Chronic Stress Alters Dendritic Morphology in Rat Medial Prefrontal Cortex

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ABSTRACT: Chronic stress produces deficits in cognition accompanied by alterations in neural chemistry and morphology. Medial prefrontal cortex is a target for glucocorticoids involved in the stress response. We have previously demonstrated that 3 weeks of daily corticosterone injections result in dendritic reorganization in pyramidal neurons in layer II–III of medial prefrontal cortex. To determine if similar morphological changes occur in response to chronic stress, we assessed the effects of daily restraint stress on dendritic morphology in medial prefrontal cortex. Male rats were exposed to either 3 h of restraint stress daily for 3 weeks or left unhandled except for weighing during this period. On the last day of restraint, animals were overdosed and brains were stained using a Golgi-Cox procedure. Pyramidal neurons in lamina II–III of medial prefrontal cortex were drawn in three dimensions, and the morphology of apical and basilar arbors was quantified. Sholl analyses demonstrated a significant alteration of apical dendrites in stressed animals: overall, the number and length of apical dendritic branches was reduced by 18 and 32%, respectively. The reduction in apical dendritic arbor was restricted to distal and higher-order branches, and may reflect atrophy of terminal branches: terminal branch number and length were reduced by 19 and 35%. On the other hand, basilar dendrites were not affected. This pattern of dendritic reorganization is similar to that seen after daily corticosterone injections. This reorganization likely reflects functional changes in prefrontal cortex and may contribute to stress-induced changes in cognition. © 2004 Wiley Periodicals, Inc. J Neurobiol 60: 236–248, 2004

Keywords: glucocorticoids; restraint stress; pyramidal neurons; dendrites; prefrontal cortex

INTRODUCTION

A wealth of data documents the adverse effects of chronic stress on physiology and behavior. Chronic stress is associated with increased risk for illness, the development of a variety of psychological disorders, and changes in cognition. For instance, chronic exposure to a stressor results in the development of gastric ulcers (Henke, 1990). In addition, individuals experiencing increased numbers of stressful life events are more likely to develop respiratory infections (Stone et al., 1987). Stress has also been hypothesized to play a causal role in several psychological disorders. For instance, depressed individuals are more likely than nondepressed individuals to have experienced at least one stressful life event prior to diagnosis (Brown and Harris, 1989), and stressful life events appear to increase the probability of a psychotic episode in schizophrenics (Ventura et al., 1989). Animal studies have also demonstrated detrimental effects of stress on many behaviors. For instance, several studies have demonstrated stress-induced deficits on a variety of cognitive tasks, including shuttle escape (Seligman

and Maier, 1967), water maze (Altenor et al., 1977), appetitively motivated operant conditioning (Rosellini, 1978), and radial maze tasks (Luine et al., 1994).

Many of the effects of chronic stress are thought to be mediated by stress-induced increases in circulating levels of glucocorticoids, the major stress hormones (e.g., Uno et al., 1989). In fact, chronic elevations of circulating glucocorticoids have been shown to produce a variety of cognitive deficits. For instance, rats given daily injections of the glucocorticoid corticosterone for 8 weeks demonstrated decreased spontaneous alternation on a T maze (Bardgett et al., 1994). Likewise, 21-day corticosterone implants that produced a two- to fourfold increase in serum corticosterone levels in rats impaired acquisition of a passive-avoidance task (Bisagno et al., 2000). In addition, chronic corticosterone treatment has been shown to impair both acquisition of a radial arm maze task (Dachir et al., 1993) and accuracy of recall of spatial information in the Morris water maze (Sousa et al., 2000) in rats. Finally, chronic administration of stress levels of cortisol has been shown to impair response inhibition in squirrel monkeys (Lyons et al., 2000).

The behavioral deficits induced by chronic corticosterone administration have typically been attributed to corticosterone-induced changes in the hippocampus, which is a primary neural target of glucocorticoids (Gerlach and McEwen, 1972) and is involved in many of the behaviors altered by chronic corticosterone administration. Both chronic corticosterone administration and chronic stress result in extensive atrophy of apical dendrites of pyramidal neurons in hippocampal area CA3 (Woolley et al., 1990; Watanabe et al., 1992; Magariños et al., 1996), and administration of cyanoketone, which blocks stress-induced increases in corticosterone, prevents the stress-induced atrophy of CA3 apical dendrites (Magariños and McEwen, 1995).

However, prefrontal cortex is involved in many of the tasks that are influenced by chronic elevations of circulating glucocorticoids. For instance, lesions of prefrontal cortex impair spontaneous alternation, radial maze performance, and passive avoidance (see Kolb, 1984) in rats and impair inhibition of the line-of-sight response in primates (e.g., Dias et al., 1996). Importantly, prefrontal cortex is also a target for glucocorticoids involved in the stress response: [^{3}H]dexamethasone binds to receptors in frontal and prefrontal cortex at about 75% of the concentration found in hippocampus. In addition, [^{3}H]dexamethasone binding in frontal cortex is altered by both corticosterone treatment and adrenalectomy, indicating the presence of endogenously regulated corticosterone receptors (Meaney and Aitken, 1985). However, despite the fact that prefrontal cortex is a major target for glucocorticoids and mediates many of the behaviors influenced by stress, the effect of chronic stress on prefrontal cortical plasticity has been essentially ignored.

Chronic corticosterone administration produces a variety of neurochemical changes in prefrontal cortex, including decreased 5-HT$_{1A}$ (Crayton et al., 1996) and 5-HT$_2$ receptor binding (Takao et al., 1997), as well as decreased serotonin levels (Luine et al., 1993). In addition, chronic elevations of corticosterone result in decreased expression of the neural cell adhesion molecule (NCAM; Sandi and Loscertales, 1999), a cell-surface macromolecule involved in regulating aspects of synapse stabilization, which suggests the possibility of structural changes as a result of chronic stress levels of corticosterone. Therefore, to determine if glucocorticoid-induced morphological changes also occur in medial prefrontal cortex, we previously assessed the effects of chronic corticosterone administration on dendritic morphology of layer II–III pyramidal neurons in this corticolimbic structure (Wellman, 2001). Chronic corticosterone administration dramatically reorganized apical arbors of layer II–III neurons in medial prefrontal cortex, with an increase of 21% in dendritic material proximal to the soma, along with a decrease of up to 58% in dendritic material distal to the soma.

In the present study, we have begun to examine the morphological sensitivity of prefrontal cortex to chronic stress by assessing whether chronic restraint stress alters dendritic morphology in medial prefrontal cortex. Chronic restraint is a standard stress manipulation that has been employed across many studies (e.g., Henke, 1990; Imperato et al., 1991; Gonzalez and Pazos, 1992; Luine et al., 1994). In addition, restraint stress (6 hours daily for 3 weeks) has been shown to produce morphological changes in hippocampal neurons (Watanabe et al., 1992). Given that neurons in medial prefrontal cortex undergo dendritic remodeling as a result of chronic elevations of the stress hormone corticosterone, we hypothesized that they would also undergo dendritic changes in response to chronic stress.

METHODS

Animals

Adult male Sprague-Dawley rats (175–200 g, approximately 50 days old at the start of the experiment; $n = 15$) were either exposed to chronic restraint stress or served as controls. Restraint-stressed rats ($n = 7$) were placed in a

plastic rat restrainer in their home cages for 3 h daily for 21 days. In addition, stressed rats were weighed every other day. The remainder of the rats ($n = 8$) served as unstressed controls, and were not handled except for weighing every other day. All rats were group housed in a vivarium with a 12:12 h light/dark cycle (lights on at 7 a.m.), ambient temperature of 23–25°C, and free access to food and water. All experimental procedures occurred between 11:00 a.m. and 4:00 p.m., and were approved by the Bloomington Institutional Animal Care and Use Committee.

Corticosterone Assay

Elevations in corticosterone titers due to restraint were assessed by collecting blood from the tail vein immediately after being placed in the restrainer, 1 h later (a duration sufficient to obtain peak plasma corticosterone levels), and immediately before removal from the restrainer on days 1, 7, 14, and 21. Blood was collected in heparinized microcapillary tubes, centrifuged at $2000 \times g$ for 15 min to obtain plasma, and corticosterone titers were assessed using a competitive enzyme immunoassay kit (R&D Systems, Minneapolis, MN). This assay has low cross-reactivity with other major steroid hormones, sensitivity typically less than 27.0 pg/mL, and coefficients of variation within and across assays of approximately 7.7 and 9.7%, respectively. Stress-induced changes in corticosterone concentrations were evaluated across time using a two-way repeated-measures ANOVA (duration of restraint × day) followed by appropriate planned comparisons.

Histology and Dendritic Analysis

Approximately 24 h after the final session of restraint stress, animals were overdosed with urethane and then perfused with 0.9% saline. Brains were removed and processed using Glaser and Van der Loos' modified Golgi stain (Glaser and Van der Loos, 1981). The tissue was immersed in Golgi-Cox solution (a 1:1 solution of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with 5% potassium chromate) for 14 days. Brains were then dehydrated in 1:1 absolute ethanol:acetone (3 h), followed by absolute ethanol and then 1:1 ethanol:ether (30 min each). Brains were then infiltrated with a graded series of cellodins before being embedded in 8% celloidin [8% (w/v) parlodion in 1:1 absolute ethanol:ether]. Coronal sections were cut at 150 μm on a sliding microtome (Leica Histoslide 2000). Free-floating sections were then alkalinized in 18.7% ammonia, developed in Dektol (Kodak), fixed in Kodak Rapid Fix (prepared as per package instructions with Solution B omitted), dehydrated through a graded series of ethanols, cleared in xylene, mounted, and coverslipped (Glaser and Van der Loos, 1981).

Pyramidal neurons in layer II–III of medial prefrontal cortex (Zilles and Wree, 1995) were drawn. The Cg1-3 area of medial prefrontal cortex is readily identified by its position on the medial wall of rostral cortex, and its location dorsal to infralimbic cortex, which is markedly thinner than the Cg1-3 area and has fewer, less well-defined layers (Zilles and Wree, 1995). Within Cg1-3, layer II–III is readily identifiable in Golgi-stained material based on its characteristic cytoarchitecture. Its position is immediately ventral to the relatively cell-poor layer I (which also contains the distal dendritic tufts of layer II–III pyramids) and immediately dorsal to layer IV; in medial prefrontal cortex, this boundary is pronounced because of the greater cell-packing density and smaller somata of pyramidal cells in layer II–III relative to layer IV (Cajal, 1995; Zilles and Wree, 1995). Pyramidal neurons were defined by the presence of a basilar dendritic tree, a distinct, single apical dendrite, and dendritic spines. Neurons with somata in the middle third of sections were chosen to minimize the number of truncated branches. For each animal, 10 neurons were drawn; this number yields a within-animal error of less than 15% (mean within-animal SEM for total branch number and length = 12.99 ± 0.61%), and thus was considered to provide a representative sample of layer II–III pyramidal neurons in medial prefrontal cortex. All neurons were drawn at 600X and morphology of apical and basilar arbors was quantified in three dimensions using a computer-based neuron tracing system (Neurolucida; MicroBrightField, Williston, VT) with the experimenter blind to condition.

Several aspects of dendritic morphology were examined. To assess overall changes in dendritic morphology, total length and number of basilar and apical dendrites were compared across groups using *t* tests. To assess differences in the amount and location of dendritic material, a three-dimensional version of a Sholl analysis (Sholl, 1956) was performed. A Sholl analysis estimates the amount and distribution of dendritic material by counting the number of intersections of dendrites with an overlay of concentric rings centered on the soma. In the present study, the number of intersections of dendrites with concentric spheres at 10 μm intervals was assessed; these numbers were then summed over 20 μm intervals. These data were compared using two-way repeated-measures ANOVAs (group × distance from soma) followed by appropriate planned comparisons. In addition, the number and length of apical and basilar branches of varying branch orders were compared in unstressed and stressed animals using two-way repeated-measures ANOVAs (group × branch order) followed by appropriate planned comparisons. For all analyses, planned comparisons consisted of *F* tests done within the context of the overall ANOVA (Hays, 1994), comparing unstressed versus stressed groups at each interval or branch order. Finally, the number and length of terminal branches were compared between groups using *t* tests.

RESULTS

Corticosterone Assay

Chronic restraint stress resulted in both a significant attenuation of weight gain [$F(1, 9) = 10.24$; $p < 0.01$; Fig. 1(A)] and increases in corticosterone titers [Fig.

A
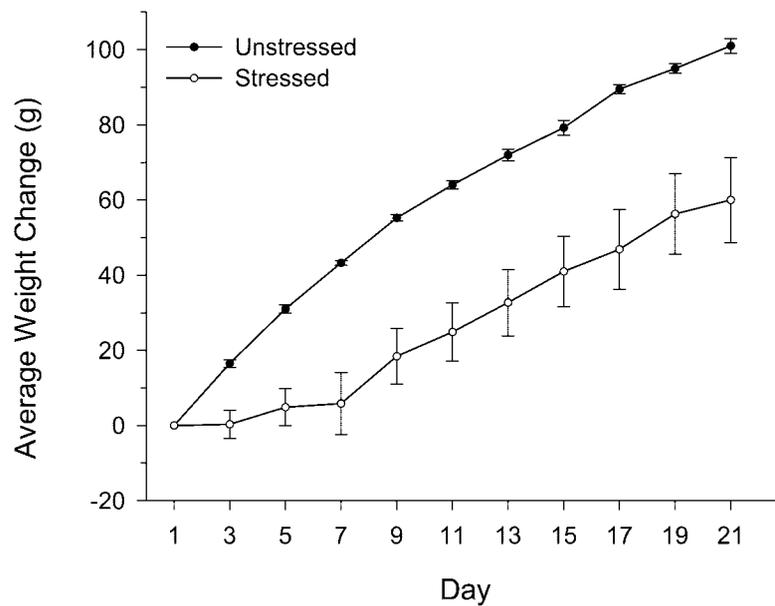
B
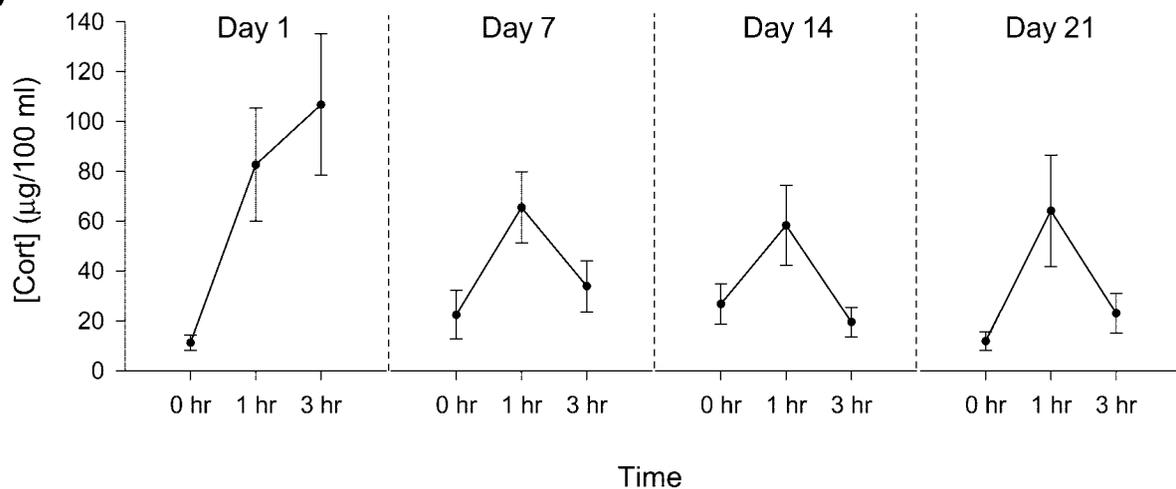
Figure 1 (A) Mean weight change in rats that either were not handled except for weighing (Unstressed; $n = 8$) or received 3 h of daily restraint stress for 3 weeks (Stressed; $n = 7$). Reductions in weight gain in restrained rats were pronounced in the first week of stress. Rates of weight gain were similar thereafter. Vertical bars represent SEM values. (B) Mean plasma corticosterone concentration after 0, 1, or 3 h of restraint on the 1st , 7th, 14th, and 21st day of restraint stress ($n = 7$ rats). Vertical bars represent SEM values.

1(B)]. Two-way repeated-measures ANOVA revealed a significant effect on plasma corticosterone titers of both duration of restraint [$F(2, 6) = 12.19$; $p < 0.01$] and day [$F(3, 6) = 4.23$; $p < 0.02$], as well as a significant interaction of duration and day [$F(6, 6) = 6.62$; $p < 0.01$]. Planned comparisons revealed that on the initial day of restraint as well as on days 7, 14,

and 21, plasma corticosterone concentration increased significantly after 1 h of restraint stress [all Fs$(1, 6)$ > 2.39; $p < 0.05$]. While plasma corticosterone concentration remained significantly elevated after 3 h on day 1 [$F(1, 6) = 3.64$; $p < 0.01$], on days 7, 14, and 21, corticosterone titers had dropped by the 3rd h of restraint, and were not significantly different from
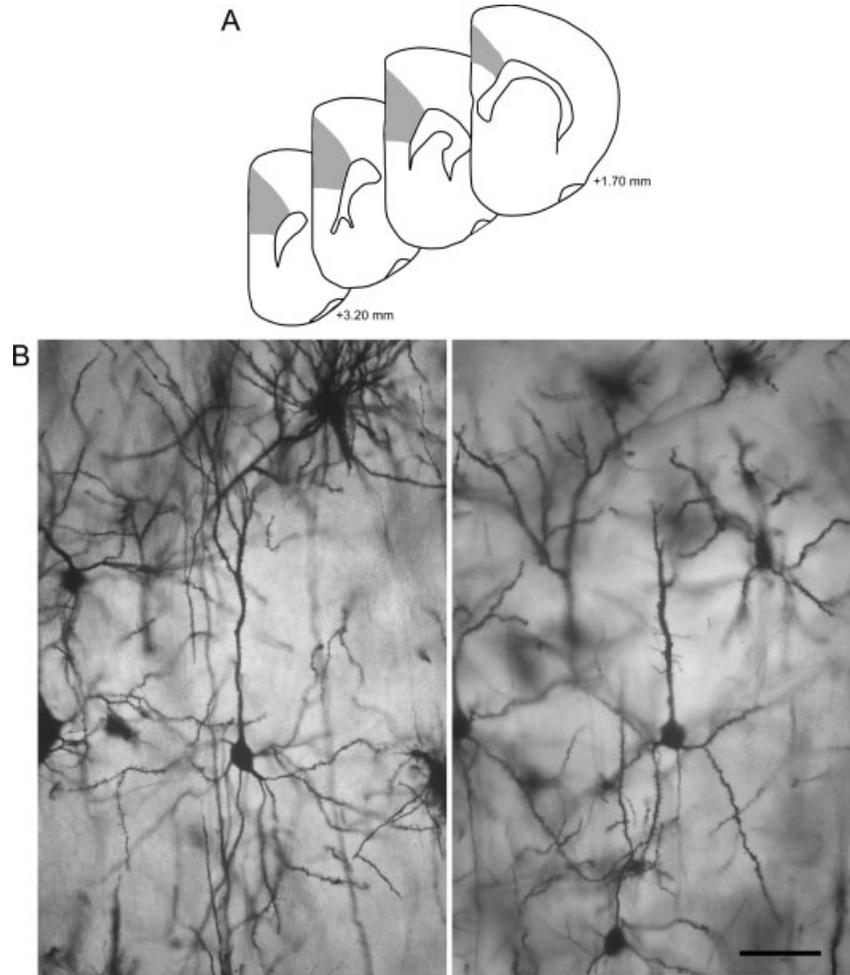
Figure 2 (A) Schematic diagram of coronal sections through rat prefrontal cortex. The portions of area Cg1-3 from which neurons were sampled is shown (shaded areas). Coordinates indicate position relative to bregma (Paxinos and Watson, 1998). (B) Digital light micrographs of Golgi-stained neuron in layer II–III of medial prefrontal cortex in an unstressed (left) and stressed (right) rat. Scale bar = 50 μm.

baseline [all $Fs(1, 6) < 1.84$, ns]. Thus, over the course of the study, while stress-induced elevations of corticosterone were present, rats appeared to habituate somewhat to the stressor.

Dendritic Analyses

In all treatment groups, complete impregnation of numerous cortical pyramidal neurons was apparent (Fig. 2), and both Cg1-3 and layer II–III were readily identifiable. Because relatively thick sections were taken through prefrontal cortex, the apical and basilar arbors of essentially all neurons selected were completely contained within a single section.

To rule out potential artifactual differences in dendritic morphology due to differential sampling in layer II and III, the distance from the soma to the pial surface of cortex was measured in each neuron. Average distance to the cortical surface was then compared across groups using a t test. Average distance to the cortical surface did not vary across groups [for unstressed rats, $M = 274.61 \pm 10.57$ μm; for stressed rats, $M = 250.58 \pm 12.79$ μm; $t(13) = 1.46$, ns]. Thus, neurons were sampled from equivalent laminar depths across groups.

Apical Dendrites. To assess overall changes in dendritic morphology, total length and number of apical dendrites were compared across groups. Chronic stress significantly decreased both mean apical branch number [$t(13) = -2.56$; $p \leq 0.02$] and mean apical
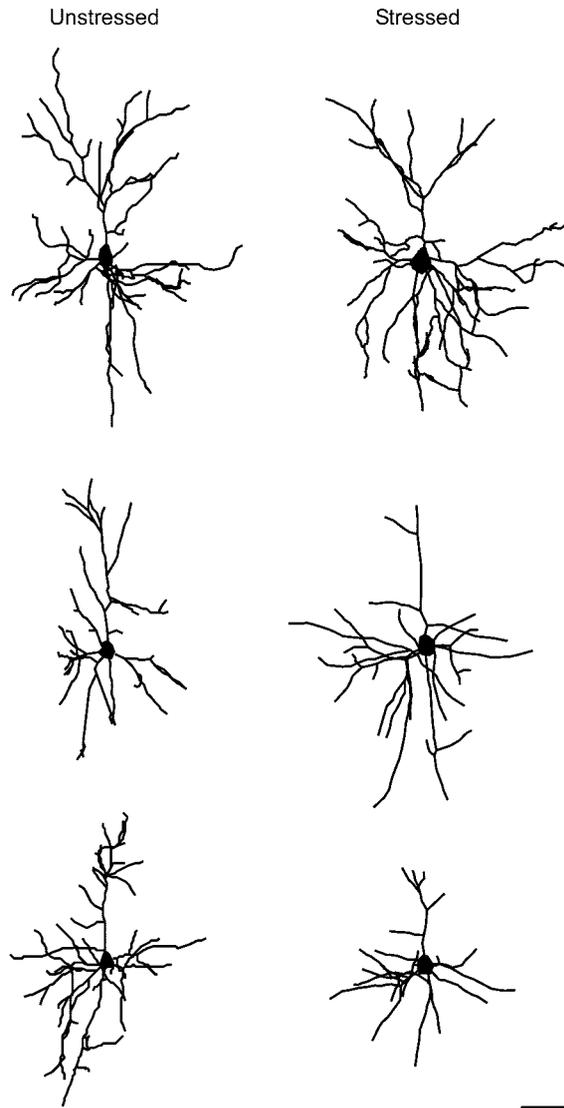
Figure 3 Computer-assisted reconstructions of Golgi-stained neurons in layer II–III of medial prefrontal cortex in unstressed (left) and stressed (right) rats. Scale bar = 50 μm. These neurons were selected because they are representative of apical dendritic lengths above (top), near (middle), and below (bottom) their respective group means.

branch length [$t(13) = -3.50$; $p \leq 0.01$] by 18 and 32%, respectively (Figs. 3 and 4).

To more closely examine changes in the distribution of dendritic material, Sholl analyses were performed. Chronic stress significantly influenced both overall apical dendritic arbor [for main effect of group, $F(1, 13) = 11.03$; $p \leq 0.01$] and the distribution of apical dendritic material [for interaction of group and distance from soma, $F(1, 10) = 3.35$; $p \leq 0.01$]. Planned comparisons indicated that chronic stress significantly decreased apical dendritic material distal to the soma by 29 to 83% [for 90–100, 110–

120, 130–140, 150–160, and 170–180 μm from the soma, all Fs(1, 13) ≥ 5.33; $p \leq 0.04$; for 190–200 μm from the soma, $F(1, 13) = 4.33$; $p \leq 0.06$; all other Fs(1, 13) ≤ 2.28, ns; Fig. 5].

To assess whether potential stress-induced differences varied across branch orders, the number and length of basilar and apical dendritic branches were compared across groups. Stress significantly altered apical branch number [$F(1, 13) = 12.34$; $p \leq 0.01$], and this effect varied across branch order [for interaction of group and branch order, $F(1, 8) = 2.96$; $p \leq 0.01$]. Planned comparisons indicated that the stress effect was restricted to higher-order branches, decreasing the number of seventh- and eighth-order branches by 42 and 50%, respectively [for branch orders 7 and 8, $F(1, 13) = 6.08$ and 5.28, respectively, $p \leq 0.04$; all other Fs(1, 13) ≤ 4.00, ns; Fig. 6]. Similarly, stress significantly altered apical branch length [$F(1, 13) = 12.34$; $p \leq 0.01$], and this effect again varied across branch orders [$F(8, 13) = 2.96$; $p \leq 0.01$; Fig. 7]. Planned comparisons indicated that, as for branch number, the effect of stress on apical branch length was restricted to higher-order branches, significantly decreasing the length of fifth-, six-, and seventh-order branches by 41 to 52% [for branch orders 5 through 7, $F(1, 13) \geq 7.10$; $p \leq 0.02$; all other Fs(1, 13) ≤ 3.88, ns].

Finally, because terminal branches may be more plastic than other parts of the arbor (Coleman and Flood, 1987; Rosenzweig and Bennett, 1996), and thus may be more sensitive to the effects of stress, the length and number of terminal dendritic branches were also compared across groups. Apical terminal branch number and length were significantly reduced by stress, by 19 and 35%, respectively [for apical terminal number, $t(13) = -2.89$; $p \leq 0.01$; for apical terminal length, $t(13) = -3.78$; $p \leq 0.01$; Fig. 8].

Basilar Dendrites. In contrast, chronic restraint stress failed to significantly alter any measure of basilar dendritic arbor. Mean basilar branch number and length were not significantly altered by chronic restraint stress [for branch number, $t(13) = 0.20$, ns; for branch length, $t(13) = -0.79$, ns; Figs. 3 and 4]. Furthermore, the distribution of basilar dendritic material did not vary overall across groups [main effect of group, $F(1, 13) = 0.86$, ns]. Although a significant group $\times$ distance from soma interaction was present [$F(1, 7) = 2.30$; $p \leq 0.03$], planned comparisons revealed no significant effect of stress at any distance from the soma [all Fs(1, 13) ≤ 2.86, ns]; thus, the significant interaction may reflect a nonsignificant trend toward decreased dendritic material distal to the soma (Fig. 5). Two-way repeated-measure ANOVAs
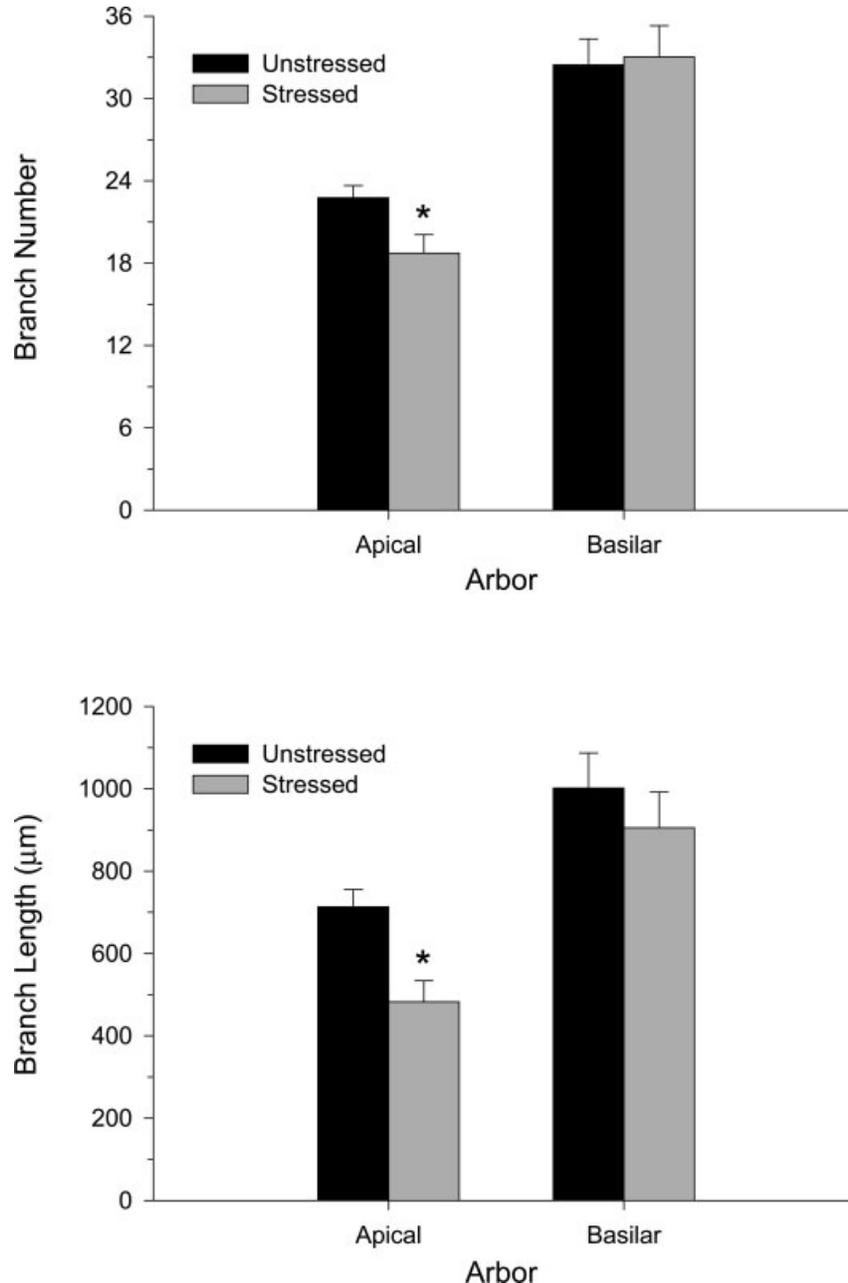
Figure 4 Mean apical and basilar branch number (top) and length (bottom) for unstressed ($n = 8$) and stressed rats ($n = 7$). Overall apical branch number and length were significantly reduced in stressed animals, whereas basilar branch number and length did not vary across groups. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unstressed rats.

indicated that stress did not significantly alter basilar branch number or length at any order [for main effect of group, Fs(1, 13) $\leq$ 0.62, ns; for interaction of group and branch order, Fs(5, 13) $\leq$ 0.54, ns; Figs. 6 and 7]. Likewise, basilar terminal branches were not significantly altered by stress [for basilar terminal number, t(13) = 0.09, ns; for basilar terminal length, t(13) = −1.07, ns; Fig. 8].

DISCUSSION

The present study demonstrates pronounced changes in the dendritic morphology of layer II–III pyramidal neurons in medial prefrontal cortex as a result of chronic stress. While restraint stress failed to significantly alter basilar dendritic arbors, it resulted in
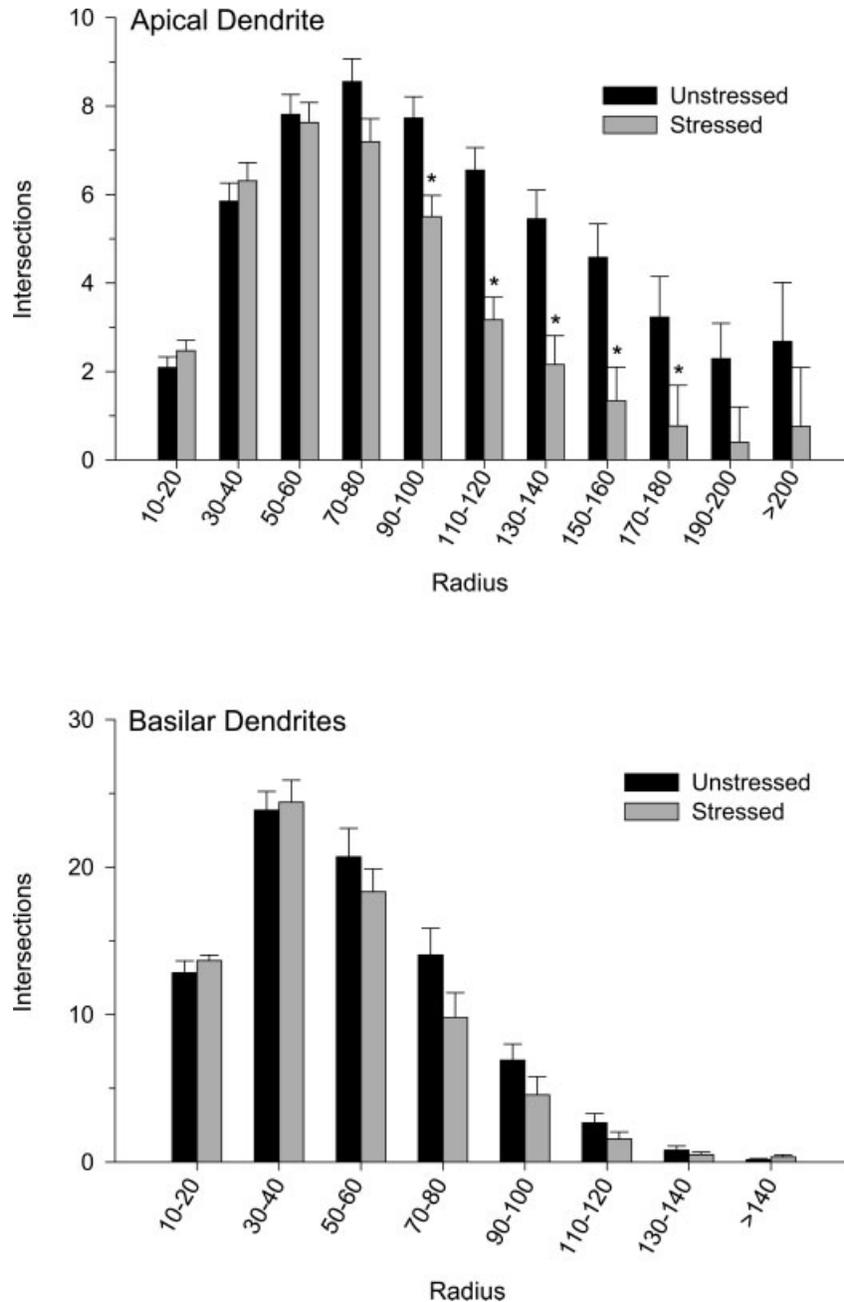
Figure 5 Mean intersections of apical (top) and basilar dendrites (bottom) with 10 μm concentric spheres in unstressed ($n = 8$) and stressed rats ($n = 7$). Data have been summed into 20 μm bins. Apical dendritic material distal to the soma was reduced in stressed rats, whereas the distribution of basilar dendritic material did not differ across groups. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unstressed rats.

significant atrophy of the apical arbors. Three weeks of daily restraint stress resulted in a pronounced decrease in both apical branch number and apical branch length. Interestingly, this decrease was restricted to the more distal portion of the apical arbor, where the amount of dendritic material was reduced by up to 83%, and higher-order branches, which showed decreases in both number and length of up to 50%. This restriction may reflect the differential atrophy of terminal branches: overall, apical branch number and length were decreased by 18 and 32%, respectively, which was paralleled by 19 and 35% decreases in terminal branch number and length.

Previously, we found that 3 weeks of daily corti-
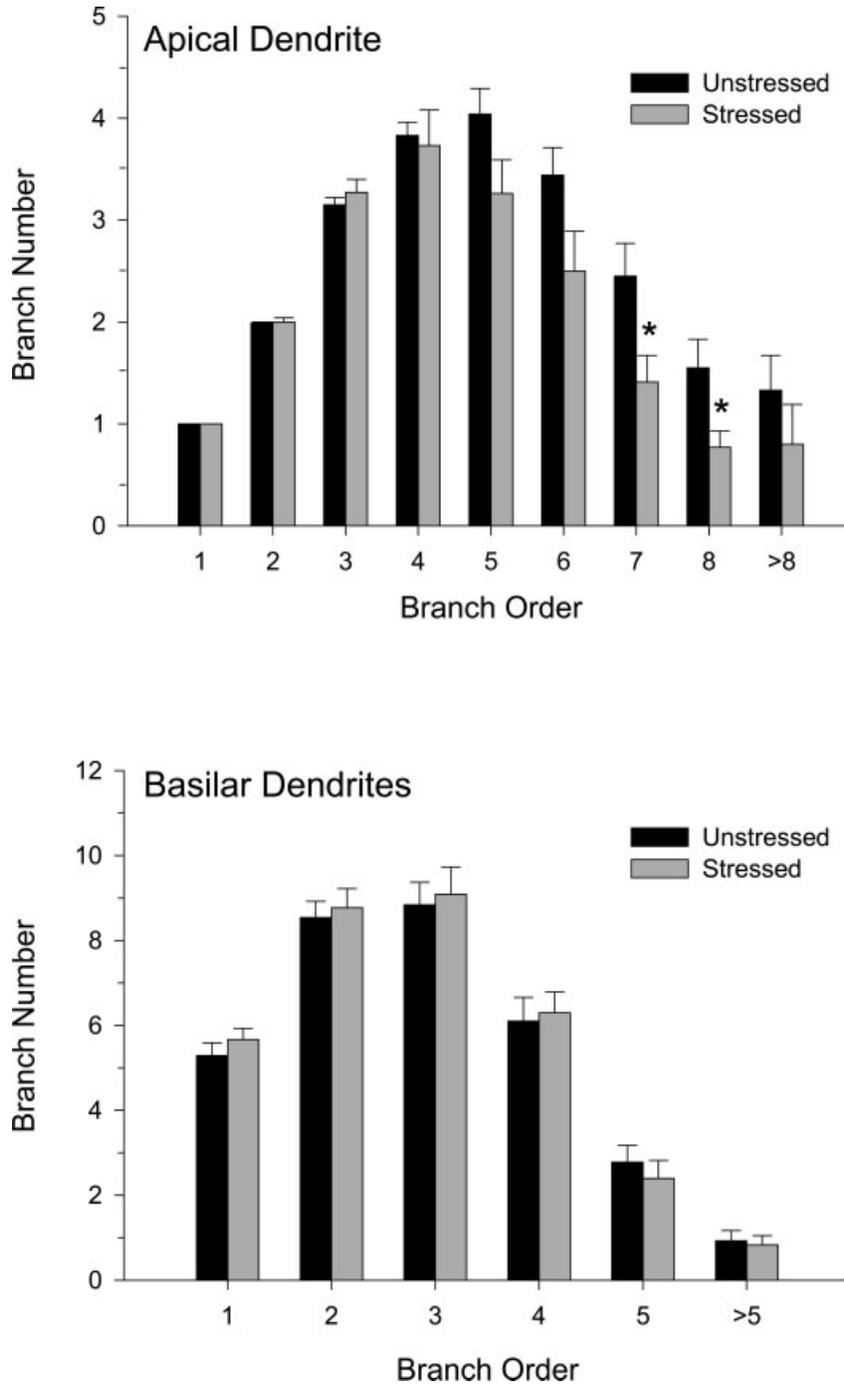
Figure 6 Mean number of branches at each order for apical (top) and basilar dendrites (bottom) in unstressed (*n* = 8) and stressed rats (*n* = 7). Stress significantly reduced the number of higher-order apical branches. Basilar branch number did not differ significantly across groups at any order. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unstressed rats.

costerone injections resulted in a significant reorganization of apical dendrites in layer II–III pyramidal neurons in medial prefrontal cortex, with an increase in dendritic material proximal to the soma and a decrease in dendritic material distal to the soma (Wellman, 2001). The stress-induced changes documented here partially parallel these corticosterone-induced changes: while apical dendrites of stressed
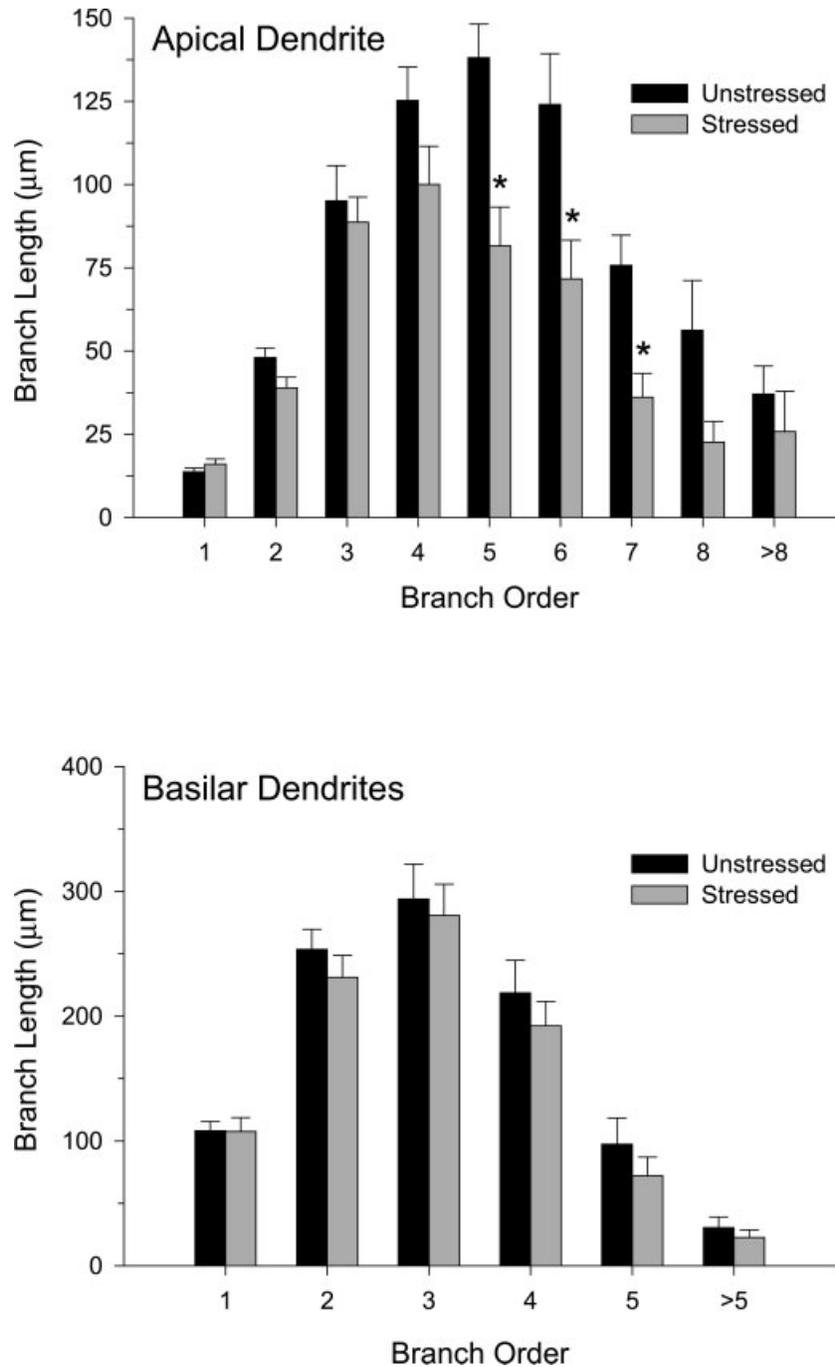
Figure 7 Mean length of branches at each order for apical (top) and basilar dendrites (bottom) in unstressed (*n* = 8) and stressed rats (*n* = 7). Stress significantly reduced the length of higher-order apical branches. Basilar branch length did not differ significantly across groups at any order. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unstressed rats.

animals undergo marked atrophy distally, the increase in dendritic material proximal to the soma is not significant. This inconsistency may reflect differences in the magnitude of stress-induced increases in corticosterone titers relative to those seen in the cortico-

sterone-injected animals. The dose of corticosterone that was used previously (10 mg/day) results in peak plasma corticosterone concentration of approximately 95 μg/100 mL (Hauger et al., 1987). Three hours of restraint stress initially elicited a similar increase in
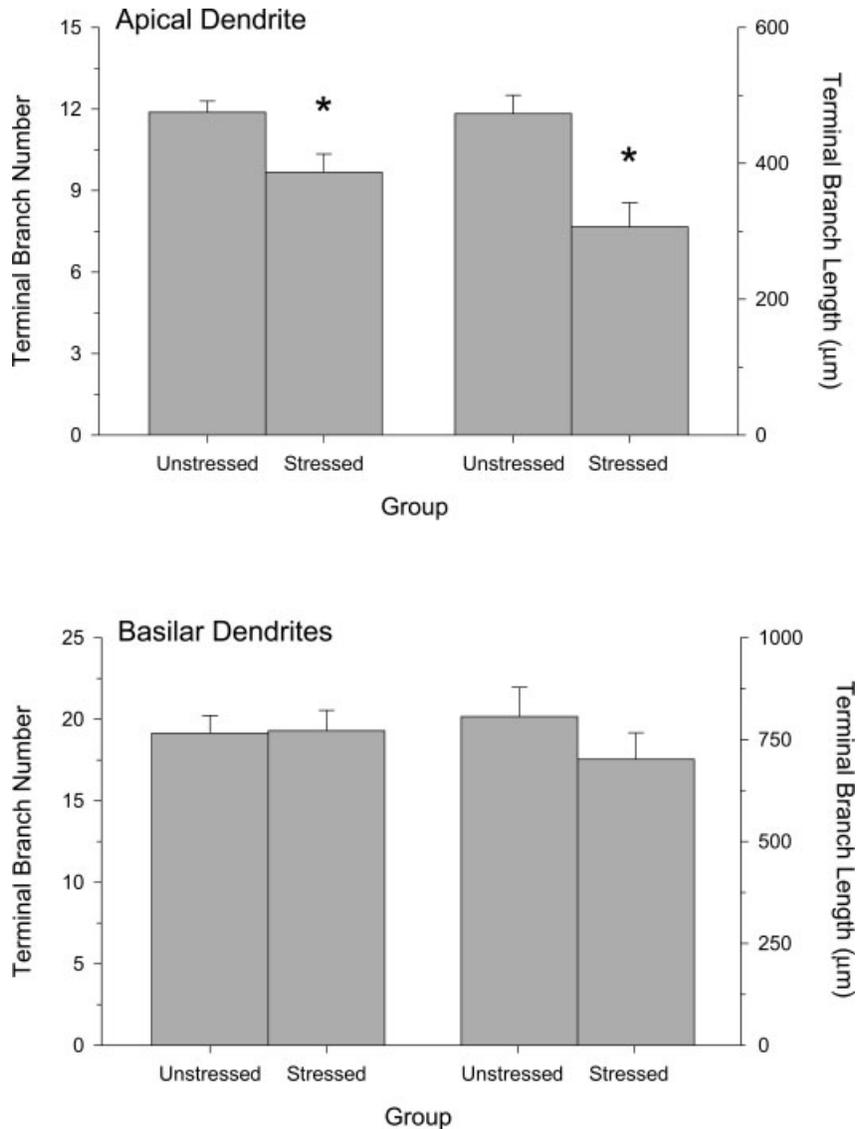
Figure 8 Mean number and length of apical (top) and basilar (bottom) terminal branches for unstressed ($n = 8$) and stressed rats ($n = 7$). Apical terminal branch number and length were both significantly reduced in stressed rats. Basilar terminals were not affected. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unstressed rats.

corticosterone concentration. However, while peak corticosterone concentrations were significantly elevated throughout the 3 week period, in the second and third weeks the increase was less pronounced. Thus, the difference in the pattern of dendritic changes induced by 3 weeks of restraint stress versus corticosterone injections may reflect lower stress-induced corticosterone titers. Alternatively, stress produces a variety of neurochemical changes in prefrontal cortex (e.g., Moghaddam, 1993; Gresch et al., 1994; Mark et al., 1996; Mizoguchi et al., 2000), a subset of which may not be mediated by corticosterone. Thus, the differential pattern of dendritic changes in the two

studies may reflect stress-induced processes not mediated by corticosterone.

Interestingly, in our previous study, we found that daily vehicle injections also resulted in atrophy of the distal portion of the apical arbor of layer II–III neurons in medial prefrontal cortex (Wellman, 2001). This atrophy was less pronounced than that seen in the present study. Thus, the relatively mild stress of daily injections alone appears to alter the morphology of medial prefrontal cortex, and this morphological alteration is more pronounced with a more severe stressor. Given that both stress (e.g., Brown and Birley, 1968; Brown and Harris, 1989; Ventura et al., 1989)

and dysfunction of prefrontal cortex (e.g., Baxter et al., 1989; Mayberg, 1997; Berman and Weinberger, 1999) are hypothesized to play important roles in psychological disorders such as depression and schizophrenia, the morphological sensitivity of prefrontal cortex to chronic stress has important implications for the etiology of these disorders.

Finally, dendrites are a major site of synaptic connectivity, with adult cortical neurons receiving approximately 15,000 synaptic inputs (Huttenlocher, 1994). Given that the geometry of the dendritic arbor (e.g., dendritic branching patterns, distribution, and overall shape) determines many functional properties of neurons (e.g., Rall et al., 1992; Mainen and Sejnowksi, 1996; Koch and Segev, 2000; Lu et al., 2001; Grudt and Perl, 2002), the pronounced stress-induced dendritic changes documented here likely result in important functional changes and may have consequences for the behaviors mediated by medial prefrontal cortex. Thus, the stress-induced changes in dendritic morphology of neurons in medial prefrontal cortex may contribute to stress-induced cognitive changes.

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