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Amelioration of pain and anxiety in sleep-deprived rats by intra-amygdala injection of cinnamaldehyde

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ABSTRACT

Background: Sleep disorders are accompanied by increased anxiety and somatic pain. In addition, it has been observed that anxiety and pain have a boosting effect on each other, resulting in continued sleep disturbances. Amygdala's (CeA) central nucleus plays a crucial role in these processes. Cinnamaldehyde (Cinn) is an aromatic compound with anti-anxiety, antioxidant, and sleep-promoting properties. The present study uses sleep-deprived rats to examine the effects of an intra-CeA injection of Cinn on pain and anxiety.

Methods: Sleep deprivation (SD) was induced using the platform technique. 35 male Wistar rats were divided into five groups. Anxiety state and nociception were evaluated among groups using formalin test (F.T.), open field test (OFT), and elevated plus maze (EPM). Anxiety tests (OFT and EPM) were conducted in all groups. The first group was undergone FT without induction of SD ($SD^{-}FT^{+}$). The second group received SD without FT($SD^{+}FT^{-}$). The third group received both SD and FT($SD^{+}FT^{+}$). The treatment and vehicle groups have undergone both SD and FT in addition to the respectively intra-CeA injection of Cinn ($SD^{+}FT^{+}$ Cinn) and Cinn vehicle ($SD^{+}FT^{+}$ VC). The recorded behaviors were analyzed between groups using IBM SPSS 24th version.

Results: SD did not lead to any significant difference in nociceptive behaviors in FT between groups $SD^{-}FT^{+}$ and $SD^{+}FT^{+}$ ($P \geq 0.05$). At the same time, there was a considerable discrepancy in rearing behaviors ($P < 0.006$) and the number of fecal boli ($P < 0.004$) recorded in OFM between these groups. Treatment with Cinn led to decreased nociception ($P < 0.038$), decreased rearing behaviors ($P < 0.01$), and reduced defecation ($P < 0.004$) in group SD + FT+ Cinn in comparison to the group $SD^{+}FT^{+}$. There were no differences in anxiety test results between the first and second groups ($P \geq 0.05$).

Conclusion: SD can lead to elevated anxiety, while intra-CeA injection of Cinn ameliorated both perceptions of acute pain and anxiety. Besides, the conduction of FT before the anxiety test led to no disturbance in the results of anxiety tests.

1. Introduction

Today's society is plagued by insomnia, sleep disturbances, and sleep debt [1]. Sleep deprivation (SD) has been linked to a variety of adverse outcomes, including elevated anxiety levels [2], a poor state of memory recognition, cardiovascular diseases (e.g., hypertension, immune system disorders) [3], and elevated incidence of psychosomatic pains including fibromyalgia [4]. It has also been reported that both acute and chronic SD is associated with neuroinflammation [5]. There are several mechanisms leading to this outcome, including the expression of inflammatory cytokines [6], activation of non-neuronal cells, such as microglia and astrocytes [7], and activation of the hypothalamic-pituitary-adrenal axis (HPA

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axis) [8]. SD also exacerbates the development of oxidative stress and the production of reactive oxygen species (ROS) [9]. Anxiety and pain also have bidirectional effects on each other [10]. While Anxiety has been reported to increase pain perception [11], pain can be induced by an elevated anxiety state [12]. The vicious cycle of causes and consequences is compounded by these factors, which are linked to causing sleep disturbances [13,14].

Located deep in the temporal lobe, the amygdala is part of the limbic system with a pivotal role in emotional disorders [15]. Amygdala has a principal function in the anxiety circuit and the central modulatory system of pain [16,17]. Radiologic studies revealed that both shrunken and enlarged amygdalas are seen in anxiety disorders [18,19]. This phenomenon implies that the amygdala consists of several nuclei having defined functions in the anxiety circuit. The central nucleus of the amygdala (CeA) is the main terminal for the output data of the amygdala [20], and its function is linked with emotional behaviors and pain perception [21,22]. Cinnamaldehyde (Cinn) is an aromatic compound found naturally in the leaves and barks of some species of the genus *Cinnamomum*. Due to the widespread use of cinnamon in Iran, researchers have always considered this compound's properties in various studies [23]. This compound has uses in soap, cosmetics, food, detergent, and pharmaceutical industries. Cinn displays several pharmacological properties, including anti-diabetic [24], antimicrobial [25], antifungal [26], anti-inflammatory, antioxidant [27], and immunomodulatory effects [28].

These reports led to the present study's design, which was designed to investigate the influence of sleep deprivation on anxiety and nociception in sleep-deprived rats, and then to determine the effects of Cinn injection in the CeA on these two parameters. Moreover, It was hypothesized that cinn has a critical role in reducing the destructive effects of sleep deprivation on the development of anxiety and pain. Essentially, it may be able to correct the effects of anxiety caused by sleep deprivation.

2. Methods

2.1. Study design

2.1.1. Animals

Thirty-five male Wistar rats weighing 200–250 g were bought from the animal center of Pasteur Institute, Tehran, Iran. They were kept in the standard laboratory conditions, including a 12-h dark/light cycle and 20 ± 2 centigrade temperatures. The animals had free access to food and water all the time except during the experimental period of sleep deprivation. After a habitualization period of one week, the rats have tested for any possible disability and mobility dysfunctions. The exploited protocols for caring for the animals and experiments follow the standard protocols based on the protocols developed by the ethics committee of Baqiyatallah University of medical sciences and NIH guidelines with the cod of I.R.BMSU.REC.1398.143.

2.1.2. Experimental design

Male Wistar rats were divided into five groups (n = 7), as detailed in Table 1. SD was not induced for the first group, while FT was conducted ($SD^{-}FT^{+}$). The second group was undergone SD without FT($SD^{+}FT^{-}$). The third group received both FT and SD ($SD^{+}FT^{+}$). The treatment and vehicle groups received FT and SD in addition to intra-CeA injection of Cinn ($SD^{+}FT^{+}$ Cinn) and Cinn vehicle ($SD^{+}FT^{+}$ VC). OFM and EPM were respectively conducted in all study groups after FT(except for $SD^{+}FT^{-}$). Except for the $SD^{-}FT^{+}$ group, all groups were subjected to SD for three days. Cinn and VC were injected in the CeA after each session of SD All the experiments were conducted between 8 a.m. and 2 p.m. Group $SD^{-}FT^{+}$ was designed to evaluate OFM, FT, and EPM in Non-sleep deprived rats, and group $SD^{+}FT^{-}$ was pursuing the goal of assessing any disturbing effect of FTon anxiety tests (Table 1).

Table 1
Categorization of the groups.

Groups	SD	injection	FT	OFM	EPM
$SD^{-}FT^{+}$	−	−	+	+	+
$SD^{+}FT^{-}$	+	−	−	+	+
$SD^{+}FT^{+}$	+	−	+	+	+
$SD^{+}FT^{+}$Cinn	+	Cinn	+	+	+
$SD^{+}FT^{+}$VC	+	VC	+	+	+

2.1.3. Sleep deprivation (SD)

The multiple platform technique induced sleep deprivation. In a water tank (51 cm × 51 cm x 50 cm) made from transparent plexiglass, four columns with 6 cm diameter and 15 cm height were placed. The tank was then filled with water up to 1 cm below the top of the column. The water temperature was adjusted carefully to room temperature (25 °C) before the initiation of the procedure. To minimize possible social instability effects, animals from a single cage were entered into each sleep deprivation session. Before initiating each SD experimental session, animals were placed in the room for 20 min. Daily SD was then performed on three consecutive days, each session lasting 3 h [29].

2.2. Surgeries and injection

2.2.1. Stereotaxic surgery

The procedure took place one week before the initiation of sleep deprivation. A 23 G needle was implanted in the CEA using a stereotactic apparatus. The location of the implantation was chosen based on the rat brain in stereotaxic coordinates, Paxinos and Watson (anteroposterior (A.P.) = −2.56 mm, mediolateral (ML) = 4.2 mm and Dorsoventral (DV) = 8 mm) [30]. Rats were anesthetized by intraperitoneal injection of a standard cocktail comprising Ketamine/Xylazine adjusted to the measured weight of animals. After the appropriate fixation of rats on the apparatus, a midline incision was made; soft and connective tissue were removed, guide cannula was implanted bilaterally in the above location. Cannulas were fixed using dental cement with the assistance of a pin inserted in the front position of the cannulas.

2.2.2. Drug administration

Cinn from Sigma Aldrich Company with 95% purity was used for this experiment. Cinn (10 μg in 2.5 μl volume of saline solution) was injected bilaterally in the CeA using a 5 μl Hamilton syringe and 30 G needle. The injection was applied using the implanted guide cannula and conducted within 1 min.

2.3. Experiments

2.3.1. Formalin Test (F.T.) protocol

The formalin test is a common method of evaluation of nociception in animals. For this, a formalin test chamber (30 cm × 20 cm x 15 cm) was designed, which was covered with a mirror behind and the sidewall with mirrors at an angle of 45°. To eradicate other animals' odors, the chamber was washed with 70% ethanol before the initiation of each experiment. After the animals were habituated for 15 min in the chamber, they were placed on the restraining apparatus for injection (using an insulin syringe with a 28 G needle) of 50 μl of a 5% formalin solution subcutaneously in the dorsal surface of the hind paw in the space between toes and the ankle. This test is biphasic or comprised of two separate phases.

Representing the acute injury pain model, the acute phase initiates just after the injection, lasting 5 min, and is the response of the animal to the injection process and not to formalin itself. The second phase represents inflammation due to formalin and begins 20 min after the injection and lasts for up to 90 min after the injection. Right after the injection of formalin, test animals were returned immediately to the chamber, and the number of paw flinches was assayed visually by an animal expert blind to the study and rats grouping of the study. Recording occurred for a 1-min period in subsequent 10-min intervals until the 90th minute. The first-minute recording represents the first phase, while the behaviors from the 20th minute till the 90th minute after injection were counted as the second phase of the formalin test.

2.3.2. Open field test protocol

A box (50 cm × 50 cm x 50 cm) was made from the blurred plexiglass with a rough surface for the OFM test. The box was placed in the center of the room with a camera fixed on the top of the box on the ceiling and four light sources to eliminate any shadows. Rats were brought to the testing room 20 min before the experiment to acclimatize. They were then placed in the center of the box, and the camera recorded animal movement using a video tracking software system supplied by the Borj Sanat azma company. The room was kept quiet during the recording, and the animals had no visual contact with the investigator. The recording was conducted for a 10-min interval, and the total distance moved by each animal was recorded in addition to the rearing behaviors and the number of defecated fecal boli. Once again, the box was washed with 95% ethanol between experimental sessions to eradicate any remnant animal scent [31,32].

2.3.3. Elevated plus maze

A cross-like maze having two open and two closed arms (each 50 cm long) located 70 cm high from the ground was used for this test. After the acclimatization period of 15 min in the EPM room, animals were placed in the clean maze, which was monitored with a camera. Located on the top of the maze, the camera recorded the movements of the rats and the time spent in each arm of the maze. The experiment started by placing the rats in the center of the maze facing the open arm and opposite to where the investigator was. The recording was done for 5 min' period. Animals with high anxiety states prefer to be in the closed arm more than the open arm, and the time spent in the open arm and the ratio of the time spent in the open arm to the closed arm have been used as indicators of anxiety.

2.3.4. Statistical analysis

The IBM SPSS software 24th version has been used for the statistical analysis of the data. The Kolmogorov-Smirnov test was assigned to determine the distribution pattern of records in each group. Based on the distribution pattern, parametric variables have been shown with Mean ± SEM, while for non-parametric variables, median ± SEM has been demonstrated. For the parametric variables, a *t*-test was conducted; for non-parametric variables, Mann Whitney u test was used to assess any significant difference among groups. Significant statistical difference was considered when a p-value was ≤0.05. Graph pad prism software (version 8) was used for plotting graphs.

3. Results

3.1. Formalin test

In the formalin nociception assay, the recorded values were 40.67 ± 1.647, and 45.43 ± 3.316, respectively, for groups $SD^{-}FT^{+}$ and $SD^{+}FT^{+}$ for the acute phase ($P > 0.249$), while in the late phase, recorded values were respectively 96.33 ± 8.577 and 101.714 ± 8.839 ($P > 0.673$). These recording for the treatment group ($SD^{+}FT^{+}$ Cinn) was 37.14 ± 1.262 for the acute phase and 103.428 ± 10.535 for the late phase, while these values for the vehicle group ($SD^{+}FT^{+}$ VC) was respectively 46.33 ± 3.18 for acute phase and 117.00 ± 16.82 for the late phase. There was a statistically significant difference between groups $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ Cinn ($P < 0.038$) in the acute phase but not in the late phase ($P > 0.903$). At the same time, there were no significant differences between groups $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ VC in both acute and late phases ($P > 0.875$ and $P > 0.471$, respectively). These data are also presented in Fig. 1.

3.2. Open field maze

The locomotor activity assay recorded values of 23.44 ± 1.99 for group SD-FT+, 33.93 ± 2.643 for group $SD^{+}FT^{-}$, and 39.062 ± 4.621 for group $SD^{+}FT^{+}$. These results demonstrate a significant difference between groups $SD^{+}FT^{+}$ and $SD^{-}FT^{+}$ ($P < 0.01$) and between groups $SD^{+}FT^{-}$ and $SD^{-}FT^{+}$ ($P < 0.01$). The recorded values for groups $SD^{+}FT^{+}$ Cinn and $SD^{+}FT^{+}$ VC were 23.668 ± 2.625 and 33.835 ± 0.675, significantly different from group $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ Cinn ($P < 0.013$). At the same time, there was no significant difference between the group SD + FT+ and SD + FT + VC ($P > 0.583$) (Fig. 2). In addition, the recordings for rearing behaviors

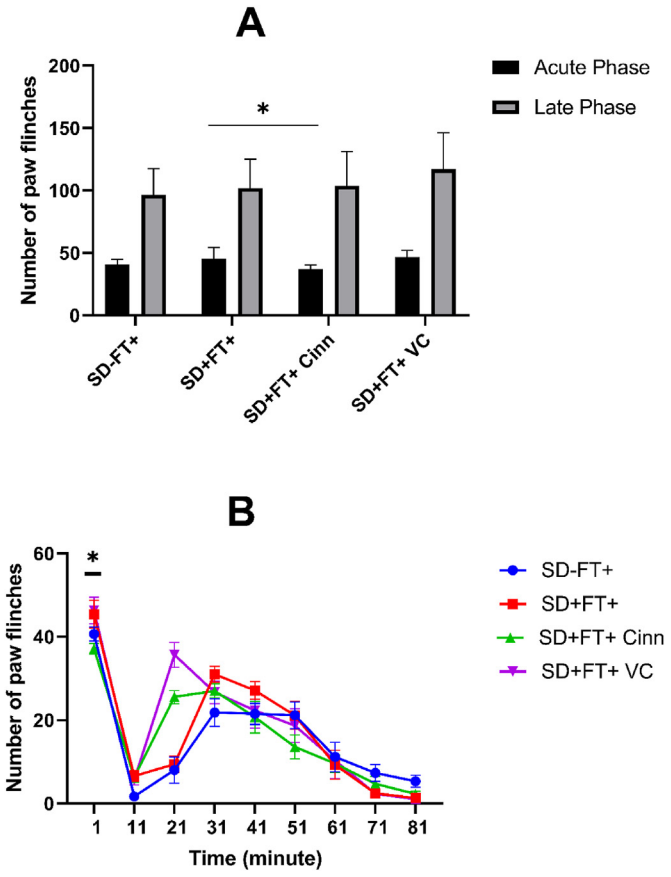

Fig. 1. Results of the formalin test. (A) depicts the data between study groups in the acute and late phase of FT nociception behavior, which was lower in group $SD^{+}FT^{+}$ Cinn than group $SD^{+}FT^{+}$. (**B**) illustrates the data on the recordings of FT in 90 min between groups. **SD** sleep deprivation, **FT** formalin test, **Cinn** injection of cinnamaldehyde in the central nucleus of **the** amygdala, **VC** injection of Cinn solvent (Vehicle) in the central nucleus of the amygdala. *P value ≤ 0.05, **P value ≤ 0.01.

were 9.83 ± 1.995 for group $SD^{-}FT^{+}$, 20.86 ± 3.62 for group $SD^{+}FT^{-}$, 29.43 ± 4.603 for group $SD^{+}FT^{+}$, 13.29 ± 2.81 for group $SD^{+}FT^{+}$ Cinn and 20.50 ± 1.5 for group $SD^{+}FT^{+}$ VC. A significant difference was found between groups SD+FT+ and SD-FT+ ($P < 0.006$) and also between groups $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ Cinn ($P < 0.01$) in rearing behaviors (Fig. 3). The number of fecal boli was 1 ± .00 for group $SD^{-}FT^{+}$, 2.00 ± 0.756 for group $SD^{+}FT^{-}$, 2.86 ± 0.459 for group SD + FT+, 0.57 ± 0.369 for group SD + FT + Cinn and 1.5 ± 1.5 for group $SD^{+}FT^{+}$ VC. A significant difference was spotted between group $SD^{+}FT^{+}$ and $SD^{-}FT^{+}$ ($P < 0.004$) and also between groups $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ Cinn ($P < 0.004$). There was no significant difference between group $SD^{+}FT^{-}$ and $SD^{+}FT^{+}$ ($P > 0.514$) and also between groups $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ VC ($P > 0.26$) (Fig. 4).

3.3. Elevated plus maze

The open arm time was 48.666 ± 7.639 for group $SD^{-}FT^{+}$, 52.833 ± 11.742 for group $SD^{+}FT^{-}$, 31.00 ± 5.247 for group $SD^{+}FT^{+}$, 61.285 ± 9.633 for group $SD^{+}FT^{+}$ Cinn and 37.750 ± 13.918 for group SD + FT + VC. A significant difference was noted between groups $SD^{+}FT+$ and SD + FT + Cinn ($P < 0.03$). At the same time, there was no statistical difference between group SD-FT+ and group $SD^{+}FT^{+}$ ($P > 0.08$), between $SD^{+}FT^{-}$ and $SD^{+}FT^{+}$ ($P > 0.134$), and also between $SD^{+}FT^{+}$ and $SD^{+}FT^{+}$ VC ($P > 0.33$) (Fig. 5).

4. Discussion

In the central pain modulatory system (Fig. 6), the amygdala plays a significant role in various inputs and outputs (Fig. 7). Additionally, a sleep-promoting neuronal population has been identified in the CeA, which receives connections from the thalamus [33]. Light of the fact that lack of sleep can lead to anger by stimulating the amygdala [34].According to Yuki Motomua's research in 2013, sleep deprivation leads to dysfunction between the amygdala and the ventral anterior cingulate cortex (vACC) and an increase in negative emotional reactions in the brain, namely, the amygdala's response to more negative stimuli [35]. Thus, CeA is involved in anxiety, pain, and sleep processes, making it an appropriate therapeutic intervention target.

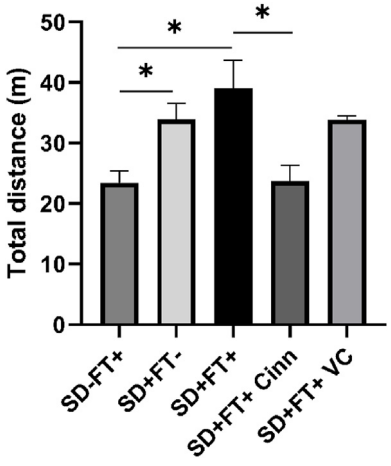

Fig. 2. The locomotor activity of the subjects. The activity of the subjects in the $SD^{-}FT^{+}$ group was meaningfully lower than groups $SD^{+}FT^{-}$ and $SD^{+}FT^{+}$, while group $SD^{+}FT^{+}$ Cinn showed lower activity than group SD + FT+. **SD** sleep deprivation, **FT**formalin test, **OFM** open field maze, **Cinn** injection of cinnamaldehyde in the central nucleus of the amygdala, **VC** injection of Cinn solvent (Vehicle) in the central nucleus of the amygdala. *P value ≤ 0.05, **P value ≤ 0.01.

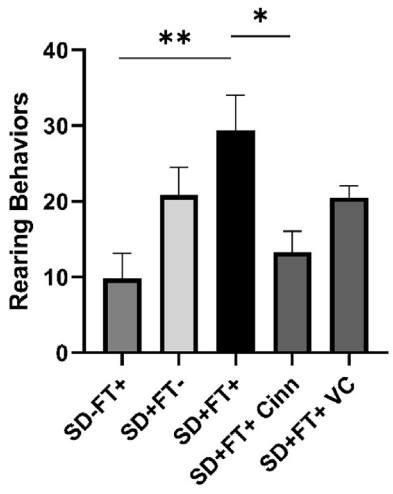

Fig. 3. Rearing behaviors of the groups. Group $SD^{+}FT^{+}$ showed increased rearing behaviors compared to group $SD^{-}FT^{+}$ while group $SD^{+}FT^{+}$ Cinn showed decreased rearing behaviors compared to $SD^{+}FT^{+}$. **SD** sleep deprivation, **FT** formalin test, **OFM** open field maze, **Cinn** injection of cinnamaldehyde in the central nucleus of the amygdala, **VC** injection of Cinn solvent (vehicle) in the central nucleus of the amygdala. *P value ≤ 0.05, **P value ≤ 0.01.

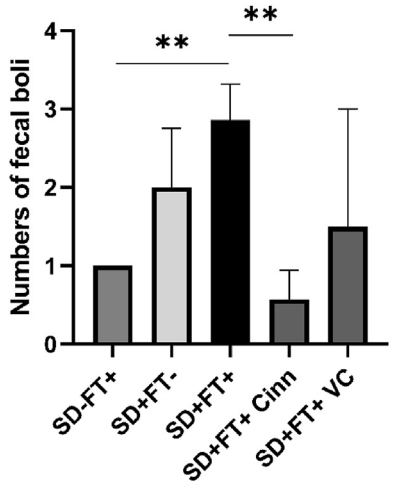

Fig. 4. The number of fecal boli defecated. During the test among groups, the number of fecal boli during OFM was meaningfully higher in $SD^{+}FT^{+}$ compared to $SD^{-}FT^{+}$ while group $SD^{+}FT^{+}$ Cinn showed decreased defecation compared to $SD^{+}FT^{+}$. **SD** sleep deprivation, **FT** formalin test, **OFM** open field maze, **Cinn** injection of cinnamaldehyde in the central nucleus of the amygdala, **VC** injection of Cinn solvent (Vehicle) in the central nucleus of the amygdala. *P value ≤ 0.05, **P value ≤ 0.01.

Analysis of the OFM results showed a significant difference among the groups in locomotor activity. This issue relates to using measured activity as a measure of anxiety. However, other behaviors, such as animal rearing and defecation, are vital in assessing anxiety. It has been documented that elevated anxiety level is accompanied by increased rearing behaviors and elevated excretion [36]. Rearing behaviors were significantly higher in group $SD^{+}FT^{+}$ in comparison to group $SD^{-}FT^{+}$. Although it was statistically not significant, in the EPM test, group $SD^{-}FT^{+}$ spent more time in the open arm in comparison to group $SD^{+}FT^{+}$, both of which highlight the role of SD in promoting anxiety. Conduction of FT before OFM and EPM did not lead to any significant findings in both tests of anxiety among group SD + FT- and SD + FT+, indicating that FT has no significant disturbing effects on anxiety tests. After treatment with Cinn, the group $SD^{+}FT^{+}$ Cinn showed decreased

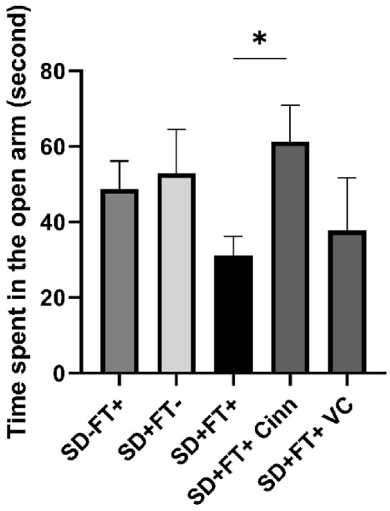

Fig. 5. Results of EPM test. Group SD $^{+}$ FT^{+} Cinn had increased time spent in the open arm compared to SD^{+}FT^{+}. **SD** sleep deprivation, **FT**formalin test, **OFM** open field maze, **EPM** elevated plus maze, **Cinn** injection of cinnamaldehyde in the central nucleus of the amygdala, **VC** injection of Cinn solvent (Vehicle) in the central nucleus of the amygdala. *P value $\leq$ 0.05, **P value $\leq$ 0.01.

records in rearing behaviors and defecation compared to group SD^{+}FT^{+} in the OFM test. Also, the results of the EPM test illustrated increased time spent in the open arm for group SD^{+}FT^{+} Cinn compared to group SD^{+}FT^{+}. All these significant recordings are indicative of the anxiolytic effects for Cinn.

There are reports on the anxiolytic effects of cinnamaldehyde and cinnamon oil [37,38], although these reports were based on oral or intraperitoneal administration of Cinn. However, the exact mechanism by which Cinn regulates anxiety is unclear. Numerous studies have shown Cinn to have anti-inflammatory and antioxidant properties [39]. Cinnamaldehyde has been shown to possess an anti-insomnia effect, however it is also able to improve berberine's absorption and distribution in the brain and testes [40–42]. In addition, Erfani et al.'s findings showing its healing properties in increasing REM sleep in response to stress. Cinnamaldehyde's healing properties are therefore attributed to its antioxidant properties in the brain [43,44]. Ly thi Houng (2020) also demonstrated the anti-anxiety effects of cinn inhalation [45].

It has been documented that many depressive and anxiety disorders are linked to oxidative stress in the central nervous system [46], and treatment with some antioxidant agents has effectively relieved anxiety [47]. In addition, disruption of the antioxidant defense system and neuronal cell death has been accompanied by anxiety-related and mental disorders [48]. According to research by Alexandra et al., in 2020, lack of sleep can even kill biological organisms due to increased reactive oxygen species (ROS) [49]. In 2005, Carol A. and colleagues demonstrated that sleep deprivation is fundamentally responsible for disorders caused by disruptions in antioxidant status in the brain and peripheral tissues [50]. Mathangi et al., In 2012, researchers discovered that REM deprivation also disrupted the antioxidant status of the brain. It is possible to resolve and revive several metabolic needs during sleep [51].

It has been debated that Cinn treatment not only has protective effects against ROS [52] and lipids [53] but also reduces the release of ROS from macrophages [54]. Also, it has modulatory effects on the expression of peroxisome proliferator-activated receptor gamma (PPAR-γ) [55] and nuclear factor kappa-light-chain-enhancer of activated B cells (N.F.-κB) (Ho 2013) [56], leading to reduced production and release of proinflammatory cytokines.

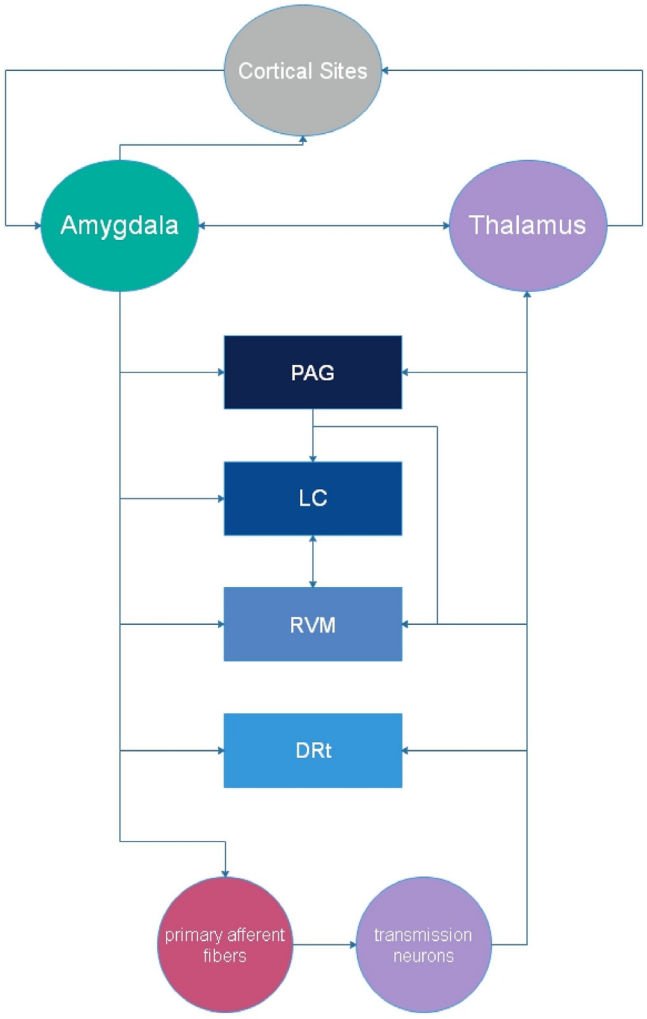

Fig. 6. The modulatory circuit of pain. Primary afferent fibers synapse with transmission neurons, and the projections ascend through the spinothalamic tract to the thalamus. In addition, collateral synapses form with mesencephalic nuclei, including DRt and RVM, and PAG in the midbrain. The thalamus emits projections to cortical sites and the amygdala, which enters the input information to CeA through the LA-BLA axis. Also, CeA receives information directly from the spinal cord and midbrain, in which the descending projection from CeA exerts the modulatory effects of CNS on the perception of pain. In addition, CeA emits projection to the thalamus and cortical sites, which leads to an emotional aspect of the perception of pain [17]. **PAG** periaqueductal grey, **LC** locus coeruleus, **RVM** rostral ventromedial medulla, **DRt** dentato-rubro thalamic tract.

Moreover, Cinn treatment could augment the level of indigenous antioxidants, including glutathione, and increase the activity of antioxidant enzymes [57].

In a study on cognition, memory, and behaviors of diabetic rats, treatment with Cinn showed positive outcomes in reducing anxiety-like behaviors. They proposed the regulatory effect of Cinn on the glutamate-GABA cycle as a potential way to exert its impact [58]. Since both the neurotransmitters glutamate and GABA play pivotal roles in the anxiety circuit in the amygdala [59,60], it seems that this mechanism also attributes to the anxiolytic effect of Cinn in the CeA. In addition, the GABAergic sleep-promoting population of neurons has been recently identified in the CeA receiving input projections from the glutamatergic neuron of the thalamus [61]. Since Cinn has modulatory effects on the glutamate-GABA cycle, it can be concluded that Cinn might also have a role in neutralizing the impact of SD. In this regard, this explanation aligns with the sleep-promoting characteristics of Cinn [62]. Moreover, Cinn is a

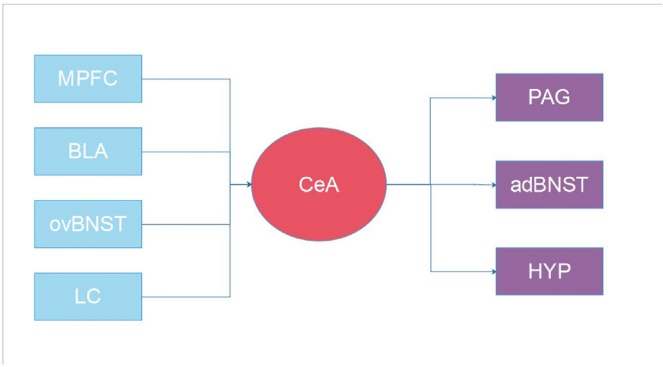

Fig. 7. connections of CeA in the anxiety circuits. CeA receives polysynaptic, excitatory, and excitatory modulatory afferent fibers from MPFC, BLA, ovBNST, and L.C. The first is increased anxiety. In contrast, the second dampens it, and the other two functions still need investigation. Also, it emits efferent inhibitory fibers to PAG and adBNST and excitatory fibers to Hypothalamus; the first has increasing effects on the anxiety state while the last decreases it, and the second needs further research [16]. **CeA** central nucleus of the amygdala, **HYP** hypothalamus, **MPFC** medial prefrontal cortex, **PAG** periaqueductal grey, **BNST** bed nucleus of stria terminalis, **ovBNST** oval nucleus of BNST, **adBNST** anterodorsal nucleus of BNST, **BLA** basolateral amygdala, **LC** locus coeruleus.

neuroprotective agent [63]; this characteristic may be specifically prominent against the production of ROS. Also, it plays a role in neurogenesis processes [64].

In the nociception testing, there had been no significant difference in both phases of the formalin test between groups $SD^{+}FT^{+}$ and $SD^{-}FT^{+,}$ implying that SD had no effects in the nociception testing. Treatment with Cinn led to a significant decrease in pain behaviors observed in groups SD + FT+ Cinn compared to group $SD^{+}FT^{+}$ during the acute phase of the formalin test.

Although the role of ROS in the spinal [65], peripheral [66], and the pathophysiology of pain [67]has been well-documented, little is known about the role of ROS in the central nervous system during pain mechanisms. Electrophysiological studies have revealed the activation of neurons in the amygdala with a painful stimulus [20]. In the pain circuit, CeA receives nociception information directly through the parabrachial area and spinal cord [68]. Also, the lateral amygdala-basolateral amygdala axis (LA-BLA axis) emit highly processed emotions combined with sensory information into CeA [69,70], and afterward, this nucleus acts as the output gateway.

Indigenous mitochondrial ROS are reported to affect the amygdala's neuronal activation during pain [71]. In this regard, intra-amygdala administration of ROS scavengers has been shown to suppress nociceptive behaviors [72]. Also, injection of ROS in the amygdala has been shown to result in the hyperactivity of the neuronal population in the amygdala through activation of metabotropic glutamate receptors [71,73], all of which highlight the involvement of ROS and oxidative stress in the pain process of the amygdala. Injection of monomethyl fumarate in the CeA due to its antioxidant features has been linked with diminished nociceptive behaviors [21]. Based on the antioxidant characteristics of Cinn along with its anti-inflammatory effects, it can be assumed that Cinn acts against ROS and neutralizes oxidative stress, leading to lower activation of neuronal cell activation and diminished perception of pain during the formalin test.

The different effects of Cinn on the acute and late phases of the formalin test can be explained by the difference in the selectiveness of other amygdala nuclei in answering various kinds of painful stimuli. Nakagawa T et al. showed that intraperitoneal administration of acetic acid is followed by activation of neuronal cells in the CeA [74]. On the other hand, Adedoyin M et al. reported that injection of formalin in the hind paw predominantly activates neuronal population in the BLA and L.A. rather than CeA [75]. Similarly, in the current study, no difference was noted among the groups in the nociceptive behaviors during the late phase of the formalin test. It should be stressed that the first phase of the formalin test happens not as a response to formalin but to the injury caused by the injection [76].

5. Conclusion

To conclude, sleep deprivation is an anxiogenic stimulus whose effects can be alleviated by Cinn's treatment. Sleep deprivation did not appear to affect nociception during the formalin test significantly. Furthermore, intra-CeA treatment with Cinn ameliorated acute pain behaviors. Further studies are needed to confirm Cinn's anti-nociceptive and anxiolytic effects, which could be attributed to its antioxidant properties against oxidative stress, its modulatory effects on the glutamate-GABA cycle, and its anti-inflammatory properties.

Ethical approval and consent to participate

The ethics committee of Baqiyatallah University of medical sciences approved the study with the cod of I.R.BMSU.R-EC.1398.143.We confirm that the survey is reported following ARRIVE guidelines.

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Consent of publication

Not applicable.

Declaration of availability of data and materials

All data are used in the article.

CRediT authorship contribution statement

Seyed Kaveh Hadeiy: Study design, Conceptualization, Writing – original draft, All authors read and approved the study. **Solomon Habtemariam:** participating in the experimental section and literature review, All authors read and approved the study. **Zeinab Shankayi:** participating in the experimental section and literature review, All authors read and approved the study. **Shima Shahyad:** participating in the experimental section and literature review, All authors read and approved the study. **Hedayat Sahraei:** data collection, All authors read and approved the study. **Milad asqardoost Rezaei:** data collection, Formal analysis, All authors read and approved the study. **Farideh Bahrami:** Study design, Conceptualization, Writing – original draft, All authors read and approved the study.

Declaration of competing interest

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Abbreviations

CeA	central nucleus of the amygdala
Cinn	Cinnamaldehyde
SD	sleep deprivation
F.T.	formalin test
OFT	open field test
EPM	elevated plus maze

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