



MLC901 during sleep deprivation rescues fear memory disruption in rats

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Abstract

Several lines of evidence suggest that sleep deprivation disrupts cognitive and emotional abilities and changes the expression of distinctive categories of genes in the brain. In the present study, saline- or MLC901 (a traditional Chinese medicine)-treated male Wistar rats were first submitted to a modified water box (for 24-h sleep deprivation) and then trained in contextual and tone fear conditioning tasks with the purpose to evaluate the effect of MLC901 during sleep deprivation on fear memory retention. Hippocampal mRNA measurement was performed by reverse transcription-polymerase chain reaction (RT-PCR). We found that the exposure of rats to 24 h of sleep deprivation impaired contextual and tone fear memory retention, while administration of MLC901 (0.2, 0.4, and 0.8 mg/kg, once/12 h; i.p.) during sleep deprivation abolished memory deficits. Meanwhile, different doses of MLC901 alone had no effect on performance in both tasks. We observed that MLC901 increased the expression levels of proapoptotic BAD, anti-apoptotic Bcl-xL, and Tfam as an index of mitochondrial biogenesis compared to sleep-deprived rats, while MLC901 during sleep deprivation increased BAX, BAD, and Bcl-xL compared to the control group. Sleep deprivation decreased BAX and Tfam, by itself. MLC901 only decreased BAX and Tfam and increased BAD level compared to the non-sleep-deprived control group. It is suggested that MLC901 might be a therapeutic option for memory impairment during sleep deprivation.

Keywords Sleep deprivation · MLC901 · Fear memory conditioning · Rat

Introduction

There is a reciprocal relationship between sleep and memory (Walker and Stickgold 2006). Human and animal studies have demonstrated that the training in various memory tasks increases sleep time and sleep deprivation interferes with different phases of memory (Rossi et al. 2014; Krause et al. 2017). Pavlovian fear conditioning is a useful paradigm for the exploring of the role of sleep and memory, because it is rapidly acquired and can dissociate hippocampus (context)-dependent memory processing from that processed by the amygdala (cue) (Walker and Stickgold 2006). It has been reported that pre-training sleep deprivation impairs contextual memory encoding assessed 24 h later, whereas cued learning was largely unaffected (Ruskin et al. 2004). These findings propose that pre-training sleep deprivation may affect memory encoding by neuroanatomical distinct systems, and impairing hippocampal encoding processes while having only minor effects on amygdala encoding (McDermott et al. 2003). A number of animal studies have reported that sleep deprivation reduces neuronal excitability and inhibits long-term potentiation

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(LTP) of synaptic strength as well as alters expression of genes related to metabolic processes. The studies also indicated that sleep deprivation decreases response to stress and inflammation, circadian sleep/wake cycles, apoptosis and mitochondrial biogenesis, and various signaling pathways in the hippocampal CA1 region (McDermott et al. 2003; Andrezza et al. 2010; Havekes et al. 2016).

In contrast, a positive effect for MLC901, as a traditional Chinese medicine, on the motor and cognitive function of ischemic and traumatic brain-injured rodents as well as normal mice has been suggested (Heurteaux et al. 2010; Quintard et al. 2011; Quintard et al. 2014; Lorivel et al. 2015). The beneficial effects of MLC901 are probably a result of the combination of neuroprotective and neurorepair mechanisms induced by nine herbal compounds acting on different targets (Lorivel et al. 2015). This compound increases dentate gyrus neurogenesis and expression of the cortical brain-derived neurotrophic factor (BDNF) (Heurteaux et al. 2010). Given the well-recognized cognitive changes associated with sleep deprivation and the positive effect of MLC901 on brain function, we decided to investigate the effect of MLC901 administration on the fear memory deficit of sleep-deprived rats. In addition, we examined pro-apoptotic gene of BAX and BAD, the anti-apoptotic gene of Bcl-xL expression levels and the expression change of genes related to mitochondrial biogenesis, PGC-1 α , and Tfam, in the hippocampus.

Material and methods

Subjects

Sixty-four adult male Wistar rats, weighing 200–220 g, born and housed in the Institute for Cognitive Science Studies (ICSS) vivarium, and were treated in accordance with the guidelines for or care and Use of Laboratory Animals (National Institutes of Health Publication No. 85-23, revised 2010) and were approved by the Animal Care and Use

Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.IAU.PS.REC.1397.182). Rats were kept in groups of four in Plexiglas cages and maintained on a 12 h/12 h light/dark schedule with lights on at 7 am and off at 7 pm and a temperature controlled 22 ± 2 °C, while they had free access to water and food.

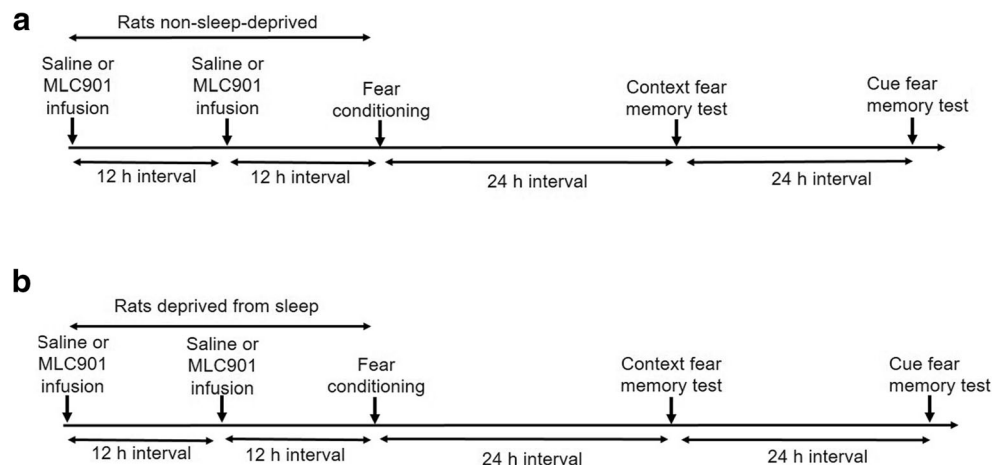
Drug treatment

MLC901 (Moleac, Singapore) combines nine herbal components with the following composition per capsule (Heurteaux et al. 2010): 0.57 g Radix astragali, 0.114 g Radix salvia miltiorrhizae, 0.114 g Radix paeoniae rubra, 0.114 g Rhizoma chuanxiong, 0.114 g Radix angelicae sinensis, 0.114 g *Carthamus tinctorius*, 0.114 g *Prunus persica*, 0.114 g Radix polygalae, and 0.114 g Rhizoma acori tatarinowii. Animals received saline (1 ml/kg) or different doses of MLC901 (0.2, 0.4, and 0.8 mg/kg, intraperitoneally) for two times with a 12-h interval. The volume of infusion was 1 ml/kg. The doses range was chosen based on preliminary experiments.

Experimental design

Animals were randomly divided into two sets of four groups: set (1), four groups of rats were placed in the water box when the apparatus was off (rats non-sleep-deprived in the apparatus) during 24 h, while received two administrations of saline or different doses of MLC901. Immediately after the first administration, rats were submitted to the water box. After 12-h interval, they received the second administration. Then, rats were kept in the water box for 12 h before behavioral training (Fig. 1a). Set (2), four groups of rats were deprived from sleep in the water box during 24 h while received saline or different doses of MLC901, as mentioned for non-sleep-deprived groups (Fig. 1b). Behavioral data collection was done from eight animals in each experimental group.

Fig. 1 Experimental design. **a** shows that non-sleep-deprived rats received saline (1 ml/kg) or various doses (0.2, 0.4, and 0.8 mg/kg; once/12 h) of MLC901 in the water box. Fear conditioning was conducted immediately after water box submission, and the rats were tested for their conditioned contextual fear response the following day. After 24 h, the rats were tested for their conditioned cue fear memory response. **b** shows the same design for sleep-deprived rats in the water box



Sleep deprivation procedure

Rats were sleep deprived by the modified automatic total sleep deprivation method using the water box for 24 h (Norzpour et al. 2016; Javad-Moosavi et al. 2017). The water box was made from four clear Plexiglas equal boxes (30 × 30 × 30 cm) filled with room-temperature water. Four rats were placed in the tank (one rat in each box) so that social stability could be kept. Two small platforms (each of 15 cm diameter and 3 mm edge high) were located next to each other in the middle of each box. The contrived holes (2 mm in diameters) on the surface of platforms evacuate water during upward movements which help rats avoid slipping or getting wet. Platform movements were done independently and automatically. Initially, both platforms were a little immersed in the water surface. Then each platform moved upward and downward to force rats to move continuously in order to avoid contact with water. The speed of movement was 1 m/s. Each platform rotation needed 30 s to complete. During this period, each platform remained at the highest elevation (10 cm) over water surface for 10 s (in order to get water and food) (holding time). After this period of time, platform moved 60 mm down for 2.5 s and immediately came up with 2.5 s to the initial position. The rats became familiar with water box 1 day before application of different sleep deprivation protocols so that their stress level was reduced for 30 min. Therefore, the rats learned to stay at the junction of two platforms and go from the sinking platform to the other one in a short movement. Behavioral observations which were obtained during daily sleep deprivation showed that rats were awake 100% of the time when platform technique was used. Potential effects of stress due to the new environment were evaluated using non-sleep-deprived groups in similar situations when the apparatus was off. To minimize stress and discomfort, animals had free access to food pellets and fresh drinking water during sleep deprivation. Rats were conditioned immediately after 24 h of sleep deprivation or control treatment.

Test of contextual versus cued memory

Freezing behavior was conditioned by placing a rat in an operant conditioning chamber with a grid floor. After a 150-s period, four 150-s blocks of tone–shock pairings were administered as follows: a tone (80 dB) was presented for 5 s; at the termination of the tone, a 2-s foot shock was administered (0.4 mA); A 30-s inter-trial interval occurred during which no shock or tone was administered. After four tone–shock pairings, the animal was returned to its home cage. Twenty-four hours after conditioning, the animal was placed in the same operant chamber as above for 5 min, during which no

tone or shock was presented. Freezing behavior, which in this setting is indicative of contextual memory, was recorded. One day after the test of contextual memory, the animal was placed in a novel chamber, which differed from the conditioning chamber by shape, texture, color, and complexity. After 150 s, the conditioning tone was presented as described in the conditioning phase, and the animal was left in the chamber for another 2.5 min. The freezing behavior after the tone, indicative of cued memory, was scored as described above (Nasehi et al. 2018). Previous research has shown that the neural processes underlying contextual memory are mediated primarily via the hippocampus, whereas those underlying cued memories are mediated by several structures, most prominently the amygdala (McDermott et al. 2003).

Tissue sample collection

After behavioral tests, rats belong to saline/non-sleep-deprived, MLC901 (0.8 mg/kg)/non-sleep-deprived, saline/sleep-deprived, and MLC901 (0.8 mg/kg)/sleep-deprived groups were decapitated and their brains removed and washed with saline solution. The manipulation of the brain was made on the ice. To dissect the hippocampus, we cut at the brain's midline to separate the cerebral hemispheres, and then cut the fimbria-fornix bundle and with the aid of a spatula removed it quite easily. Tissue samples were immediately homogenized and frozen at $-20\text{ }^{\circ}\text{C}$.

RNA isolation and RT-PCR

Total hippocampal RNA was extracted using RNeasy lipid tissue mini kit (Qiagen, USA) according to the manufacturer's instructions. Purity and quantity of extracted RNA were checked by gel electrophoresis (1% agarose, Gibco/BRL) and spectrophotometry. One-microgram RNA was used to synthesize 20- μl first-strand cDNA with the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's protocol. Diluted cDNA was added to SYBR Green PCR Master Mix (Life Technologies) in addition to the respective primers for BAX, Bcl-xL, PGC-1 α , and Tfam (Table 1). The expression of the targeted genes was normalized using GAPDH as the endogenous control. Quantitative RT-PCR assays were performed using 96-well plates sealed with optical quality film (Applied Biosystems) on a DNA thermal cycler (Bio-Rad, Hercules, CA, USA) using the CFX96 Real-Time System. Gene expression was analyzed by the $2^{-\Delta\Delta Ct}$ method and is expressed in arbitrary units.

Data analysis

The data were analyzed using two-way ANOVA, followed by the Tukey comparison post hoc test. The data were expressed as the means \pm SEM. The results were considered statistically

Table 1 The obtained results from the gene expression levels (BAX, BAD, Bcl-xL, PGC-1 α , and Tfam) in non-sleep-deprived rats received MLC901 (0.8 mg/kg), sleep-deprived rats received saline (1 ml/kg), sleep-deprived rats received MLC901 (0.8 mg/kg) compared to the

non-sleep-deprived control group, and sleep-deprived rats received MLC901 (0.8 mg/kg) compared to sleep-deprived rats received saline (1 ml/kg)

	MLC901 group		SD group		MLC901-SD related to control		MLC901-SD related to SD group	
	Regulation by the factor	<i>P</i> value	Regulation by the factor	<i>P</i> value	Regulation by the factor	<i>P</i> value	Regulation by the factor	<i>P</i> value
BAX	Down-regulation 5.5	0.001	Down-regulation 10.818	0.001	Down-regulation 10.56	0.001	Up-regulation 1.024	0.8854
BAD	Up-regulation 1.704	0.016	Down-regulation 1.092	0.4565	Up-regulation 2.263	0.016	Up-regulation 2.472	0.001
Bcl-xL	Up-regulation 1.724	0.0744	Up-regulation 1.075	0.5225	Up-regulation 1.799	0.001	Up-regulation 1.673	0.0509
PGC-1 α	Up-regulation 1.511	0.1101	Down-regulation 1.217	0.3041	Down-regulation 1.003	0.9779	Up-regulation 1.214	0.4617
Tfam	Down-regulation 1.363	0.001	Down-regulation 1.531	0.001	Down-regulation 1.067	0.5786	Up-regulation 1.436	0.001

significant when $P < 0.05$. Data of gene expression experiments were analyzed by Relative Expression Software Tool (REST)-XL version 2 (Pfaffl 2002) which is used to determine significant differences in relative expression levels between sample and control groups. The software uses pair-wise-fixed reallocation randomization test to define the significance of results. One of the advantages of this software is that gene quantification and normalization are done at the same time. Data are shown as fold differences of mean normalized expression values \pm SEM. $P < 0.05$ is considered statistically significant.

Results

Sleep deprivation impaired contextual fear memory retention, and this effect blocked by MLC901

This experiment sought to determine whether pre-training administration of MLC901 during sleep deprivation would rescue contextual fear memory retention. Two-way ANOVA revealed a significant interaction between different doses of MLC901 and MLC901 plus sleep deprivation on the percentage of freezing time [effect of MLC901: $F(3, 56) = 2.75$, $P = 0.05$; effect of sleep deprivation: $F(1, 56) = 3.38$, $P = 0.07$; MLC901, sleep deprivation interaction: $F(3, 56) = 13.12$, $P < 0.001$; Fig. 2A] but not latency to the freezing [effect of MLC901: $F(3, 56) = 5.25$, $P = 0.003$; effect of sleep deprivation: $F(1, 56) = 1.77$, $P = 0.18$; MLC901, sleep deprivation interaction: $F(3, 56) = 0.11$, $P = 0.95$; Fig. 2B]. The analysis also indicated that sleep deprivation decreased the percentage of freezing time compared to control non-sleep-deprived group, suggesting contextual fear memory retention impairment. All doses of MLC901 when administered during sleep deprivation blocked contextual fear memory retention deficit, while the drug had no effect on performance of contextual memory task in non-sleep-deprived rats. None of groups showed a significant alteration in latency to freezing.

Sleep deprivation impaired tone fear memory retention, and this effect blocked by MLC901

This experiment sought to determine whether pre-training administration of MLC901 during sleep deprivation would rescue tone fear memory retention. Two-way ANOVA revealed a significant interaction between different doses of MLC901 and MLC901 plus sleep deprivation on the percentage of freezing time [effect of MLC901: $F(3, 56) = 4.16$, $P = 0.01$; effect of sleep deprivation: $F(1, 56) = 6.94$, $P = 0.01$; MLC901, sleep deprivation interaction: $F(3, 56) = 5.69$, $P = 0.002$; Fig. 3A] but not latency to the freezing [effect of MLC901: $F(3, 56) = 1.04$, $P = 0.38$; effect of sleep deprivation: $F(1, 56) = 1.30$, $P = 0.26$; MLC901, sleep deprivation interaction: $F(3, 56) = 1.08$, $P = 0.37$; Fig. 3B]. The analysis also indicated that sleep deprivation decreased the percentage of freezing time compared to control non-sleep-deprived group, suggesting a tone fear memory retention impairment. All doses of MLC901 when administered during sleep deprivation blocked tone fear memory retention deficit, while the drug had no effect on the performance of tone memory task in non-sleep-deprived groups. None of the groups showed a significant alteration on latency to freezing.

Hippocampal BAX and Tfam mRNA expressions decreased following sleep deprivation. MLC901 decreased BAX and increased BAD and Bcl-xL mRNA expressions during sleep deprivation compared to the control group, while it increased BAD, Bcl-xL, and Tfam compared to non-sleep-deprived rats

This experiment sought to determine whether pre-training administration of MLC901 during sleep deprivation would alter mRNA expression levels of BAX, BAD, Bcl-xL, PGC-1 α , and Tfam compared to control non-sleep-deprived or sleep-deprived rats. The pro-apoptotic gene of BAX was down-regulated, while the apoptotic gene of the BAD and anti-apoptotic gene of Bcl-xL was up-regulated in sleep-deprived

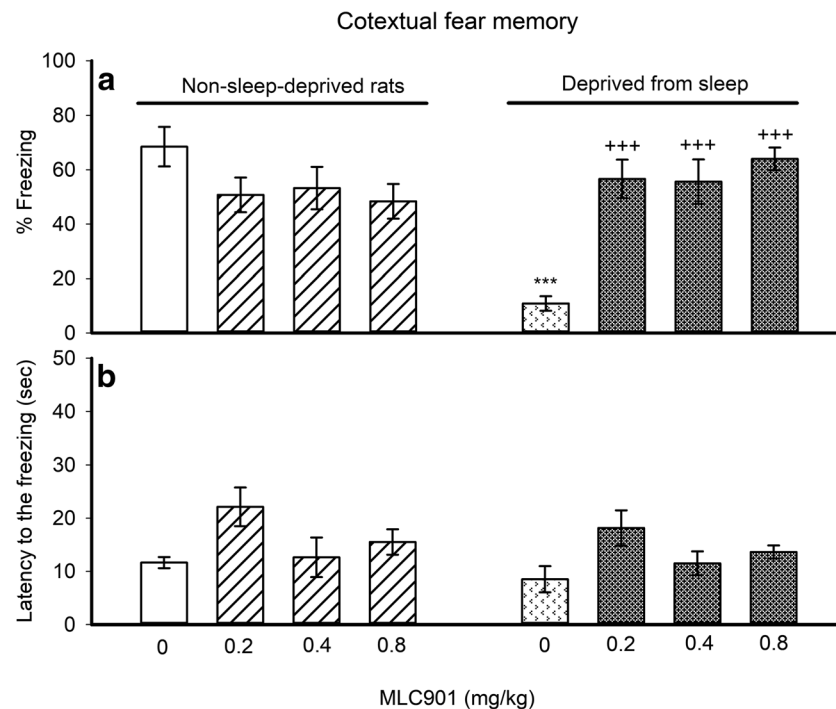


Fig. 2 Effects of intraperitoneal administration of saline (1 ml/kg) or different doses of MLC901 (0.2, 0.4, and 0.8 mg/kg; once/12 h) alone or during 24 h of sleep deprivation on contextual fear recall. Two-way ANOVA and post hoc analysis revealed that sleep deprivation decreases the percentage of freezing time compared to the control non-sleep-deprived group, suggesting contextual fear memory retention impairment. All doses of MLC901 when administered during sleep deprivation

blocked contextual fear memory retention deficit, while the drug had no effect on performance of contextual memory task in awake groups. Percent freezing (A) and latency to the freezing (B). Values are expressed as mean \pm S.E.M ($n = 8$ in each group). *** $P < 0.001$ different from saline non-sleep-deprived group; **** $P < 0.001$ different from the respective MLC901/non-sleep-deprived group

rats that received MLC901 compared to the control group. BAD, Bcl-xL and Tfam were up-regulated in sleep-deprived rats that received MLC901 compared to the sleep-deprived group. The expression levels of BAX and Tfam was down-regulated in sleep-deprived rats compared to the control non-sleep-deprived group. The mRNA expressions of BAX and Tfam were down-regulated in the MLC901 group compared to the control group (Fig. 4 and Table 1).

Discussion

This study, as far as we know, is the first to show the effects of MLC901 on fear memory deficits in sleep-deprived rats. Behavioral and genetic aspects investigated here provide evidence for the protective effect of MLC901 on cognitive impairments induced by sleep deprivation in rats.

Sleep deprivation during 24-h impairs context and cue fear memory retention

Our data show that 24 h of sleep deprivation impaired contextual and cued fear memory recall in male Wistar rats. However, rats deprived from sleep did not alter latency to the freezing compared to non-sleep-deprived rats. Evidence

from various human and rodent studies supports the importance of sleep in learning and memory (Oudiette et al. 2013; Halonen et al. 2016). Although the functions of sleep remain largely unknown, one of the most exciting hypotheses is that sleep contributes importantly to processes of memory and brain plasticity (Walker and Stickgold 2006). In animals, pre-training sleep deprivation has been demonstrated to impair the encoding of numerous memory tasks. For example, Guan et al. reported that 6 h of sleep deprivation prior to training results in a severe disruption of spatial memory encoding, as assessed by retention 24 h later (Guan et al. 2004). Similarly, two previous studies in our laboratory showed that 24 h of pre-training sleep deprivation impairs passive avoidance memory retention in step-through task that is used to evaluate emotional memory (Norzpour et al. 2016; Javad-Moosavi et al. 2017). Ruskin et al. demonstrated that 72 h of pre-training sleep deprivation profoundly impairs contextual memory encoding measured 24 h later, whereas cued learning is largely unaffected. Therefore, they suggested that pre-training sleep deprivation impairs hippocampal encoding processes but not amygdala memory encoding (Ruskin et al. 2004). On the other hand, Rossi et al. reported that 96 h of sleep deprivation impairs contextual and tone fear memory encoding but not memory consolidation (Rossi et al. 2014). The findings about the effect of sleep deprivation on hippocampal-dependent

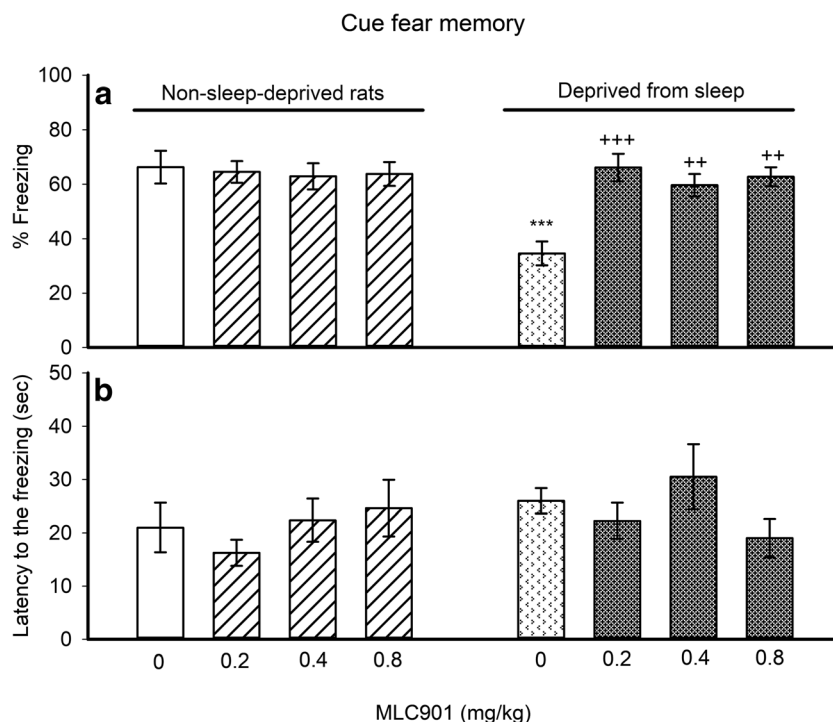


Fig. 3 Effects of intraperitoneal administration of saline (1 ml/kg) or different doses of MLC901 (0.2, 0.4, and 0.8 mg/kg; once/ 12 h) alone or during 24 h of sleep deprivation on cue fear recall. Two-way ANOVA and post hoc analysis indicated that sleep deprivation decreases the percentage of freezing time compared to control non-sleep-deprived group, suggesting a tone fear memory retention impairment. All doses of MLC901 when administered during sleep deprivation blocked tone fear

memory retention deficit, while the drug had no effect on the performance of tone memory task in non-sleep-deprived groups. Percent freezing (A) and latency to the freezing (B). Values are expressed as mean \pm S.E.M ($n = 8$ in each group). ^{***} $P < 0.001$ different from the saline non-sleep-deprived group; ⁺⁺⁺ $P < 0.001$ and ⁺⁺ $P < 0.01$ different from the respective MLC901/non-sleep-deprived group

contextual fear memory are the same, whereas the data on the effect of sleep deprivation on amygdala-dependent cued fear memory are controversial, with studies showing this task to be resistant to the disturbing effect of sleep deprivation and others showing that it is also affected by sleep deprivation (Rossi et al. 2014). One possible reason for this discrepancy might stem from the differences in deprived sleep stage, period of sleep deprivation, the effect of sleep deprivation on different phases of memory formation, the number of shock-tone pairing.

Ineffective doses of MLC901 block context and cue fear memory retention deficits induced by sleep deprivation

Our results reveal that intraperitoneal administration of different doses of MLC901 (twice/24 h) abolished memory impairment induced in sleep-deprived rats. Meanwhile, non-sleep-deprived rats treated with MLC901 showed a successful fear memory retention compared to the control group. It should be noted that MLC901 had no effect on latency to the freezing in sleep-deprived or awake rats. MLC901 has previously been shown to exert neuroprotective and neuroregenerative effects in rodent models of stroke (Siow 2008). Recently, MLC901

has been shown to improve cognition in normal C57BL/6 mice by promoting hippocampal neurogenesis, raising the possibility that it may be useful against cognitive impairment in neurodegenerative diseases (Lorivel et al. 2015). A number of molecules in the different herbs extracted to manufacture MLC901 has been identified that are neurobeneficial, including butylidenephthalide, tanshinone, salvianolic acid B, ferulic acid, and tetramethylpyrazine (Chen et al. 2012; Zhuang et al. 2012; Tang et al. 2012; Koh 2013; Kao et al. 2013; Nam et al. 2013). Pharmacological data obtained in rodents have established that MLC901 prevents death of threatened neuronal tissues, decreases cognitive deficits, and improves functional outcome by restoring neuronal circuits (Heurteaux et al. 2010; Quintard et al. 2011). It was therefore expected that the beneficial effects of MLC901 would be due to “multitarget” effects. Several mechanisms of action have been proposed for MLC901. 1, MLC901 acts as an activator of K_{ATP} channels (Moha Ou Maati et al. 2012); 2, MLC901 activates the serine/threonine kinase Akt (protein kinase B) pathway in the model of global ischemia (Franke et al. 1997); and 3, MLC901 decreases the level of the Bax protein and involves a decrease of apoptotic pathways (Quintard et al. 2011). This findings show that MLC901 can induce both peripheral and central effects on the nervous system.

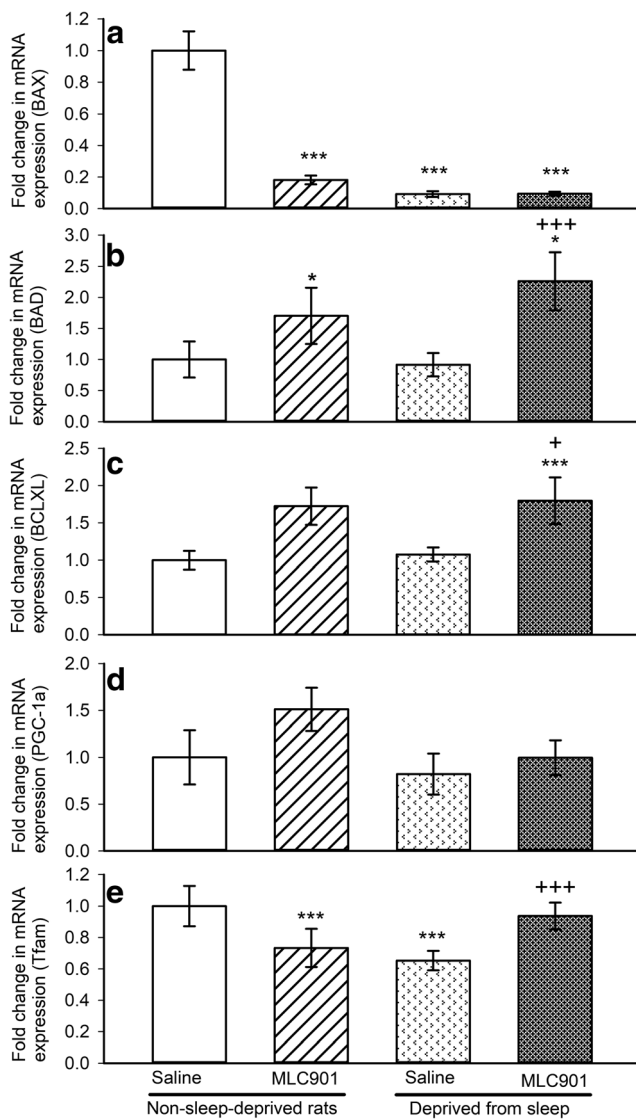


Fig. 4 The mRNA expression of BAX (A), BAD (B), Bcl-xL (C), PGC-1 α (D), and Tfam (E) in the hippocampus of saline/non-sleep-deprived, MLC901 (0.8 mg/kg)/non-sleep-deprived, saline/sleep-deprived, and MLC901 (0.8 mg/kg)/sleep-deprived groups. Bars represent fold differences of mean normalized expression values \pm S.E.M. ($n = 4$). *** $P < 0.001$ and * $P < 0.05$ from the saline/non-sleep-deprived group; +++ $P < 0.001$ and + $P < 0.05$ different from the respective MLC901/non-sleep-deprived group

MLC901 changes expression levels of some apoptotic or mitochondrial biogenesis-related genes in sleep-deprived rats

A number of animal studies have explored the potential cellular and molecular underpinnings of sleep deprivation-induced encoding deficits. It has been reported that sleep deprivation induces the apoptosis of hippocampal neurons. Sleep deprivation might decrease membrane excitability by affecting mitochondrial function and initiate apoptosis of neurons by increasing pro-apoptotic BAX protein level in mitochondria and promoting cytochrome *c* release (Yang et al. 2008). In

addition, some studies at the molecular level reveal that prior sleep deprivation induces a significant reduced level of hippocampal extracellular signal-regulated kinase (ERK), a protein intimately linked to LTP formation and learning (Guan et al. 2004). In the present study, sleep deprivation decreased the hippocampal expression levels of pro-apoptotic gene of BAX and Tfam, as a gene involved in mitochondrial biogenesis. It should be noted that the changes in the level of gene expression were investigated 24 h after sleep deprivation. In other words, rats are non-sleep-deprived during 24 h in an interval between sleep deprivation and memory test retention. However, most of the sleep deprivation studies have failed to document a consistent cellular damage or apoptosis in the brain of animals subjected to different periods of sleep deprivation (D'Almeida et al. 1997; Cirelli et al. 1999; Hipolide et al. 2002). In agreement with our results, Montes-Rodriguez et al. pointed out that hippocampal BAX level decreased and BCL-2 level increased by 24-h total sleep deprivation and 24-h sleep rebound. They also reported that an increased BCL-2:BAX ratio (Montes-Rodriguez et al. 2009). We know that the balance between pro-apoptotic and anti-apoptotic genes may determine whether a cell will respond to a pro-apoptotic factor or not (Dlugosz et al. 2006; Montes-Rodriguez et al. 2009). It is suggested that 24 h of sleep rebound may restore the brain homeostasis altered by the 24 h of sleep deprivation. However, we did not examine the changes of the level of gene expression, immediately after sleep deprivation. Meanwhile, there is a report showing that 72 h of sleep deprivation prior to training reduces phosphorylated cAMP responsive element binding protein (pCREB) expression in the amygdala (Pinho et al. 2013). This finding helps to explain the amygdala-mediated cued fear memory deficit in our study. Recently, Lee and co-workers stated that MLC901 decreases tau phosphorylation, suggesting that targeting tau hyperphosphorylation may be therapeutically effective in neurodegenerative diseases (Lee et al. 2017). Our data show that MLC901 decreased BAX and Tfam and increased BAD expression levels in the hippocampus. We did not find any published papers that report the effect of MLC901 on the expression of mentioned genes in our study in control rodents.

One limitation of the present study is that various behaviors that can induce changes in freezing, such as attention, emotion, and motivation have not been tested. It is possible that sleep deprivation and MLC901 affect all of them. Several studies have reported a hyperalgesic effect related to total sleep deprivation and an analgesic effect related to sleep recovery (Onen et al. 2000; Onen et al. 2001). In agreement with this finding, our previous study showed that sleep recovery after a 24-h total sleep deprivation increases the thermal pain threshold was assessed by the hot plate test (Javad-Moosavi et al. 2017; Eydi-pour et al. 2017), indicating a decrease in pain sensitivity. In addition, Bessey et al. reported that anxiety status levels increase across two nights of total sleep deprivation

and return to baseline following a single night of recovery sleep (Bessey et al. 2018). Some animal experiments are in accordance with the results observed in humans, describing an anxiogenic behavior due to sleep deprivation (Nair et al. 2011; Pires et al. 2013), others reported anxiolysis (Andersen et al. 2005; Zielinski et al. 2013) opposite to what would be expected based on human studies. On the other hand, Lorivel et al. reported that MLC901 did not alter the time spent in the open arms, as an index of anxiety-like behavior, in the elevated plus maze test (Lorivel et al. 2015). Unfortunately, we did not find any article about the effect of the MLC901 on the sensitivity of the pain, by searching on scientific sites. We try to investigate the role of the MLC901 on the pain sensitivity and its effect on fear memory in the future studies.

Another limitation of present study is that cellular and molecular mechanisms involved in memory retrieval by MLC901 have not been investigated, and we cannot suggest the exact mechanism of the effect of MLC901 in memory deficit abolishment during sleep deprivation. Despite these limitations, it is worth highlighting that our findings suggest a protective role for MLC901 in fear memory retention during sleep deprivation.

Conclusions

Our results show that all doses of MLC901 abolished fear memory deficits induced by sleep deprivation. In other words, sleep-deprived rats retained fear memory following MLC901 administration. This is the first study that reports the positive effect of MLC901 on memory disruption induced by sleep deprivation. Sleep-deprived rats received the higher dose of MLC901 decreased BAX and increased BAD and Bcl-xL expression levels compared to control rats. Moreover, MLC901 during sleep deprivation increased BAD, Bcl-xL, and Tfam expression levels compared to sleep-deprived rats. In agreement with our results, a decrease in BAX expression of CA1 neurons of global ischemic rat model after MLC901 administration was reported, indicating a protective effect of MLC901 on apoptosis process (Quintard et al. 2011). In conclusion, MLC901 might be a therapeutic option for cognitive deficits induced by prolonged waking. However, it needs more investigations in the level of cellular and molecular mechanisms.

Author's contribution AM acquired the animal data and wrote the manuscript. MN, ME, MH, and MZ were responsible for the study concept, design, and proteomics analysis as well as assisted with the data analysis and interpretation of findings. All authors critically reviewed the content and approved the final version for publication.

Compliance with ethical standards The study was carried out in accordance with ethical standards in all aspects.

Conflict of interest The authors declare that they have no conflict of interest.

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