

Research report

Effects of different kinds of acute stress on nerve growth factor content in rat brain

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Abstract

Nerve growth factor (NGF) has several effects on the central nervous system; on the one hand NGF fosters survival and function of cholinergic neurons of the basal forebrain, on the other hand this protein is implicated in the stress response of the hypothalamic–pituitary–adrenocortical axis (HPAA). In this study we tested the influence of threatening and painful stress treatments in three different intensities as well as forced motoric activity on NGF content in different brain areas in adult rats. We found that threatening treatment with or without painful stimuli was followed by a significant decrease of NGF concentration in the amygdala (44.5%; $P=0.03$) and the frontal cortex (–45.5%; $P=0.02$). We also observed that after stress of forced motoric activity NGF content in the frontal cortex (–32%; $P=0.01$) and the hippocampus (–32%; $P=0.006$) was significantly reduced. Thus, NGF content in distinct brain regions is decreased, following different forms of acute stress. This might be relevant for the pathophysiological understanding of psychiatric diseases, such as depression, which are associated with stress.

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1. Introduction

Nerve growth factor (NGF), which belongs to a family of neurotrophic proteins, is responsible for development and survival particularly of cholinergic neurons of the basal forebrain (for reviews, see Refs. [25,26,19]). In the mature central nervous system (CNS) NGF ameliorates detrimental effects of experimental lesions of cholinergic neurons (for reviews, see Refs. [19,39]). Apart from a trophic function, NGF seems to be implicated in the stress response and the function of the hypothalamic–pituitary–adrenocortical axis (HPAA) (for a review, see Ref. [25]). Previous studies have shown that exogenous NGF treat-

ment activates the HPAA in rats [29]. Conversely, the serum NGF concentration was found to be elevated in response to different stressors in humans [4,12]. In animals, e.g., in mice an increase of serum and hypothalamic NGF levels has been observed after the experience of a social stress involving inner male aggression [2,3]. Thus, NGF appears to play a role in the regulation of the HPAA-mediated stress response, which exceeds its well known neurotrophic function within the nervous system.

The stress response of the HPAA is activated by direct stressors, e.g., hypoxia or hypotension, via adrenergic neurons of the brainstem [31]. Cholinergic neurons of the frontal cortex, the septum and the amygdala, are important for the activation of the HPAA in response to indirect stressors, which require further cognitive processing [20]. During stress cholinergic neurons of the septohippocampal system are highly active [9,23]. Since NGF is particularly important for survival and function of the cholinergic system as well as being involved in the stress reaction, the question is, whether stress influences NGF concentration in

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central cholinergic areas. So far previous experiments have yielded different and conflicting results. For instance, NGF mRNA in the hippocampus was found to be increased after cold stress in rats [10] and reduced after stress of immobilization or foot shocks in rats [36,40].

Possible explanations for these conflicting results are the different nature of stressors employed in each experiment and the duration of exposure to stress, which might differ in their efficacy to initiate a HPA axis response. In this experiment we report the effect of a threatening experimental treatment on NGF content in several brain areas, which are important for the activation of the HPA axis, such as amygdala, hippocampus, limbic forebrain and frontal cortex. To answer the question, whether stimulus intensity has an impact on NGF content, stress treatment was graded to range from very threatening and highly painful to mildly threatening and not painful. Furthermore, we employed two different forms of stress: acutely threatening treatment and a forced motoric activity paradigm.

2. Materials and methods

2.1. Materials

Chemicals of analytical grade were purchased from Merck (Darmstadt, Germany). Special reagents were obtained from Sigma (Deisenhofen, Germany). Anti-mouse-NGF antibodies (clone 27/21) and anti-mouse-NGF (clone 27/21)- β -galactosidase conjugate were acquired from Chemicon (USA, formerly Boehringer Mannheim, Germany); mouse-NGF (2.5 S) was donated by Professor Dr. Rohrer, Max-Planck-Institute for Brain Research, Frankfurt/Main, Germany.

2.2. Animal treatments

In this study 106 adult male Sprague–Dawley rats, weighing between 250 and 300 g, were used. Two animals were housed in one cages under standard conditions in a 12 h light–12 h dark cycle (light period between 6.00 a.m. and 6.00 p.m., dark period between 6 p.m. and 6 a.m.). Rats had free access to laboratory chow and tap water. Treatment started after an adaptation period of 1 week.

Stress experiments started at 7 p.m. at the beginning of the active period. Two forms of stress, namely an acutely threatening treatment and forced motoric activity, were tested. The impact of threatening treatment was tested in three different stress intensities: high, moderate or mild. Forced motoric activity was tested for a period of 2 or 10 h. The experimental protocol was approved by the Landesamt für Arbeitsschutz, Gesundheitsschutz und Technische Sicherheit Berlin (G 0156/98). All efforts were made to minimize the suffering and the number of animals used. The experimental groups are detailed as follows.

2.2.1. Group 1 (high stress)

Our laboratory rats were grasped individually with one hand by the scruff of the neck and taken out of the cage. With the other hand the tail was pulled and the rat was turned upside down for 30–60 s. The abdomen wall was stretched in order to administer an intraperitoneal injection of a physiological saline solution. After rats had received a painful intraperitoneal injection they were placed back into the cage. The stress of rough handling and the painful injection caused the rats to vocalize loudly. Rats were decapitated without anesthesia 1 h later, i.e., at 8 p.m.

2.2.2. Group 2 (moderate stress)

Rats were grasped by the scruff at 7 p.m., lifted out of the cage, turned upside down and the tail was pulled for 30–60 s as for preparation of an intraperitoneal injection, yet the injection was not administered. After this rough treatment rats were placed back into their cage. They were decapitated without anesthesia 2 h later.

2.2.3. Group 3 (mild stress)

At 7 p.m. rats were grasped cautiously by the tail and transferred from their own cages to adjacent cages three times within 2 min. They were decapitated without anesthesia 2 h later.

2.2.4. Group 4 (motor stress, 2 h)

Motoric activity was enforced by placing rats at 7 p.m. into a running wheel, which rotated at a speed of one revolution per 45 s. This device forced the rats to walk slowly and continuously. Rats were killed after 2 h of continuous walking at 9 p.m. by decapitation without anesthesia.

2.2.5. Group 5 (motor stress, 10 h)

Rats were placed into a rotating wheel from 7 p.m. to 5 a.m. They were killed 10 h later at 5 a.m. as described above.

2.2.6. Control groups

All control animals were kept under the same conditions as animals in the treatment group. Control animals, which were used for the groups of mild, moderate and strong forms of threatening treatment (groups 1–3), did not experience stress through experimental handling and were killed at 9 p.m. on the same day with the same procedure as rats of the treatment groups. Rats in the control group of the 2 h motor stress treatment (group 4) were killed as described above prior without walking stress at 9 p.m. Rats in the control group for the 10 h motor stress treatment (group 5) were killed without prior walking stress at 5 a.m.

Rat brains were dissected as described by Glowinski and Iversen [11] and dissected tissues were immediately frozen and stored until further processing at 70 °C. The fact that animals from group 1 were killed just 1 h after stress exposure is due to the circumstance that our samples were

collected within a larger study that measured rat brain type II 5'-iodothyronine deiodinase activity during acute stress exposure [6]. Nevertheless, it seems reasonable to us, to compare these NGF values with animals killed 1 h later because several data suggest, that NGF brain concentrations show no circadian rhythm [27].

2.3. Homogenization procedure and determination of cerebral NGF levels

Tissue samples were individually homogenized on ice in 5–6 volumes of 0.25 mol/l sucrose, 10 mmol/l 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid (HEPES; pH 7.0) containing 10 mmol/l dithiothreitol (DTT), immediately frozen in a dry ice/acetone bath and stored at -80°C until NGF measurement. The homogenates were centrifuged at 10,000 g for 10 min at 15°C . The remaining pellets were each dissolved in 750 μl NGF-homogenization buffer, treated with ultrasound for 3 min and processed for quantification of endogenous NGF as described in detail elsewhere [15–17]. The protein quantity of this resuspension was quantified by Bio-Rad[®] protein assays [7]. NGF was then measured in two separate probes using a highly sensitive ELISA (enzyme-linked immunosorbent assay). To one probe a set amount of external NGF (the so-called recovery) was added. After NGF measurement and correction of the NGF recovery rate of each ELISA measurement, NGF content was calculated per (pg/mg) protein of the homogenate.

2.4. Statistical analysis

All data are presented as means and standard deviation and standard error of mean (S.E.M.). The Kolmogorov–Smirnov test showed that the NGF concentrations measured in our samples were not normally distributed and differ considerably from a normal distribution. On that account, individual comparisons between the control group and each of the treatment groups were carried out using the Mann–Whitney *U*-test. Statistical significance was accepted when $P < 0.05$.

3. Results

3.1. NGF content of the frontal cortex after threatening stress (groups 1, 2, 3)

One hour after exposure to the high stress condition NGF concentrations in the frontal cortex were significantly reduced by 45.5% ($P = 0.02$) in comparison to the control group. A significant NGF reduction by 37% was observed after the moderate stress condition ($P = 0.04$) and even a highly significant reduction of NGF concentration by 38% was seen after the induction of mild stress condition ($P = 0.01$) (see Fig. 1).

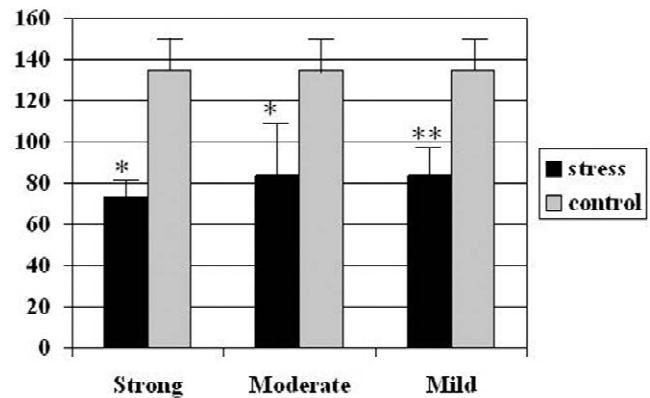


Fig. 1. NGF concentrations (pg/mg) of the frontal cortex (FC) as mean \pm one standard deviation. Asterisks indicate a significant (*) ($P < 0.05$) or highly significant (**) ($P < 0.01$) difference between control animals ($n = 4$) or animals after strong ($n = 5$), mild ($n = 4$) and moderate ($n = 4$) stress conditions.

3.2. NGF content of the amygdala after threatening stress (groups 1, 2, 3)

We observed a significant reduction of NGF concentration by 34% compared to the control group ($P = 0.04$) in the high stress condition. The mild stress condition induced a NGF reduction by 44.5% ($P = 0.03$). A substantial, yet not significant reduction of NGF concentration was observed after the moderate stress condition by 34% ($P = 0.07$). This moderate stress group contained only three animals, so the statistical analysis of this group is limited (see Fig. 2).

3.3. NGF content of the hippocampus and limbic forebrain after threatening stress (groups 1, 2, 3)

NGF content neither in the hippocampus nor in the limbic forebrain changed after the experience of threatening stress (see Figs. 3 and 4).

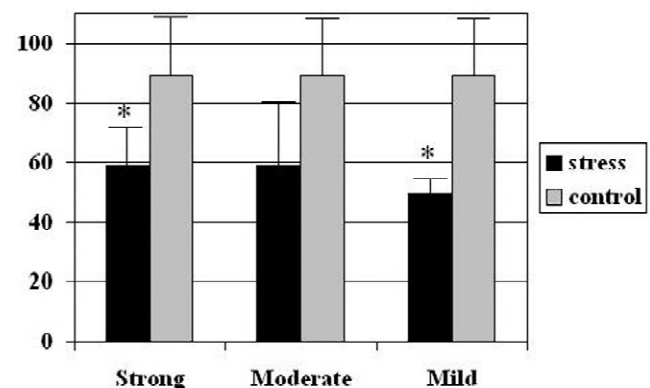


Fig. 2. NGF concentrations (pg/mg) of the amygdala (AMY) as mean \pm one standard deviation. Asterisks indicate a significant (*) ($P < 0.05$) difference between control animals ($n = 6$) or animals after strong ($n = 5$), mild ($n = 3$) and moderate ($n = 4$) stress conditions.

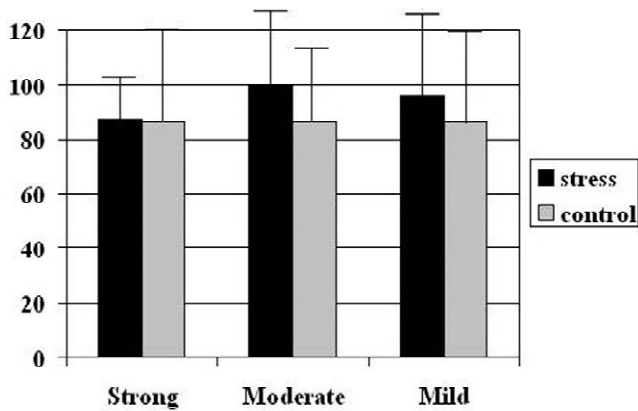


Fig. 3. NGF concentrations (pg/mg) of the hippocampus as mean±one standard deviation. No significant differences between control animals ($n=5$) or animals after strong ($n=5$), mild ($n=5$) and moderate ($n=5$) stress conditions.

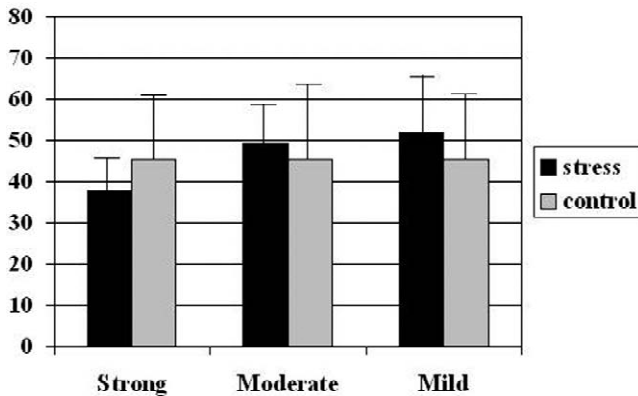


Fig. 4. NGF concentrations (pg/mg) of the limbic forebrain as mean±one standard deviation. No significant differences between control animals ($n=6$) or animals after strong ($n=4$), mild ($n=5$) and moderate ($n=5$) stress conditions.

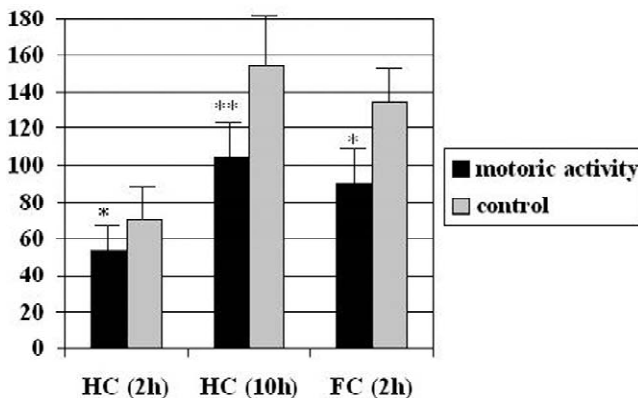


Fig. 5. NGF concentrations (pg/mg) of the frontal cortex (FC) and hippocampus (HC) as mean±one standard deviation. Asterisks indicate a significant (*) ($P<0.05$) or highly significant (**) ($P<0.01$) difference between control animals ($n=5$) or animals after 2 h motoric activity (FC: $n=5$; HC: $n=5$) and 10 h motoric activity (HC: $n=5$).

3.4. NGF content of the frontal cortex and hippocampus after forced movement (groups 4, 5)

After the induction of 2 h of forced movement in a running wheel, NGF content was found to be significantly decreased in the frontal cortex by 32% ($P=0.01$) and hippocampus by 25% (0.047). After 10 h of motor activity NGF content in the hippocampus was significantly reduced by 32% ($P=0.006$) (see Fig. 5).

3.5. NGF content of the limbic forebrain and the amygdala after forced movement (groups 4, 5)

NGF content neither in the amygdala nor in the limbic forebrain changed after the experience of motoric stress (data not shown).

4. Discussion

We found that acute stress, i.e., the experience of a physical threat and pain, significantly reduced NGF content in the frontal cortex as well as in the amygdala but not in the limbic forebrain or the hippocampus. Similarly, NGF content was found to be reduced after forced running in the frontal cortex and in the hippocampus, however, NGF was unaffected in the amygdala or limbic forebrain.

Since the NGF content of brain tissues is known to be several fold higher than generally reported and largely associated with sedimentable fractions [22], the homogenates of our tissue samples and the NGF content was quantified in the remaining pellets after centrifugation. Our results obtained are highly consistent with those previously reported by Hoener and co-workers [21,22].

4.1. NGF reduction in frontal cortex and amygdala after acute physical threat

Rats were exposed to an acutely threatening as well as painful treatment, which we graded by using a painful injection of saline in the high stress group, rough but only mildly painful handling in the moderate group and no pain but repeated exposure to a new environment in the mild stress group. NGF content of the frontal cortex of rats was reduced in the high, medium and mild stress condition by 45, 37 and 38%, respectively. Thus, even the mild stress of handling and exposure to a novel environment was suffice to induce a robust and significant reduction of cerebral NGF.

One possible explanation for the conflicting reports of stress-related NGF changes is that the effect of stress on the NGF system depends on the nature of stressors used in the experiment. When an acute threat is perceived and

physical pain is aroused, comparable to immobilization or the foot shocks used in other experiments, NGF concentration in the central cholinergic system, e.g., in the hippocampus, seems to be reduced [36]. In our experiment, we observed a significant reduction of NGF content in amygdala and frontal cortex only. Interestingly both brain regions, the amygdala and the frontal cortex, are implicated in processing of fear responses as well as in the activation of the HPAA [13,20]. The observed change of NGF concentration in the amygdala after the experience of an acute threat is a new finding. The relevance of NGF changes in the amygdala for the processing of fear is not understood, yet it may be speculated that NGF plays an important role.

One possible explanation for this finding is that glucocorticoids, which are elevated during stress, reduce NGF synthesis [5,40]. However, contrary to this hypothesis, the change of glucocorticoid concentration during stress experiments does not always correspond to the reduction of measured NGF concentrations. While the level of glucocorticoids in the bloodstream showed adaptation after chronic stress of foot shocks, the observed decrease of NGF after acute and chronic stress was similar [36].

Another explanation for a reduced NGF concentration after stress could be that the NGF content is influenced by changes in neuronal activity during stress. In support of this theory, it has been demonstrated that the expression of hippocampal NGF is dependent on neuronal activation and release of cholinergic neurotransmitters [18,24].

In contrast to experiments delineated above by Ueyama et al. [40] and Scaccianoce et al. [36], we did not observe a reduction of NGF protein concentration in the hippocampus after our physical threat treatment. One explanation for these divergent results may be that our experimental stress situation, which lasted about 3 min, was too short to induce changes in hippocampal NGF content. Correspondingly, a 5-min restraint stress did not significantly change NGF levels in the hippocampus [36]. Furthermore, the hippocampal NGF protein level may be influenced by serum NGF levels to a greater extent than other brain regions, since uptake of serum NGF is highest in the hippocampus [32] and serum NGF is elevated during the stress reaction [3].

Since NGF might be important to counteract the neurotoxic effects of glucocorticoids, which are elevated during stress [30], the observed reduction of NGF after the experience of stress could be of pathophysiological relevance. Chronically elevated glucocorticoids lead to atrophy and neuronal death [34,35], by making the nerve cell vulnerable to oxidative stress [30]. NGF, however, can reduce negative effects of oxidative stress on the nerve cell [33]. NGF serves as a survival protein for cholinergic neurons, it can reverse cellular damage and reduce vulnerability to toxic influences [19]. Thus, a reduction of NGF content by stress could have detrimental consequences for the survival of cholinergic neurons.

4.2. NGF reduction in frontal cortex and hippocampus after forced motoric activity

In our second experiment we found that forced motoric activity of 2 h in a running wheel induced a significant reduction of NGF concentration in the frontal cortex and in the hippocampus. After a longer duration of motor activity a more pronounced decrease in hippocampal NGF was observed. By contrast no change in NGF levels in the amygdala or the limbic forebrain was detected. A similar result was reported by Scaccianoce et al. [36], who found a reduction of NGF in the hippocampus after 1 h of rotatory stress for 1 or 10 consecutive days.

In contrast to our finding, an increase of NGF expression in the hippocampus and cortex has been reported after 12 h of motoric activity in a running wheel [28]. However, one important difference to our experimental procedure was that rats moved voluntarily (in a running wheel without set rotation speed). Furthermore, rats in this experiment were adapted to the apparatus and experimental conditions for several days in order to reduce stress effects. Thus it can be hypothesized that NGF is regulated differently in motoric activity, when the situation is novel, unpredictable and motoric activity is forced.

Our results could also be relevant for the course of NGF in Alzheimer's disease, where NGF might follow a distinctive pattern [19,37,38]. In this disease, an initial deficit of NGF at the onset of the pathological process might be followed by its temporary elevation, during which some neuronal deficits may be partially ameliorated. Although the mechanisms of this time course of NGF regulation are widely unknown, it may be speculated that disease-related stressors may be responsible for an impaired supply with neurotrophins leading to functional consequences in neurotrophin-dependent neurons [15,18,19,37]. Accordingly a disturbed utilization of endogenous NGF has been demonstrated in the brain of cognitively impaired aged rats [14] which can be restored by administration of exogenous NGF [1].

In conclusion, we have observed a decrease in NGF concentration in the frontal cortex and the amygdala after a stress of physical threat and a decrease of NGF after forced motoric activity in the frontal cortex and the hippocampus. The finding of reduced intracerebral NGF levels after acute fear and stress may have pathophysiological implications in the pathogenesis and treatment of depression. This view would be in line with the increasing evidence that depression may be associated with a disruption of mechanisms that regulate cell survival and neural plasticity in the brain [8].

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