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Effect of Environmental Factors on Depressive-like Behavior and Memory Function in Adolescent Rats

Song, Min Kyung[1] · Lee, Jae-Min[2] · Kim, Yoon Ju[1] · Lee, Joo Hee[1] · Kim, Youn-Jung[3]

[1]Department of Nursing, Graduate School, Kyung Hee University, Seoul; [2]Department of Physiology, College of Medicine, Kyung Hee University, Seoul; [3]College of Nursing Science, Kyung Hee University·East-West Nursing Research Institute, Seoul, Korea

Purpose: The aim of this study was to identify the effects of environmental factors on depressive-like behavior and memory function during adolescence. We performed behavior tests in adolescent rats exposed to environmental enrichment, handling, and social deprivation for eight weeks. **Methods:** Wistar rats were randomly assigned to control, environmental enrichment, handling, and social deprivation groups at the age of four weeks. **Results:** In the forced swim test, the immobility time in the environmental enrichment group was decreased than that in the control group ($p=.038$), while the immobility time in the social deprivation group was increased than that in the control group ($p=.035$), the environmental enrichment group ($p<.001$), and the handling group ($p=.001$). In the Morris water maze test, the social deprivation group had an increased latency time than the control group ($p=.013$) and the environmental enrichment group ($p=.001$). In the passive avoidance test, the environmental enrichment group had an increased latency time than the control group ($p=.005$). However, the social deprivation group had reduced latency time than the socially housed groups (control: $p=.030$; environmental enrichment: $p<.001$; handling: $p<.001$). **Conclusion:** These findings suggest that environmental factors play an important role in emotion and memory function during adolescence.

Key Words: *Adolescent; Social environment; Depression; Cognition*
국문주요어: 청소년, 사회적 환경, 우울, 인지

INTRODUCTION

1. Necessity of the research

Adolescence is a transition period from childhood to adulthood, and the brain continues to mature suffering structural and functional changes. This period is referred to as the second critical period because a variety of changes in physical, emotional, and cognitive development happens and susceptible to the external stimulus. Even though the gross structure of the brain is developed during prenatal, the connection among brain regions is not perfectly formed until adulthood. In human brain study with structural MRI from childhood to adulthood, cortical thickness kept thinning, but white matter volume was increased with dependent on age [1]. Cortical thickness is associated with pruning that eliminate unused neuron, and increased white matter means connection among brain regions become sophisticated as frequently stimulated. These structural changes accompany functional brain changes. During adolescence, functional MRI displayed working memory and self-regulation were gradually improved with age [2]. In teenagers, positive stimuli lead to emotional and cognitive maturation to improve the skills that they need to function independently in adulthood, meanwhile, harmful stimuli can result in fatal damage [3,4]. Early life stress not only impairs neurobiological and neuroendocrinal function but also induces

Corresponding author: **Kim, Youn-Jung**
College of Nursing Science, Kyung Hee University·East-West Nursing Institute, 26 Kyungheedae-ro, Dongdaemun-gu, Seoul 02447, Korea
Tel: +82-2-961-0311 Fax: +82-2-961-9398 E-mail: yj129@khu.ac.kr

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anxiety, depression and memory impairment [5]. Social relationships are expanded in this period, so social interaction with peer groups plays an important role in the development of emotion and cognition. In particular, as adolescents spent a lot of time with their peers rather than their parents, the importance of peer groups gradually increases [6].

The definition of environmental enrichment is 'a combination of complex inanimate and social stimulation' [7]. Environmental enrichment improves the quality of life of laboratory animals by providing sensory stimulation, increased physical activity, and enhanced social interactions by using large cages and groups [8]. In previous studies of the elevated plus maze test, rats reared in an enriched environment displayed a greater number of entries into open arm and more time spent in open arm than rats reared in a standard environment, which would suggest reduced anxiety [9]. In the forced swim test, when rats reared in an enriched environment faced stressors, they tried to escape with increased swimming and climbing time. In other words, environmentally enriched housing helped improve their stress-coping strategies [10]. Regarding spatial learning and memory, environmental enrichment showed positive effect by reducing the escape latencies in the Morris water maze test [11,12].

The affective quality of handling by experimenters influenced various behavior in rats, the tickling method, which induced a positive affective state by reducing the fear of humans in laboratory rats [13]. Some researchers considered handling as social enrichment and experimented [14]. Handling of rats reduced anxiety-like behavior, increased the number of entries into open arms and time spent in open arms [15,16]. In a previous study, when handling was treated in the two different environments of an isolated condition and enriched condition, anxiety-like behavior was reduced in isolated rats with handling, but increased in enriched rats with handling [17]. The behavioral experiments using handling methods have been mainly related to anxiety, and other behavioral tests have not been performed.

Social deprivation that diminished social interaction between individuals was considered environmental impoverishment. The social deprivation of rats during developmental stage was used as a potent chronic stressor [18]. Previous studies have reported that rats raised in socially deprived environments exhibited diverse behavioral changes that showed hyperactivity in novel environments [19]. Regarding the emotional aspect, social deprivation has shown an increase in abnormal forms of the aggression [20], anxiety-like and depressive-like behavior [21]. With regard to cognitive function, rats reared in social deprivation have shown impaired spatial learning and memory in the Morris water maze test [22].

During adolescence, sensory, motor, and social experience influence brain development, leading to emotional and cognitive maturation. It is crucial to verify positive or harmful environmental factors for developing the appropriate intervention. However, to verify each of environmental effects, the reality is not appropriate for evaluating its effect due to numerous external stimuli, so we designed animal study. It is necessary to confirm the effects of social environment on behavioral changes related to emotion and cognition in adolescences. We investigated the effect of environmental factors on depressive-like behavior and memory function during adolescence by performing the forced swim test, the Morris water maze test, and the passive avoidance test in adolescent rats exposed to environmental enrichment, handling, and social deprivation.

2. Purpose of the research

The research's purpose was to identify the effect of depressive-like behavior and memory function related to various environmental factors including environmental enrichment, handling, and social deprivation in adolescent rats. The specific purposes in this study are as follow:

1) Evaluate depressive-like behavior in forced swim test.

2) Evaluate memory function in the Morris water maze test and passive avoidance test.

METHODS

1. Experimental design

This study used a randomized control group posttest-only design to identify the effect of environmental factors on depressive-like behavior

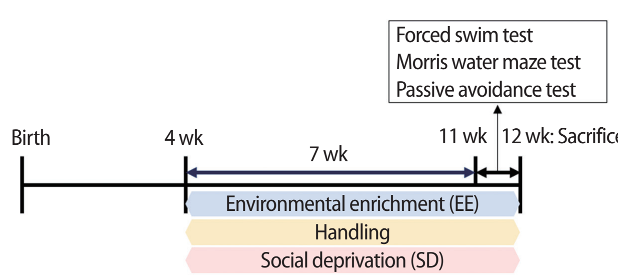

Figure 1. Experimental design.

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and cognitive function in adolescent rats. The experimental design was as shown in Figure 1. Wistar male rats were randomly assigned to control, environmental enrichment, handling, and social deprivation groups (n = 10 in each group) at the age of four weeks. Environmental enrichment, handling, and social deprivation protocols were applied to indicated groups for eight weeks; behavioral tests were performed at 11 weeks of age and all rats were sacrificed after the behavioral tests. The rat of six months old is 18 years old in human, and sexually mature in rats occurs postnatal 38-42 days. So, we provided environmental interventions for 4 to 12 weeks [23].

2. Animals

We used Wistar rats (60 ± 10 g, three weeks old) which are commonly used species in the laboratory. The influence of stress following transport from the animal supplier was minimized by providing a one-week acclimatization period. The number of samples in this study was based on the resource equation method, which is used when there is no information on the effect size [24]. When eight rats were selected for each group, the E value was measured as 28. Considering exclusion during the experimental process, a total of 40 rats with 10 rats per group were used in the study. Animals were housed in an air-conditioned room (temperature 23 ± 2°C, humidity 50 ± 10%), with 12 hours on/off cycle (07:00-19:00). Food and water were available *ad libitum*.

3. Methods

1) Environmental enrichment

Rats were housed in larger cages (70 × 45 × 42 cm) and large groups (5 animals per cage) with the opportunity for greater sensory stimulation, voluntary activities, and social interaction compared to the standard cages (40 × 26 × 18 cm, 3-4 animals per cage). The cage of the environmental enrichment group consisted of running wheels, wooden objects to gnaw, balls, ramps, ladders and various differently shaped animal toys. The water and food locations were changed frequently to encourage explorative behaviors [7,8].

2) Handling

The handling protocol was performed by modifying the previously described method [15] and was conducted by an individual experimenter in a handling group cage for 15-20 minutes each day, except weekends. Handling consisted of touching the fur of the neck and the dorsal surface of rat. The experimenter stopped the procedure if the rats displayed signs of discomfort such as defecation, urination, or startle response.

3) Social deprivation

Rats were individually reared in a small cage (26 × 20 × 13 cm) compared to the standard cage (40 × 26 × 18 cm). The cages of the social deprivation group were wrapped with black paper except for the cage lid to minimize stimulation. The rats reared in social deprivation were chronically stressed by social isolation, and were only handled when weighed once per week [18].

4) Forced swim test

The forced swim test was the most common method for assessing depressive-like behavior in rodents [25,26]. Rats were dropped individually into a transparent cylindrical tank (of height 100 cm and diameter 20 cm) filled with water at a temperature of 23-25°C to a depth of approximately 50 cm. On the first day, all rats had a 15 minutes training session to adapt to the water. On the second day, the rats were tested for 10 minutes. Mobility and immobility time were analyzed during this test session using a Smart ver. 2.5 video tracking system (Panlab, Barcelona, Spain). Mobility was considered as the ability to swim and climb and willingness to cope with stressors, while immobility was when they floated on the water's surface in hopelessness without the will to overcome the stressors. Immobility time meant depressive-like behavior.

5) Morris water maze test

The Morris water maze test is a common method of evaluating spatial learning and memory in rodent [27,28]. The water maze consisted of a circular pool (200 cm in diameter) filled with water (23 ± 1°C) to a depth of 30 cm. Imaginary lines divided the maze into four equal-sized quadrants (each 25% of the pool surface area). A platform (15 cm in diameter) was submerged 2 cm in the center of the northeastern quadrant. There were several visual cues on the room's walls. Hidden platform training was performed for four consecutive days for all groups, with three trials per day. Each rat was randomly placed into the water at one of four starting positions facing the wall of the water pool in each trial and was allowed to swim for 60 seconds. If the rats did not find the platform within 60 seconds, the rats were gently guided towards it and the escape latency time each required to reach the platform was recorded. Following the fi-

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nal training trial, a probe test was carried out and the platform was removed. Rats were allowed to swim in the pool for 60 seconds and the time spent in the target quadrant was recorded. In the Morris water maze, a short escape latency time and a lot of time spent in the target quadrant meant better spatial learning and memory.

6) Passive avoidance test

The passive avoidance test is a popular method for measuring short-memory in rodents [29]. The test uses the characteristics that rats prefer darker places over brighter places. The experimental instrument was the Gemini automation system (San Diego Instruments, San Diego, CA, USA) with chambers composed of bright and dark spaces. When rats crossed from a bright compartment to a dark compartment, they were delivered a shock via the feet of 0.3 mA for two seconds. The escape latency time was measured 24 hours after the foot shock.

4. Statistical analysis

All data were shown as mean ± SEM (standard error of mean). One-way ANOVA with Tukey's multiple comparisons test was conducted to determine the significance of differences in body weight, immobility time, mobility time, escape latency time, and time spent in the target quadrant among the four groups. A level of $p < .05$ was set as the criterion for statistical significance.

5. Ethical considerations

The 3Rs were first mentioned in 1959 by British scientists William Lassell and Rex Burch in the principles of human experimental technique [30], and have still widely applied in ethics of animal experimentation. First, replacement means that animal research should be conducted in case of absence of other experimental methods. Refinement is to improve welfare by minimizing pain and stress on animals. Reduction is to reduce the number of animals used in the experiment [31]. We conducted experiments based on 3Rs. The experimental procedures were performed in accordance with the animal care guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences.

RESULTS

1. Change in body weight

Body weights were measured once a week on Thursday between 10:00 and 11:00 am. Figure 2 shows the changes in weight gain among rats in the four groups throughout 12 weeks of age. All rats gradually increased in weight, and there were no differences between the four groups. In addition, it was confirmed that environmental factors did not affect weight change.

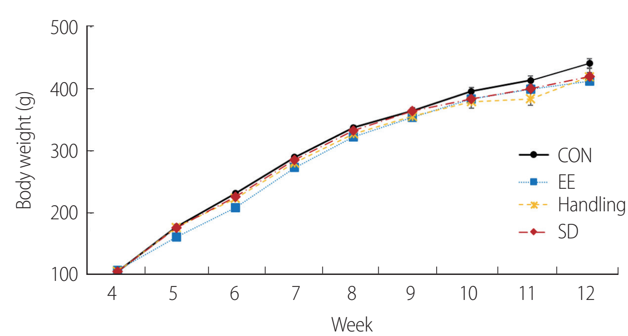

Figure 2. Weekly body weight gain.
The data are presented as the mean ± SEM.
CON = Control; EE = Environmental enrichment; SD = Social deprivation.

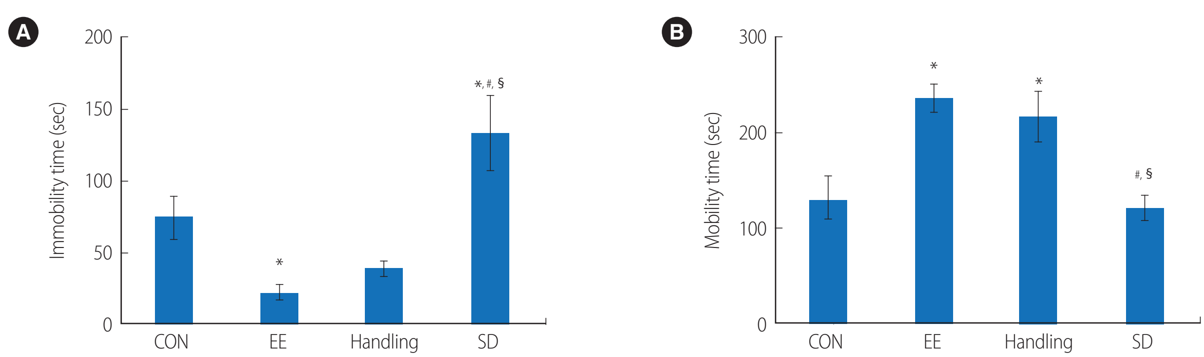

Figure 3. Results of the forced swim test. (A) Immobility time (sec). (B) Mobility time (sec).
The data are presented as the mean ± SEM.
*$p < .05$ compared to CON group; $^{\#}p < .05$ compared to EE group; $^{\S}p < .05$ compared to Handling group.
CON = Control; EE = Environmental enrichment; SD = Social deprivation.

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2. Effects of environmental factors on depressive-like behavior in the forced swim test

Figure 3 shows the results of the immobility time and mobility time in the forced swim test. There was a significant difference between the four groups for immobility time and mobility time (immobility time: F = 12.00, $p < .001$; mobility time: F = 6.85, $p = .002$). In immobility time, the environmental enrichment group was significantly decreased compared to the control group ($p = .038$), while the social deprivation group was significantly increased compared to the control group ($p = .035$), environmental enrichment group ($p < .001$), and handling group ($p = .001$). In the mobility time, the environmental enrichment group and handling group were significantly increased compared to control ($p = .009$; $p = .040$), while the social deprivation group was significantly decreased compared to the environmental enrichment group ($p = .012$) and handling group ($p = .044$). Environmental enrichment and handling increased the mobility time, which confirmed that the stress-coping strategy improved. The immobility of the social deprivation group was significantly increased compared to the socially housed groups, which confirmed that social deprivation induced depressive-like behavior.

3. Effects of environmental factors on spatial learning ability in the Morris water maze test

Figure 4 shows the results of the Morris water maze test. The escape latency time was gradually reduced in all four groups during the four days of training, and there was a significant difference in the escape latency time among the four groups on the fourth day (F = 6.60, $p = .001$). The social deprivation group had a significantly increased latency time compared to the control group ($p = .013$) and environmental enrichment group ($p = .001$). There was a significant difference between the four groups in the probe test performed on the fifth day (F = 3.56, $p = .031$), and the social deprivation group was significantly reduced compared to the environmental enrichment group ($p = .022$). Our findings showed that social deprivation impaired the spatial learning and memory.

4. Effects of environmental factors on short-term memory in the passive avoidance test

Figure 5 shows the results of the escape latency time in the passive avoidance test. There was a significant difference between the four groups for the escape latency time (F = 17.21, $p < .001$). The environmen-

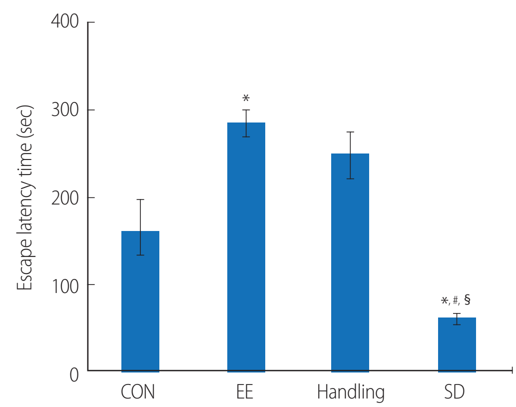

Figure 5. Results of escape latency time (sec) in the passive avoidance test.
The data are presented as the mean ± SEM.
*$p < .05$ compared to CON group; #$p < .05$ compared to EE group; §$p < .05$ compared to Handling group.
CON = Control; EE = Environmental enrichment; SD = Social deprivation.

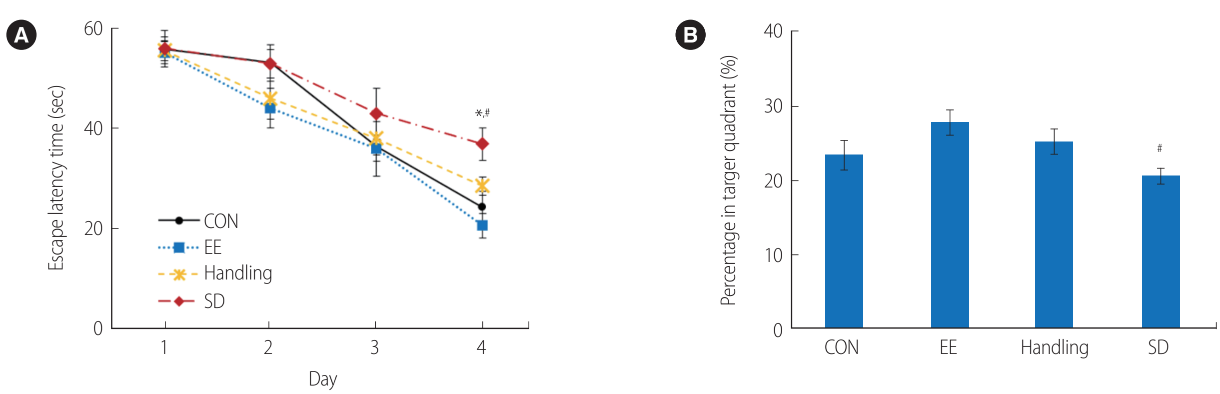

Figure 4. Results of the Morris water maze test. (A) Escape latency time (sec) during training 4 days. (B) Percentage spent in target quadrant (%) at 5 days.
The data are presented as the mean ± SEM.
*$p < .05$ compared to CON group; #$p < .05$ compared to EE group.
CON = Control; EE = Environmental enrichment; SD = Social deprivation.

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tal enrichment group had a significantly increased escape latency time compared to control group ($p = .005$). However, the social deprivation group had significantly reduced latency time compared to the socially housed groups (control group: $p = .030$; environmental enrichment: $p < .001$; handling group: $p < .001$), showing that social deprivation aggravated short-term memory.

DISCUSSION

The present study confirmed that changes in depressive-like behavior and memory ability were caused by environmental factors, such as environmental enrichment, handling, and social deprivation in adolescent rats. Environmental enrichment induced a positive effect on emotion and memory function, which alleviated depressive-like behavior and improved short-term memory rather than spatial learning and memory. Handling decreased depressive-like behavior, but did not affect memory function. Social deprivation negatively affected both emotion and memory function, which increased depressive-like behavior and aggravated spatial memory and short-term memory.

There was no significant difference in body weight among the four groups during the experimental intervention. The level of social interaction factors, such as environmental enrichment, handling, and social deprivation, did not affect the body weight in adolescent rats. Social deprivation was used as a chronic stress condition [18], and previous studies on body weight changes in rats reared in social deprivation have shown no change [32,33]. Our results showed that the social deprivation group was slightly lower than the control group for body weight, but not to a statistically significant difference.

In our experiment, rearing condition affected depressive-like behavior in the forced swim test. In animal behavior study, immobility time means the hopelessness and depressive-like behavior. Environmental enrichment showed antidepressant-like effects by significantly reducing immobility time and increasing mobility time in forced swim test, and these results were consistent with previous studies [10]. In previous studies on handling and emotion, some have shown handling reduced anxiety-like behavior [15,16], but there are few studies related to depressive-like behavior. Our data showed that handling slightly reduced immobility time in the forced swim test, but there was no significant difference. Meanwhile, it was confirmed that handling significantly increased the mobility time, and improved the stress-coping strategy. We need to repeat the experiment to verify the effects of handling. Social deprivation induced depressive-like behavior by significantly increasing immobility time in the forced swim test. These results are consistent with several previous studies that showed social deprivation induces depressive-like behavior [10,21]. We believe that social deprivation induces chronic stress and results in depressive-like behavior. As a basis for this behavioral test, there are limitations in not identifying physiological indicators related to stress or depression such as corticosterone levels.

In previous studies using environmental enrichment, they have shown to improve spatial memory in the Morris water maze test [11,12]; however, our results showed improved short-term memory rather than spatial learning and memory. We performed the probe test on the fifth day after training for four consecutive days in the Morris water maze test. Our results did not show the statistical difference in the environmental enrichment group compared to the control group. But we observed that the environmental enrichment group found the hidden platform the fastest on the fourth day and stayed for the longest time in the target quadrant on the fifth day. Also, in the passive avoidance test, environmental enrichment showed improved short-term memory. Based on these results, we confirmed that environmental enrichment has a positive effect on memory function. Unlike the results of a previous study in which handling improved learning and memory [16], our data showed that handling slightly increased the escape latency time in the passive avoidance test, but not to a statistically significant degree. There was the difference using behavior test with the previous study; further study should consider evaluating memory function in handling with more various assessment tool. In social deprivation, consistent with previous studies [22], our data showed that social deprivation decreased memory function in the Morris water maze test and passive avoidance test. Social deprivation induced structural damage in brain related to cognitive function, and it is considered as a chronic stress condition [18]. Corticosterone which is highly increased stress condition was increased in social deprivation [20], it has been reported increased corticosterone in adolescence impairs memory performance with in rats [34].

The environmental enrichment paradigm has emerged as a possible method for successful intervention to improve emotion and memory function during adolescence. Handling is less effective than environmental enrichment, but is expected to have a positive impact on emotion and memory function. It is necessary to revise and supplement the handling method for further study. We found that social deprivation nega-

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tively affected emotion and memory function, and showed a need to intervene in socially isolated adolescents using appropriate methods.

CONCLUSION

The brain of adolescence is immature and has structural and functional changes in the corticolimbic and frontal regions related to emotion and cognition, and social relationships are essential factors for the development of the brain during adolescence. Our findings confirm that social relationships during adolescence have important effects on the emotional and cognitive maturation. In particular, environmental enrichment and handling, which extended social relationships, decreased depressive-like behavior and improved cognitive function, whereas social deprivation, which removed the social relationships, increased depressive-like behavior and decreased cognitive function. It is worthwhile to apply the methods of environmental enrichment and handling to consider both the characteristics of adolescents and the tendency of individuals so that ostracized teenagers can establish constructive social relationships with their peers.

CONFLICTS OF INTEREST

The authors declared no conflict of interest.

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