Original Article

Different Role of CA1 5HT3 Serotonin Receptors on Memory Acquisition Deficit Induced by Total (TSD) and REM Sleep Deprivation (RSD)

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Abstract

Background: Serotonin receptors such as 5-HT3 plays critical role in regulation of sleep, wake cycle and cognitive process. Thus, we investigated the role of CA1 5HT3 serotonin receptors in memory acquisition deficit induced by total sleep deprivation (TSD; for 24 hour) and REM sleep deprivation (RSD; for 24 hour). Pain perception and locomotor activity were also assessed as factors that may affect the memory process.

Methods: Modified water box and multi-platform apparatus were used to induce TSD or RSD, respectively. Passive avoidance, hot plate and open field devices were used for assessment of memory acquisition, pain and locomotor activity, respectively.

Results: Totally, 152 male Wistar rats were used in the study. Pre-training, intra-CA1 injection of 5-HT3 receptor agonist Chlorophenylbiguanide (Mchl; 0.01 and 0.001 µg/rat; $P < 0.001$) and antagonist Y-25130 (0.1 µg/rat; $P < 0.001$) reduced memory acquisition and did not alter pain response, while higher dose of both drugs increased locomotor activity in normal rats. Both TSD and RSD reduced memory acquisition ($P < 0.001$) and did not alter locomotor activity, while TSD ($P < 0.001$) but not RSD induced analgesia effect. The amnesia induced by TSD was restored by subthreshold dose of Y25130 (0.001 µg/rat; $P < 0.001$) but not Mchl (0.0001 µg/rat), while both drugs reversed TSD-induced analgesia effect ($P < 0.01$ for Mchl and $P < 0.05$ for Y25130), and Y25130 increased locomotor activity in TSD rats ($P < 0.05$). In RSD rats, subthreshold dose of both drugs did not alter memory acquisition deficit and increased locomotor activity ($P < 0.001$ for Mchl and $P < 0.01$ for Y25130), while the Y25130 ($P < 0.001$), but not Mchl induced analgesia in the RSD rats.

Conclusion: Based on the above data, CA1 5HT3 receptors seem to play a critical role in cognitive and non-cognitive behaviors induced by TSD and RSD.

Keywords: 5-HT3, CA1, hippocampus, memory, pain, sleep deprivation

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Introduction

Several investigations have indicated that sleep plays an important role in human health. In fact, sleep regulates the performance of neurons during memory formation.[1] Disruption in the sleep-wake process will be accompanied by different underlying diseases such as memory disorders, daily fatigue, depression, reduced motor skills, slow thinking, irritability, and even psychotic behaviors as well as behavioral problems.[2,3] In general, sleep stages are divided into two types of rapid eye movements (REM) and non-rapid eye movements (NREM).[3] NREM sleep is also divided into some stages including N1, N2, and N3.[4] Numerous studies have shown the importance of NREM sleep in declarative memory, while the significance of REM sleep in emotional memory has been also reported.[5]

Serotonin (5-HT; 5-Hydroxytryptamine) is a main monoamine neurotransmitter,[6] which is involved in memory,[7,8] locomotion,[9] and sleep.[10] 5-HT has been introduced as an important sleep-related neurotransmitter that is expressed in human and animal tissues. As a neurotransmitter, 5-HT also acts as vessel constrictor, smooth muscle stimulant, regulator of the cycle of biological processes, anxiety, memory, learning, sleep and sexual activities.[11] Raphe nucleus neurons are the main source of 5-HT production.[11–13] 5-HT has seven types of receptors which are G protein-paired receptors, except for 5-HT3 receptor (an inotropic receptor). 5-HT3 receptor may be formed by a combination of 5 different subunits labeled as 5-HT3A-E; however, at present only the subunits 5-HT3A and 5-HT3B have been studied more comprehensively.[11,14] The highest density of 5-HT3 in brain is associated with brain stem nucleus including dorsal vagal nerve cells, postrema region, and secluded group nucleus. 5-HT3 receptor is also expressed in hippocampus,

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amygdala, caudate nuclei and putamen.[11,12,15] In mammals, the serotonergic pathways originate from the raphe nuclei, and the ascending serotonergic fibers innervate the brain areas (e.g., cortex and hippocampus) involved in learning and memory processes.[16] Serotonergic inputs act preferably through 5-HT3 receptors from brain stem to hippocampus.[12,14] For example, it has been shown that deactivation of 5-HT3 receptor by selective antagonist induces LTP in hippocampal CA1 region.[17] According to evidence, using 5-HT3 receptor antagonists will prevent memory disorders and will also facilitate learning in situations that need high mental learning process such as strengthening mind, memory, and learning, as well as cognitive functions.[18,19] Moreover, investigations have shown that 5HT3 plays a role in the sleep-wake cycle in human, particularly in regulation of REM sleep and memory consolidation.[20] Studies have demonstrated high levels of serotonergic neuronal activity during active waking, a further reduction during non-rapid eye movement sleep (non-REMS) and absence of neuronal activity during rapid eye movement sleep (REMS).

Serotonin also plays an important role in regulation of pain through valve inhibitory mechanisms for pain in dorsal horn (downstream inhibitory path).[21] Dysfunction of these downstream inhibitory paths can lead to high sensitivity to pain and even painful reaction to drivers which are not naturally unfavorable.[21] It seems that combinations which increase serotonin nervous transmission are effective in pain control.[22] Numerous studies have indicated the role of 5-HT1, 5-HT2, and 5-HT3 receptors in regulation of pain transmission and have shown that activities of these receptors at spinal cord lead to painlessness through formalin and other pain models.[23–25]

In mammals, hippocampus plays a role in acquisition and consolidation stages of memory formation.[26] One of the most important parts of hippocampus with a basic role in learning and memory is the posterior hippocampus area (CA1) that is involved in the effect of SD on cognitive behaviors.[27] Sleep deprivation (SD) or disruption of sleep leads to sensitivity to painful drivers;[28] however, it is not easy to clarify which mechanisms exactly lead to this damage and many aspects are still ambiguous. Investigation of findings about each question leads to some hypotheses whose demonstration or rejection requires design of separate research models. Finally and according to the above studies, there may be a close relationship between sleep and wake, serotonergic system, pain and memory. Therefore, the present study aims to investigate the effects of CA1 5HT3 receptors on memory acquisition deficit induced by total SD (TSD) and REM SD (RSD) with respect to analgesia and locomotor activities.

Materials and Methods

Animals

Animals used in experiments were male Wistar rats with a weight range of 200 – 250 grams. Animals were kept in environmental conditions under standard temperature (22 + 2°C) and light/dark cycle (12/12 hours), while they had free access to water and food without being exposed to any kind of noise pollution. All steps of maintaining and performing of tests were done in the Institute for Cognitive Science Studies (ICSS), Tehran, Iran. Each group that will be explained in the experimental design section included eight male rats and each rat was used only once. Behavioral tests were done during light phase, and all experimental operations were performed between 8 A.M. and 2 P.M. while all ethical issues were followed during experiments.

Stereotaxic surgery

Animals were anesthetized using intra-peritoneal injection of ketamine hydrochloride (50 mg/kg) and Xylazine (5 mg/kg); subsequently, the hair at the back of their head was removed (incision site) and they were located in stereotactic apparatus (Stoelting Company, Illinois, USA). Then, 22-guage guide cannula (with a diameter of 0.7 mm) was placed one millimeter above the intended site for injection (bilaterally) according to Paxinos and Watson's Atlas.[29] Stereotactic coordinates for hippocampus CA1 areas included AP = -2 mm from bregma, ML= ±1.6 sagittal suture, and DV: -1.5 mm from skull surface. Cannulae were fixed with dental acrylic. In order to prevent contamination, stainless steel stylets (27-gauge) were inserted into the guide cannulas. Animals' recovery after surgery was 5 – 7 days.[30–32]

Apparatus for total sleep deprivation (TSD)

Sleep deprivation included total SD (TSD; animals that were deprived from sleep for 24 continuous hours). An apparatus composed of a water tank made of clear Plexiglas (120 cm × 30 cm × 50 cm) and divided into four equal boxes (30 cm × 30 cm × 50 cm) was used to induce sleep deprivation in rats, while water temperature was set at 30°C (BorjeSanat Azma Co. Tehran, Iran). Four rats were put together in the tank (one rat in each box) so that social stability was kept. Two small platforms (with diameter of 15 cm) with a 3-mm deep edge were located next to each other in the middle of the tank. Some holes were placed on the surface of each platform (with diameter of 2 mm) to facilitate water discharge during upward movements which helped rats avoid slipping or getting wet. Platform movements were done independently and automatically. In the starting position, both platforms were a little immersed in 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 seconds to complete. During this period, each platform remained in the highest elevation (10 cm) over water surface for 10 seconds in order to get water and food (holding time). After this period of time, platform moved 60 mm down for 2.5 seconds and immediately came up for 2.5 seconds to the initial position. The rats became familiar with water box one day before application of different sleep deprivation protocols so that their stress level was reduced for 30 minutes. 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. During this period, the rats had free access to water and food which was always located above the box. Behavioral observation of video tapes during daily sleep deprivation showed that the rats were awake 100% of the time using the platform technique.[27,33]

REM sleep deprivation (RSD) apparatus

For REM sleep deprivation, a multiple-platform apparatus was used. In this method, the rats were located in a water tank (90 cm × 50 cm × 50 cm) while 12 small transparent Plexiglas platforms with a diameters of 7 cm and 10 cm height were provided for SD groups and platforms with a diagonal of 15 cm were provided for sham groups. Platforms were surrounded by water which was about 2 cm below the platforms. Animals were located on these platforms and when they went into REM sleep,[34] their axial

muscles of the neck relaxed, muscle tone decreased, and the animals touched water which caused them to wake up. Animals could go from one platform to the other platforms inside the multiple-platform chamber. During RSD period, the rats had sufficient food and water. The time period of RSD was considered 24 hours.[33,35]

Intra-CA1 injection

Injections were done using 2 μL Hamilton syringe 7 days after surgery.[36,37] Stainless steel stylets were removed from the guide cannula and replaced with 27-gauge injection needles (1 mm below the tip of the guide cannula). Injections were done manually in total volume of 1 μL/rat (0.5 μL per each hemisphere) during a period of 60 seconds and needles were put in the site for 60 seconds to facilitate drug injection. Animals were transferred into their cages after the stylets were returned.[38]

Passive avoidance memory test

Learning box included a shuttle box and included two partitions: light partition (20 cm × 20 cm × 30 cm) and dark partition (20 cm × 20 cm × 30 cm) connected through a guillotine door in the middle of the wall with dimensions of 7 cm × 9 cm. The floor was covered by stainless steel wires with a diameter of 2.5 cm and a distance of 1 cm. Slight periodic electric shocks (50 Hz, 3 s, 1 mA intensity) were delivered to the grid floor of the dark compartment and were applied only once to the animal's paws.[27,36]

Training

Training was done based on previous studies.[37,39,40] Animals were first transferred to test room at least 30 minutes before the experiments. First, animals were placed in the shuttle box with open guillotine door for five minutes to become familiar with the apparatus (training) and move between two compartments freely. Then, each animal was placed gently in the light compartment of the apparatus. The guillotine door was opened after ten seconds and the animal could enter the dark compartment. Animal's delay to enter into the dark compartment was recorded (initial delay time as learning). Animals that waited more than 120 seconds to enter into the dark compartment were excluded from the experiments. When four paws of the animal were completely in the dark compartment, the guillotine door was closed and the rat returned to its cage after 10 seconds (habituation trial). Learning trial was performed 30 minutes after habituation trial. The animal was placed in the light compartment and the guillotine door was opened 10 seconds later. As soon as the animal entered into the dark compartment, the door was closed and an electric shock (50 Hz, 1 mA, 3 s) was immediately delivered to the animal's feet through the floor. The rat was removed from the apparatus after 20 seconds and returned to its cage temporarily. After 2 minutes, the procedure was repeated. When the rat remained in the light compartment for 120 consecutive seconds, learning trial was ended. All animals learned with 3 trials at the most. This section was performed 5 minutes after drug injection.

Retrieval test

Retrieval test was done 24 hours (one day) after training in order to determine long-term memory. Each animal was placed in the light compartment for 20 seconds after which the door was opened and delay in entrance into the dark compartment (which delivered a shock before but not this time) was recorded as passive avoidance memory based on seconds. This part of experiment finished when the animal entered into the dark part. A maximum stop time of 300 seconds was applied for animals that had remained in the light part. No electric shock was applied during these sessions. This was done for all rats in all research groups.[27]

Hot plate apparatus for pain test

Hot plate apparatus is a sheet which gets hot by electric current (BorjSanatAzma Co, Tehran, Iran). In our experiment, hot plate sheet was first cleaned with 70% ethanol and then, all the rats were put on the plate. The start time (zero) was determined and as soon as the rats started to lick their paws or change their steps, their basic tolerance was recorded. In hot plate experiments, the temperature of apparatus was set at 50 + 1°C, while reaction time to thermal pain was recorded as licking paws or special changes in rats' steps. Cut-off time of the test was 60 seconds. This test was performed for each rat half an hour after drug injection.[41]

Locomotor activity evaluation

Locomotion apparatus (BorjSanatAzma Co, Tehran, Iran) was composed of a transparent Perspex container (with a height of 30 cm × 30 cm × 40 cm). This apparatus included a gray Perspex panel (with a thickness of 30 cm × 30 cm × 2.2 cm) with 16 photocells which divided the container into 16 squares of equal sizes. Locomotor activities were evaluated as the number of movements from one square to another during 5 minutes. This test was done on training day of memory.[13]

Drugs

Drugs used in the present study included M-Chlorophenylbiguanide (M-Chl) (5-HT3 receptor agonist at doses 0.01, 0.001 and 0.0001 μg/rat) and Y-25130 (5-HT3 receptor antagonist at doses 0.1, 0.01 and 0.001 μg/rat) which were purchased from Tocris (Tocris Bioscience, United Kingdom). Drugs were dissolved in sterile 0.9% saline solution immediately before injection. 5-HT3 receptor agents were administered into the CA1 at the volume of 0.5 μL/site.

Histological evaluation to confirm establishment of injection cannula tip in brain

After test sessions, each rat was anesthetized irremeably and 0.5 μL/solution of 4% methylene-blue was injected into CA1. Then, the rat's head was separated and the brain was fixed in formaldehyde (10%). Five days later, the brain was cut at hippocampus and the sites of injections were verified using stereomicroscope based on Paxinos and Watson's atlas. Data from rejected cut brains were excluded.

Experimental design

Experiment 1: Effects of pre-training M-Chl or Y-25130 on memory acquisition, pain and locomotor activity

In this experiment, 7 groups of animals were used. The animals received saline (1 μL/rat), different doses of M-Chl (0.0001, 0.001 and 0.01 μg/rat) or Y-25130 (0.001, 0.01 and 0.1 μg/rat) in the control group (not sham TSD or sham RSD). All drug injections were done as pre-train 5min before the 1st trial of the passive avoidance test. The aim of this experiment was to determine subthreshold and effective doses of each drug in normal conditions.

Experiment 2: Effects of TSD on memory acquisition, pain and locomotor activity in presence and absence of M-Chl or Y-25130

In this experiment, 6 groups of animals were used. The animals

received intra-CA1 injection of saline (μL/rat), M-Chl (0.0001 μg/rat) or Y-25130 (0.001 μg/rat) 5 min before memory training in the sham of TSD and TSD. The purpose of this experiment was to determine if subthreshold dose of drugs in the sham condition can alter the responses induced by TSD.

Effects of RSD on memory acquisition, pain and locomotor activity in presence and absence of M-Chl or Y-25130

In this experiment, 6 groups of animals were used. The animals received intra-CA1 injection of saline (μL/rat), M-Chl (0.0001 μg/rat) or Y-25130 (0.001 μg/rat) 5 min before memory training in the sham RSD and RSD. The purpose of this experiment was to determine if subthreshold dose of drugs in the sham condition can alter the responses induced by RSD.

Statistical analysis

Considering the normal distribution of data and variance homogeneity in each group (according to the Kolmogorov–Smirnov goodness of fit test by SPSS 19), the results are presented as mean ± S.E.M. One-way ANOVA and independent *t*-test were performed to analyze the data. More analyses were done to compare two groups using Tukey's post-hoc test. In all comparisons, $P < 0.05$ indicated statistical significance.

Results

Effects of Mchl or Y-25130 on memory acquisition, pain and locomotor activity

One-Way ANOVA and post-hoc Tukey's test showed that intra-CA1 injection of Mchl in the control rats at doses of 0.01 and 0.001 but not 0.0001 μg/rat reduced memory acquisition [F (3, 28) = 38.79, $P < 0.001$; Figure 1, panel 1A], while at dose of 0.01 but not 0.0001 and 0.001 μg/rat increased locomotor activity [F (3, 28) = 6.67, $P < 0.01$; Figure 1, panel 1C]. Furthermore, Mchl did not alter pain response [F (3, 28) = 3.08, $P > 0.05$; Figure 1, panel 2A] at any doses used.

One-way ANOVA and Tukey' test showed that intra-CA1 injection of Y-25130 in the control rats at dose of 0.1 but not 0.01 and 0.001 μg/rat reduced memory acquisition [F (3, 28) = 43.98, $P < 0.001$; Figure 1, panel 1B]. Similar analysis showed that the Y25130 did

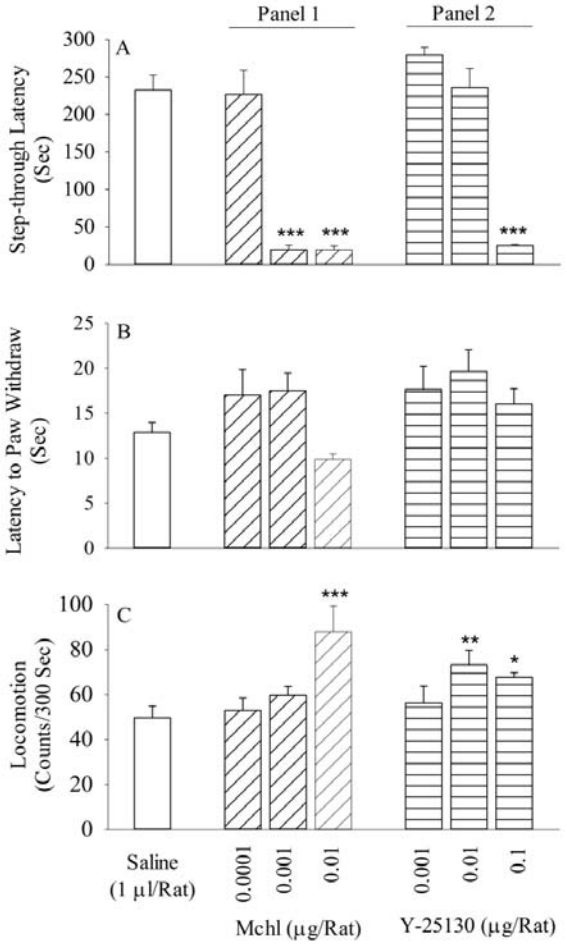

Figure 1. The effect of pre-training intra-CA1 injection of Mchl (panels 1) or Y25130 (panels 2) on memory acquisition (panels A), Hot plate latency (panels B) and locomotor activity (panels C). The data are expressed as Mean ± S.E.M for eight rats per group. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ as compared to saline control group.

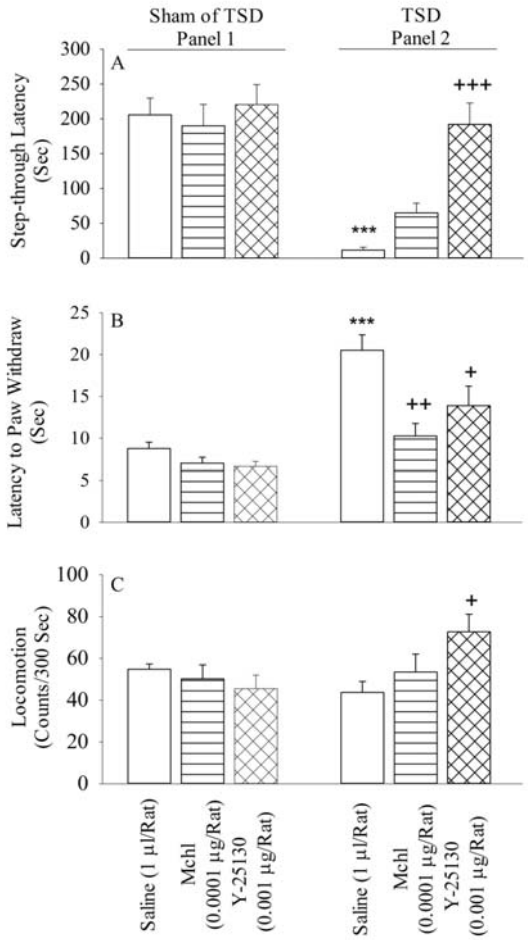

Figure 2. The effects of pre-training intra-CA1 injection of Mchl or Y25130 on memory acquisition (panels A), hot plate latency (panels B) and locomotor activity (panels C) on sham of TSD (panels 1) or TSD (panels 2) rats. The data are expressed as Mean ± S.E.M for eight rats per group. ***$P < 0.001$ as compared to saline/sham of TSD control group. +$P < 0.05$, ++$P < 0.01$ and +++$P < 0.001$ as compared to saline/TSD control group.

not alter pain response [F (3, 28) = 1.91, $P > 0.05$; Figure 1, panel 2B], while the drug increased locomotor activities at doses of 0.1 and 0.01 µg/rat [F (3, 28) = 3.17, $P < 0.05$; Figure 1, panel 2C].

According to these results, we selected Mchl at dose of 0.0001 µg/rat and Y25130 at dose of 0.001 µg/rat as subthreshold dose of these drugs in next experiments.

The effects of TSD on memory acquisition, pain and locomotor activity in presence and absence of Mchl or Y25130.

One-way ANOVA and Tukey' test showed neither drug altered memory acquisition [F (2, 21) = 0.29, $P > 0.05$; Figure 2, panel 1A], pain response [F (2, 21) = 2.72, $P > 0.05$; Figure 2, panel 2B] and locomotor activity [F (2, 21) = 0.69, $P > 0.05$; Figure 2, panel 2C] in the sham of TSD.

Independent *t*-test analysis demonstrated that TSD decreased memory acquisition (t = 8.06, $P < 0.001$; Figure 2, panel 2A), while increased time interval was required for reaction to pain (t = 5.83, $P < 0.001$; Figure 2, panel 2B) compared to sham control group. TSD did not alter locomotor activity (t = 1.89, $P > 0.05$; Figure 2, panel 2C).

Furthermore, one-way ANOVA showed that Y25130 but not Mchl restored impairment of memory acquisition induced by TSD [F (2, 21) = 22.47, $P < 0.001$; Figure 2, panel 2A], while Y25130 but not Mchl increased locomotor activity induced by TSD [F (2, 21) = 3.77, $P < 0.05$; Figure 2, panel 2C]. Moreover, both drugs reversed TSD-induced analgesia effect [F (2, 21) = 7.23, $P < 0.01$; Figure 2, panel 2B].

The effects of RSD on memory acquisition, pain and locomotor activity in presence and absence of MchlorY25130

The results of one-way ANOVA indicated that Mchl and Y25130 did not alter memory acquisition [F (2, 21) = 0.65; $P > 0.05$; Figure 3, panel 1A], hot plate latency [F (2.21) = 0.46; $P > 0.05$; Figure 3, panel 2B] and locomotor activity [F (2, 21) = 2.19, $P > 0.05$; Figure 3, panel 3C] in the sham of RSD.

Independent *t*-test showed that RSD significantly decreased

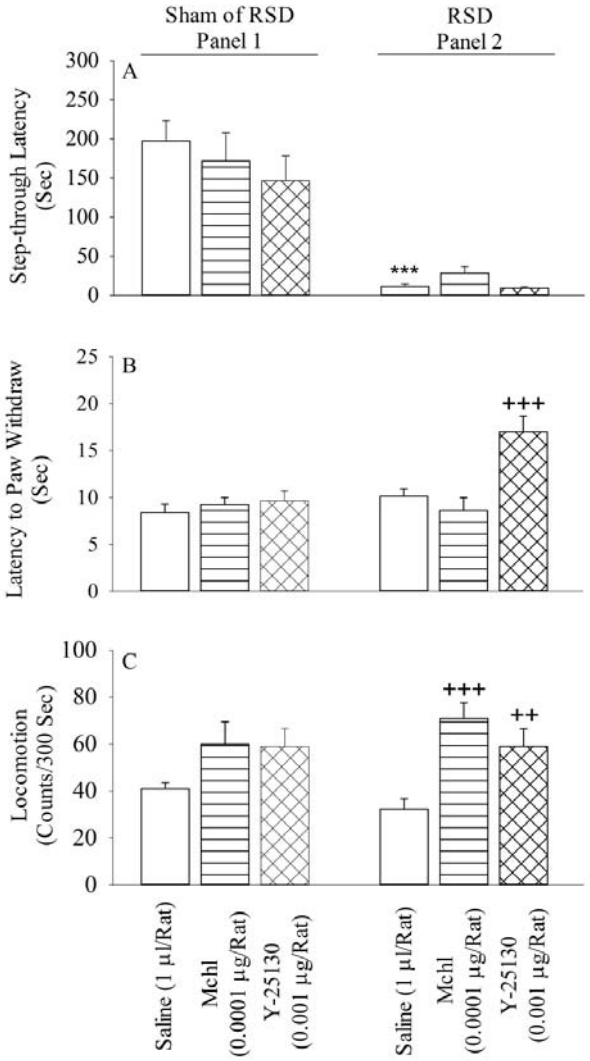

Figure 3. The effects of pre-training intra-CA1 injection of Mchl or Y25130 on memory acquisition (panels A), hot plate latency (panels B) and locomotor activity (panels C) on sham of RSD (panels 1) or RSD (panels 2) rats. The data are expressed as Mean±S.E.M for eight rats per group. ***$P < 0.001$ as compared to saline/sham of TSD control group. ++$P < 0.01$ and +++$P < 0.001$ as compared to saline/RSD control group.

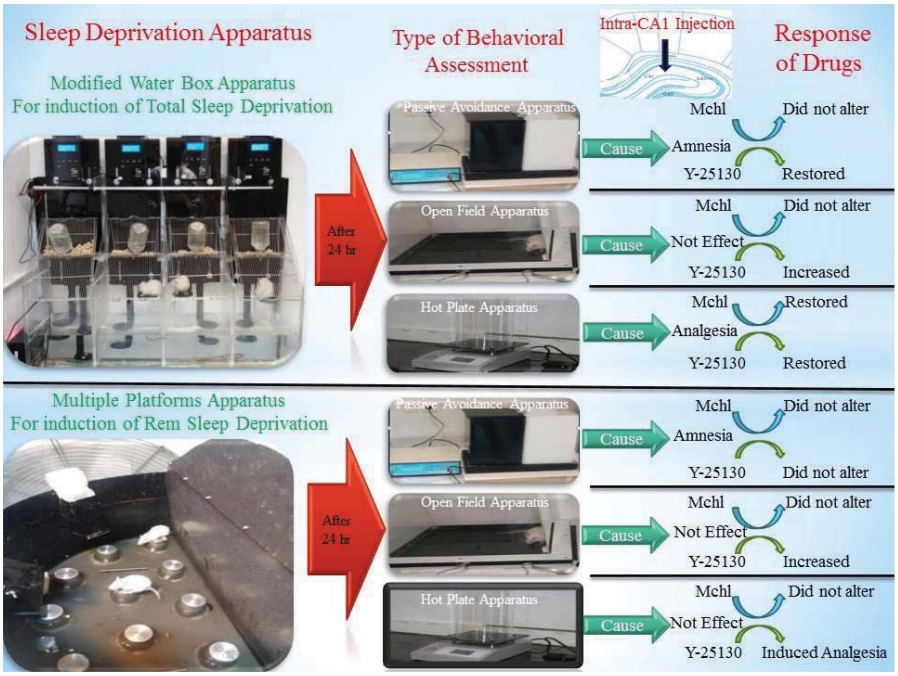

Figure 4. A graphical abstract for order of behavioral assessments and their results.

memory acquisition ($t = 7.09$, $P < 0.001$; Figure 3, panel 2A) but did not alter hot plate latency ($t = 1.67$, $P > 0.05$; Figure 3, panel 2B) and locomotor activity ($t = 1.42$, $P > 0.05$; Figure 3, panel 2C).

Similar analysis of one-way ANOVA showed that Mchl and Y25130 did not restore memory impairment induced by RSD [$F(2, 21) = 2.77$, $P > 0.05$; Figure 3, panel 2A], while both drugs increased locomotor activity induced by RSD [$F(2, 21) = 9.81$, $P < 0.001$; Figure 3, panel 3C). Y25130 but not Mchl increased hot plate latency [$F(21, 21) = 11.36$, $P < 0.001$; Figure 3, panel 2B].

Figure 4 shows the summery of the study protocol, apparatus and results by a graphical abstract.

Discussion

The effects of 5-HT3 agents on behaviors

According to the results of the present study, it was shown that intra-CA1 injection of both agonist (Chlorophenylbiguanide; Mchl) and antagonist (Y-25130) of 5-HT3 receptor reduced memory acquisition and increased locomotor activity in higher doses. Moreover, Mchl and Y25130 did not alter pain response in normal rats. Our results are consistent with the findings of Nasehi and colleagues (2015) who reported that pre-training intra-CA3 administration of Mchl and Y25130 leads to impaired memory acquisition, but does not affect locomotor activity.[42]

The 5-HT3Rs are non-selective cation channels whose activation induces a rapid membrane depolarization mediated by cation flow.[43] It seems that Ca^{2+} influx through the 5-HT3Rs probably facilitates GABA release from these interneurons.[43,44] 5-HT and 5-HT3R agonists induced inhibitory postsynaptic potentials (IPSPs) and increased GABA-mediated inhibition of pyramidal neurons in mPFC and also suppressed the development of long-term potentiation (LTP, a cellular basis for memory) in hippocampo-mPFC pathway and impaired learning and avoidance. So, it appears that 5-HT3Rs participate in inhibitory regulation of forebrain pyramidal neurons.[43]

The effects of TSD and RSD on behavior

The present results show that RSD and TSD disrupt memory acquisition, but do not alter locomotor activity. TSD but not RSD induced analgesia effect. According to previous investigations, sleep regulates the performance of neurons during memory formation.[1] Loren Graves, et al. in 2001 suggested that REM sleep is an important hippocampus modulator.[45] Previous studies have shown that RSD leads to memory impairment and LTP at hippocampal CA1.[46] Cognitive impairments which occur following SD and irregularities are due to changes in cellular and molecular levels as well as genes expression.[31] According to reports, synthesis of cAMP response element-binding protein (CREB) and signal path of protein kinase A (PKA) is important for long-term memory (LTP). REM sleep regulates synthesis of these proteins in hippocampus and leads to consolidation of LTP memory. Therefore, RSD decreases the level of expression of these proteins and consequently leads to impairment of LTP consolidation.[45,47]

In findings of the present study, increased pain threshold was intangibly observed after 24 hours of TSD but not RSD. Unlike the results of the present study, Onen, et al. (2001) showed that RSD could significantly increase pain.[48] In this regard, SD animals may experience changes in opioidergic receptor system. The opioidergic system is a part of a central path for pain processing and previous findings show that the threshold of pulling back the paws after thermal stimuli reduces after 24 hours of SD. Insufficient sleep can also affect pain perception; however, the effects of sleep disorders on pain sensitivity have not been investigated completely yet.[48]

The effects of activation and deactivation of 5-HT3 into CA1 on behavior due to SD

Present findings show that Y25130 but not Mchl restored impairment of memory acquisition by TSD, while both drugs restored TSD-induced analgesia effect. The Y25130 increased locomotor activity in this condition. Moreover, in the RSD condition, neither drug altered RSD-induced memory acquisition deficit, while they locomotor activity. Y25130 increased locomotor activity. The data presented here show a close correlation between hippocampal levels of serotonergic receptors and behavioral state in rats.

Hippocampal 5-HT levels correlated with sleep-wake behavior show high levels during wakefulness, intermediate levels during non-REMS and low levels during REMS.[49] This serotonin is secreted by nuclei that originate in the median raphe of the brain stem and project to many brain and spinal cord areas, especially to the dorsal horns of the spinal cord and to the hypothalamus[14,50] and contributes to many functions including regulation of mood, and sleep.[51] It also has some cognitive functions, including memory and learning.[52] Hence, reduction of hippocampal 5-HT level is important for modulation of hippocampus function during REM and NREM sleep. However, reduced level of 5-HT in hippocampus during REM and NREM sleep might be one way through which this state contributes to memory consolidation. Therefore, it may be proposed that RSD and TSD disrupt this phenomenon and induce memory consolidation deficit. Evidence suggests high levels of 5-HT during RSD and TSD that is reasonable to have desensitized and/or down-regulated 5-HT1A somatodendritic autoreceptors. Alternative hypotheses for increased 5-HT levels after sleep deprivation include a decrease in reuptake or metabolism of 5-HT.[53]

In addition, synaptic consolidation during REMS needs LTP, and 5-HT is reported to inhibit LTP in the cortex. Serotonergic activity must strongly diminish during REMS to induce LTP and memory consolidation.[54]

In the present research, similar to the results obtained in SD groups, post-train extracellular 5-HT level and its metabolism in hippocampus increased following these changes, hippocampus-dependent memory deficit occurs. Studies performed on depression patients have shown that during TSD and RSD, the level of extracellular 5-HT increases due to increased 5-HT metabolism and also inhibition of 5-HT reuptake in dorsal hippocampus which leads to deficiency of acquisition memory.[53,55,56]

Our data show a difference between TSD and RSD. In TSD as expected, Y25130 improved amnesia, indicating that Y25130 decreased the effect of 5-HT on 5-HT3 receptor and restored memory impairment induced by TSD. While in RSD we expected that Y25130 would improve memory deficit but it did not happen; it seems that RSD increased the level of 5-HT more than TSD in hippocampus, so Y25130 could not restore amnesia induced by RSD.

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