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Exposure of Wistar rats to 24-h psycho-social stress alters gene expression in the inferior colliculus

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HIGHLIGHTS

- Psycho-social stress induces significant gene expression changes in the auditory system.
- Two neuroprotective genes – *Ngfb* and *Hsf1* are asymmetrically expressed following stress. The left–right differences were significant only immediately after stress. No other genes were expressed asymmetrically.
- Observed changes are temporary are reverse to baseline with time.
- Auditory system may be subject to allostasis.

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ABSTRACT

Recently, we have demonstrated that the exposure of Wistar rats to psycho-social stress results in a transient auditory hypersensitivity. Here, to learn more about modifications occurring in auditory brainstem, we have analyzed gene expression pattern in inferior colliculus using quantitative RT-PCR. As targets, we have chosen genes associated with: neural activity (FBJ osteosarcoma viral oncogene, *cFos*), hypoxia (nitric oxide synthase inducible, *iNos*; superoxide dismutase 2, *Sod2*), neuroprotection (nerve growth factor beta, *Ngfb*; heat shock factor 1, *Hsf1*; heat shock protein 70, *Hsp70*) and inflammation (tumor necrosis factor alpha, *Tnfa*; tumor necrosis factor alpha receptor, *Tnfar*; substance P, *Sp*; cyclooxygenase 2, *Cox2*). We found that the expression of all genes was modified following stress, as compared to the controls. Immediately after stress, the number of transcripts encoding *iNos*, *Sod2*, *Hsf1*, *Ngfb*, *Tnfa*, *Tnfar* and *Sp* was significantly increased, suggesting possible modulation during exposure to stressor. Interestingly, we found that expression of *Hsf1 and Ngfb* at this particular time was left–right asymmetrical: there were more transcripts of both genes found in the left colliculi, as compared to the right colliculi. Three hours post-stress, *iNos*, *Hsf1*, *Tnfa* and *Tnfar* were still upregulated, *Sod2*, *Ngfb* and *Sp* went back to baseline and *Cox2* was upregulated. Six hours post-stress, *cFos* mRNA became downregulated. The number of *Hsp70* mRNA increased 24 h post-stress. Except for the reduced number of *cFos* transcripts, expression of all other genes tested reached the baseline seven days post-stress. Presented results corroborate the concept of auditory system responding to the psycho-social stress. Post-stress changes in the IC gene expression could likely indicate shift from allostasis to homeostasis in the auditory brainstem.

1. Introduction

Stress can be triggered by numerous stimuli and, as a consequence, it can provoke morphological and physiological changes in different organs and systems [32]. Depending on a type of stressor used (physical or psycho-social) and the duration of stress, stress-related changes may vary [24]. Stress affects the expression of a number of genes, particularly those involved in hypoxia, regeneration and inflammation [7,4]. Both inhibitory and excitatory effects of stress on gene expression have been described. Changes in gene expression are important for stress-induced neuronal plasticity,

Abbreviations: ABR, auditory brainstem response; ACTH, adrenocorticotropic hormone (ACTH); cFos, FBJ osteosarcoma viral oncogene; Cox2, cyclooxygenase 2; Ct, crossing threshold; DPOAE, distortion product otoacoustic emission; IC, inferior colliculus; iNos, nitric oxide synthase inducible; GR, glucocorticoid receptor; Hif1a, hypoxia-inducible factor 1 alpha; Hsf1, heat shock factor 1; HPA, hypothalamic–pituitary–adrenal; Hsp70, heat shock protein 70; Ngf, nerve growth factor; Ngfb, nerve growth factor beta; NOS, nitric oxide synthase; OC, organ of Corti; Tnfa, tumor necrosis factor alpha; Tnfar, tumor necrosis factor alpha receptor; SOD, superoxide dismutase; Sod2, superoxide dismutase 2; Sp, substance P.

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which in turn is a vital mechanism of responding to altered conditions. This response process is also called *allostasis* and means a shift from usual, homeostatic status into a condition, in which the organism can cope with changes [31]. Abuse or chronic deregulation of allostatic processes (such as prolonged or repeated stress) may lead to so-called *allostatic load* that comprises negative physiological and behavioral effects of stress [31].

Auditory signals are received and processed not only by auditory but also by limbic tissues, such as lateral nuclei in the amygdala, playing a central role in the emotional processing [27] or the hypothalamus, which is connected with the auditory system *via* projections from inferior colliculus (IC) [1]. The reticular activating system, responsible for emotional arousal, projects serotonergic fibers to all levels of the auditory pathway ranging from cochlea to the auditory cortex [13,22]. Bulk of research regarding auditory and limbic systems was dedicated to study the influence of acoustic signals on emotions but little is known about emotions influencing the auditory function.

In a recent study, we have demonstrated that 24-h exposure to a psycho-social stress induces auditory hypersensitivity associated in time with elevated corticosterone concentration in serum [28]. Here, we wanted to obtain more information about the effects of stress on the auditory brainstem. Consequently, we have focused our work on the inferior colliculus and analyzed stress-provoked changes in the expression of genes associated with neural activity (*cFos*), hypoxia (nitric oxide synthase inducible, *iNos*; superoxide dismutase, *Sod2*), regeneration (nerve growth factor beta, *Ngfb*) and inflammation (*Tnfa*; tumor necrosis factor alpha receptor, *Tnfar*; heat shock factor 1, *Hsf1*; heat shock protein, *Hsp70*; substance P, *Sp*; cyclooxygenase 2, *Cox2*).

2. Materials and methods

2.1. Animals, stress exposure and tissue dissection

Animal tissues and mRNA used in the present study originated from our earlier work, where we have described all the experimental settings in detail [28]. Briefly, during each experiment, two pre-pubertal female Wistar rats (4–6 weeks old) were placed in a new cage and transferred to a separate room, whereas the control animals (5 animals per cage) stayed in their home cage. Next, activated rodent repellent (Conrad Electronics Berlin, Germany) was placed in the cage for 24 h. This particular rodent repellent produces 300 Hz and 65 dB(A) sound, which is perceived by rats just above their hearing threshold [25]. Sound and vibrations, which are also produced by the repeller, induce anxiety in rodents and cause them to flee. Under experimental conditions, escape is impossible and this situation induces anxiety in animals. Additional handling, change of cage, change of room and the reduced number of animals per cage were supplementary stressors. Control animals were not exposed to an inactive repellent, they were not removed from their cage, the number of animals per cage remained the same, and the cage was not moved. Thus, we did not study the "pure" effects of sonic/vibration stress but rather sonic/vibration stress plus handling and possibly social (reduced number of animals) stress. This sonic stress system was in the past 20 years shown to induce miscarriages, asthma attacks and hair loss in the experimental animals [35,2,3].

Six experimental groups were examined: group (1) immediately after stress (15–30 min); group (2) 3 h; (3) 6 h; group (4) 24 h; group (5) 7 days after stress and group (6) control animals. The number of animals in group (1) was $n = 5$; group (2) $n = 7$; group (3) $n = 6$; group (4) $n = 5$; group (5) $n = 6$; group (6) $n = 9$. The animals were decapitated and ICs dissected. The left and right IC tissues were processed as separate samples, based on the notion of asymmetry occurring in a steady-state and stressed auditory system [5,30] as well as based on the fact that other "identical" tissues (*e.g.* brain or gonads) despite systemic treatment demonstrate discrete properties for the left and right tissues [6,42].

2.2. Semi-quantitative RT-PCR

The RNA isolation procedure was performed with PicoPure™ RNA Isolation Kit according strictly to the manufacturer's directions (Molecular Devices, Sunnyvale, CA, U.S.A. cat. # KIT0204). RNA concentration was estimated using Ribogreen® RNA Quantitation Reagent (Molecular Probes, Göttingen, Germany). Fifty nanogram of total RNA was reverse transcribed in 25 μl reaction (Promega GmbH, Mannheim, Germany) with an oligo dT primer. Obtained cDNA was used in five consecutive PCR reactions, 5 μl per reaction (equivalent of 10 ng of total RNA per reaction).

FastStart PCR Master Mix (Roche, Mannheim, Germany) was prepared according to the manufacturer's instructions. Primers used are listed in Table 1. A control PCR reaction containing no cDNA was used as a blank. Expression of a housekeeping gene encoding ribosomal protein S16 (rS16) was used as internal control. Expression of the housekeeping gene under all experimental conditions was stabile, as per analysis of $2^{(-Ct)}$ equation and Student's-*t* test [37]. PCR consisted of 40 cycles under optimized conditions in a LightCycler® 2.0 (Roche). The crossing threshold (C_t) value was calculated automatically by the LightCycler software version 4.0 and used to calculate relative expression of target genes against the housekeeping gene rS16 and against the unstressed controls.

2.3. Statistics

Means ± standard errors of the mean (SEM) were calculated for all parameters measured. Separate specimens obtained from the left and right sides of each animal were included into the analysis. Temporal changes in the gene expression were analyzed using one-way analysis of variance (ANOVA). Significant ANOVA was followed by Fisher's LSD test to compare individual means *vs.* controls. Differences resulting in $p < 0.05$ were considered to be significant. All statistical tests and graphics were made using Statistica 7.1 (Statsoft).

3. Results

The expression of *cFos* has changed following exposure to stress (ANOVA, $F_{(5,\ 61)} = 3.82$, $p < 0.01$). Post hoc test revealed significant downregulation of *cFos* by about 2.5-fold at 6 h and 7 days after stress.

Immediately after finishing stress, expression of oxidative stress-related genes *iNos* and *Sod2* was significantly upregulated. The number of *iNos* transcripts increased 1.7-fold (ANOVA, $F_{(5,\ 61)} = 3.89$, $p < 0.01$) whereas *Sod2* transcripts increased 1.4-fold (ANOVA, $F_{(5,\ 63)} = 8.32$, $p < 0.0001$). Three hours post-stress, the number of *iNos* transcripts was still significantly higher than in the controls (1.6-fold), whereas that of *Sod2* returned to baseline.

Expression of genes associated with neuroprotection was also upregulated. In detail, *Ngfb* was upregulated 4.6-fold (ANOVA, $F_{(5,\ 62)} = 5.98$, $p < 0.001$) and *Hsf1* was upregulated 2-fold (ANOVA, $F_{(5,\ 62)} = 23.35$, $p < 0.0001$) immediately post-stress. Three hours later, the expression of *Hsf1* was still elevated, whereas *Ngfb* it was not statistically significant. Expression of *Hsp70* was upregulated 1.25-fold 24 h post-stress (ANOVA, $F_{(5,\ 63)} = 2.62$, $p < 0.05$).

Of genes associated with inflammation, the number of mRNAs encoding *Tnfa* increased 1.6-fold (ANOVA, $F_{(5,\ 62)} = 7.70$, $p < 0.0001$), *Tnfar* increased 1.6-fold (ANOVA, $F_{(5,\ 62)} = 11.27$, $p < 0.0001$) and *Sp* increased 2.9-fold (ANOVA, $F_{(5,\ 62)} = 6.91$, $p < 0.0001$) immediately post-stress. Three hours later, the number of transcripts encoding