Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons

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The hippocampus is vulnerable to the damaging actions of insults such as transient ischemia and repetitive stimulation, as well as repeated exposure to exogenous glucocorticoids. This study investigated effects of a repeated psychological stressor, restraint, on the CA3 pyramidal neurons which are vulnerable to damage by repetitive stimulation. Repeated daily restraint stress for 21 days caused apical dendrites of CA3 pyramidal neurons to atrophy, while basal CA3 dendrites did not change. Rats undergoing this treatment were healthy and showed some adaptation of the glucocorticoid stress response over 21 days; however, stress reduced body weight gain by 14% and increased adrenal weight relative to body weight by 20%. Results are discussed in relation to the possible role of adrenal steroids and excitatory amino acids.

Whereas the effects of stress on neurochemical and neuroendocrine responses are well-characterized, the role of these responses in adaptation, as well as in damaging aspects of stress[14], is not well understood; nor is it clear how the brain itself changes under conditions of repeated stress. Previous studies have examined the effects of repeated elevations of circulating glucocorticoids on the morphology and survival of neurons in the hippocampus. These studies have shown that that such hormone manipulations have destructive effects on pyramidal neurons in the CA3 region. For example, treatment of young adult rats with daily injections of corticosterone over 3 weeks induces atrophy of the apical dendrites of CA3 pyramidal neurons[21], whereas 12 weeks of daily corticosterone injections results in loss of CA3 pyramidal neurons[10]. The most intriguing aspect of these findings is the possibility that natural conditions in which glucocorticoids are elevated produce similar damage. Since stress elevates endogenous glucocorticoids, it is possible that chronic stress would produce neuronal damage in the hippocampus similar to that produced by excess glucocorticoids. Some indirect evidence suggests this is the case; a correlation between chronic social stress and loss of hippocampal neurons has been made[18,19]. How-

ever, these studies were not performed under laboratory conditions where the sole variable is stress, rendering interpretations of such data difficult. To better characterize the effects of stress on the brain, we chose to assess the morphology of dendrites in the hippocampal formation after application of a single laboratory stressor, restraint.

Male Sprague–Dawley (Charles River) rats (250–280 g) were divided into two groups: unstressed controls, which remained in their cages except for weighing (n = 8) and a chronic restraint stressed group (n = 8). Six hours (10.00–16.00 h) of restraint stress was performed daily for three weeks with wire mesh restrainers. During restraint stress, the rats were put back in their home cages. Blood samples were collected from the tail vein just before the restraint stress (0 min as a basal plasma corticosterone level), 30 min, 1 h, 3 h and 6 h after the beginning of the stress on days 1, 4, 7, 14 and 21. Plasma corticosterone (B) was measured by radioimmunoassay[17] using a commercially available rabbit antiserum raised against corticosterone-3-oxime BSA (Endocrine Sciences, Tarzana, CA). The antiserum has very low cross-reactivity with other major steroids. Assay sensitivity was 10 pg and coefficients of variation within and between assays were 5% and 10%,

respectively. For the serum corticosterone data, a two-way (time × day) analysis of variance was used. Different rats were used for blood sampling and for the morphological measures described below.

One day after the last restraint stress, the rats were deeply anesthetized with Ketamine HCl and tran-scardically perfused with 100–150 ml 4% paraformaldehyde in 0.1 M phosphate buffer with 1.5% (v/v) picric acid. The adrenal and thymus glands were removed and weighed. Brains were postfixed for 24 h in the perfusate at 4°C and processed using a modified version of the single-section Golgi impregnation method[2]. Briefly, coronal sections 100 μm thick were cut with a Vibratome in a bath of 3.0% potassium dichromate in distilled water and stored in this solution for 24 h. Following this, the sections were rinsed in distilled water, mounted onto plain slides and a cover-slip was glued over the sections at the four corners. These slide assemblies were placed in 1.5% silver nitrate in distilled water for 24 h in the dark. The slide assemblies were then dismantled, the sections were rinsed in distilled water, dehydrated, cleared and cov-erslipped. The slides containing Golgi-impregnated sections were coded prior to quantitative analysis. The code was not broken until the analysis was completed.

In order to be selected for analysis, Golgi-impregnated CA3 pyramidal neurons had to possess the fol-lowing characteristics: (1) location in the dorsal portion of the CA3c hippocampal field; (2) dark and consistent impregnation throughout the extent of all of the dendrites; (3) relative isolation from neighboring impregnated cells which could interfere with analysis. For each brain, 6 CA3c pyramidal cells were analyzed. Of these selected neurons, 3 were of the short shaft type and 3 were of the long shaft type[1,4]. Camera lucida tracings (500 ×) were obtained from selected neurons. From these tracings, the total number of dendritic branch points of each apical and basal dendritic tree was determined. In addition, the total length of the dendrites for each dendritic tree was determined using a Zeiss Interactive Digitizing Analysis System. Means of these variables were determined for each animal and these data were subjected to two-tailed Student's t-tests.

At the end of the stress experiments, all rats, even the chronically-stressed rats, appeared to be very healthy. However, there were moderate reductions in weight gain, as well as an increase in relative adrenal weights in the chronically stressed rats compared to the cage controls. The body weight was reduced in stress rats compared to controls (363 ± 16 ($n = 8$) vs. 420 ± 10 ($n = 8$), $P < 0.001$). Relative adrenal weights (adrenal wt. in mg/b. wt. in g × 100) of stressed animals were increased compared to controls (20.4 ± 2.3 ($n = 8$) vs.
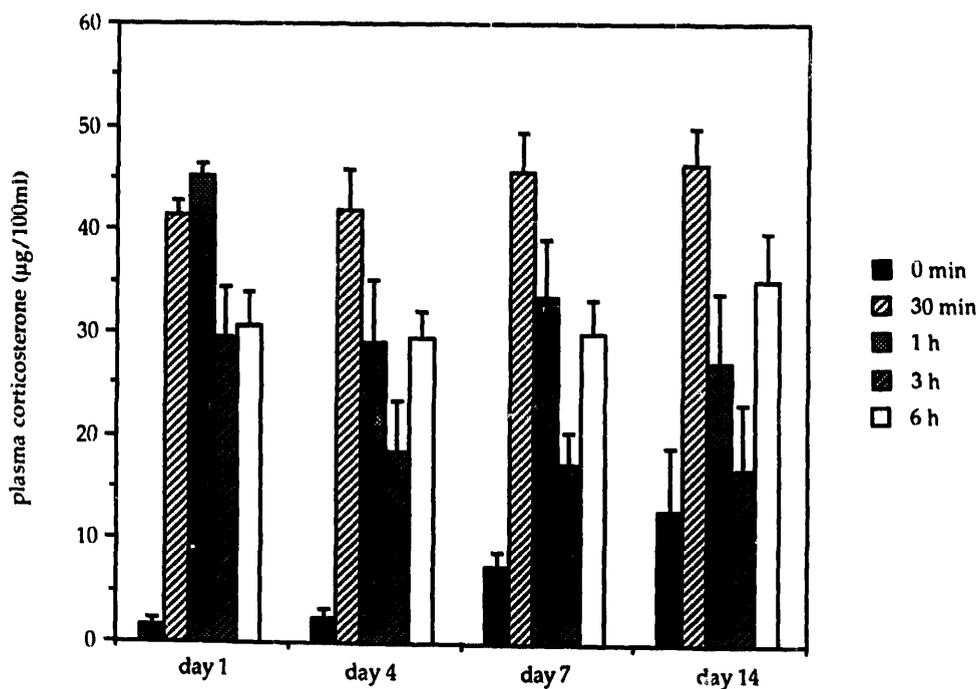
Fig. 1. Plasma corticosterone response to 6 h/day restraint stress over 14 days of daily stress repetition. Plasma corticosterone levels determined by radioimmunoassay are presented in μg/100 ml plasma. The data was analyzed by two-way ANOVA over time of stress and over days of stress repetition. There was a significant time of stress variation ($F = 34.301$, $P < 0.001$) and a significant time by days of stress interaction ($F = 2.246$, $P < 0.02$). The time by day interactions indicated that there was a significant attenuation of the corticosterone response over stress repetition, although corticosterone secretion in response to stress persisted throughout the 21 days.

16.9 ± 2.3 (n = 8), P = 0.01). These changes were replicated in a second experiment (data not shown).

Plasma corticosterone (B) responses during 6 h restraint are shown in Fig. 1 for days 1, 4, 7 and 14 of the repeated stress. The B level data was analyzed by two-way ANOVA over time of stress and over days of stress repetition; there was significant time of stress variation (F = 34.301, P < 0.001; and a significant time by days of stress interaction (F = 2.246, P < 0.02). The time by day interaction indicates that there was a significant attenuation of the B response over stress repetition. As shown in Fig. 1, the basal B level (0 min) and the peak B response at 30 min after the beginning of stress did not change in chronically-stressed rats,

although there was a trend for increased basal B levels after 7 and 14 days of daily stress. In contrast, animals showed sustained high B levels during the stress on day 1, while repeated stress attenuated the B response at 1 h and 3 h. This attenuation was evident by day 4 of chronic stress and continued to day 21. Since the 6 h stress ended at 16.00 h, when plasma B level begin to show their diurnal rise, plasma B levels at the end of the stress were higher than the 3 h stress level. This diurnal elevation showed no difference between day 1 and other stress days.

Light microscopic examination of Golgi-impregnated tissue revealed reliable and consistent staining throughout the hippocampus for all brains. Six hours of
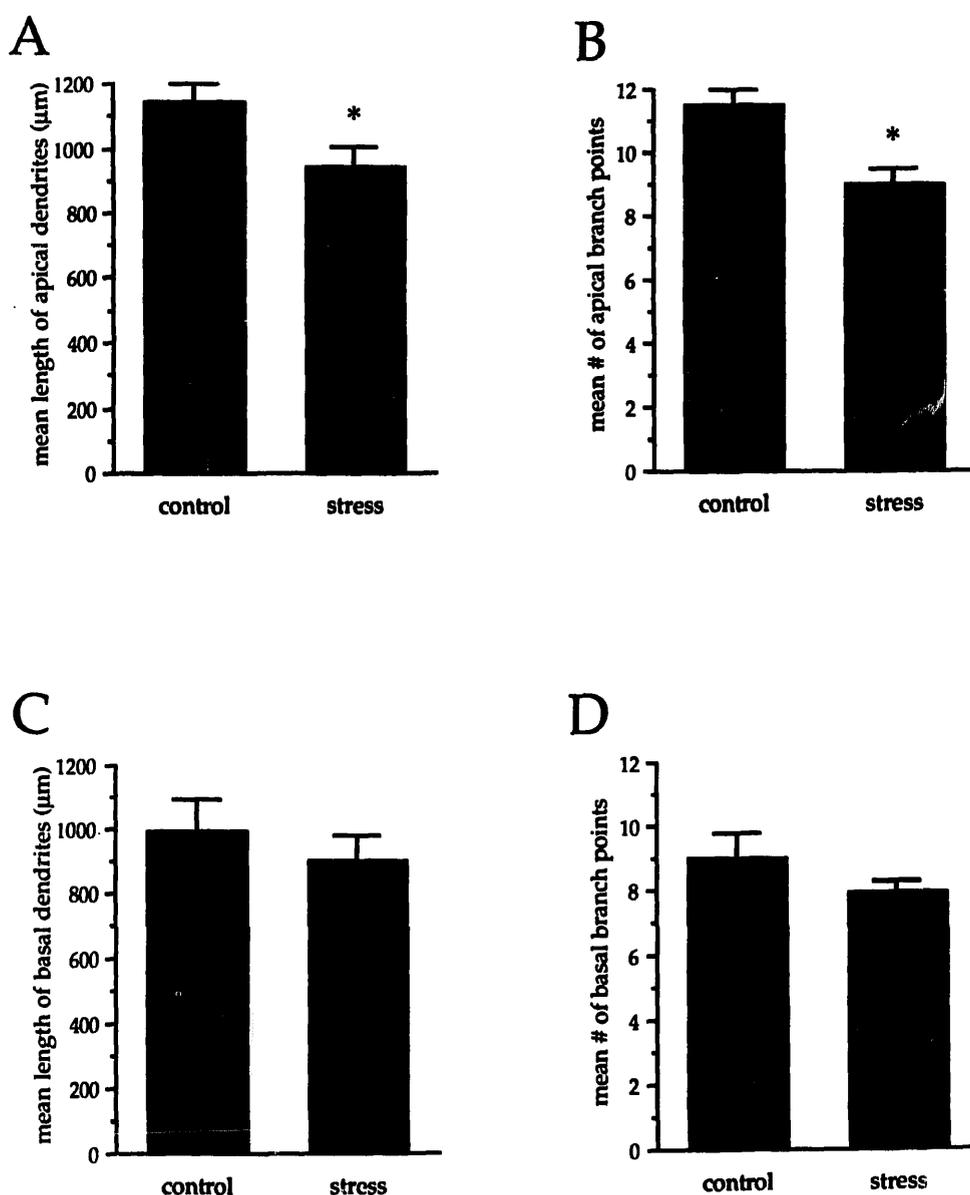
Fig. 2. Stress-induced changes in dendritic morphology of CA3c pyramidal neurons are presented for apical (A,B) and basal (C,D) dendrites. Results are presented as mean dendritic length (A,C; left) and mean number of dendritic branch points (B,D). Asterisks represent significant differences from sham (P < 0.05; two-tailed Student's t-tests).

restraint stress for 3 weeks resulted in significant decreases in both the total dendritic length (Fig. 2A; $P < 0.05$) and in the number of branch points (Fig. 2B; $P < 0.05$) in the apical dendritic trees of Golgi-impregnated CA3 pyramidal cells in 100-μm-thick sections. In contrast, CA3 pyramidal cell basal dendrites showed no significant differences in the total length of dendrites (Fig. 2C; $P > 0.1$) and the number of branch points (Fig. 2D; $P > 0.1$) as a result of the repeated restraint stress.

These results demonstrate that repeated restraint stress, which results in a moderate reduction in body weight gain and moderate changes in adrenal and thymus weights, resulted in a small but significant decrease in dendritic length and branch points of CA3 pyramidal neurons in the hippocampus. The present study is the first report directly linking repeated stress to changes in hippocampal dendritic morphology. Such decreases in dendritic parameters reported here may be indicative of neurons in the early stages of degeneration, possibly leading to neuronal death such as has been reported to result from chronic social stress[18,19] as well as from repeated corticosterone treatment[10].

The effects we report appear to be selective, in that basal CA3 dendrites did not show the significant changes found on the apical CA3 dendrites. Corticosterone effects on dendritic length and branching, described previously[21], were specific for apical dendrites of CA3 pyramidal neurons and did not occur on CA1 neurons or dentate gyrus neurons. However, the possibility of morphological changes occurring in other neural regions as a result of stress exists. Restraint stress 6 h/day for 2 weeks also results in retraction of cortical locus coeruleus axons, inferred from results using electrophysiological techniques[9].

A major reason for focusing on the hippocampus and on the CA3 region has been our previous finding that chronic glucocorticoid treatment caused atrophy of apical, but not basal, dendrites in the CA3 cell field and not in CA1[21]. The underlying mechanism of this selective damage may involve excitatory neurotransmitters such as glutamic acid. CA3 neurons are extremely sensitive to the neurotoxic effect of kainic acid as well as to repetitive stimulation of the perforant path[7,15]. Kainic acid, administered intraventricularly, selectively destroyed the CA3 cells, and this neurotoxic effect was prevented by destruction of dentate gyrus neurons with colchicine or transection of the mossy fibers[7]. The granule cell seizure activity evoked by electrical stimulation of the perforant pathway induced CA3 dendritic swelling and neuronal loss[15].

This evidence suggests that the CA3 neuronal vulnerability strongly depends on neuroanatomical char-

acteristics and in particular the mossy fiber projection from the granule neurons in the dentate gyrus[5]. CA3 neurons show an extreme excitatory responsiveness to kainic acid administered by microiontophoresis, and this responsiveness is drastically reduced with the destruction of the mossy fibers[6]. CA3 neurons may also be more vulnerable to damage because they lack both calbindin D28k and parvalbumin, calcium-binding proteins that are present in dentate granule neurons as well as in CA1 and CA2 pyramidal neurons[16].

Thus, activation of excitatory amino acid pathways could be one possible explanation of the enhanced susceptibility of the apical dendrites of CA3 neurons to stress. Restraint stress is reported to increase glutamate high-affinity uptake and release in hippocampus, frontal cortex and septum[3] and to increase hippocampal lactic acid release by an NMDA receptor mechanism[13]. These observations imply that excitatory amino acid release is activated in response to stress. Thus, it is quite possible that repeated restraint stress could damage CA3 neurons via excitation of the granule cells in the dentate gyrus. In support of this hypothesis, we have found that phenytoin (Dilantin), an anti-epileptic drug that reduces excitatory amino acid release, prevents the stress- and corticosterone-induced reductions in CA3c apical dendritic length and branch point number[20].

In conclusion, we have shown that repeated restraint stress results in atrophy of dendrites of hippocampal neurons receiving mossy fiber input from dentate gyrus. The fact that repeated stress produced a similar result to repeated corticosterone treatment is consistent with a role of glucocorticoids in the effects of stress. However, do glucocorticoids play a role? Although excess glucocorticoid treatment by itself does not increase dentate gyrus neuronal excitability[8], it does exacerbate the damaging effects of kainic acid[11]. That glucocorticoids potentiate excitatory amino acid damage is further supported by in vivo and cell culture studies using excitatory amino acids, hypoxia and kainic acid as damaging agents[12]. However, future studies need to investigate the role of glucocorticoids in stress-induced damage by interfering with glucocorticoid secretion and/or action during stress.

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