Chinese Journal of Physiology 53(5): 285-293, 2010
DOI: 10.4077/CJP.2010.AMK056

Aging Effects on the BDNF mRNA and TrkB mRNA Expression of the Hippocampus in Different Durations of Stress

Shu-Hong Shao[1,*], Shou-Sen Shi[1,*], Zun-Ling Li[2], Ming-Shan Zhao[3], Shu-Yang Xie[2], and Fang Pan[4]

[1]*Institute of Medical Psychology, Binzhou Medical College, Yantai 264003*
[2]*Institute of Biochemistry and Molecular Biology, Binzhou Medical College, Yantai 264003*
[3]*The School of Basic Medicine, Binzhou Medical College, Yantai 264003*
and
[4]*Institute of Medical Psychology, Shandong University Medical School, Jinan 250012*
Shandong, P.R. China

Abstract

Brain-derived neurotrophins factor (BDNF) belongs to the neurotrophins family which acts on neuronal survival and growth and has been associated with cognition process. TrkB is the primary signal transduction receptor for BDNF. In the present study, hippocampal BDNF and TrkB mRNA were detected by RT-PCR in 2- and 22-month rats, respectively, which were exposed to different durations of mild stress protocol of 8-day, 21-day and 28-day chronic unpredictable mild stress (CUMS). Observation of exploratory behavior in an open field (OF) test indicated stress levels and changes of spontaneous activity. We demonstrated that CUMS induced decrease of BDNF mRNA in two aged groups, but the increase change of TrkB mRNA compared with those of the control groups. Moreover, the changes of BDNF mRNA and TrkB mRNA measured in both the 21-day and 28-day stress groups represent obvious decrease than those of the 8-day stress groups, and the expression examined in young groups appeared to be higher than those of the aged group, especially in the 28-day stress groups. Results of OF test showed that explicit behaviors in two age groups decreased gradually with the process of stress revealing a depressive state under the stress condition. Meanwhile, the behaviors of young rats seemed to be more active than those of the aged rats, exhibiting weak adaptation to the stress. The study suggested that stress paradigm and aging certainly had effect on the regulation of BDNF mRNA and TrkB mRNA which might be related to damage and protection function of the hippocampus.

Key Words: BNDF, hippocampus, behavior, senescence

Introduction

Brain-derived neurotrophic factor (BDNF) is the most widely and abundantly expressed neurotrophin (NT) is involved in neuronal survival and plasticity in different brain areas (6, 9). Beside its classical function, BDNF has been suggested to be involved in stress-induced hippocampal adaptation and pathogenesis of depression in the adult animal (47). As the central neurotrophic factor (NTF) for neurons and neurogliocytes, BDNF also plays an important role in hippocampal aging. Tyrosine kinase-coupled receptor (TrkB) is the primary signal transduction receptor for BDNF. In fact, the expression of BDNF and TrkB show significant change under stress conditions and senescent state. It was reported that

Corresponding author: Fang Pan, M.D., Ph.D., Institute of Medical Psychology, Shandong University Medical School, Jinan 250012, Shandong, P.R. China. Tel: +86-531-88382039, E-mail: panfang2003@hotmail.com
*These authors contributed equally to this work. The study was carried out in the Lab of Medical Psychology and the Institution of Biochemistry and Molecular Biology.
Received: September 7, 2009; Revised: December 8, 2009; Accepted: January 17, 2010.
 ISSN : 0304-4920. http://www.cps.org.tw

significant decreases with age were detected for BDNF and TrkB mRNAs in many areas of the brain. Elevation for BDNF and TrkB mRNAs was found in stress protocol, and different levels were detected in rat hippocampus (13, 30, 59).

The hippocampus is one of the important brain areas that is connected with emotional expression and cognition function, and it is also an area that is very susceptible to stress and aging (2, 36). It is well established that stress is a conspicuous factor of neuronal injury and can trigger degenerative cellular processes in the limbic system (1, 37). Single or repeated immobilization stress treatments have been reported to decrease BDNF mRNA throughout the hippocampus (16, 32, 53). Stress is not only involved in the alternation of the hippocampus function but is also related to morphological changes of hippocampus neurons (11, 59). In contrast, BDNF mRNA expression measured by *in situ* hybridization increased as early as 15 min in most hippocampal regions and was significantly augmented after 180 min of stress exposure. Adlard and Cotman (2004) reported that the expression of BDNF obviously decreased at 5 and 10 hours after binding stress, but the expression of TrkB mRNA showed no change in dentate gyrus and hippocampus after single stress (1). Previous studies showed that the levels of BDNF and TrkB mRNAs were significantly decreased in Alzheimer's patient hippocampus compared with normal hippocampus, which may be lead to neuronal dystrophy and cognitive impairment and behavior disorder (25, 41, 50).

Although it is recognized that BDNF is the most important neurotrophic factor in critical CNS functions, time-course studies of different stress applications have not yet been investigated in terms of aging responses. In the present study, we investigated whether different time periods of stress might modify the expression of BDNF and TrkB mRNAs, or whether there was an age-related change in BDNF expression in the hippocampus of rats.

Materials and Methods

Animals

Sixty-four male Sprague-Dawley rats, young rats (2 months) and aged rats (24 months), were obtained from the Animal Experiment Center of Binzhou Medical University, and were housed for 1 week before experiments under constant temperature (21 ± 1°C with 52 ± 2% humidity) and lighting regiments (light on from 07:00 AM to 07:00 PM). Light, food pellets and water were freely available throughout the experiments except when the CUMS procedure required deprivation.

Young and aged rats were randomly divided into four subgroups respectively, 8-day stress group, 21-day stress group, 28-day stress group and control group (n = 8). Animals were housed individually in conventional caging. The experimental rats were subjected to chronic unpredictable mild stress for 8, 21 and 28 days, respectively, and at the end of the stress protocol, they were sacrificed after behaviors test (between 9 and 11 AM), convenient for the collection of hippocampus. This work followed the regulations for the administration of affairs concerning experimental animals (48) and the experiments in the present study were designed to minimize the number of animals used and their suffering.

Chronic Unpredictable Mild Stress (CUMS) Paradigm

The CUMS paradigm was designed to maximize the unpredictable nature of the stressors (40, 54) which consisted of eight stress procedures including 24-h water deprivation, 24-h food deprivation, 5-min tail suspension, 2-h restraint, 5-min forced swim in cold water (4°C), 5-min hot environment oscillation (30 min, 45°C), continuous overnight illumination and 5-second inescapable foot-shock (30 min, 30 volt intensity and 5 sin interval). During the stress period, the stressors were carried into execution in random order in the experimental animals once per day for 8 days, 21 days and 28 days, respectively. The control group unstressed animals were handled daily and on the day of the experiment they were sacrificed at the same time as the stressed ones.

Sucrose Test

Sucrose test was most commonly used to measure anhedonia in chronic stress literatures (5, 8, 28, 55). In the present study, we used the one-bottle sucrose intake test which improved on the previous basic work (20, 21, 22). Before the experiments, all rats were trained to drink sucrose solution. In this test, the rats were first deprived of food and water for 24 h and they were then provided with 1% sucrose solution, and consumption was measured by comparing the bottle weight before and after the test after 24 h. During the CUMS protocol, the sucrose intake and bodyweight were measured once a week.

Open Field (OF) Test

OF box is a 90 × 90 × 45 cm wooden box, which is used to study cognitive and emotional reactions by observing the animal's behaviors (44), with its bottom divided into 5 × 5 squares and the square in the middle of the bottom is the center square while others are peripheral squares. During the period of stress, rats of two age groups were observed by OF test performed

Table 1. Measure of body weight of young and aged animals

Groups	Young groups	Aged groups
Control	228 ± 21.60	514 ± 51.10
8-day stress	215 ± 15.20	487 ± 32.30
21-day stress	189 ± 31.80**	453 ± 21.60**
28-day stress	187 ± 19.40**	431 ± 35.50**

Data are expressed as the means ± SD (n = 8). ** $P < 0.001$ indicated a significant difference as compared with the control group (Student's *t*-test).

on day 0, 8, 15, 21 and 28 during the stress. Each animal was placed in the center of the OF box and was observed for 3 min. The indices of test included the number of square crossing; grooming numbers, time spent on the center square, vertical movement scores and numbers of stools. All indices were observed and recorded by two persons who did not know the purpose of this test. OF box was cleaned up after each test session.

Measurement of mRNA Levels by RT-PCR

The rats in every group were sacrificed by decapitation after the test. Hippocampal tissues were obtained from the rats. Total mRNA was extracted from 50-100 mg hippocampus according to the instruction of TRIzol kit (Invitrogen, Carlsbad, CA, USA). The RT-PCR kit was purchased from Promega (Madison, WI, USA). The primers of BDNF and TrkB were synthesized by Sbsgene Company (Shanghai, PRC) and the sequences of the primers were 5'AGTGATGACCATCCTTTTCCTTAC3'plus5'CCTCAAATGTGTCATCCAAGGA3' (196 bp, for BDNF) and 5'GGCCAAGAATGAATATGGTAA 3'plus 5'TTGAGCTGGCTGTTGGTGAT 3' (485 bp, for trkB) and 5'CACAGCTAGAGGGAAATCG3'plus 5'CACACAGAGTAGTTGCGCTC3' (348bp, for β-actin). Reactions were performed in 50 μl containing 1 μl AMV Reverse Transcriptase, 1 μl TflDNA polymerase, 2 μl 25 mM $MgSO_4$, 2 μl primers, 1 μl dNTP Mix, 10 μl 5 × Reaction Buffer, 31 μl Nuclease-Free Water. Then the cycling process was 45°C for 45 min (1cycle), 94°C for 2 min (1cycle), 94°C for 30 sec, 55°C for 1 min, 68°C for 2 min (30 cycle), 68°C for 7 min (1cycle), and 4°C soak. Then the PCR products were analyzed by electrophoresis and the density ratio of target genes to β-actin band delegated the expression quantity of target genes.

Statistical Analysis

SPSS 11.0 software was used in this study. Mean and SEM were calculated from 8 animals per group for BDNF and TrkB. The quantitative data were expressed as Means ± SD and statistical analysis was performed by *t*-test between the young and aged groups. One-way ANOVA was used to analyze the different expression of BDNF and TrkB mRNA during different stress groups. A difference of $P < 0.05$ was considered statistically significant.

Results

Change of Body Weight

The results showed that there was no significant difference on body weight between the 8-day and the control groups both in the young and aged groups. Whereas, it was noted that the weights of the 21-day and 28-day groups were lighter than those of the control groups, and the weights of aged rats subjected to 28-day stress were down-regulated 21.5% ± 2.5% ($P < 0.001$), and the young rats inhibited 18.0% ± 3.7% ($P < 0.001$), relative to the control rats (Table 1).

Sucrose Consumption Measured in both the Young and Aged Stress Groups

The sucrose consumption measured in both the young and aged stress rats was obviously less than that in the control groups ($P < 0.05$) which represented a downtrend during the stress period. The aged 28-day and 21-day stress rats drank significant less than the rats of the 8-day stress group, as was also detected in the young group ($P < 0.001$). Meanwhile, comparing with the basic line, the control rats increased their consumption and kept the high levels throughout the study (Figs. 1 and 2).

Changes of Behaviors in OF Test

Though the aged rats showed more weakly exploratory behavior than the young rats, there was no significant difference between the young and aged rat behaviors before stress (0 day). The CUMS paradigm increased the time spent in the center square both in the young and aged rats groups. Meanwhile, the quadrant crossing, grooming and vertical movement represented pronounced decrease compared with the control groups

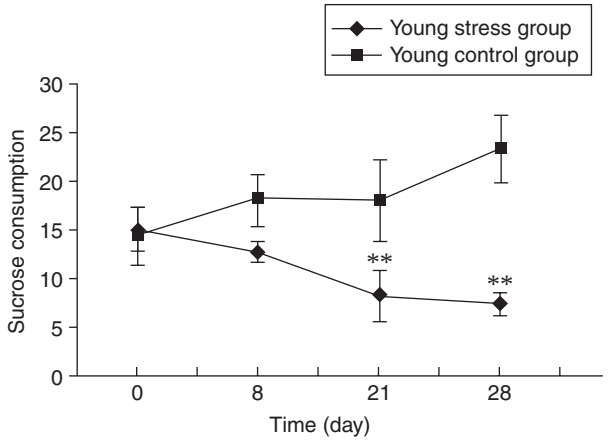

Fig. 1. Effects of different CUMS paradigms on sucrose consumption measured in the young groups (Means ± SD) (n = 8). $^{**}P < 0.001$, compared with the control group (Student's *t*-test). In the three young CUMS groups, sucrose intake was significantly diminished compared to control group, which was continuously kept at a high intake level.

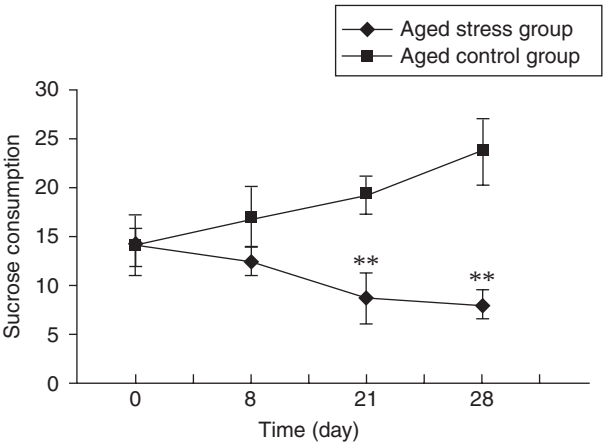

Fig. 2. Effects of different CUMS paradigms on the sucrose consumption measured in the aged groups (Means ± SD) (n = 8). $^{**}P < 0.001$, compared with the control group (Student's *t*-test). In the three aged CUMS groups, sucrose intake was significantly diminished compared to the control group, which was continuously kept at a high intake level.

Table 2. Open field test of young animals

	Control group	8 day stress	15 day stress	21 day stress	28 day stress
SQ	61.57 ± 8.23	39.16 ± 5.53*	25.42 ± 2.34**	27.24 ± 1.44**	24.06 ± 3.21**
VM	39.75 ± 5.32	26.07 ± 3.84*	21.46 ± 3.14**	17.52 ± 4.29**	16.33 ± 1.57**
GN	46.28 ± 6.44	30.67 ± 8.01*	29.56 ± 5.25**	30.91 ± 2.07**	16.57 ± 7.09**
TS	2.91 ± 0.65	3.99 ± 0.86*	4.86 ± 1.27**	5.31 ± 0.82**	4.66 ± 1.17**

Data are expressed as the means ± SD (n = 8). $^{*}P < 0.05$, $^{**}P < 0.001$ indicated a significant difference as compared with the control group (Student's *t*-test). SQ: Number of square crossing. VM: Vertical movements. GN: Grooming numbers. TS: Time spending in center square.

Table 3. Open field test of aged animals

	Control group	8 day stress	15 day stress	21 day stress	28 day stress
SQ	47.33 ± 4.08	24.16 ± 7.66*	17.21 ± 5.06**	12.64 ± 3.08**	11.47 ± 1.55**
VM	27.81 ± 4.49	16.47 ± 6.01*	11.85 ± 2.17**	6.49 ± 4.08**	6.98 ± 1.42**
NG	34.63 ± 6.24	22.07 ± 7.11*	18.35 ± 4.96**	8.45 ± 3.01**	9.06 ± 1.12**
TS	2.13 ± 0.57	3.34 ± 1.29*	5.07 ± 2.34**	5.66 ± 1.56**	5.54 ± 0.57**

Data are expressed as the means ± SD (n = 8). $^{*}P < 0.05$, $^{**}P < 0.001$ indicated a significant difference as compared with the control group (Student's *t*-test). SQ: Number of square crossing. VM: Vertical movements. GN: Grooming numbers. TS: Time spending in center square.

($P < 0.01$). The OF indices of the aged 21-day and 28-day groups were significant less than those of the young groups ($P < 0.05$), and it was noted that the aged animals represented obviously depression state after 21 days of stress, but the young ones showed weakly adaptive state to the stress representing an resumed excitatory state (Tables 2 and 3).

Age-Related Changes of the BDNF mRNA Levels Measured in the Hippocampus

RT-PCR was performed in the hippocampus to examine discrete modifications of the BDNF mRNA levels in this region in response to 8, 21 and 28 days CUMS. In this study, an increase expression of BDNF mRNA was found in both the young and aged rats compared to the control groups, respectively ($P < 0.001$). Aged rats subjected to different durations of

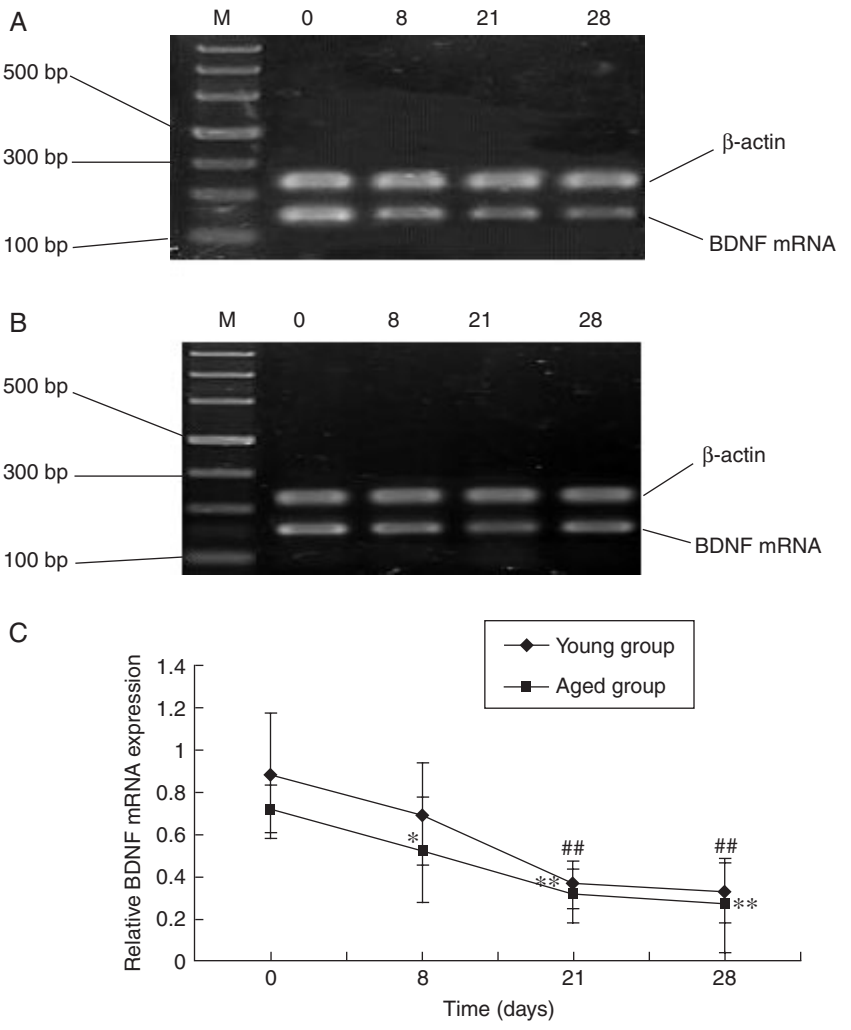

Fig. 3. BDNF mRNA expression detected by RT-PCR in the control groups (unstressed, 0 day) and in both the young and aged three CUMS model groups. Total RNA was isolated from the hippocampus and assayed for BDNF mRNA after 8, 21 and 28 days stress. A: Representative electrophoretograms illustrating the expression of BDNF mRNA in the young stress groups and the control group (0 day). B: Expression of BDNF mRNA in the aged stress groups and the control group (0 day). C: Quantitative analysis of BDNF mRNA at different time points after stress. The results were calculated as the intensity of the lane of each transcript over the intensity of the β-actin (internal standard) band and expressed as the means ± SEM. $^{\#\#}P < 0.001$ *vs.* the young control group. $^{*}P < 0.05$ and $^{**}P < 0.001$ *vs*. the aged control group. n = 7-8 rats per time point studied under independent stress conditions.

CUMS, particularly the animals exposed to 28 days stress, exhibited a significant inhibition of BDNF mRNA. The expression of BDNF mRNA also showed a significant decrease in young CUMS groups, but it represented no difference between 21-day and 28-day unpredictable stress ($P = 0.23$) reflecting a light adaptation to the stress condition. Moreover, the BDNF levels observed in aged rats displayed remarkable lower levels than those of the young rats in the three CUMS groups (Fig. 3).

Effect of Stress Duration on TrkB mRNA Expression

CUMS application resulted in a significant elevation for TrkB mRNA in both the young and aged groups. Rats exposed to 21-day and 28-day stress represented a remarkable increase of TrkB mRNA compared with the control groups ($P < 0.001$), and they also showed higher levels than those of the 8-day stress groups ($P < 0.05$). Meanwhile, the TrkB mRNA levels observed in the aged rats were lower than those measured in the young groups in different periods of CUMS conditions (Fig. 4).

Discussion

In the present study, we investigated the effect of different durations of chronic unpredictable stress

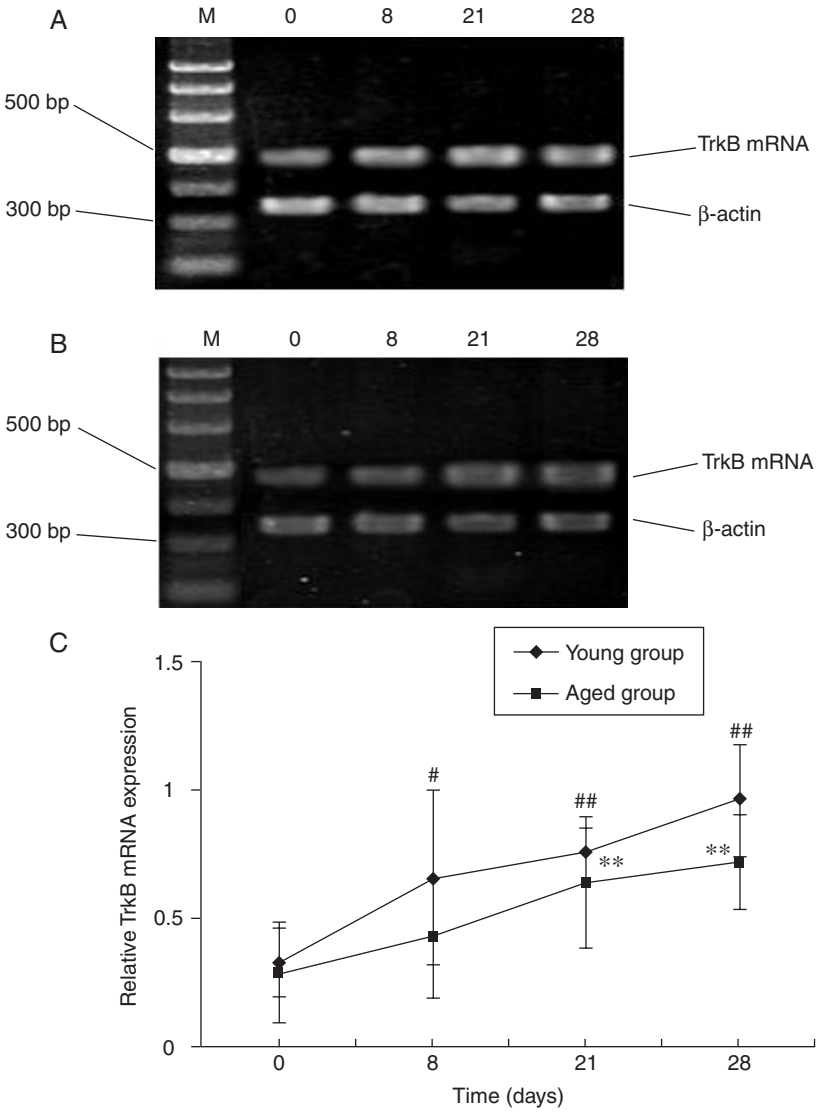

Fig. 4. TrkB mRNA expression measured by RT-PCR in the control groups (unstressed, 0 day) and in both young and aged CUMS groups. Total RNA was isolated from the hippocampus and assayed for TrkB mRNA after stress. A: Representative electrophoretograms showing the expression of TrkB mRNA in the young stress groups and the control group (0 day). B: TrkB mRNA expression in the aged stress groups and the control group (0 day). C: Line chart representing the results of quantitative analysis of TrkB mRNA after different stress sessions. The results were calculated as the intensity of the lane of each transcript over the intensity of β-actin (internal standard) band and expressed as the means ± SEM. $^{\#}P < 0.05$ and $^{\#\#}P < 0.001$ *vs*. the young control group. $^{*}P < 0.05$ and $^{**}P < 0.001$ *vs*. the aged control group. n = 7-8 rats per time point studied under independent stress conditions.

on the expression of BDNF and TrkB mRNAs in the hippocampus of both young and aged rats. An open field test was used in the evaluation of the change of rat behaviors (17, 52), and sucrose test was used to measure anhedonia. Three different durations of CUMS, 8-day, 21-day and 28-day stress, all resulted in decreased expression of BDNF mRNA but an elevation for TrkB mRNA in the hippocampus. BDNF and TrkB mRNA levels detected in the aged groups were lower than those of the young groups, and the expression levels changed more obviously in the 21-day and 28-day stress groups than in the 8-day stress group comparing with the control groups. During the CUMS protocol, animal behavior of the stress group displayed a decrease tendency, and stressed rats consumed less sucrose solution compared with the control groups.

The chronic unpredictable stress paradigm is a validated model subjected by using several stress methods in random order (56). Animal behaviors, such as exploratory behavior, cognitive reaction and excreting behavior, clearly changed in response to

stressful conditions (3, 7) and resulted in depressive stated in chronic mild stress (31). This study showed that both young and aged stress rats displayed a decrease in square crossing, grooming behavior and increased retention time in the center square compared with the control groups representing an inhibition of activities and an increase in emotional disorders. Animals showed little interest in, and weak adaptability to, new surroundings under CUMS state. Moreover, behaviors of aged animals inhibited more obviously than those observed in the young groups reflecting a weaker tolerance to stress. Stress has effects on the function of pre-frontal cortex and limbic system which are closely bound up with cognition and behavioral response (19). The hippocampus is one of the brain areas that are highly sensitive to the stress and senescence, and its dysfunction could enhance the susceptive of animals to stress, which might act on further stress injury and behaviors disorder.

Decrease consumption for sucrose solutions is considered as the maker of hyposensitivity to anhedonia (5, 21, 22, 28, 56). Our study showed that the stress time duration and senescence were two factors that tied up with the decrease of sucrose solution. The results showed that aged rats exposed CUMS consumed less sucrose than young rats and when compared with the control group. Meanwhile, the rats subjected to 21-day and 28-day stress drank more than the 8-day stress groups, and consumption of both young and aged rats was lower throughout the paradigm exhibiting distinctive anhedonia.

After the last session of 8, 21 and 28 days CUMS, respectively, BDNF mRNA in the hippocampus exhibited an obvious reduction compared to the control groups, and elevation for TrkB levels was observed in both the young and aged CUMS groups. A significant difference was observed between the young and aged stress groups, and the rats exposed to 21-day and 28-day stress resulted in lower expression of BDNF mRNA and higher TrkB levels than those of the 8-day stress groups. Such a stress-time dependent reduction in hippocampal BDNF mRNA and TrkB mRNA possibly contributed to the hippocampal neuronal atrophy and stress impairment protection.

The limbic-hypothalamopituitary-adrenal (LHPA) axis plays an important role in stress conditions and its high accommodation center is the hippocampus closely connected with cognitive function, emotion expression and explicit behaviors. It was confirmed that stress resulted in the elevation of glucocorticoids (GCs), the key hormones involved in stress adaptation in the hippocampus, and in the enhanced hippocampal sensitivity for stress (14, 29, 38). Previous studies have indicated that 3-h incubation of hippocampal neurons with glucocorticoids depresses activity-dependent expression of BDNF mRNA (12, 34). Lauterborn *et al*. reported that adrenal hormones inhibited *in vivo* BDNF expression (31). Senescence and stress both resulted in the increase of the corticosterone (24, 33) which led to the hyperfunction of hippocampus and functional deficit of learning, memory and cognition. Meanwhile, the hippocampal neuron atrophy that is induced by sustained high concentration of GCs has effect on the decrease of BDNF mRNA expression (10, 49).

BDNF is a neurotrophic factor involved in critical CNS function as well as in synaptic transmission and plasticity. Since BDNF also acts as the important NT in neurons survival, maintenance and growth (51, 57, 58), its deficiency may result in retraction or atrophy of dendrites (26). The present study showed that CUMS caused a distinct decrease BDNF mRNA response to stress compared to control rats. Previous studies showed that chronic repeated stress resulted in the down-regulation of BDNF production which might be implicated in the pathophysiology of depression (4, 15, 23, 35). In our study, we demonstrated that the long-term stress and senescence had explicit effect on the expression of BNDF mRNA both of which resulted in the decrease levels of BDNF. High density of GCs could be induced by both stress and aging and they could interact with alkaline phosphatase (AP) and nuclear transcription factor-κ B (NF-κB), which possibly made contribution to the down-regulation of BNDNF mRNA expression (24, 43, 60).

Now it is acknowledged that the decreased expression of BDNF production and the increased expression of TrkB mRNA in response to chronic repeat stress could have beneficial effect on neuronal function and survival (18, 42). Acute stress acts as the rapidly increasing expression of BDNF mRNA and protein to provide an excited organism with a degree of protection for hippocampus in a short time. If the excitation was not attenuated, secondary neuronal damage would occur (39) which would involved in neuronal atrophy and degeneration and in the reduction of BDNF. Thus, the observed up-regulation of TrkB mRNA might be a further compensatory adaptation to prolonged stress-induced down-regulation of BDNF. Up-regulation of TrkB could possibly make the neurons of the hippocampus more responsive to lower levels of BNDF than are induced by chronic stress (42, 45).

Previous studies showed that the induction of BDNF and TrkB mRNA detected in the hippocampus of AD was substantially lower than that seen in normal adult cellular tissues (25, 27, 46), and the spread of BDNF in the AD hippocampus was similar to that in naturally aging animals suggesting that a low content of BDNF in the brain was one of the reasons for cognitive disorder and weak responsiveness to stress. In

the present study, we demonstrated that the expressions of BNDF mRNA and TrkB mRNA detected in the aged stress groups were significantly contrary to those measured in the young groups, confirming previous work.

In conclusion, stress paradigm and aging certainly had effect on the regulation of BDNF mRNA and TrkB mRNA which might be involved in the damage and protection function for hippocampus. Clearly, further study will be necessary to explore the mechanisms in up/down-regulation of BDNF expression in different brain areas in order to further understand the complexities of neuronal responses to stress.

Acknowledgments

This work was supported by the Technological Study Project of Shandong Province (2007GG2000 2018), PRC.

References

1. Adlard, P.A. and Cotman, C.W. Voluntary exercise protects against stress-induced decreases in brain- derived neurotrophic factor protein expression. *Neuroscience* 124: 985-992, 2004.
2. Aggleton, J. P., Vann, S.D., Oswald, C.J. and Good, M. Identifying cortical inputs to the rat hippocampus that subserve allocentric spatial processes: a simple problem with a complex answer. *Hippocampus* 10: 466-474, 2000.
3. Alberts, S.C., Sapolsky, R.M. and Altmann, J. Behavioral, endocrine, and immunological correlates of immigration by an aggressive male into a natural primate group. *Horm. Behav*. 26: 167-178, 1992.
4. Altar, C.A. Neurotrophins and depression. *Trends Pharmacol. Sci*. 20: 59-61, 1999.
5. Baker, S.L., Kentner, A.C., Konkle, A.T., Santa-Maria Barbagallo, L. and Bielajew, C. Behavioral and physiological effects of chronic mild stress in female rats. *Physiol. Behav*. 87: 314-322, 2006.
6. Barde, Y.A. Neurotrophins: a family of proteins supporting the survival of neurons. *Prog. Clin. Biol. Res*. 390: 45-56, 1994.
7. Beck, K.D. and Luine, V.N. Food deprivation modulates chronic stress effects on object recognition in male rats: role of monoamines and amino acids. *Brain Res*. 830: 56-71, 1999.
8. Benelli, A., Filaferro, M., Bertolini, A. and Genedani, S. Influence of S-adenosyl-L-methionine on chronic mild stress-induced anhedonia in castrated rats. *Br. J. Pharmacol*. 127: 645-654, 1999.
9. Berg-von der Emde, K., Dees, W.L., Hiney, J.K., Hill, D.F., Dissen, G.A., Costa, M.E., Moholt-Siebert, M. and Ojeda, S.R. Neurotrophins and the neuroendocrine brain: different neurotrophins sustain anatomically and functionally segregated subsets of hypothalamic dopaminergic neurons. *J. Neurosci*. 15: 4223-4237, 1995.
10. Bremner, J.D. Stress and brain atrophy. *CNS Neurol. Disord. Drug Targets* 5: 503-512, 2006.
11. Chourbaji, S., Hellweg, R., Brandis, D., Zorner, B., Zacher, C., Lang, U.E., Henn, F.A., Hortnagl, H. and Gass, P. Mice with reduced brain-derived neurotrophic factor expression show decreased choline acetyltransferase activity, but regular brain monoamine levels and unaltered emotional behavior. *Mol. Brain Res*. 121: 28-36, 2004.
12. Cosi, C., Spoerri, P.E., Comelli, M.C., Guidolin, D. and Skaper, S.D. Glucocorticoids depress activity-dependent expression of BDNF mRNA in hippocampal neurons. *Neuroreport* 4: 527-530, 1993.
13. Croll, S.D., IP, N.Y., Lindsay R.M. and Wieqand, S.J. Expression of BDNF and TrkB as a function of age and cognitive performance. *Brain Res*. 812: 200-208, 1998.
14. De Kloet, E.R., Vreugdenhil, E., Oitzl, M.S. and Joels, M. Brain corticosteroid receptor balance in health and disease. *Endocr. Rev*. 19: 269-301, 1998.
15. Duman, R.S., Heninger, G.R. and Nestler, E.J. A molecular and cellular theory of depression. *Arch. Gen. Psychiatry* 54: 597-606, 1997.
16. Fuchikami, M., Morinobu, S., Kurata, A., Yamarmoto, S. and Yamawaki, S. Single immobilization stress differentially alters the expression profile of transcripts of the brain-derived neurotrophic factor (BDNF) gene and histone acetylation at its promoters in the rat hippocampus. *Int. J. Neuropsychopharmacol*. 12: 73-82, 2009.
17. Fukumoto, T., Morinobu, S., Okamoto, Y., Kagaya, A. and Yamawaki, S. Chronic lithium treatment increases the expression of brain-derived neurotrophic factor in the rat brain. *Psychopharmacology* 158: 100-106, 2001.
18. Givolois, L., Marmigere, F., Rage, F., Ixart, G., Arancibia, S. and Tapia-Arancibia, L. Immobilization stress rapidly and differentially modulates BDNF and TrkB mRNA expression in the pituitary gland of adult male rats. *Neuroendocrinology* 74: 148-159, 2001.
19. Gregus, A., Wintink, A.J., Davis, A.C. and Kalynchuk, L.E. Effect of repeated corticosterone injections and restraint stress on anxiety and depression-like behavior in male rats. *Behav. Brain Res*. 156: 105-114, 2005.
20. Gronli, J., Bramham, C., Murison, R., Kanhema, T., Fiske, E., Bjorvatn, B., Ursin, R. and Portas, C.M. Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. *Pharmacol. Biochem. Behav*. 85: 842-849, 2006
21. Gronli, J., Murison, R., Bjorvatn, B., Sorensen, E., Portas, C.M. and Ursin, R. Chronic mild stress affects sucrose intake and sleep in rats. *Behav. Brain Res*. 150: 139-147, 2004.
22. Gronli, J., Murison, R., Fiske, E., Bjorvatn, B., Sorensen, E., Portas, C.M. and Ursin, R. Effects of chronic mild stress on sexual behavior, locomotor activity and consumption of sucrose and saccharine solutions. *Physiol. Behav*. 84: 571-577, 2005.
23. Hashimoto, K., Shimizu, E. and Iyo, M. Critical role of brain-derived neurotrophic factor in mood disorders. *Brain Res. Rev*. 45: 104-114, 2004.
24. Hibberd, C., Yau, J.L.W. and Seckl, J.R. Glucocorticoids and the aging hippocampus. *J. Anat*. 197: 553-562, 2000.
25. Hock, C., Heese, K., Hulette, C., Rosenberg, C. and Otten, U. Region specific neurotrophin imbalances in Alzheimer's disease: decreased levels of nerve growth factor on hippocampus and cortical areas. *Arch. Neurol*. 57: 846-851, 2000.
26. Horch, H.W., Kruttgen, A., Portbury, S.D. and Katz, L.C. Destabilization of cortical dendrites and spines by BDNF. *Neuron* 23: 353-364, 1999.
27. Hosinger, R.M.D., Schnarr, J., Henry, P., Castelo, V.T. and Fahnestock, M. Quantitation of BDNF mRNA in human parietal cortex by competitive reverse transcription-polymerase chain reaction: decreased levels in Alzheimer's disease. *Mol. Brain Res*. 76: 347-354, 2000.
28. Jayatissa, M.N., Bisgaard, C., Tingstrom, A., Papp, M. and Wiborg, O. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. *Neuropsychopharmacology* 31: 2395-2404, 2006.
29. Korte, S.M. Corticosteroids in relation to fear, anxiety and psychopathology. *Neurosci. Biobehav. Rev*. 25: 117-142, 2001.
30. Laifenfeld, D., Kerry, R., Grauer, E., Klein, E. and Ben-Shacher, D. Antidepressant and prolonged stress in rats modulate CAM-L1, laminin, and pCREB, implicated in neuronal plasticity. *Neurobiol. Dis*. 20: 432-441, 2005.

31. Lauterborn, J.C., Poulsen, F.R., Stinis, C.T., Isackson, P.J. and Gall, C.M. Transcript-specific effects of adrenalectomy on seizure-induced BDNF expression in rat hippocampus. *Brain Res. Mol. Brain Res.* 55: 81-91, 1998.
32. Lee, T., Saruta, J., Sasaquri, K., Sato, S. and Tsukinoki, K. Allowing animals to bite reverses the effects of immobilization stress on hippocampal neurotrophin expression. *Brain Res*. 1195: 43-49, 2008.
33. Lupien, S.J., de Leon, M., de Santi, S., Convit, A., Tarchish, C., Nair, N.P.V., Thakur, M., McEwen, B.S., Hauger, R.L. and Meaney, M.J. Cortisol levels during human aging predict hippocampal atrophy and memory deficits. *Nat. Neurosci*. 1: 69-73, 1998.
34. Makino, S., Kaneda, T., Nishiyama, M., Asaba, K. and Hashimoto, K. Lack of decrease in hypothalamic and hippocampal glucocorticoid receptor mRNA during starvation. *Neuroendocrinology* 74: 120-128, 2001.
35. Mazure, C.M., Maciejewski, P.K., Jacobs, S.C. and Bruce, M.L. Stressful life events interacting with cognitive/personality styles to predict lateonset major depression. *Am. J. Geriatr. Psychiatry* 10: 297-304, 2002.
36. McEwen, B.S. Stress and hippocampal plasticity. *Annu. Rev. Neurosci*. 22: 105-122, 1999.
37. McEwen, B.S. Protective and damaging effects of stress mediators: central role of the brain. *Dialogues Clin. Neurosci*. 8: 367-381, 2006.
38. McEwen, B.S. and Magarinos, A.M. Stress effects on morphology and function of the hippocampus. *Ann. N. Y. Acad. Sci*. 821: 271-284, 1997.
39. Murakami, S., Imbe, H., Morikawa, Y., Kubo, C. and Senba, E. Chronic stress, as well as acute stress, reduces BDNF mRNA expression in the rat hippocampus but less robustly. *Neurosci. Res*. 53: 129-139, 2005.
40. Murua, V.S., Gomez, R.A., Andrea, M.E. and Molina, V.A. Shuttlebox deficits induced by chronic variable stress: reversal by imipramine administration. *Pharmacol. Biochem. Behav*. 38: 125-130, 1991.
41. Murer, M.G., Boissiere, F., Yan, Q., Hunot, S., Villares, J., Fauchex, B., Agid, Y., Hirsch, E. and Raisman-Vozari, R. An immunohistochemical study of the distribution of brain-derived neurotrophic factor in the adult human brain, with particular reference to Alzheimer's disease. *Neuroscience* 88: 1015-1032, 1999.
42. Nibuya, M., Takahashi, M., Russell, D.S. and Duman, R.S. Repeated stress increases catalytic TrkB mRNA in rat hippocampus. *Neurosci. Lett*. 267: 81-84, 1999.
43. Rage, F., Givalois, L., Marmigere, F., Tapia-Arancibia, L. and Arancibia, S. Immobilization stress rapidly modulates BDNF mRNA expression in the hypothalamus of adult male rats. *Neuroscience* 112: 309-318, 2002.
44. Sarkisova, K.Yu. and Kulikov, M.A. Prophylactic actions of the antioxidant agent AEKOL on behavioral (psychoemotional) disturbances induced by chronic stress in rats. *Neurosci. Behav. Physiol*. 31: 503-508, 2001.
45. Scaccianoce, S., Del Bianco, P., Caricasole, A., Nicoletti, F. and Catalani, A. Relationship between learning, stress and hippocampal brain-derived neurotrophic factor. *Neuroscience* 121: 825-828, 2003.
46. Silhol, M., Bonnichon, V., Rage, F. and Tapia-Arancibia L. Age-related changes in brain-derived neurotrophic factor and tyrosine kinase receptor isoforms in the hippocampus and hypothalamus in male rats. *Neuroscience* 132: 613-624, 2005.
47. Siuciak, J.A., Lewis, D.R., Wiegand, S.J. and Lindsay, R.M. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol. *Biochem. Behav*. 56: 131-137, 1997.
48. Smith, J.A. and Boyd, K.M. Lives in the Balance. The Ethics of Using Animals in Biomedical Research. Oxford. Oxford University Press, 1991, pp. 296-297.
49. Smith, M.A., Makino, S., Kvetnansky, R. and Post, R.M. Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci*. 15: 1768-1777, 1995.
50. Sun, C.Y., Qi, S.S., Lou, X.F., Sun, S.H., Wang, X., Dai, K.Y., Hu, S.W. and Liu, N.B. Changes of learning, memory and levels of CaMKII, CaM mRNA, CREB mRNA in the hippocampus of chronic multiple-stressed rats. *Chinese Med. J*. 119: 140-147, 2006.
51. Thoenen, H. Neurotrophins and activity-dependent plasticity. *Prog. Brain Res*. 128: 183-191, 2000.
52. Toth, E., Gersner, R., Wilf-Yarkoni, A., Raizel, H., Dar, D.E., Richter-Levin, G., Levit, O. and Zangen, A. Age-dependent effects of chronic stress on brain plasticity and depressive behavior. *J. Neurochem*. 107: 522-532, 2008.
53. Ueyama, T., Kawai, Y., Nemoto, K., Sekimoto, M., Tone, S. and Senba, E. Immobilization stress reduced the expression of neurotrophins and their receptors in the rat brain. *Neurosci. Res*. 28: 103-110, 1997.
54. Willner, P., Sampson, D., Phillips, G., Fichera, R., Foxlow, P. and Muscat, R. Effects of isolated housing and chronic antidepressant treatment on cooperative social behaviour in rats. *Behav. Pharmacol*. 1: 85-90, 1989.
55. Willner, P., Sanger, D. and Oglesby, M. The behavioural pharmacology of anxiety and depression. *Behav. Pharmacol*. 8: 475-476, 1997.
56. Willner, P., Towell, A., Sampson, D. Sophokleous, S. and Muscat, R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology* 93: 358-364, 1987.
57. Yamada, K., Mizuno, M. and Nabeshima, T. Role for brain-derived neurotrophic factor in learning and memory. *Life Sci*. 70: 735-744, 2002.
58. Yamasaki, Y., Shigeno, T., Furukawa, Y. and Furukawa, S. Reduction in brain-derived neurotrophic factor protein level in the hippocampal CA1 dendritic field precedes the delayed neuronal damage in the rat brain. *J. Neurosci. Res*. 53: 318-329, 1998.
59. Yun, S.J., Park, H.J., Yeom, M.J., Hahm, D.H., Lee, H.J. and Lee, E.H. Effect of electroacupuncture on the stress induced changes in brain derived neurotrophic factor expression in rat hippocampus. *Neurosci. Lett*. 318: 85-88, 2002.
60. Zhou, J., Zhang, F. and Zhang, Y. Corticosterone inhibits generation of long-term potentiation in rat hippocampal slice: involvement of brain derived neurotrophic factor. *Brain Res*. 885: 182-191, 2000.