ± SEM (n=6-8). *p<0.05 vs. control group (CTL), $^{\#}p$<0.05 vs. vehicle group.

session is always indicated by rodents with cognitive deficits such as aging, chronic stress or sleep deprivation (Huang *et al*., 2011). In the present study, it was demonstrated that 72 h of REM-sleep deprivation induced a memory deficit in passive avoidance retention in accordance with previous reports. Although differences in performances were observed among the groups following REM-sleep deprivation, no differences were found in the training session performance (Fig. 2C). The latency to enter the dark chamber was significantly lower in the REM-sleep-deprived group than the control group. Groups treated with linalool (1 or 3 mg/kg), however, presented a significantly higher latency than the REM-sleep-deprived group in the test session (Fig. 2D). These results demonstrate that linalool improved the learning memory deficit that was induced by REM-sleep deprivation.

The effect of linalool on depression and behavioral impairment in REM-sleep-deprived mice

To explore the anti-stress and antidepressant activity of linalool, we used the FST. The FST is a widely used behavioral test for the evaluation of potential anti-stress and antidepressant activity in rodents (de la Pena *et al*., 2014). In the current study, we explored whether 72 h of REM-sleep deprivation caused stress or a depressant state in mice. It was observed that the immobility time in the sleep-deprived group in the FST was significantly higher than that observed in the control group (CTL: 98 ± 10 sec, Veh: 192 ± 10 sec). Linalool administration (1 or 3 mg/kg) significantly reduced the immobility time of the REM-sleep-deprived mice in the FST (1 and 3 mg/kg; Veh: 119 ± 9 sec and Linalool: 107.75 ± 10.04 sec, respectively; Fig. 3). These results suggest that linalool has an antidepressant and anti-stress effect in 72 h REM-sleep-deprived mice.

The effect of linalool on the changing of the HPA axis-related stress hormone in REM-sleep-deprived mice

As shown in Fig. 4A, the plasma cortisol levels in the sleep-deprived group (veh) were significantly higher than those of the control group (ctl). After 72 h REM-sleep deprivation and linalool treatment (1 or 3 mg/kg), the cortisol levels were significantly lower than those of the untreated group. Plasma ACTH levels in REM-sleep-deprived mice were not significantly different between the linalool treated group and the untreated group (Fig. 4B).

The effect of linalool on hippocampal and plasma serotonin levels in REM-sleep-deprived mice

It has been reported that stress increases the serotonin turnover in animals and increases the serotonin levels in the hippocampus. As shown in Fig. 5A, hippocampal serotonin levels in the sleep-deprived group (veh) were significantly higher than in the control group. Following the administration of 3 mg/kg linalool, the serotonin levels were significantly higher than in the vehicle groups during REM-sleep deprivation. However, treatment with 0.3 or 1 mg/kg linalool produced no change in the serotonin levels compared to the untreated group. These results suggest that the plasma serotonin level is significantly reduced in REM-sleep-deprived mice, and that this reduction is inhibited by linalool treatment at 3 mg/kg (Fig. 5B).

DISCUSSION

In the present study, we demonstrated that linalool may protect mice from REM-sleep deprivation-induced stress, as demonstrated by a reduction in the behavioral impairment accompanied by memory loss. These anti-stress effects of linalool can be further substantiated by the observed decrease in cortisol levels and changes in serotonin levels.

REM-sleep is a mentally active phase of sleep. During REM-sleep, our bodies function in many ways that are similar to when we are awake. Our minds are active, with increased respiration and heart rate with more variability than is observed in other sleep stages. REM-sleep is thought to be essential for memory formation and storage, while most of our muscles shut down, severely restricting movement during this phase. REM-sleep deprivation is known to increase basal arousal and affect physiological and psychological processes as well as neurotransmitter levels, but the mechanism through which these effects are caused is not fully understood. During the initial stages of REM-sleep deprivation, plasma corticosterone levels increase, indicating that sleep deprivation results in stress on the process of maintaining body homeostasis (Mathangi *et al*., 2012). In addition, REM-sleep depriva-

tion changes the levels of HPA axis-related hormones, and the level of corticosteroid-releasing hormone (CRH). The effects of REM-sleep deprivation varies depending on the brain area (Fadda and Fratta, 1997). In the current study, linalool treatment was shown to decrease the immobility time as well as the plasma cortisol levels following REM-sleep deprivation. These results are in agreement with a previous report, which suggests that linalool has anti-stress and anti-depressant activity (Guzman-Gutierrez *et al*., 2012). However, we found that plasma ACTH levels remained unchanged during REM-sleep deprivation with or without linalool treatment (Fig. 4B), suggesting the presence of a negative feedback loop, in which the secretion of CRH from the pituitary gland is decreased as a result of a rise in cortisol levels (Andersen *et al*., 2005).

It may be assumed that the mechanism of action of linalool in improving the depression that is caused by stress during REM-sleep deprivation may involve the serotonergic pathway, because the levels of hippocampal serotonin appear to be altered by linalool. The relationship between stress and serotonin has been studied previously. It has been reported that stress increases serotonin turnover in the hypothalamus, the tonsils, and the hippocampus in rats. Additionally, serotonin-induced hyperglycemia is associated with a decrease in serotonin levels in stress patients (Dunn and Welch, 1991). Furthermore, the role of serotonin in mental disorders, such as depression, is well known. Notably, it has been suggested that serotonin may be directly or indirectly involved in depression-induced behavioral impairment, as SSRIs (selective serotonin reuptake inhibitors) are known to be protective against depression. As displayed in Fig. 5, we found that REM-sleep deprivation induced an increase in hippocampal serotonin levels, indicating that sleep deprivation-induced stress led to increased serotonin turnover, resulting in a temporary increase in serotonin levels. In addition, linalool treatment led to higher serotonin levels than treatment with a vehicle. Increased serotonin levels may have a positive effect in the short term, while maintaining a high level of serotonin in the hippocampus for a long period without a rapid decrease may have other effects. Furthermore, in our results, linalool attenuated the reduction of plasma serotonin levels that were induced by REM-sleep deprivation (Fig. 5B), consistent with previous reports that higher plasma serotonin levels lead to a better mood (Williams *et al*., 2006). Thus, serotonin levels in plasma, as well as in the hippocampus, may be closely related to mood disorders such as depression and anxiety. The effects of REM-sleep deprivation on depression are controversial. As in previous reports, our results demonstrate that REM-sleep deprivation induces depressive behavior. Clinical evidence suggests that the relationship between sleep deprivation and depression is complex, and that sleep deprivation aggravates depression (Roberts and Duong, 2014). Conversely, time-lapse treatment of sleep deprivation may be used to treat depression (Giedke and Schwarzler, 2002). However, the anti-depressive symptoms caused by REM-sleep deprivation do not persist, they are a temporary phenomenon, so clear identification of the mechanism is necessary.

We first reported that linalool improves the behavioral impairments caused by memory loss following REM-sleep deprivation. It is possible that linalool has the ability to ameliorate the behavioral impairments of REM-sleep deprivation via the serotonergic system. Serotonin has not only been related to the inhibition of sleep and the promotion of wakefulness, but also plays a role in the initiation and maintenance of sleep (Zhang *et al*., 2014). Because of the role of serotonin in sleep and wakefulness, serotonin may be a candidate for the neural mechanism of the observed learning and memory impairment following REM-sleep deprivation (Silvestri, 2005). In some cases, the serotonin receptors in the CNS are co-localized with other neurotransmitters such as glutamate and γ-aminobutyric acid (GABA) at the same nerve terminals, leading to mutual interactions in the neurobiological control of learning and memory and behavioral alteration (Ma, 2001). Recently, several studies have reported that glutamate release is reduced by serotonin receptor activation in various brain regions, while serotonergic sensitization is regulated through the activation of N-methyl-D-aspartate (NMDA) receptors (Higuchi *et al*., 2008). Linalool has been shown to regulate glutamate activation *in vitro* and *in vivo* through competitive antagonism of NMDA receptors (Silva Brum *et al*., 2001). It is known that NMDA receptors plays a crucial role in the induction of activity-dependent synaptic plasticity and memory formation (de Lima *et al*., 2005). In contrast, we observed that linalool treatment improved memory loss in REM-sleep-deprived mice. Previous studies have shown that REM-sleep deprivation temporarily increases the long-term potentiation (LTP) of EPSPs and subsequently maintains increasing long-term depression (LTD) in the hippocampal CA1 region. Because LTD plays a role in the regulation of memory consolidation, the enhancement of LTD through REM-sleep deprivation can have an adverse effect on memory consolidation (Tadavarty *et al*., 2009). Accordingly, it is possible that linalool attenuates the transient enhancement of LTP caused by REM-sleep deprivation, which may modulate the serotonergic pathways, resulting in an improvement in behavioral alteration and memory consolidation. However further studies are required in order to fully identify the efficacy of linalool in the treatment of the memory loss and behavioral alteration, and the involved changes in neurotransmitters, during REM-sleep deprivation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ACKNOWLEDGMENTS

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REFERENCES

Andersen, M. L., Martins, P. J., D'Almeida, V., Bignotto, M. and Tufik, S. (2005) Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats. *J. Sleep Res.* **14**, 83-90.

Anjos, P. J., Lima, A. O., Cunha, P. S., De Sousa, D. P., Onofre, A. S., Ribeiro, T. P., Medeiros, I. A., Antoniolli, A. R., Quintans-Junior, L. J. and Santosa, M. R. (2013) Cardiovascular effects induced by linalool in normotensive and hypertensive rats. *Z. Naturforsch., C, J. Biosci.* **68**, 181-190.

de la Pena, J. B., Kim, C. A., Lee, H. L., Yoon, S. Y., Kim, H. J., Hong, E. Y., Kim, G. H., Ryu, J. H., Lee, Y. S., Kim, K. M. and Cheong, J. H. (2014) Luteolin mediates the antidepressant-like effects of Cirsium japonicum in mice, possibly through modulation of the GABAA receptor. *Arch. Pharm. Res.* **37**, 263-269.

de Lima, M. N., Laranja, D. C., Bromberg, E., Roesler, R. and Schroder, N. (2005) Pre- or post-training administration of the NMDA receptor blocker MK-801 impairs object recognition memory in rats. *Behav. Brain Res*. **156**, 139-143.

Dimitrov, S., Lange, T., Tieken, S., Fehm, H. L. and Born, J. (2004) Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans. *Brain Behav. Immun.* **18**, 341-348.

Dunn, A. J. and Welch, J. (1991) Stress- and endotoxin-induced increases in brain tryptophan and serotonin metabolism depend on sympathetic nervous system activity. *J. Neurochem.* **57**, 1615-1622.

Fadda, P. and Fratta, W. (1997) Stress-induced sleep deprivation modifies corticotropin releasing factor (CRF) levels and CRF binding in rat brain and pituitary. *Pharmacol. Res.* **35**, 443-446.

Giedke, H. and Schwarzler, F. (2002) Therapeutic use of sleep deprivation in depression. *Sleep Med. Rev.* **6**, 361-377.

Guzman-Gutierrez, S. L., Gomez-Cansino, R., Garcia-Zebadua, J. C., Jimenez-Perez, N. C. and Reyes-Chilpa, R. (2012) Antidepressant activity of Litsea glaucescens essential oil: identification of beta-pinene and linalool as active principles. *J. Ethnopharmacol.* **143**, 673-679.

Higuchi, M., Suzuki, Y., Yatani, Y., Kitagawa, Y., Nagayasu, K., Shirakawa, H., Nakagawa, T. and Kaneko, S. (2008) Augmentation of serotonin release by sustained exposure to MDMA and methamphetamine in rat organotypic mesencephalic slice cultures containing raphe serotonergic neurons. *J. Neurochem.* **106**, 2410-2420.

Hu, Y., Liu, M., Liu, P., Yan, J. J., Liu, M. Y., Zhang, G. Q., Zhou, X. J. and Yu, B. Y. (2013) Effect of kai xin san on learning and memory in a rat model of paradoxical sleep deprivation. *J. Med. Food* **16**, 280-287.

Huang, L. Z., Wei, L., Zhao, H. F., Huang, B. K., Rahman, K. and Qin, L. P. (2011) The effect of Eleutheroside E on behavioral alterations in murine sleep deprivation stress model. *Eur. J. Pharmacol.* **658**, 150-155.

Huo, M., Cui, X., Xue, J., Chi, G., Gao, R., Deng, X., Guan, S., Wei, J., Soromou, L. W., Feng, H. and Wang, D. (2013) Anti-inflammatory effects of linalool in RAW 264.7 macrophages and lipopolysaccharide-induced lung injury model. *J. Surg. Res.* **180**, e47-e54.

Kwon, S. H., Ma, S. X., Joo, H. J., Lee, S. Y. and Jang, C. G. (2013) Inhibitory effects of eucommia ulmoides Oliv. Bark on scopolamine-induced learning and memory deficits in mice. *Biomol. Ther. (Seoul)* **21**, 462-469.

Linck, V. M., da Silva, A. L., Figueiro, M., Piato, A. L., Herrmann, A. P., Dupont Birck, F., Caramao, E. B., Nunes, D. S., Moreno, P. R. and Elisabetsky, E. (2009) Inhaled linalool-induced sedation in mice. *Phytomedicine* **16**, 303-307.

Ma, Q. P. (2001) Co-localization of 5-HT(1B/1D/1F) receptors and glutamate in trigeminal ganglia in rats. *Neuroreport* **12**, 1589-1591.

Mabunga, D. F., Gonzales, E. L., Kim, H. J. and Choung, S. Y. (2015) Treatment of GABA from fermented rice germ ameliorates caffeine-induced sleep disturbance in mice. *Biomol. Ther. (Seoul)* **23**, 268-274.

Mathangi, D. C., Shyamala, R. and Subhashini, A. S. (2012) Effect of REM sleep deprivation on the antioxidant status in the brain of Wistar rats. *Ann. Neurosci.* **19**, 161-164.

Mirescu, C., Peters, J. D., Noiman, L. and Gould, E. (2006) Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. *Proc. Natl. Acad. Sci. U.S.A.* **103**, 19170-19175.

Park, H., Seol, G. H., Ryu, S. and Choi, I. Y. (2016) Neuroprotective effects of (-)-linalool against oxygen-glucose deprivation-induced neuronal injury. *Arch. Pharm. Res.* **39**, 555-564.

Roberts, R. E. and Duong, H. T. (2014) The prospective association between sleep deprivation and depression among adolescents. *Sleep* **37**, 239-244.

Sabogal-Guaqueta, A. M., Osorio, E. and Cardona-Gomez, G. P. (2016) Linalool reverses neuropathological and behavioral impairments in old triple transgenic Alzheimer's mice. *Neuropharmacology* **102**, 111-120.

Scheer, F. A., Hilton, M. F., Mantzoros, C. S. and Shea, S. A. (2009) Adverse metabolic and cardiovascular consequences of circadian misalignment. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 4453-4458.

Silva Brum, L. F., Emanuelli, T., Souza, D. O. and Elisabetsky, E. (2001) Effects of linalool on glutamate release and uptake in mouse cortical synaptosomes. *Neurochem. Res.* **26**, 191-194.

Silva, R. H., Abilio, V. C., Takatsu, A. L., Kameda, S. R., Grassl, C., Chehin, A. B., Medrano, W. A., Calzavara, M. B., Registro, S., Andersen, M. L., Machado, R. B., Carvalho, R. C., Ribeiro Rde, A., Tufik, S. and Frussa-Filho, R. (2004) Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. *Neuropharmacology* **46**, 895-903.

Silvestri, A. J. (2005) REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiol. Behav.* **84**, 343-349.

Suchecki, D., Duarte Palma, B. and Tufik, S. (2000) Sleep rebound in animals deprived of paradoxical sleep by the modified multiple platform method. *Brain Res.* **875**, 14-22.

Tadavarty, R., Kaan, T. K. and Sastry, B. R. (2009) Long-term depression of excitatory synaptic transmission in rat hippocampal CA1 neurons following sleep-deprivation. *Exp. Neurol.* **216**, 239-242.

van Hulzen, Z. J. and Coenen, A. M. (1981) Paradoxical sleep deprivation and locomotor activity in rats. *Physiol. Behav.* **27**, 741-744.

Williams, E., Stewart-Knox, B., Helander, A., McConville, C., Bradbury, I. and Rowland, I. (2006) Associations between whole-blood serotonin and subjective mood in healthy male volunteers. *Biol. Psychol.* **71**, 171-174.

Zhang, C., Zhao, X., Mao, X., Liu, A., Liu, Z., Li, X., Bi, K. and Jia, Y. (2014) Pharmacological evaluation of sedative and hypnotic effects of schizandrin through the modification of pentobarbital-induced sleep behaviors in mice. *Eur. J. Pharmacol.* **744**, 157-163.