

Mild, Short-term Stress Alters Dendritic Morphology in Rat Medial Prefrontal Cortex

Sarah M. Brown¹, Shannon Henning¹ and Cara L. Wellman^{1,2}

¹Department of Psychology, Indiana University, Bloomington, IN, USA and ²Program in Neural Science, Indiana University, Bloomington, IN, USA

Prefrontal cortex is a target for glucocorticoids, shows neurochemical changes in response to stress and mediates many of the behaviors that are altered by chronic corticosterone administration. Three weeks of either daily corticosterone injections or 3 h daily restraint stress result in dendritic changes in pyramidal neurons in medial prefrontal cortex. Interestingly, vehicle injection results in similar but less pronounced changes. Thus, the mild stress of daily injections alone may alter morphology of medial prefrontal cortex, suggesting an exquisite sensitivity to chronic stress. To further examine this morphological sensitivity, we assessed the effect of 1 week of daily brief restraint stress on dendritic morphology in medial prefrontal cortex. Male rats were restrained 10 min per day for one week, handled daily or left unhandled. Rats were then overdosed and brains were stained using a Golgi-Cox procedure. Layer II–III pyramidal neurons in medial prefrontal cortex were drawn and dendritic morphology was quantified. One week of daily brief restraint resulted in selective remodeling of apical dendrites, with atrophy of up to 22–35% in distal branches and sparing of proximal branches. This pattern of reorganization is similar to that seen after either corticosterone injections or 3 weeks of daily 3 h restraint stress. Thus, the stress-induced dendritic changes in medial prefrontal cortex occur rapidly, and in response to a mild stressor.

Keywords: dendritic morphology, medial prefrontal cortex, rat, restraint stress

Introduction

Chronic stress adversely affects both physiology and behavior. A variety of studies have documented its association with increased risk for illness, the development 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). Likewise, 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 increases in glucocorticoids (e.g. Uno *et al.*, 1989),

and in fact, both chronic stress and chronic elevations of glucocorticoids have been shown to produce a variety of cognitive deficits. For instance, rats exposed to daily corticosterone injections for 8 weeks demonstrated decreased spontaneous alternation on a T maze (Bardgett *et al.*, 1994). Likewise, 21 day corticosterone implants that produced a 2- to 4-fold 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 stress and elevations of glucocorticoids. For instance, lesions of prefrontal cortex impair spontaneous alternation, radial maze performance and passive avoidance in rats (see Kolb, 1984) and 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: [³H]dexamethasone binds to receptors in frontal and prefrontal cortex at ~75% of the concentration found in hippocampus. In addition, [³H]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). Accordingly, we examined dendritic morphology of layer II–III pyramidal neurons in medial prefrontal cortex after chronic restraint stress (Cook and Wellman, 2004). The results of this study demonstrated that 3 weeks of 3 h daily restraint stress resulted in marked dendritic changes in the apical dendrites of pyramidal neurons in medial prefrontal cortex. While restraint stress failed to significantly alter basilar dendritic arbor, it resulted in significant atrophy of the distal portion of the apical arbor. This effect has been replicated using

a more severe but equally prolonged stressor (6 h of daily restraint for 3 weeks; Radley *et al.*, 2004).

The effect of chronic stress on prefrontal dendritic morphology is probably mediated by corticosterone: chronic corticosterone administration dramatically reorganized apical arbors of layer II–III neurons in medial prefrontal cortex, with an increase in dendritic material proximal to the soma, along with a decrease in dendritic material distal to the soma (Wellman, 2001). Interestingly, vehicle-treated animals showed similar but less pronounced changes in apical arbors. The similarity of the vehicle- and corticosterone-treated animals suggests that the stress of daily subcutaneous injections alone may have altered apical dendritic arbors of layer II–III pyramidal neurons in medial prefrontal cortex, and that this effect is corticosterone-mediated.

These findings contrast with the morphological changes seen in hippocampal area CA3 after chronic corticosterone administration. Whereas others have found that chronic injection of vehicle has no effect on the morphology of hippocampal neurons (Woolley *et al.*, 1990), we found that these same vehicle injections resulted in changes in layer II–III neurons in medial prefrontal cortex that were parallel to, but less pronounced than, the corticosterone-induced changes (Wellman, 2001). Thus, the relatively mild stress of daily injections alone appears to alter the morphology of medial prefrontal cortex, suggesting an exquisite sensitivity to chronic stress — perhaps even greater than that seen in the hippocampus.

Moreover, our previous studies assessed the effects of 3 weeks of daily injections or restraint stress (Cook and Wellman, 2004; Wellman, 2001), a duration chosen because it has been shown to produce alterations in hippocampal neuron morphology (Woolley *et al.*, 1990). However, if prefrontal cortical morphology is in fact extremely sensitive to mild chronic stress, then dendritic changes may occur after a shorter duration of stress. Our previous data suggest that the largest stress effects may in fact occur within 1 week: stress-induced reductions in weight gain and elevations in corticosterone were most pronounced in the first 7 days of chronic stress (Cook and Wellman, 2004). Therefore, to further examine the morphological sensitivity of prefrontal cortex to chronic stress, we assessed the effect of just 1 week of daily brief (10 min) restraint stress on dendritic morphology in medial prefrontal cortex.

Materials and Methods

Animals

Adult male Sprague–Dawley rats (175–200 g, ~50 days old at the start of the experiment; $n = 15$) either were exposed to chronic brief restraint stress or served as controls. Restraint-stressed rats ($n = 5$) were placed in a plastic rat restrainer in their home cages for 10 min daily for 7 days. Another group of rats ($n = 5$) were handled twice daily for 7 days. In addition, stressed and handled rats were weighed every other day. The remainder of the rats ($n = 5$) served as unhandled controls, and were neither handled nor weighed during this period. 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 10:00 a.m. and 4:00 p.m., were carried out in accordance with the guidelines published in the NIH *Guide for the Care and Use of Laboratory Animals*, and were approved by the Bloomington Institutional Animal Care and Use Committee.

Corticosterone Assay

To assess whether the brief restraint was stressful, plasma corticosterone titers were measured in a separate set of restraint-stressed animals

($n = 60$). Elevations in corticosterone titers due to restraint were assessed by collecting blood from the tail vein either immediately, or 10, 30 or 60 min after being placed in the restrainer [a duration sufficient to obtain peak plasma corticosterone levels (De Souza and Van Loon, 1982)] on days 1, 3 and 7. To ensure that the effects of mild restraint stress were not confounded with the effects of the potentially stressful blood-drawing procedure, a cross-sectional design was employed, in which each rat only had blood drawn once. To establish baseline corticosterone levels, on days 1, 3 and 7, rats were placed in restrainers and had blood collected immediately ($n = 15$; 5 per group). Other groups of rats were restrained and had blood drawn after 10 min (the duration of the mild stressor) on day 1, 3 or 7 ($n = 15$; 5 per group). Rats from which samples were taken at either 30 or 60 min ($n = 30$; 5 per group) were restrained for 10 min, returned to their home cages, and then replaced in the restrainers for blood collection. These final groups were used to assay peak elevations in corticosterone resulting from the 10 min of restraint stress. Tail vein blood was collected in heparinized microcapillary tubes, centrifuged at 2000 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 <27.0 pg/ml, and coefficients of variation within and across assays of ~7.7% and ~9.7%, respectively. Stress-induced changes in corticosterone concentrations were evaluated across time using a two-way ANOVA (time after initiation of restraint \times day) followed by appropriate planned comparisons. Planned comparisons consisted of two-group *F*-tests done within the context of the overall ANOVA (Hays, 1994), comparing average corticosterone concentration at either 10, 30 or 60 min to baseline (0 min).

Histology and Dendritic Analysis

On the last day 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 celloidins before being embedded in 8% celloidin (8% v/v parlodion in 1:1 absolute ethanol:ether). Coronal sections were cut at 150 μ m 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 Cg1–3 area 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 pyramidal cells) 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. In addition, to minimize both variability of apical dendritic data and the probability of potential artifactual differences due to sampling neurons from varying cortical depths, only neurons whose somata were between 150 and 455 μ m from the pial surface were chosen. Thus, the pyramidal neurons drawn were located in either deep layer II or superficial layer III; short-shaft pyramidal cells from superficial layer II were excluded. For each animal, 10 neurons were drawn; this number yields a within-animal error of <15% (mean within-animal SEM

for total branch length = $12.99 \pm 0.61\%$), and thus was considered to provide a representative sample of deeper layer II-III pyramidal neurons in medial prefrontal cortex. All neurons were drawn at 600 \times 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 one-way ANOVAs. 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 radii (10 μm from soma radius, 20 μm from soma radius, etc., to encompass the most distal dendrites) was counted; for statistical and graphical purposes, the counts of intersections were summed over pairs of radii (i.e. 10 and 20 μm radii, 30 and 40 μm radii, etc.). These data were compared using two-way repeated-measures ANOVAs (group \times distance from soma) followed by appropriate planned comparisons. In addition, the number and length of terminal branches were compared across groups using one-way ANOVAs followed by appropriate planned comparisons. For all analyses, planned comparisons consisted of two-group *F*-tests done within the context of the overall ANOVA (Hays, 1994), comparing unstressed versus either handled or stressed groups at each interval.

Results

Corticosterone Assay

Chronic brief restraint stress resulted in significant increases in corticosterone titers (Fig. 1). Two-way ANOVA (time after initiation of restraint \times day) revealed a significant effect on plasma corticosterone titers of time after initiation of restraint [$F(3,48) = 24.14, P < 0.01$] but not day of restraint [$F(2,48) = 1.43, \text{NS}$], as well as a significant interaction of time post-restraint and day [$F(6,48) = 2.33, P < 0.05$]. Planned comparisons revealed that, averaged across days, corticosterone titers were significantly increased relative to baseline at 10 and 30 min after being placed in the restrainer [$F(1,28) = 51.47$ and 25.36 ,

respectively, $P < 0.01$], but had returned to baseline by 60 min [$F(1,28) = 0.38, \text{NS}$].

Dendritic Analyses

In all treatment groups, complete impregnation (defined as the absence of dendrites trailing off into a series of dots: see Buell, 1982; Coleman and Flood, 1987; Williams *et al.*, 1978) 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 one-way ANOVA. Average distance to the cortical surface did not vary across groups [for unhandled rats, mean = $283.58 \pm 6.16 \mu\text{m}$; for handled rats, mean = $257.75 \pm 8.48 \mu\text{m}$; for restraint-stressed rats, mean = $253.72 \pm 13.42 \mu\text{m}$; $F(2,12) = 2.66, \text{NS}$]. 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. One-way ANOVAs revealed that chronic brief restraint stress significantly altered both branch number and length [$F(2,12) = 4.81$ and 3.75 , respectively, $P \leq 0.05$]. Planned comparisons indicated that whereas handling did not significantly alter apical branch number or length compared to unstressed controls [$F(1,8) = 0.65$ and 0.64 , respectively, NS], 10 min restraint stress significantly decreased apical branch number and length by $\sim 20\text{--}28\%$ relative to both handled and unhandled rats [all $F(1,8) > 6.10, P < 0.05$; Figs 3 and 4].

To more closely examine changes in the distribution of dendritic material, Sholl analyses were performed. Chronic brief stress significantly altered the distribution of apical dendritic

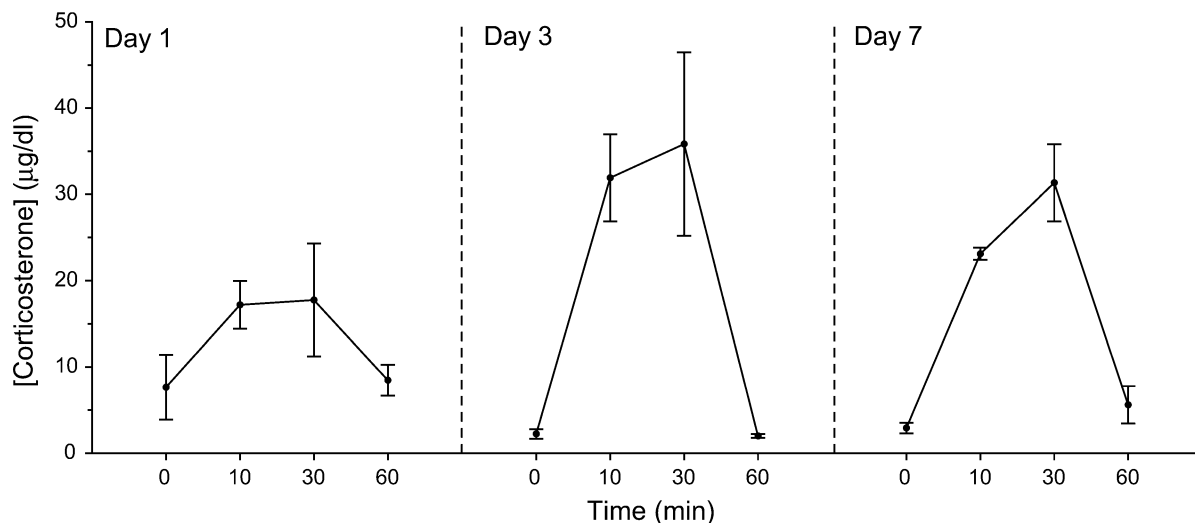


Figure 1. Mean plasma corticosterone concentration at either 0, 10, 30 or 60 min after initiation of 10 min of restraint on the first, third or seventh day of restraint stress ($n = 5$ rats per group). Vertical bars represent SEM values.

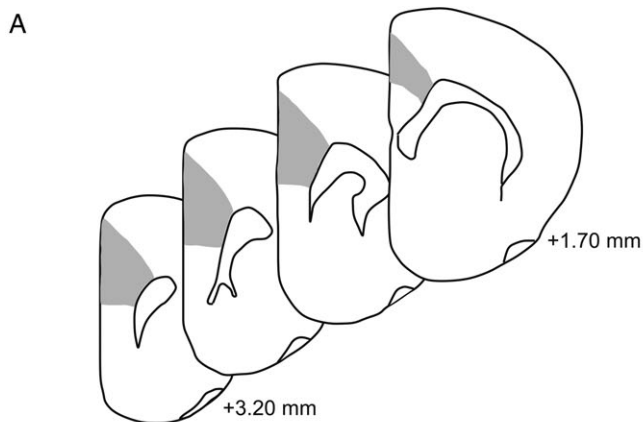


Figure 2. (A) Schematic diagram of coronal sections through rat prefrontal cortex. The portions of area Cg1-3 from which neurons were sampled is shown (shaded areas). Coordinates indicate position relative to bregma (Paxinos and Watson, 1998). (B) Digital light micrograph of Golgi-stained neuron in layer II-III of medial prefrontal cortex in an unstressed rat. Scale bar = 50 μ m.

material [for main effect of group, $F(2,12) = 3.66$, $P \leq 0.06$; for interaction of group and distance from soma, $F(2,22) = 2.61$, $P \leq 0.01$]. Planned comparisons indicated that handling alone failed to significantly alter apical dendritic material at any distance from the soma [all $F_s(1,8) \leq 3.37$, NS]. On the other hand, relative to unhandled controls, chronic brief restraint stress significantly decreased apical dendritic material in the middle portion of the apical arbor by 22-31% [for 90-100, 110-120, and 130-140 μ m from the soma, all $F_s(1,8) \geq 6.47$, $P \leq 0.04$];

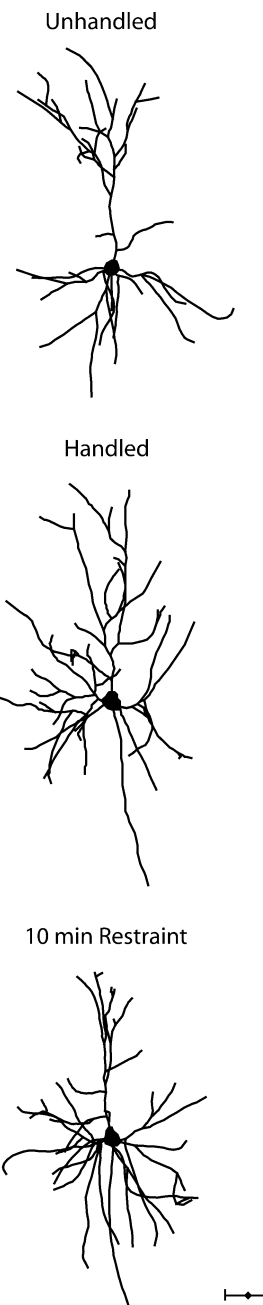


Figure 3. Computer-assisted reconstructions of Golgi-stained neurons in layer II-III of medial prefrontal cortex in unhandled (top), handled (middle) and restraint-stressed (bottom) rats. Scale bar = 50 μ m. These neurons were selected because they are representative of apical dendritic lengths near their respective group means.

decreases in dendritic material more distally approached significance [for 150-160 μ m from the soma, $F(1,8) = 3.57$, $P \leq 0.09$; for 210-220 and >220 μ m from the soma, $F_s(1,8) \geq 4.44$, $P \leq 0.07$; all other $F_s(1,13) \leq 2.75$, NS; Fig. 5]. Similarly, dendritic material in the middle portion of the apical arbor in restraint-stressed rats was decreased relative to handled rats [for 70-80, 90-100, 110-120, and 130-140 μ m from the soma, all $F_s(1,8) \geq 7.71$, $P \leq 0.02$], but was not significantly different either closer to or further away from the soma [all $F_s(1,8) \leq 3.16$, NS; Fig. 5].

Finally, because terminal branches may be more plastic than other parts of the arbor (Coleman and Flood, 1987; Rosenzweig

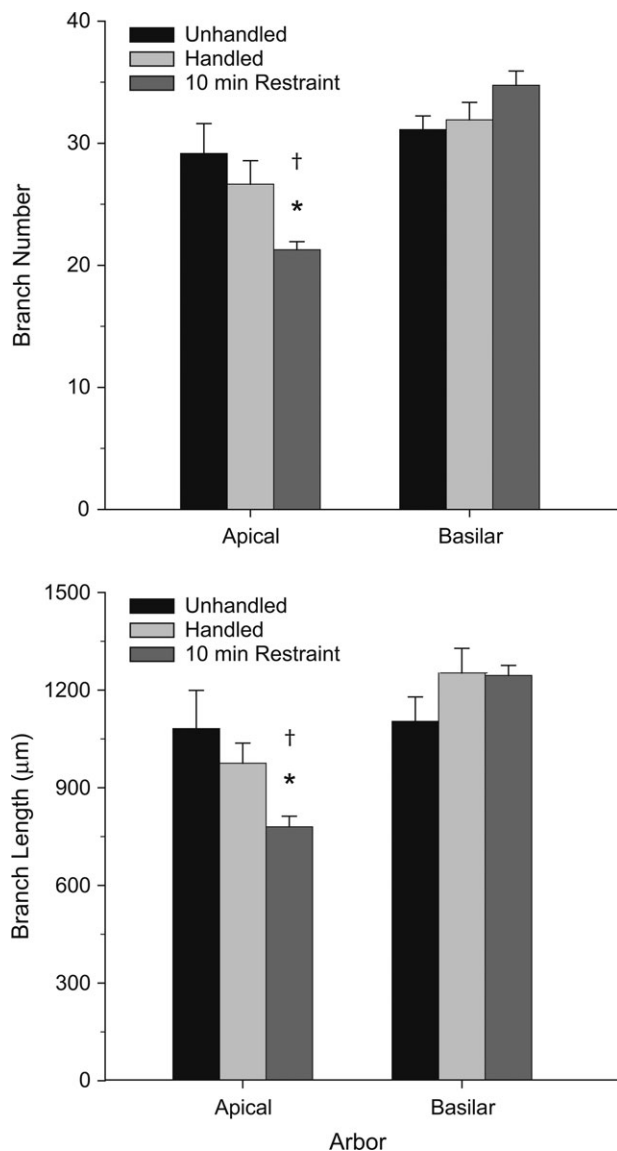


Figure 4. Mean apical and basilar branch number (top) and length (bottom) for unhandled, handled and restraint-stressed rats ($n = 5$ per group). Overall apical branch number and length were significantly reduced in stressed animals relative to both handled and unhandled animals, whereas basilar branch number and length did not vary across groups. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unhandled rats; daggers (†) indicate significant differences relative to handled rats.

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. One-way ANOVA revealed a significant effect of group on apical branch number [$F(2,12) = 4.71$, $P \leq 0.03$]. Planned comparisons demonstrated that while handling failed to significantly alter apical terminal branch number [$F(1,8) = 0.61$, NS], the number of apical terminal branches was significantly reduced in restraint-stressed animals relative to both unhandled and handled controls, by 26 and 19%, respectively [$F(1,8) \geq 6.94$, $P \leq 0.03$; Fig. 6]. Similarly, one-way ANOVA demonstrated a significant effect of group on apical terminal branch length [$F(2,12) = 4.04$, $P \leq 0.05$], with subsequent planned comparisons demonstrating no difference between handled and unhandled rats [$F(1,8) = 0.45$, NS], but a significant reduction in the length of

apical terminal branches in restraint-stressed animals relative to both unhandled and handled controls [29 and 23%, respectively; $F(1,8) \geq 6.94$, $P \leq 0.03$; Fig. 6].

Basilar Dendrites

In contrast, handling and brief restraint stress failed to significantly alter any measure of basilar dendritic arbor. Mean basilar branch number and length were not significantly altered by either handling or restraint stress [for branch number, $F(2,12) = 2.35$, NS; for branch length, $F(2,12) = 1.72$, NS; Figs 3 and 4]. Furthermore, the distribution of basilar dendritic material did not vary across groups [main effect of group, $F(2,12) = 1.92$, NS; interaction of group by distance from soma, $F(2,10) = 1.52$, NS; Fig. 5]. Likewise, basilar terminal branches were not significantly altered by stress [for basilar terminal number, $F(2,12) = 2.29$, NS; for basilar terminal length, $F(2,12) = 0.98$, NS; Fig. 6].

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 a short-duration, mild stressor: 1 week of daily brief restraint resulted in a selective remodeling of apical dendrites, with atrophy of up to 31% occurring in more distal branches and relative sparing of proximal branches. These effects replicate our previous work using a longer-term, more severe stressor (3 weeks of daily 3 h restraint). In our previous study, the longer-term, more severe stress produced a pronounced decrease in both branch number and branch length in the apical dendrites of layer II–III pyramidal neurons in medial prefrontal cortex (Cook and Wellman, 2004). Sholl analyses demonstrated that 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%. This restriction may reflect the differential atrophy of terminal branches: terminal branch number and length throughout the arbor were decreased by 19 and 35%, respectively (Cook and Wellman, 2004). In the present study, a short-term mild stressor produced a similar pattern of dendritic changes. As with the effects of the longer-term, more severe stressor used in the previous study, the dendritic atrophy resulting from the short-term, mild stressor used in the current study was restricted to relatively distal portions of the apical arbor and appeared to differentially affect terminal branches: apical terminal branch number and length were decreased by 26 and 29%, respectively, relative to unhandled rats. Thus, our results suggest that the morphology of medial prefrontal cortex is exquisitely sensitive to stress, with changes occurring in as little as 1 week in response to even a mild stressor.

Importantly, this remodeling of the apical arbors of prefrontal pyramidal neurons is likely due to the stressful nature of the restraint: 1 week of brief restraint produced robust increases in corticosterone levels, which did not habituate over the course of the study. Interestingly, in a previous study (Wellman, 2001), 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 the more severe stressor used in the present study. Indeed, our present data, combined

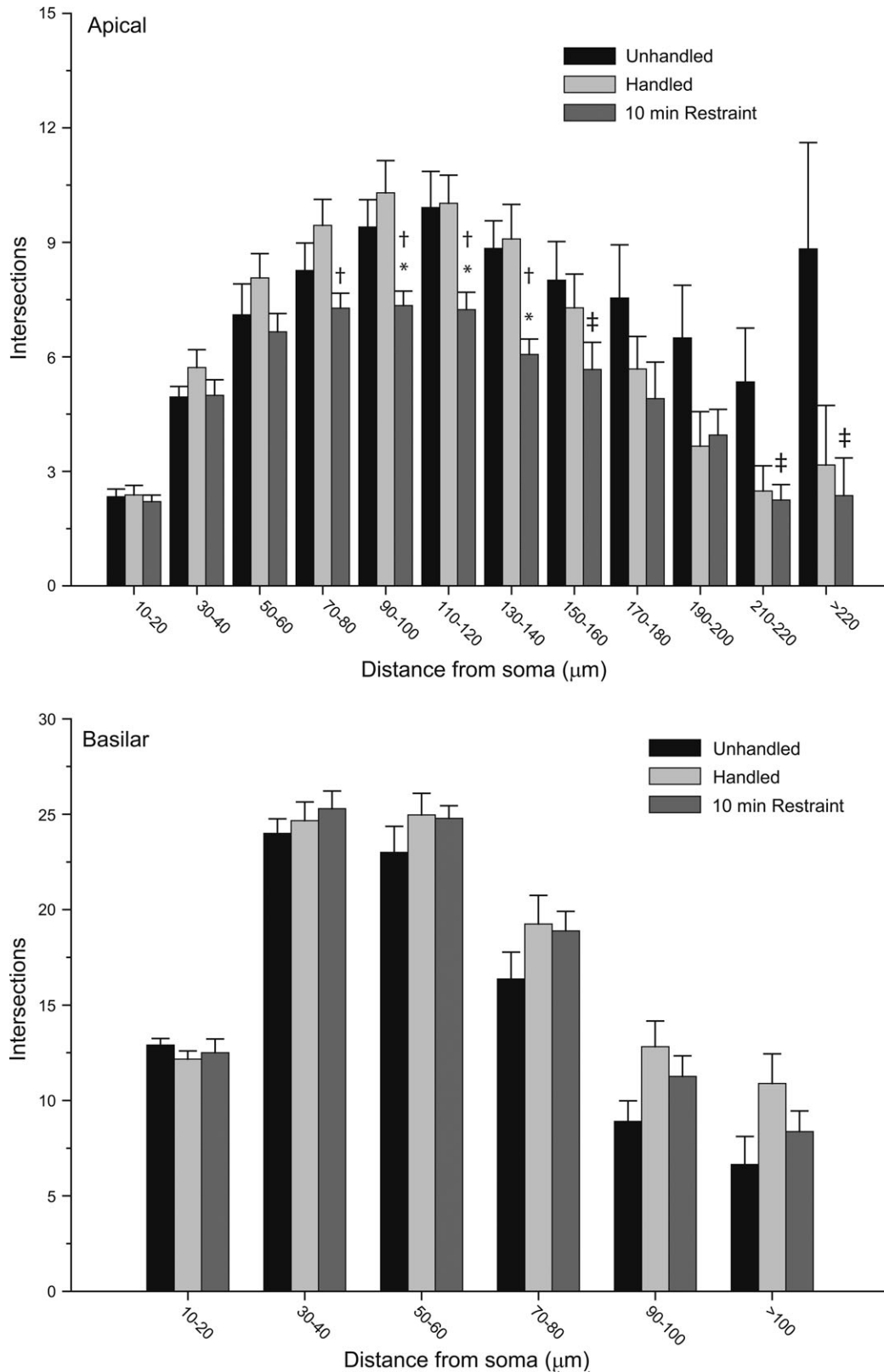


Figure 5. Mean intersections of apical (top) and basilar dendrites (bottom) with 10 μm concentric spheres in unhandled, handled and restraint-stressed rats ($n = 5$ per group). Data have been summed into 20 μm bins. Apical dendritic material distal to the soma was reduced in stressed rats relative to both unhandled and handled rats, whereas the distribution of basilar dendritic material did not differ across groups. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unhandled rats; daggers (†) indicate significant differences relative to handled rats; double daggers (‡) indicate differences that approach significance relative to unhandled rats.

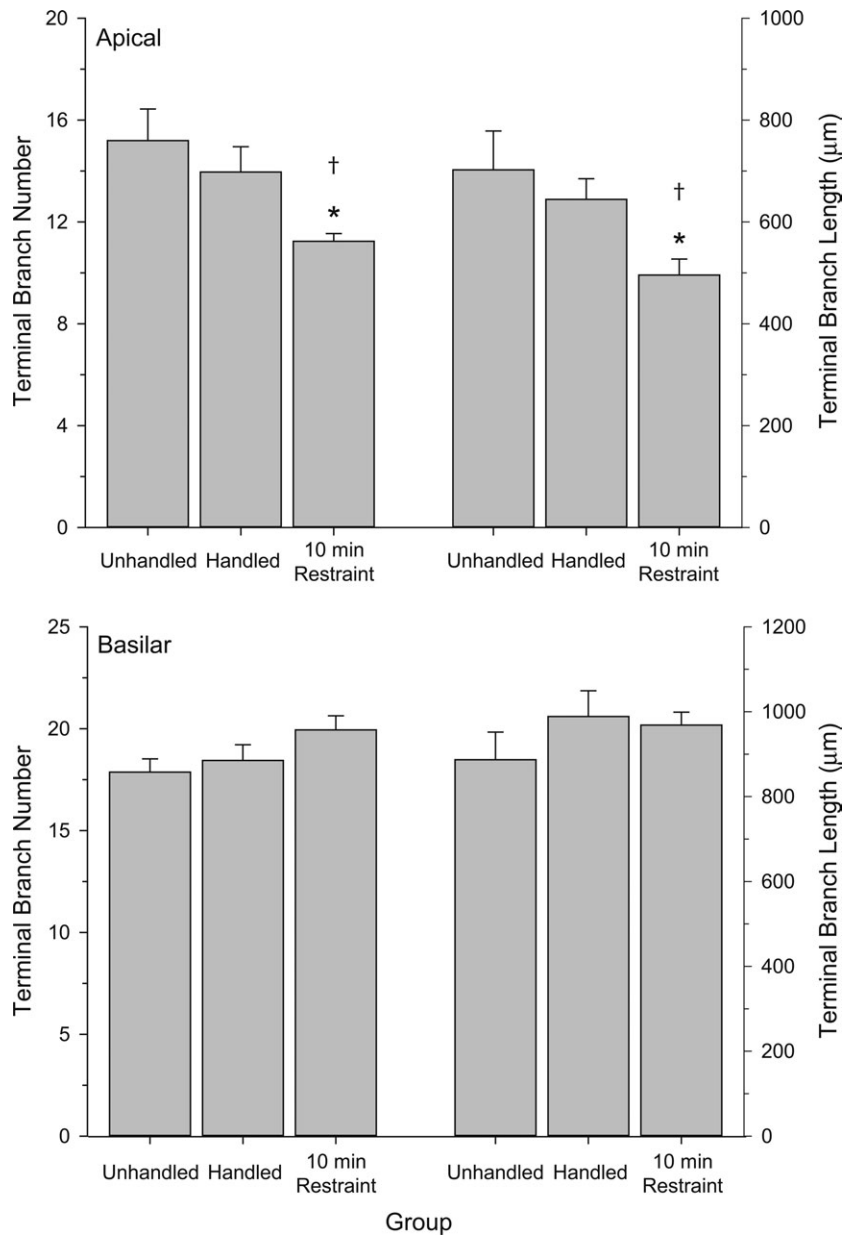


Figure 6. Mean number and length of apical (top) and basilar (bottom) terminal branches for unhandled, handled and stressed rats ($n = 5$ per group). Apical terminal branch number and length were both significantly reduced in stressed rats. Basilar terminals were not affected. Vertical bars represent SEM values. Asterisks (*) indicate significant differences relative to unhandled rats; daggers (†) indicate significant differences relative to handled rats.

with our previous studies, suggest a consistent dose-effect relationship between stress, stress-induced corticosterone levels and dendritic changes in medial prefrontal cortex. Daily vehicle injections, which presumably would produce relatively small increases in corticosterone titers, produced moderate decreases in apical arbor (Wellman, 2001); 10 min of daily restraint, resulting in average peak corticosterone titers of ~ 28 $\mu\text{g}/\text{dl}$, produced similarly moderate but robust alterations in apical arbor; 3 h of daily restraint, resulting in average peak corticosterone titers of ~ 74 $\mu\text{g}/\text{dl}$, produced more pronounced decreases in distal apical arbor (Cook and Wellman, 2004); and daily corticosterone administration, which typically produces average peak corticosterone titers of ~ 95 $\mu\text{g}/\text{dl}$ (Hauger *et al.*, 1987), produced similarly pronounced decreases in distal

dendritic material accompanied by increases in dendritic material proximal to the soma (Wellman, 2001).

However, it is important to note that, while our results suggest that the chronic stress effects we have documented in medial prefrontal cortex are corticosterone-mediated, we have not yet directly tested this hypothesis. In fact, stress produces a variety of neurochemical and physiological changes in prefrontal cortex (e.g. Moghaddam, 1993; Gresch *et al.*, 1994; Mark *et al.*, 1996; Mizoguchi *et al.*, 2000; Rocher *et al.*, 2004; Tan *et al.*, 2004), which are not necessarily mediated by corticosterone. Indeed, stress-induced changes in dendritic morphology of hippocampal area CA3 neurons have been shown to be mediated at least in part by glutamatergic NMDA receptors (Magariños and McEwen, 1995). Thus, the dendritic

reorganization in medial prefrontal cortex resulting from chronic stress could be due to stress-induced processes not mediated by corticosterone.

Finally, dendrites are a major site of synaptic connectivity, with adult cortical neurons receiving ~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 Sejnowski, 1996; Koch and Segev, 2000; Lu *et al.*, 2001; Grudt and Perl, 2002), the dendritic changes resulting from even the short-term, mild stressor documented here probably result in important functional changes and may have consequences for the behaviors mediated by medial prefrontal cortex. 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 disorders such as depression and schizophrenia, the morphological sensitivity of prefrontal cortex to chronic stress has important implications for the etiology of these disorders.

Notes

We thank Ms Anthea C. Diamondis for her technical assistance and Dr Dale R. Sengelaub for his helpful comments on the manuscript. This research was supported by the HHMI Biological Sciences Education Program (S.M.B.) and MH067607 (C.L.W.).

Address correspondence to C.L. Wellman, Psychology Department, 1101 E. 10th Street, Indiana University, Bloomington, IN 47405, USA. Email: wellmanc@indiana.edu.

References

- Altenor A, Kay E, Richter M (1977) The generality of learned helplessness in the rat. *Learn Motiv* 8:54–61.
- Bardgett ME, Taylor GT, Csernansky JG, Newcomer JW, Nock B (1994) Chronic corticosterone treatment impairs spontaneous alternation behavior in rats. *Behav Neural Biol* 61:186–190.
- Baxter LR, Schwartz JM, Phelps ME, Mazziotta JC, Guze BH, Selin CE, Gerner RH, Sumida RM (1989) Reduction of prefrontal cortex glucose metabolism common to three types of depression. *Arch Gen Psychiatry* 46:243–250.
- Berman KF, Weinberger DR (1999) Neuroimaging studies of schizophrenia. In: *Neurobiology of mental illness* (Charney DS, Nestler EJ, Bunney BS, eds), pp. 246–257. New York: Oxford University Press.
- Bisagno V, Ferrini M, Rios H, Zieher LM, Wikinski SI (2000) Chronic corticosterone impairs inhibitory avoidance in rats: possible link with atrophy of hippocampal CA3 neurons. *Pharmacol Biochem Behav* 66:235–240.
- Brown GW, Birley JLT (1968) Crises and life changes and the onset of schizophrenia. *J Health Social Behav* 9:203–214.
- Brown GW, Harris TO (1989) Depression. In: *Life events and illness* (Brown GW, Harris TO, eds), pp. 49–93. New York: Guilford.
- Buell SJ (1982) Golgi-Cox and rapid Golgi methods as applied to autopsied human brain tissue: widely disparate results. *J Neuropathol Exp Neurol* 41:500–507.
- Cajal Sry (1995) *Histology of the nervous system*. New York: Oxford University Press.
- Coleman PD, Flood DG (1987) Neuron numbers and dendritic extent in normal aging and Alzheimer's disease. *Neurobiol Aging* 8: 521–545.
- Cook SC, Wellman CL (2004) Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol* 60:236–248.
- Dachir S, Kadar T, Robinzon B, Levy A (1993) Cognitive deficits induced in young rats by long-term corticosterone administration. *Behav Neural Biol* 60:103–109.
- De Souza EB, Van Loon GR (1982) Stress-induced inhibition of the plasma corticosterone response to a subsequent stress in rats: a nonadrenocorticotropin-mediated mechanism. *Endocrinology* 110:23–36.
- Dias R, Robbins TW, Roberts AC (1996) Primate analogue of the Wisconsin Card Sorting Test: effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behav Neurosci* 110: 872–886.
- Gerlach J, McEwen B (1972) Rat brain binds adrenal steroid hormone: radioautography of hippocampus with corticosterone. *Science* 175:1133–1136.
- Glaser EM, Van der Loos H (1981) Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high-quality Golgi-Nissl stain. *J Neurosci Methods* 4:117–125.
- Gresch PJ, Sved AF, Zigmond MJ, Finlay JM (1994) Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *J Neurochem* 63:575–583.
- Grudt TJ, Perl ER (2002) Correlations between neuronal morphology and electrophysiology features in the rodent superficial dorsal horn. *J Physiol* 540:189–207.
- Hauger RL, Millam MA, Catt KJ, Aguilera G (1987) Differential regulation of brain and pituitary corticotropin-releasing factor receptors by corticosterone. *Endocrinology* 120:1527–1533.
- Hays WL (1994) *Statistics*. Fort Worth, TX: Harcourt Brace.
- Henke PG (1990) Granule cell potentials in the dentate gyrus of the hippocampus: coping behavior and stress ulcers in rats. *Behav Brain Res* 36:97–103.
- Huttenlocher PR (1994) Synaptogenesis in human cerebral cortex. In: *Human behavior and the developing brain* (Dawson G, Fischer KW, eds), pp. 137–152. New York: Guilford Press.
- Koch C, Segev I (2000) The role of single neurons in information processing. *Nat Neurosci* 3:1171–1177.
- Kolb B (1984) Functions of the frontal cortex of the rat: a comparative review. *Brain Res Rev* 8:65–98.
- Lu Y, Inokuchi H, McLachlan EM, Li J-S, Higashi H (2001) Correlation between electrophysiology and morphology of three groups of neurons in the dorsal commissural nucleus of lumbosacral spinal cord of mature rats studied *in vitro*. *J Comp Neurol* 437:156–169.
- Luine V, Villegas M, Martinez C, McEwen BS (1994) Stress-dependent impairments of spatial memory: role of 5-HT. *Ann NY Acad Sci* 746:403–404.
- Lyons DM, Lopez JM, Yang C, Schatzberg AF (2000) Stress-level cortisol treatment impairs inhibitory control of behavior in monkeys. *J Neurosci* 20:7816–7821.
- Magariños AM, McEwen BS (1995) Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* 69:89–98.
- Magariños AM, McEwen BS, Flugge G, Fuchs E (1996) Chronic psychosocial stress causes apical dendritic atrophy of hippocampal CA3 pyramidal neurons in subordinate tree shrews. *J Neurosci* 16:3534–3540.
- Mainen ZF, Sejnowski TJ (1996) Influence of dendritic structure on firing pattern in model neocortical neurons. *Nature* 382:363–366.
- Mark GP, Rada PV, Shors TJ (1996) Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. *Neuroscience* 74:767–774.
- Mayberg HS (1997) Limbic-cortical dysregulation: a proposed model of depression. *J Neuropsychiatry* 9:471–481.
- Meaney MJ, Aitken DH (1985) [³H]Dexamethasone binding in rat frontal cortex. *Brain Res* 328:176–180.
- Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui D-H, Tabira T (2000) Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *J Neurosci* 20:1568–1574.
- Moghaddam B (1993) Stress preferentially increases extraneuronal levels of excitatory amino acids in the prefrontal cortex: comparison to hippocampus and basal ganglia. *J Neurochem* 60:1650–1657.
- Paxinos G, Watson C (1998) *The rat brain in stereotaxic coordinates*. New York: Academic Press.

- Radley JJ, Sisti HM, Hao J, Rocher AB, McCall T, Hof PR, McEwen BS, Morrison JH (2004) Chronic behavioral stress induces apical dendritic reorganization in pyramidal neurons of the medial prefrontal cortex. *Neuroscience* 125:1-6.
- Rall W, Burke RE, Holmes WR, Jack JJ, Redman SJ, Segev I (1992) Matching dendritic neuron models of experimental data. *Physiol Rev* 72:S159-S186.
- Rocher C, Spedding M, Munoz C, Jay TM (2004) Acute stress-induced changes in hippocampal/prefrontal circuits in rats: effects of antidepressants. *Cereb Cortex* 14:224-229.
- Rosellini RA (1978) Inescapable shock interferes with the acquisition of a free appetitive operant. *Anim Learn Behav* 6:155-159.
- Rosenzweig MR, Bennett EL (1996) Psychobiology of plasticity: effects of training and experience on brain and behavior. *Behav Brain Res* 78:57-65.
- Seligman MEP, Maier SF (1967) Failure to escape traumatic shock. *J Exp Psychol* 74:1-9.
- Sholl DA (1956) *The organization of the cerebral cortex*. London: Methuen.
- Sousa N, Lukoyanov NV, Madeira MD, Almeida OFX, Paula-Barbosa MM (2000) Reorganization of the morphology of hippocampal neurites and synapses after stress-induced damage correlates with behavioral improvement. *Neuroscience* 97:253-266.
- Stone AA, Reed BR, Neale JM (1987) Changes in daily event frequency precede episodes of physical symptoms. *J Hum Stress* 13:70-74.
- Tan H, Zhong P, Yan Z (2004) Corticotropin-releasing factor and acute stress prolongs serotonergic regulation of GABA transmission in prefrontal cortical pyramidal neurons. *J Neurosci* 24:5000-5008.
- Uno H, Tarara R, Else JG, Suleman MA, Sapolsky RM (1989) Hippocampal damage associated with prolonged and fatal stress in primates. *J Neurosci* 9:1705-1711.
- Ventura J, Neuchterlein KH, Lukoff D, Hardesty JD (1989) A prospective study of stressful life events and schizophrenic relapse. *J Abnorm Psychol* 98:407-411.
- Watanabe Y, Gould E, McEwen BS (1992) Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res* 588:341-345.
- Wellman CL (2001) Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *J Neurobiol* 49:245-253.
- Williams RS, Ferrante RJ, Caviness VS Jr (1978) The Golgi rapid method in clinical neuropathology: the morphological consequences of suboptimal fixation. *J Neuropathol Exp Neurol* 37:13-33.
- Woolley C, Gould E, McEwen BS (1990) Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res* 531:225-231.
- Zilles K, Wree A (1995) Cortex: areal and laminar structure. In: *The rat nervous system* (Paxinos G, ed.), pp. 649-685. San Diego: Academic Press.