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Roles of Hypothalamic Subgroup Histamine and Orexin Neurons on Behavioral Responses to Sleep Deprivation Induced by the Treadmill Method in Adolescent Rats

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Abstract. Sleep deprivation induces several negative effects on behavior, emotion, attention, and learning ability. Sleep appears to be particularly important during adolescent brain development. In the present study, we examined the effects of sleep deprivation on behavior and hypothalamic neurotransmission including histamine and orexin neurons in adolescent rats using the treadmill method. Adolescent male rats were divided into three groups: treadmill sleep-deprived, treadmill control, and cage control groups. Energy expenditure, anxiety-like behavior, and locomotor activity were examined among the three groups. Histamine concentration in the cortex and diencephalon and the number of c-Fos–positive neurons in the hypothalamus were also examined. In addition, histamine and orexin neurons in the hypothalamus were simultaneously identified using rat histidine decarboxylase and orexin-A immunohistochemistry, respectively. Both energy expenditure and anxiety-related behavior significantly increased by the experimental 3-day sleep deprivation, while exploratory locomotor activity significantly decreased. Histamine contents did not change in the cortex, but significantly decreased in the diencephalon of sleep-deprived rats. Increased expression of c-Fos–positive neurons, including subgroup histamine and orexin neurons, was observed in the hypothalamus. These findings indicate that sleep deprivation increases energy expenditure and anxiety in adolescent rats and provide evidence for the pivotal role of hypothalamus subgroup histamine and orexin neurons in the behavioral response to sleep deprivation.

Keywords: sleep deprivation, anxiety, energy expenditure, hypothalamus, histamine

Introduction

Sleep is essential for all living beings. It helps keep physical and psychosocial stability and plays a key role in preventing diseases and injury. Sleep deprivation can cause negative effects on behavior, emotion, attention, learning, and memory and may subsequently give rise to psychiatric disorders (1 – 3). Since sleep time is longer in childhood, the hypothesis that sleep promotes brain development has recently been gaining support (4 – 6). Due to the lack of appropriate animal models, little is known about how sleep deprivation affects brain functions in adolescent animals, particularly those of the hypothalamus. However, recent studies have reported the treadmill method as a useful way to evaluate the negative effects of sleep deprivation on brain functions in rats. This method, which compares the effects of sleep deprivation on brain function among a treadmill sleep-deprived group, treadmill control group, and cage control group, is considered as a mild stressor that allows rats to move freely and avoid the severe stressful conditions of the previously used 'platform-over-water' method (7 – 9).

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Evidence has shown the role of histamine as a neurotransmitter in various brain functions, including modulation of sleep–wake cycle and cortical arousal, learning and memory, emotion, and feeding (10, 11). Histamine neurons are located in the tuberomammillary nucleus and adjacent areas of the posterior hypothalamus, which are known to be important in the mechanism of wakefulness (12, 13). Histamine neurons send widespread projections to almost all brain regions, including the cortex, thalamus, posterior and preoptic/anterior hypothalamus, forebrain, and brainstem (14, 15), which are known to be important in the control of the sleep–wake cycle. Indeed, evidence has shown that increased histamine neuron activity is associated with wakefulness (16 – 21). For example, it is known that drugs that enhance histamine neurotransmission produce arousal (17 – 21). While drugs, such as sedative H_1 antagonists and histamine synthesis inhibitors, interfere with histamine neurotransmission, H_3 receptor agonists can enhance sleep (21). It has also been reported that microinjection of histamine in the preoptic area increases wakefulness and decreases both the slow wave sleep (SWS) and the rapid eye movement (REM) sleep (19), while inhibition of histamine synthesis in the same area increases SWS and inhibition of histamine degradation elicits long-lasting arousal (17, 18). In addition, histidine decarboxylase knockout mice have been reported to show increased paradoxical sleep, decreased cortical EEG power in θ-rhythm during wakefulness, and decreased EEG SWS/waking power ratio (22). However, it is still unknown how sleep deprivation affects the histaminergic neuron system in animals.

Another important neuropeptide in the hypothalamus is orexin (23, 24). Orexin neurons are known to be located in the sub-regions of the hypothalamus, such as the perifornical nucleus, lateral hypothalamic area, dorsomedial hypothalamic nucleus, and posterior hypothalamus area, and their fibers overlap in part with histaminergic fibers (25, 26). Several studies have reported that orexin plays a critical role in the regulation of the sleep–wake cycle and reward seeking behavior (25 – 28). In particular, it has recently been shown that orexin induces arousal response by stimulating histamine neurons (29, 30), although this finding could not be supported by a recent report (31).

The present study was therefore designed to investigate how sleep deprivation affects behaviors and hypothalamic neurotransmission in adolescent rats using the treadmill method.

Materials and Methods

Animals preparation and experimental procedure

Male adolescent Sprague Dawley rats weighing 140 – 200 g (5 – 8 weeks old) were used in this study. The animals were housed individually with continuous access to food and water under a 12 h : 12 h light–dark cycle in a room kept at 23°C and 65% – 70% humidity. All experimental protocols were approved by the Animal Care Committee of Tohoku University School of Medicine, and all experiments were performed in compliance with relevant laws and institutional guidelines.

Three groups of rats were studied: treadmill sleep-deprived, treadmill control, and cage control. Rats for sleep deprivation were placed inside a Plexiglas enclosure (70D × 30W × 35D cm) and subjected to 72 h sleep deprivation using forced locomotion in a treadmill apparatus for rats and mice (Model MK-680S; Muromachi Kikai Co., Ltd., Tokyo). The treadmill was set at a speed of 6 m/min (10 cm/s) and timed to move with either 3 s on / 12 s off (sleep deprivation) or 15 min on / 60 min off (treadmill control) periodicity during the entire recording session (72 h) according to a previous report (32). For the cage control group, rats were housed individually in Plexiglas cages. Bodyweight and food intake were also measured throughout the experiment. The means ± S.E.M. of the body weight at the start of the experiments are 152 ± 5, 141 ± 3.3, and 152 ± 6.41 g, respectively, in the treadmill sleep-deprived, treadmill control, and cage control groups.

Plus-maze test

The apparatus used for the plus-maze test is essentially the same as that described previously (33). The maze was elevated to a height of 70 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 cm) arranged so that the arms of the same type were opposite each other and connected by an open central area (10 × 10 cm). The experiment was performed under dim light conditions. At the beginning of the experiment, maze-naïve rats were placed in the center of the maze facing the same open arm and observed for 5 min. All experiments were performed immediately after sleep deprivation. The time spent in the open arms (defined as entry of three limbs into an arm of the maze) and the number of entries into the open arms were taken as a measure of anxiety, while the number of stretched attend posture, entries into the enclosed arms, and the total number of entries were taken as a measure of locomotor activity.

Open field locomotor activity

Exploratory locomotor activity in an open field was measured in a square area (60 × 60 cm) by a photo-beam apparatus (BTA-1, Muromachi Kikai) linked to a computer that monitored the animal's movements. The apparatus included a Plexiglas cage consisting of 64 photocells (8L × 8W, spaced 56.25 cm^2 apart). A frame of

sensors, which continuously tracks the animal's movements, surrounded the cage. The peripheral region was defined as 15 cm from any wall, while the center was defined as the 30 × 30 cm region in the middle of the field. At the time of testing (9:00 – 13:00), animals were placed individually into the center of the open field and their movements recorded continuously for 25 min (5 min × 5 sessions). The apparatus was cleaned with ethanol (70%) and air dried at the end of each session. Measurements included overall activity (beam crossing and rearing), total distance traveled (cm) as locomotor activity, and percent time spent in the center within the first 10 min of the test.

Brain dissection and HPLC measurement of histamine content

Immediately after the 72-h sleep deprivation, rats were sacrificed and their brains were removed and dissected on ice. The forebrains were divided into two parts: the cortex and the diencephalon. Brain samples were stored at −80°C until use. The brains were homogenized with 0.2 M perchloric containing 100 μM EDTA–2Na and centrifuged at 20,000 × g for 15 min at 0°C. The supernatant was adjusted to pH 3.0 using 1 M sodium acetate, and histamine content was assayed by HPLC using ion exchange separation, post-column derivation, and fluorescence detection (34).

Immunohistochemistry

In the present study, several coronal sections through the hypothalamus were stained by c-Fos immunoreactivity, the immediate early gene marker, and analyzed according to a previous report (35). Briefly, sections were immersed in a freshly prepared solution of 4% paraformaldehyde in phosphate-buffered saline (PBS) for 20 min and then washed several times in PBS. The sections were then incubated in methanol containing 0.3% hydrogen peroxide for 30 min to quench endogenous peroxidase followed by several washes in PBS-T, and endogenous proteins were blocked in a blocking solution (3% normal goat serum in PBS-T) for 1 h. Finally, the sections were incubated with primary antibody overnight at room temperature. The incubation solution contained c-Fos polyclonal antibody (Ab-2, rabbit polyclonal; Calbiochem, Darmstadt, Germany) diluted 1:1000 in the blocking solution. The sections were rinsed in PBS-T for 1 h before being incubated in a secondary antibody solution (Vector Laboratories, Burlingame, CA, USA) diluted 1:1000 in 0.4% Triton X-100 and 1.5% normal goat serum in PBS. After rinsing for 60 min, the sections were incubated for 1 h in a Vectastain ABC Elite kit (Vector Laboratories, diluted 1:500 in PBS), rinsed, and incubated again in a 0.05% 3,3′ diaminobenzidine hydrochloride (DAB) solution containing 0.003% H_2O_2 and 0.05% nickel chloride to get a dark blue reaction product.

Double staining for c-Fos and histidine decarboxylase / orexin was performed on the other two series of sections as described previously (36). After c-Fos staining, the sections were rinsed overnight in PBS with 0.02% sodium and then incubated by immunocytochemistry against a marker enzyme of rat histidine decarboxylase [anti-rat histidine decarboxylase serum, diluted 1:1000, 60 μg/ml (15)] or by an anti-orexin antibody (rabbit polyclonal, 1:1000; from Peninsula Laboratories Inc., Belmont, CA, USA). The sections were next rinsed in a secondary antibody solution (Vector Laboratories) diluted 1:1000 in 0.4% Triton X-100 and 1.5% normal goat serum in PBS. After rinsing for 60 min, the sections were incubated for 1 h in a Vectastain ABC Elite kit (diluted 1:500 in PBS, Vector Laboratories), rinsed, and stained in a 0.05% DAB without 0.05% nickel chloride to get a cytoplasmic brown reaction product in cytoplasmic areas.

Quantification of c-Fos–immunoreactive expression

Activation of the different brain regions was assessed by bilaterally counting c-Fos–immunoreactive neurons in three coronal sections of the hypothalamus. We counted c-Fos–positive neurons in three sections (10-μm-thick) from each nucleus. All cell counts were calculated for constant rectangular grids (100 × 100 μm) corresponding to the area of interest. Comparison among the three groups (cage control, treadmill control, sleep deprivation) allowed us to separate c-Fos expression by sleep deprivation from that by treadmill exercise. Double-labeled neurons for c-Fos and histidine decarboxylase or orexin were shown in the tuberomammillary nucleus and posterior hypothalamus area at approximate levels of −4.16 mm and in the ventromedial hypothalamus nucleus and the lateral hypothalamic area at approximate levels of –2.85mm, according to the atlas of George Paxinos & Charles Watson.

Statistics

Results are expressed as the mean ± S.E.M. Data were analyzed with one-way ANOVA. Subsequent comparisons between the treatment groups and the control were carried out by the Dunnett test.

Results

Energy expenditure during sleep deprivation

Body weight in each rat was measured during the sleep deprivation period. As shown in Fig. 1A, all rats increased their body weight during the experiment. However, the increase in body weight in sleep deprivation and treadmill control groups was significantly less than that in the cage

control group ($P < 0.05$). In contrast to body weight, food intake in the sleep deprivation group was much more than that in the treadmill control or cage control group (Fig. 1B, $P < 0.05$). These results indicate that sleep deprivation increases energy expenditure in adolescent rats.

Plus-maze test

Parameters related to rats' behavior on the plus maze are shown in Fig. 2, ANOVA analysis revealed a significant effect of group on plus-maze conventional measures. As shown in Fig. 2A, sleep-deprived adolescent rats showed less entries into the open arms of the maze. In addition, the numbers of stretch attend exhibited by rats in the sleep deprivation group were significantly less than those in the treadmill control group (Fig. 2C, $P < 0.05$). Finally, entries to the closed arms and all arms of the maze in the sleep deprivation group were signifi-

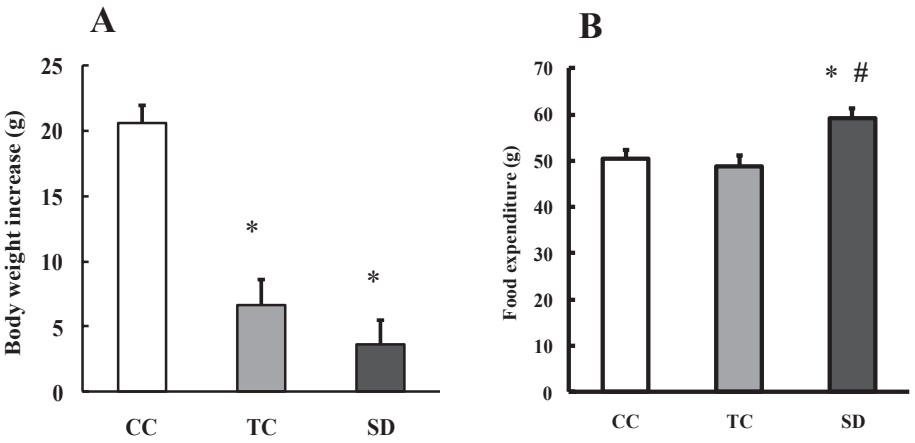

Fig. 1. Effects of 3-day SD on body weight and food intake in adolescent rats. A: Change in body weight after 3-day SD. Body weight increased less in the TC and SD groups than in the CC group. The average body weights of the rats at the start of the experiment are not statistically different between the groups. B: Food expenditure after 3-day SD. Food intake in the SD group was higher than that in the CC or TC group. $^{*}P < 0.05$, compared to CC and $^{\#}P < 0.05$, compared to TC. CC: cage control, TC: treadmill control, SD: treadmill sleep-derived. Data are expressed as the mean ± S.E.M. (N = 12 – 14).

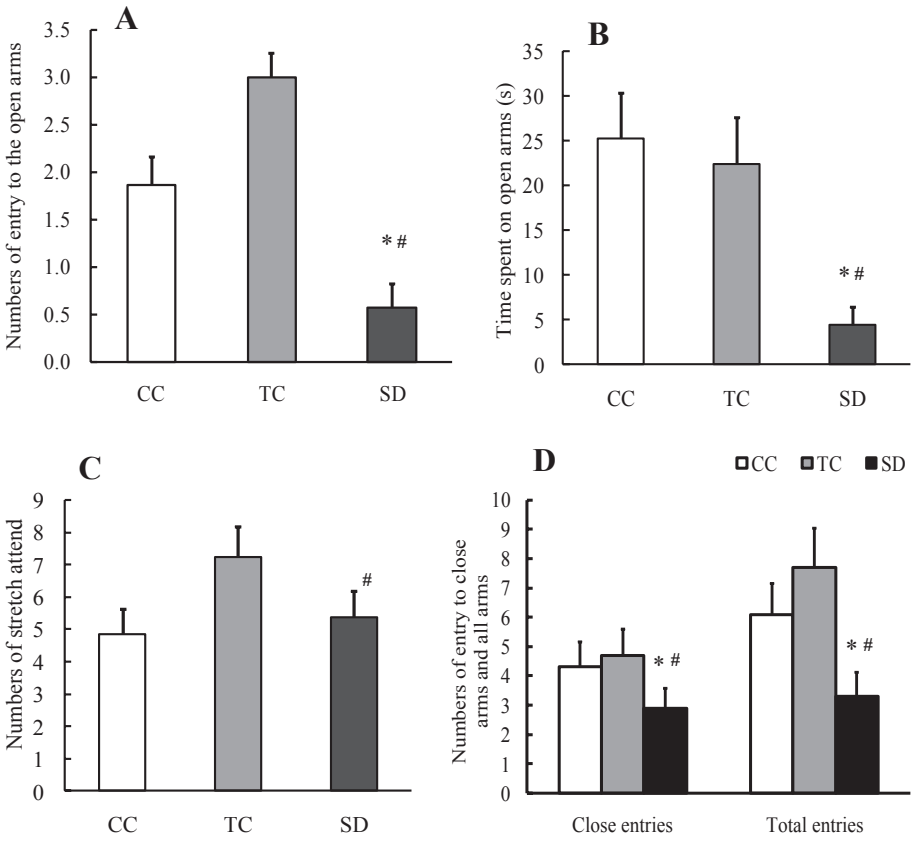

Fig. 2. Elevated plus-maze test. A: Number of entries to the open arms. B: Time spent on the open arms. C: Number of stretch attend. D: Number of entries to the closed arms and entries to all arms. A significant decrease in the number of entries and time spent on the open arms was observed in the SD group. No significant difference was observed in the stretch attend between the SD group and the CC group. The number of entries to the closed and all arms was smaller in the SD group than in the CC or TC group. $^{*}P < 0.05$, compared to CC and $^{\#}P < 0.05$, compared to TC. CC: cage control, TC: treadmill control, SD: treadmill sleep-derived. Data are expressed as the mean ± S.E.M. (N = 12 – 14).

cantly less than those in the treadmill control or cage control group (Fig. 2D, $P < 0.05$), indicating that sleep deprivation decreases locomotor activity in adolescent rats. The time spent on the open arms in the sleep-deprived rats was significantly less than that in the treadmill control or cage control group as shown in Fig. 2B, suggesting increased level of anxiety in the sleep-deprived rats. In order to confirm this, we next examined the behavior of the rats in the open field test.

Open field test

To confirm decreased locomotion and increased anxiety in sleep-deprived adolescent rats, rats were examined in an open field test, which is based on spontaneous exploratory behavior under novel circumstances. Generally, normal animals spend more time exploring the center area during the first 10 min of the test. Results of the open field test conducted in this study are shown in Fig. 3. Significant treatment effects on beam crossing and rearing between sleep deprivation and cage control groups were demonstrated (Fig. 3: A and B, $P < 0.01$). The total distance (cm) traveled, especially in the 3 – 5 sessions, was shorter in sleep deprivation and treadmill control rats than in cage control rats (Fig. 3C, $P < 0.01$). Further analysis showed that sleep deprivation significantly decreased the time spent in the center area during

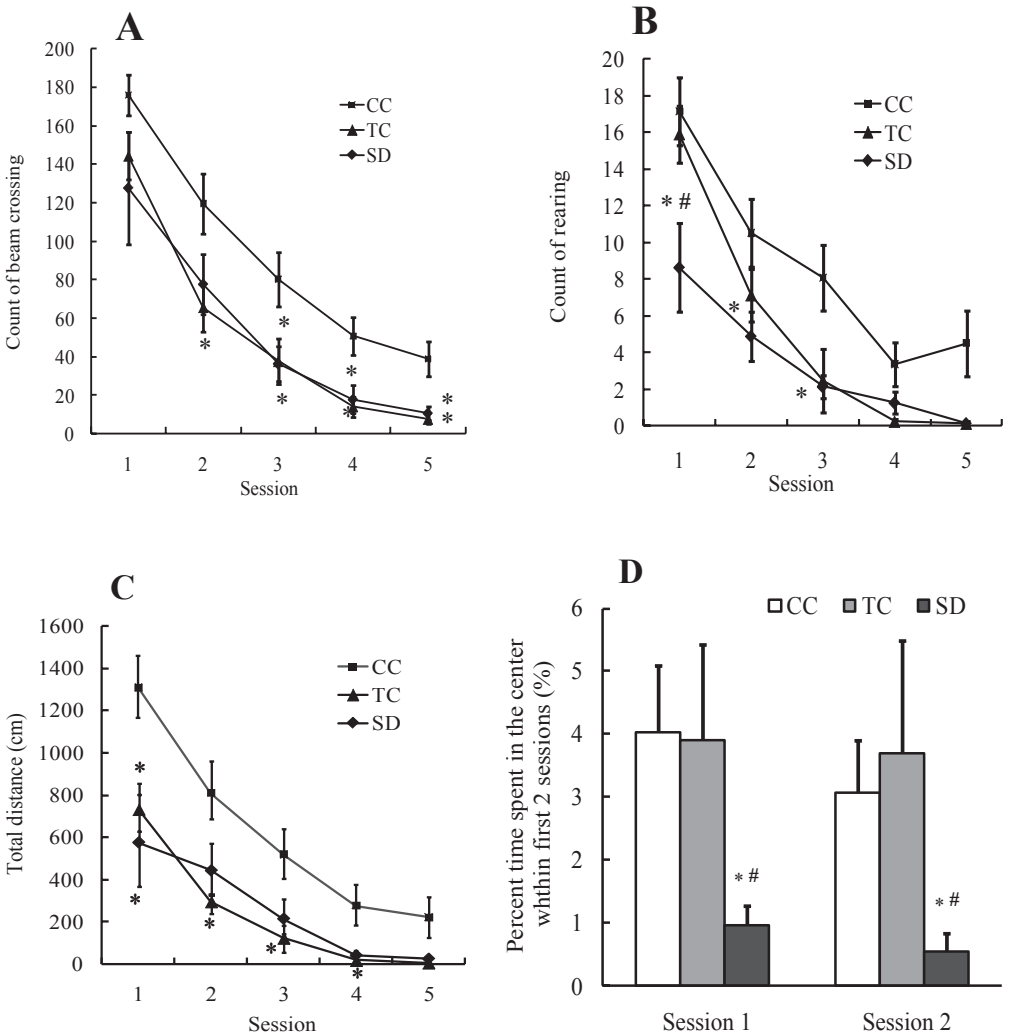

Fig. 3. Locomotion in the open-field test. A: Beam crossing. B: Rearing. C: Total distance traveled. D: Percent time spent in the center within the first 2 sessions. Data are expressed as the mean ± S.E.M. Significant difference was observed in the number of crossing (A) and rearing (B) among the three groups. The total distance traveled and number of crossing during test sessions were significantly smaller in the SD and TC groups than in the CC group. The number of rearing and percent time spent in the center within the first 10 min of the test were smaller in the SD group than in the CC or TC group ($P < 0.05$). *$P < 0.05$, compared to CC and $^{\#}P < 0.05$, compared to TC. CC: cage control, TC: treadmill control, SD: treadmill sleep-derived. Data are expressed as the mean ± S.E.M. (N = 12 – 14).

the first 10 min of the test (Fig. 3D, $P < 0.01$), suggesting a anxiety-like response in the sleep deprivation rats.

HPLC analysis of histamine in the brain

Histamine content decreased significantly in the diencephalons of sleep deprivation rats compared to that in the diencephalons of cage control rats (Fig. 4, $P < 0.05$). However, in the cortex, histamine content tended to increase in sleep deprivation rats, but this change was not statistically significant. These findings suggest that sleep deprivation can affect histamine neurotransmission.

c-Fos expression in response to sleep deprivation

The number of c-Fos–positive neurons in the hypothalamus is illustrated in Figs. 5 and 6. Sleep deprivation significantly increased the number of c-Fos–positive neurons in the lateral hypothalamic area, dorsomedial hypothalamic nucleus, and ventromedial hypothalamus nucleus, although different subgroups of hypothalamic neurons were differently activated by sleep deprivation. The number of c-Fos–positive neurons in the premammillary nucleus, ventral premammillary nucleus, and dorsal and posterior hypothalamus area increased markedly, but the effects of sleep deprivation on the tuberomammillary nucleus were not prominent. In addition, significant difference in the number of c-Fos–positive neurons was observed in the lateral hypothalamic area, posterior hypothalamus area, ventromedial hypothalamus nucleus, and dorsomedial hypothalamic nucleus between sleep deprivation and treadmill control groups. Furthermore, activated histamine and orexin neurons were confirmed by orexin-A and histidine decarboxylase immunocytochemistry as shown in the lateral hypothalamic area (Fig. 6A) and posterior hypothalamus area (Fig. 6B) and in the tuberomammillary nucleus (Fig. 6C) and posterior hypothalamus area (Fig. 6D), respectively. These results indicate that sleep deprivation can change the pathway of several neurotransmitters, including those of the orexinergic and histaminergic neurotransmission.

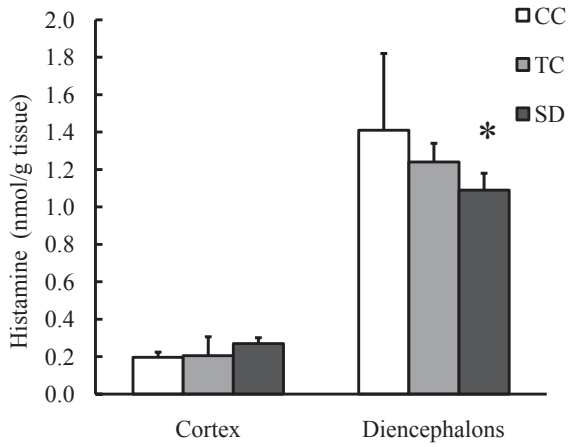

Fig. 4. Histamine concentration in the brain. Histamine content significantly decreased in the diencephalons of SD rats ($P < 0.05$), but showed a tendency to increase in the cortex (statistically insignificant). *$P < 0.05$, compared to CC. CC: cage control, TC: treadmill control, SD: treadmill sleep-derived. Values (nmol/g brain tissue) are expressed as the mean ± S.E.M. of 6 – 8 determinations.

Discussion

In this study we used the treadmill method to elucidate the effects of sleep deprivation on energy expenditure, anxiety-like behavior, and hypothalamus neurotransmission in adolescent rats. The advantage of using this method is its ability to induce long-term sleep deprivation in total sleep, NREM, and REM. In addition, treadmill sleep-deprived rats appear healthy and suffer no lesions. Another merit of the treadmill method is that the effects of sleep deprivation can be separated from those of forced locomotion, allowing a valid comparison of the effects of sleep deprivation among treadmill sleep-deprived, treadmill control, and cage control rats. Finally, the treadmill method allows rats free movement, thereby

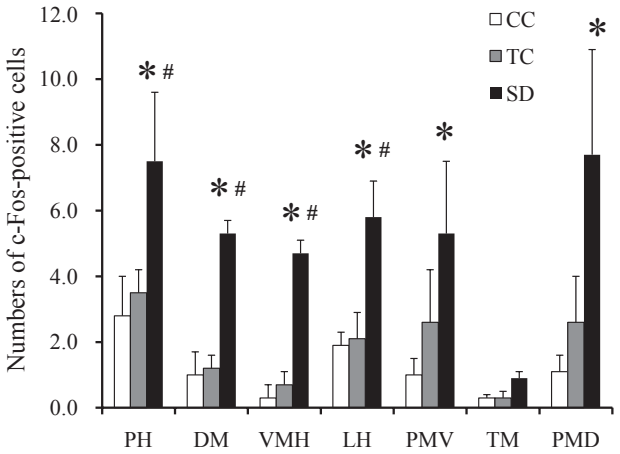

Fig. 5. Numbers of total c-Fos–positive neurons in the hypothalamic nuclei. LH, DM, and VMH: −2.80 mm; TM, PH, PMV, and PMD: −4.16 mm. Significant difference in the number of c-Fos–positive neurons was observed between the SD and CC groups in the PH, LH, DM, VMH, PMV, and PMD. Significant difference was also observed between the SD and TC groups in the PH, LH, DM, and VMH. There was no significant difference in the number of c-Fos–positive neurons in TM among the SD, CC, and TC groups. *$P < 0.05$, compared to CC and $^{\#}P < 0.05$, compared to TC. CC: cage-control, TC: treadmill control, SD: treadmill sleep-derived. DM: dorsomedial hypothalamic nucleus; VMH: ventromedial hypothalamus nucleus; LH: lateral hypothalamic area; PH: posterior hypothalamus area; TM: tuberomammillary nucleus; PMD: premammillary nucleus, dorsal; PMV: premammillary nucleus, ventral.

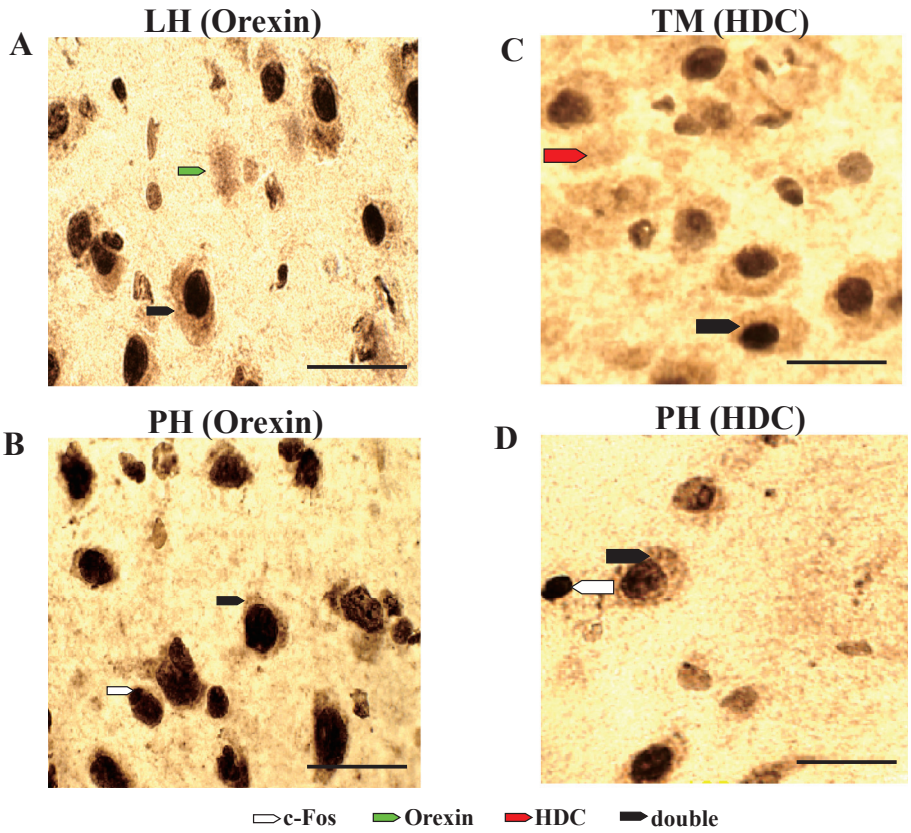

Fig. 6. Photomicrographs of double immunostaining with c-Fos and orexin in LH (A) and PH (B) and with c-Fos and HDC in TM (C) and PH (D). Black arrowheads indicate double-labeled cells, and white arrowheads indicate examples of adjacent single-labeled c-Fos-ir cells. Green arrowheads indicate examples of single-labeled orexin-positive cells, and red arrowheads indicate examples of single-labeled HDC-positive cells. Scale bar, 25 μm. LH: lateral hypothalamic area, PH: posterior hypothalamus area, TM: tuberomammillary nucleus, HDC: histidine decarboxylase.

avoiding stress induced by the conventional 'platform-over-water' method, which has previously been used in many studies. Indeed several studies using a treadmill have shown that serum corticosterone levels were not elevated in these rats (32).

Our results in this study show an increase in energy expenditure, which was calculated from food intake and bodyweight change, in sleep-deprived rat. The exact mechanism of this increased energy expenditure in sleep-deprived rats is still not clear, even though this phenomenon has been confirmed by indirect calorimetry (37). We also found in this study that sleep-deprivation increased c-Fos expression in the hypothalamus. It is well known that the lateral hypothalamus, where histamine and orexin neurons are localized, is involved in feeding behavior (24, 38). In addition, previous studies have shown that hypothalamic neurons are glucose-sensitive neurons that can be activated by glucopenia and thus are implicated in the positive short-term regulation of feeding and energy expenditure (39). It has also been shown that hypothalamic neurons can be inhibited by glucose mediating tandem-pore K^+ (K_{2p}) channels (40). Moreover, a previous study has demonstrated that microinjection of H_1-receptor antagonists into the ventromedial hypothalamus nucleus of rats induces a transient increase in food intake in the early light period (41), suggesting that ventromedial hypothalamus nucleus is important in feeding. In accordance with the results of these previous reports, our observations suggest that increased activity in the hypothalamus is associated with increased energy expenditure inducing by sleep deprivation.

We also examined in this study the effect of sleep deprivation on activity-related behavior in the elevated plus maze, which is frequently used to assess anxiety-like behavior. This test is based on naturally occurring conflicting behavior exhibited by rodents: the avoidance of open space and the tendency to explore novel environments. Thus, rodents exhibiting less anxiety-like behavior will enter and spend more time in the open arms of the maze. Following the previous studies on sleep deprivation induced with the platform method (42, 43), we observed that sleep deprivation produces anxiogenic effects in adolescent rats in the elevated plus maze. Even though the elevated plus maze test is used to assess anxiety-like behavior, the number of stretched attend posture, entries into the enclosed arms, and the total number of entries can be taken as a measure of locomotor activity. The present results suggest that sleep deprivation decreases overall activity in the elevated plus maze (Fig. 2: C and D). In order to clarify whether the observed anxiety-like

behavior was due to decreased activity, we examined the effect of sleep deprivation on exploratory locomotor activity in an open field, which is usually used to evaluate overall activity based on spontaneous exploratory behavior under novel circumstances. Interestingly, even after a 72-h period of treadmill sleep deprivation, the distance covered by sleep-deprived rats was equal to that covered by the treadmill control rats. This finding demonstrates that walking on the treadmill decreased locomotor activity in both the treadmill control group and sleep deprivation group, while anxiolytic-like behavior was observed only in the sleep deprivation group.

Previous studies have demonstrated that sleep restriction can give rise to anxiety (44, 45) and that this anxiety-related behavior might be related to unbalanced levels of several neurotransmitters, including serotonin and dopamine (45 – 47). Previous studies also demonstrated that sleep restriction for 120 h using the 'platform-over-water' method increases histamine levels in the brain stem with no apparent changes in the cortex (48). On the other hand, results that contradict these findings have indicated that histamine concentration does not change after REMS deprivation induced by the platform-over-water method and that this concentration increases in the anterior hypothalamus only during the rebound sleep in REMS-deprived rats (49). Neural histamine also plays a pivotal role in maintaining wakefulness (16), and the histamine–orexin interaction regulates vigilance in both normal and pathological conditions (29). It is also well known that cell bodies of histaminergic neurons are distributed in several hypothalamic nuclei, including the tuberomammillary nucleus, premammillary nucleus, ventral and premammillary nucleus, and dorsal and adjacent area (14, 15, 17 – 19). Early anatomical studies described histaminergic neurons as a homogeneous group of neurons, but recent evidence indicates that histaminergic neurons are heterogeneous and organized into distinct circuits (50). Therefore, it could be possible that a subgroup of histamine neurons were distinctly activated during sleep deprivation. In the present study, we found that histamine concentration was significantly decreased in the diencephalons after 72-h sleep deprivation with increased c-Fos expression in the histidine decarboxylase–positive neurons of the hypothalamus.

We recently reported the heterogeneous properties of histaminergic neurotransmission, in which deficiency in H_3 receptor has an inhibitory effect on psychostimulants-induced increase in locomotion, but negligible effect on the reward (51). In that study, we compared psychostimulants-induced behavioral changes to the expression of c-Fos protein as an indicator of neuronal activity. In this study, we examined whether sleep deprivation markedly increases the expression of c-Fos–positive neurons in the hypothalamus. Our results show that marked increase in the expression of c-Fos–positive neurons occurred in subgroups of the hypothalamic nuclei, suggesting the heterogeneity. In fact, the expression of c-Fos–positive neurons was markedly increased in several hypothalamic nuclei except for the tuberomammillary nucleus as shown in Fig. 5. Consistent with this finding, a previous study has demonstrated that c-Fos–positive cells associated with forced wakefulness in the rat hypothalamus were not GABAergic (52).

Several studies suggest that orexin induces the arousal response by stimulating histamine neurons (29, 30). Accordingly, we carried out in this study an immunocytochemical experiment using anti–orexin A and anti–histidine decarboxylase antibodies combined with Ab-2 antibody in the hypothalamus. We found that c-Fos–activated neurons were orexin A or histidine decarboxylase positive in part. This finding suggests that certain orexin and histamine subgroup neurons in the hypothalamus are activated by sleep deprivation.

In conclusion, our findings demonstrate that sleep deprivation increases energy expenditure and anxiety-like behavior and decreases locomotor activity in adolescent rats. The mechanisms of these behavioral changes probably involve hypothalamic subgroup orexin and histamine neurons in the brain.

Acknowledgments

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