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Effects of increasing durations of immobilization stress on plasma corticosterone level, learning and memory and hippocampal BDNF gene expression in rats

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ABSTRACT

Stress effects on learning and memory are widely recognized, but less agreement exists on whether they are positive or negative as well as on their neuronal and neuromolecular correlates. Stress involves expression of certain genes such as neurotrophin BDNF (brain derived neurotrophic factor), which is also involved in learning, but results are not consistent. Here effects of stress on memory and BDNF expression were studied using on adult male rats exposed to "immobilization stress" for various "short" durations, i.e., 1-h, 3-h, 5-h and "long-term" ones (2-h/day for 1 week). Learning and memory was measured using passive avoidance testing (STL = step-through-latency scores) as well as plasma corticosterone (CSt) levels and hippocampal BDNF gene expression. CSt increased in the 3-h and longer stressed groups but differences were significant in the 5-h and 1-week stressed subgroups. Three and 5-h of stress markedly and significantly (60–69%, $p < 0.01$) decreased memory retention in the stressed animals, while 1-h of stress had no effect; prolonged stress (2-h daily for 1-week) increased memory significantly (33%, $p < 0.05$). Hippocampal BDNF gene expression increased in the 1-h and 3-h stressed groups (44%, $p < 0.05$ and 71%, $p < 0.01$); but this parameter steadily declined in the 5-h stressed group (26%, $p < 0.05$) and weeklong stressed group (6%, not significant). Statistical analysis revealed an apparent but significant *negative* correlation between changes in memory and those of BDNF gene expression, indicating that BDNF may possibly play a compensatory role, reversing deleterious effects of stress on hippocampal memory functions.

Effects of stress on cognitive and neuronal functions, particularly memory, have long been studied [5,27] but results vary, some reporting enhancement of memory [2], while others find a decline [32]. Some authors suggest a complex relationship between stress, corticosterone (CSt) secretion and memory (21) while others emphasize the adaptive aspects of stress on learning and memory [27]. Stress influences gene expression for some brain proteins but it is not clear how neuronal gene expression is involved in stress effects on learning and memory [14].

The neurotrophin BDNF (brain derived neurotrophic factor) is involved in synaptic function and plasticity, neuronal growth and survival, apoptosis and cell death [3,11,15,20,30]. The specific roles of BDNF and other neurotrophins depend on mediation of particular receptors (TrkB, p75) [3,17]. BDNF has two receptors, TrkB and p75. TrkB mediates signals related to plasticity, survival and differentiation of neurons, while p75 relays signals related to apoptosis, although this topic requires further studies [10]. TrkB expression is important in proliferation and maturation of neurons in mouse dentate gyrus [6].

Although BDNF shows significant responses to stresses [28], the direction of these effects (increase or decrease) varies in different investigations [16,17]. Recent studies support BDNF involvement in molecular correlates of learning and memory. Specifically LTP (long-term potentiation), a well-known electrophysiological indicator of learning and memory in hippocampal circuits, shows alterations upon changes in BDNF mRNA activity [13,30]. BDNF is expressed at high levels in hippocampus, a brain structure known for its responsiveness and vulnerability to chronic stress (6) and for its critical roles in memory consolidation and recall [1,16]. Meisami and collaborators [7–9] have shown responses of hippocampal NGF (nerve growth factor) and its p75 receptor to thyroid hormones during development and in recovery from early brain retardation [8,9].

In view of the differing results of stress on learning and memory and on involvement of BDNF, we investigated the effects of short-

Abbreviations: BDNF, brain derived neurotrophic factor; CSt, corticosterone; HC, hippocampus; LTP, long-term potentiation; PA, passive avoidance; STL, step through latency.

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and long-term immobilization stress on passive avoidance learning and memory, as well as on hippocampal BDNF gene expression in adult rats, to clarify some of these important issues.

Sixty adult (4 months old) male Wistar rats, obtained from the animal colony of the Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, were used in this project. Initially, 30 rats were randomly assigned to 1 control (6 rats) and 4 experimental subgroups (6 rats/subgroup). Three of the experimental subgroups were exposed to the followings short-term durations of immobilization stress: subgroup 1: 1-h, subgroup 2: 3 h, and subgroup 3: 5-h. Rats of the fourth experimental subgroup were exposed to the same immobilization stress for 2-h per day, every day for 1 week. Each rat's restraining procedure started at 8.00 a.m. The rats of the control subgroup received no immobilization treatment. To apply immobilization stress, each experimental rat was placed in a restrainer device (length 20 cm, width 5 cm, height 6 cm). A portable door allowed adjusting the box length for each rat; thus rats were held completely immobilized with no space to move for the duration of immobilization. Control rats were kept in standard rat cages (approximately 30 inches length, 20 inches width and 12 inches height) allowing rats ample movements around the cage. Immediately following each stress duration regiment, rats were tested for memory as described below.

Learning and memory testing. Immediately at the end of each stress period, rats were tested for passive-avoidance (PA) procedure by measuring STL (Step Through Latency in seconds) for each animal and obtained group means. STL testing is widely used as a behavioral measure to model learning and memory in a variety of experimental paradigms because it allows quick and easy training of subjects (rats) and results are quantifiable [24,26]. The PA apparatus (shuttle box, I maze) and details of behavioral studies employed were the same as those used by Lashgari et al. [14] from the same Institute as this study, with one difference: here rats received 50 Hz square wave, 0.3 mA constant current shock for 2 s, as was done in a recent study by Nooshinfar and Lashgari [19].

Due to inherent high variance in the learning and memory testing of rats, we carried out an additional series of experiments using 30 more rats, which were again divided similarly into the same number of control and stressed subgroups, but were utilized for learning and memory (STL testing) only, without measuring CSt and BDNF. Data from the first and second series of STL testing were combined and statistically treated as a single larger group, having a total 60 rats, 12 controls and 12 for each of the 4 stressed subgroups.

Corticosterone measurement and BDNF gene expression. Following STL measurement, 2 ml of blood was collected from each rat to determine plasma CSt level, using standard kits. CSt is released in stressed rats and its release is a physiological sign of applied stress [4,12,29]. Lastly brains were removed and hippocampus extracted to measure BDNF gene expression (mRNA for BDNF protein) by RT-PCR method (reverse transcription-polymerase chain reaction), involving extraction of total RNA followed by synthesis of complimentary DNA (cDNA) and DNA replication by the PCR (polymerase chain reaction). Final material was loaded onto agarose gel using a UVI Transilluminator device and the resultant bands were photographed. Then the intensity of the different bands on the agarose gels was determined using the UV-SOFT-V-99 software. Next the ratios of BDNF band intensity to β-actin bands were determined for normalization purposes and the results were analyzed using the SPSS15 software and averaged per subgroup to determine the means, standard deviation and standard error of the means. Materials for the PCR procedures were obtained from Fermetas (Lithuania) and the procedures were according to protocols provided by the supplier.

To determine differences between the various means in the unstressed control and stressed subgroups, the methods of one-way analysis of variance (ANOVA) and the Tukey HSD follow-up

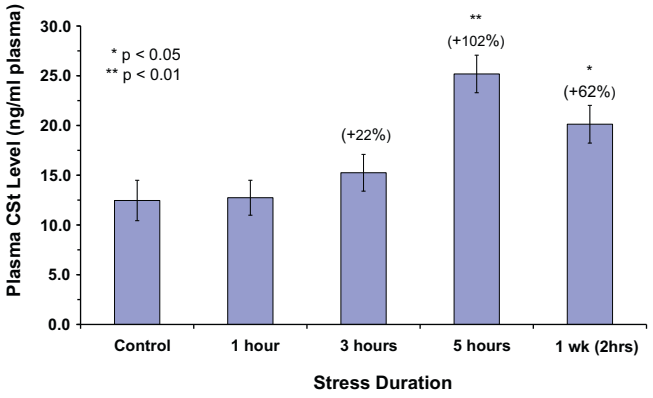

Fig. 1. Effects of immobilization stress for 1-, 3-, and 5-h and for 1 week (2-h/day) on plasma corticosterone (CSt) levels in adult male rats compared to unstressed controls. Bars are group means ± SEM; $N = 6$ animals per control group and each of the experimental subgroups (total of 30 rats in all); $^{*}p < 0.05$ and $^{**}p < 0.01$, compared to controls, based on ANOVA and Tukey Follow-Up test; 1wh2(2 h) = 2-h of stress/day for 1 week.

test were utilized using the SPSS-15 software. To determine the statistical significance of the difference between the means of STL (step through latency) and the changes in BDNF gene expression results, the Spearman correlation coefficient was utilized.

This project was approved by the Committee on Bioethics and Animal Care of the Shahid Beheshti University and followed the recommendation of international conventions.

Fig. 1 compares CSt levels in the control and experimental subgroups. Variation in hormone level in various groups was statistically significant ($p < 0.001$). Stress caused marked and significant increases in hormone level in the 5-h (102%, $p < 0.01$) and the long-term (1 week-2 h/day) stressed subgroups (62%, $p < 0.01$) but not in subgroups stressed for shorter durations of 1- and 3-h (2% and 22%). There is a tendency for CSt secretion to increase in the 3-h subgroup but the change was not significant.

Fig. 2 compares the effects of stress on learning and memory (PA task test) between the control and various stressed subgroups in terms of STL. The 1-h stressed subgroup showed a small (2%) decrease in STL scores compared to controls, while the 3- and 5-h stressed subgroups showed marked and highly significant decreases in the STL scores (60% and 69% respectively, $p < 0.01$), indicating a critical decline in memory retention. Interestingly, as seen in Fig. 2 (last bar), prolonged exposure to stress (2 h/day for

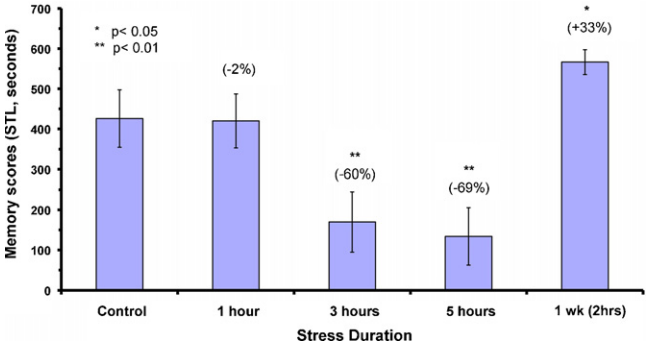

Fig. 2. Effects of immobilization stress for 1-, 3-, and 5-h and also for 1-week (2-h/day) on learning and memory, measured by STL (Step Through Latency) scores in a passive avoidance (PA) test, in adult male rats compared to unstressed controls. 1 h of stress had little effect on memory, while 3- and 5-h of stress markedly and significantly diminished memory. Memory (STL) scores in the weeklong stressed animals (2 h/day) improved significantly. Bars are group (subgroup) means ± SEM; $N = 12$ animals per control group and each of the 4 experimental subgroups (total of 60 rats in all); other details, same as in Fig. 1 sub-legend.