

Specific Configuration of Dendritic Degeneration in Pyramidal Neurons of the Medial Prefrontal Cortex Induced by Differing Corticosteroid Regimens

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We previously demonstrated that hypercorticalism induces pronounced volumetric reductions in the rat medial prefrontal cortex (mPFC) and that these structural changes correlate with deficits in executive function. By applying 3-dimensional analysis of Golgi-Cox-stained material, we now demonstrate that corticosteroids can exert differential effects on dendritic arborizations of pyramidal neurons in lamina II/III of the mPFC. Treatment with the glucocorticoid receptor-selective agonist dexamethasone and with the natural adrenosteroid, corticosterone (CORT), results in significant reductions in the total length of apical dendrites in the pyramidal neurons in lamina II/III of the anterior cingulate/prelimbic and infralimbic cortices. Interestingly, although these treatments do not affect the number of dendritic branches, they are associated with impoverished arborizations in their distal portions and, in CORT-treated animals, with increased branching in the middle portions of the apical dendritic tree. Deprivation of corticosteroids by adrenalectomy leads to decreases in total apical dendritic length and spine number, but in this case, dendritic impoverishment was restricted to the middle/proximal segments of the dendritic trees. None of the treatments influenced the architecture of the basal dendrites. These results add to our knowledge of the morphological substrates through which corticosteroids may disrupt mPFC-dependent behaviors.

Keywords: adrenalectomy, cingulate cortex, corticosteroid, dendritic morphology, stress

Introduction

Prefrontal cortex (PFC) dysfunction is a hallmark of several psychiatric disorders, and a number of studies have implicated functional and structural abnormalities in this region as a probable basis of cognitive impairment in schizophrenia and mood disorders (Rajkowska 2000; Rauch et al. 2003; Ballmaier et al. 2004; Lewis et al. 2004; Rogers et al. 2004; Shad et al. 2004; Yamasue et al. 2004; Cullen et al. 2006; Nugent et al. 2006). Stress is a major contributor to the etiology, pathophysiology, and treatment outcome of mood and affective disorders, and corticosteroids released in response to stress have been causally linked to psychopathology. Both chronic stress and disturbances in the homeostatic mechanisms that regulate corticosteroid secretion are known to affect the structure and function of the rat medial prefrontal cortex (mPFC) (Mizoguchi et al. 2000, 2004; Wellman 2001; Coburn-Litvak et al. 2003; Cook and Wellman 2004; Radley et al. 2004, 2005; Roozendaal et al. 2004; Cerqueira, Catania, et al. 2005; Cerqueira, Pego, et al. 2005). Among others, the structural changes include volumetric reductions (Cerqueira, Catania, et al. 2005; Cerqueira, Pego, et al. 2005) in the most superficial layers and dendritic

remodeling of pyramidal cells located in layer II/III of the mPFC (Cook and Wellman 2004; Radley et al. 2004, 2005).

Corticosteroid actions in the brain are mediated by mineralocorticoid (MR) and glucocorticoid (GR) receptors (de Kloet et al. 2005). The mPFC contains both MR and GR (Chao et al. 1989; Patel et al. 2000; Herman et al. 2005) that become activated by stress (Cullinan et al. 1995; Figueiredo et al. 2003) and contribute to the neural control of the endocrine response and behavioral adaptation to stress (Diorio et al. 1993; Sullivan and Gratton 2002; Mizoguchi et al. 2003; Herman et al. 2005).

We previously showed that differential activation of MR and GR results in distinct structural rearrangements in the mPFC (Cerqueira, Pego, et al. 2005), resembling those seen in the hippocampal formation in several respects (reviewed in Sousa and Almeida 2002). In Cerqueira, Pego, et al. (2005), we reported that exposure to the GR-selective agonist dexamethasone (DEX) results in significant reductions in the volume of layer II and cell number in layer II pyramidal neurons that are paralleled by behavioral impairments. Strikingly, the concomitant activation of GR and MR by the physiological adrenosteroid, corticosterone (CORT), produces only mild cognitive deficits; this treatment paradigm results in a reduction in the volume of layer II without influencing neuronal numbers. In that same study (Cerqueira, Pego, et al. 2005), we observed deficits in behavioral flexibility in rats deprived of endogenous corticosteroids by adrenalectomy (ADX); the behavioral changes occurred in the absence of morphological changes in the mPFC.

In an extension of our previous studies, we have now assessed dendritic morphology in pyramidal layer II/III of ADX rats, in rats in which GRs were persistently activated through DEX administration, and in animals experiencing concomitant occupation of MR and GR through exogenous CORT therapy. All treatments lasted 1 month. Golgi-Cox-stained dendritic trees (apical and basal arborizations) of pyramidal neurons located in lamina II/III of the different subdivisions of the mPFC (infralimbic [IL], prelimbic, and cingulate [Cg] cortex regions) were quantitatively examined by applying 3-dimensional (3D) morphometry.

Experimental Procedures

Animals and Treatments

Experiments were conducted in accordance with European regulations (European Union Directive 86/609/EEC) and National Institutes Health guidelines on animal care and experimentation.

Adult male Wistar rats (Charles River Laboratories, Barcelona, Spain) were group housed under standard laboratory conditions (lights on from 8 AM–8 PM, room temperature 22 °C, ad libitum access to food and drink). Treatments were initiated when animals were 8 weeks old and

continued over a period of 4 weeks. All drugs were from Sigma (St Louis, MO). To compare the influence of the corticosteroid milieu, rats were randomly assigned to one of 4 treatment groups ($n = 5$), with equal average weights: 1) Controls (VEH), receiving daily subcutaneous injections of sesame oil (vehicle), but otherwise maintained as described above; 2) Adrenalectomized (ADX, performed under pentobarbital anesthesia), receiving daily subcutaneous injections of vehicle and provided with 0.9% saline as drinking solution; 3) Corticosterone (CORT) treated, receiving one daily subcutaneous injection of CORT (25 mg/kg in sesame oil); and 4) DEX treated, receiving a daily subcutaneous injection of DEX (300 $\mu\text{g}/\text{kg}$ dissolved in sesame oil containing 0.01% ethanol). All injections were administered 1 h before "lights off." At the end of the experimental period, 22 h after the last injection, blood (tail venepuncture) samples were collected for basal measurements of CORT (1 h after "lights on"). Serum CORT levels were subsequently measured by radioimmunoassay (MP Biochemicals, Costa Mesa, CA).

Histological Procedures

One day after the last injection, rats were transcardially perfused with 0.9% saline under deep pentobarbital anesthesia and processed according to the protocol described by Gibb and Kolb (1998). Briefly, brains were removed and immersed in Golgi-Cox solution (a 1:1 solution of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with 5% potassium chromate—Glaser and Van der Loos 1981) for 14 days; brains were then transferred to a 30% sucrose solution (minimum 3 days), before being cut on a vibratome. Coronal sections (200 μm thick) were collected in 6% sucrose and blotted dry onto cleaned, gelatin-coated microscope slides. They were subsequently alkalized 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.

Dendrite Analysis

Pyramidal neurons in layers II–III of the Cg1–3 and IL areas of the mPFC (Zilles and Wree 1995) were analyzed. Both areas of the mPFC, which are comparable to prefrontal areas in the primate (Uylings et al. 2003), are located on the medial wall of the rostral cortex and are readily identifiable: the IL cortex, which lies ventral to area Cg1–3 (i.e., anterior Cg and prelimbic cortices, which are indistinguishable in Golgi-Cox-stained preparations), is markedly thinner and has fewer, less well-defined layers (Van Eden and Uylings 1985; Zilles and Wree 1995). Within the mPFC, layers II–III are readily identifiable in Golgi-stained material on the basis of its characteristic cytoarchitecture. It is positioned immediately ventral to the relatively cell-poor layer I (which also contains the distal dendritic tufts of layer II/III pyramidal cells) and immediately superficial to layer V; this boundary is pronounced because of the greater cell-packing density and smaller somata of pyramidal cells in layers II–III relative to layer V in this region of the brain (Van Eden and Uylings 1985; Cajal 1995; Zilles and Wree 1995).

Golgi-impregnated pyramidal neurons of the mPFC were readily identified by their characteristic triangular soma, apical dendrites extending toward the pial surface, and numerous dendritic spines. The criteria used to select neurons for reconstruction were those described by Uylings et al. (1986) and Radley et al. (2004): 1) location of the cell soma in layer II/III of the mPFC, approximately in the middle third of the section; 2) full impregnation of the neurons; 3) apical dendrite without truncated branches (except on the most superficial layer); 4) presence of at least 3 primary basal dendritic shafts, each of which branched at least once; and 5) no morphological changes attributable to incomplete dendritic impregnation of Golgi-Cox staining. In order to minimize selection bias, slices containing the region of interest were randomly searched and the first 10 neurons fulfilling the above criteria (maximum of 3 neurons per slice) were selected.

For each selected neuron, all branches of the dendritic tree were reconstructed at 600 \times magnification using a motorized microscope (Axioplan 2, Carl Zeiss, Germany), with oil objectives, and attached to a camera (DXC-390, Sony Corporation, Tokyo, Japan) and Neurolucida software (MicroBrightfield, VT). A 3D analysis of the reconstructed neurons was performed using NeuroExplorer software (MicroBrightfield).

Forty neurons were studied for each animal of the 4 experimental groups, and neurons from the same animal were averaged. Several aspects of dendritic morphology were examined. To assess overall changes, total length of basal and apical trees and number of basal dendrites and apical dendritic branches were compared across groups using 1-way analysis of variance (ANOVA) (Uylings and van Pelt 2002). To assess differences in the arrangement of dendritic material, a 3D version of a Sholl analysis (Sholl 1956; Uylings and van Pelt 2002) was performed. For this, we counted the number of intersections of dendrites with concentric spheres positioned at radial intervals of 20 μm ; in addition, we also measured the length of the dendritic tree located between 2 consecutive spheres.

The method for sampling dendritic branches for spine density (i.e., spines per micron dendritic length) was designed as follows: only branches that 1) were either parallel or at acute angles to the coronal surface of the section and 2) did not show overlap with other branches that would obscure visualization of spines were considered. Because treatment-induced changes in the apical dendritic branches varied with distance to soma, segments were randomly selected in the proximal (Cg: 140–200 μm ; IL: 60–120 μm) and distal (Cg: 240–300 μm ; IL: 140 μm –200 μm) parts of the tree, where maximal effects were observed; basal dendrites selection was done at radial distances between 50 and 100 μm .

In Golgi-impregnated material, the spines emerging toward the surface or directly into the section are not well visualized. Thus, to minimize bias, only spines that emerged perpendicular to the dendritic shaft were counted. Furthermore, an attempt to correct for hidden spines (Feldman and Peters 1979) was not made because the use of visible spine counts for comparison between different experimental conditions had been validated previously (Horner and Arbuthnott 1991). To assess treatment-induced changes in spine morphology, spines in the selected segments were classified according to Harris et al. (1992) in mushroom, thin, wide, and ramified categories and the proportion of spines in each category compared using 1-way ANOVA.

Statistics

All data were compared using 1-way ANOVA followed by appropriate post hoc comparisons using Tukey's honestly significant differences (HSDs) test. Sholl analysis data were compared across groups using 1-way repeated measures ANOVA (group \times distance from soma) followed by post hoc comparisons (Tukey's HSD). Differences were considered to be significant if $P < 0.05$.

Results

Serum CORT Levels

The steroid treatment protocols resulted in the following serum CORT level (CONTROLS [CONT]: 45.3 ± 12.0 ng/mL; CORT: 283.6 ± 22.6 ng/mL; DEX: nondetectable; ADX: nondetectable).

Anterior Cg and Prelimbic Cortices (Cg1-3)

All treatments produced significant reductions in total length of apical dendrites of pyramidal cells in layer II/III of the anterior Cg and prelimbic areas (i.e., Cg1-3) ($F_{3,16} = 7.509$, $P = 0.002$; CONT: 2100.0 ± 88.6 μm ; CORT: 1384.9 ± 138.6 μm [less 34%], $P = 0.004$; DEX: 1476.5 ± 108.7 μm [less 30%], $P = 0.012$; ADX: 1434.0 ± 146.3 μm [less 32%], $P = 0.007$) without affecting the total number of apical branches per neuron ($F_{3,16} = 0.128$, $P = 0.942$; CONT: 20.25 ± 1.63 ; CORT: 19.83 ± 1.85 ; DEX: 19.57 ± 1.33 ; ADX: 18.88 ± 1.58) (Fig. 1). Sholl analysis of apical dendritic segment lengths and the number of intersections as a function of their distance from the soma revealed an overall effect of treatment (length: $F_{3,16} = 7.834$, $P = 0.002$; intersections: $F_{3,16} = 4.888$, $P = 0.013$) (Fig. 2). CORT animals had increased branching in the middle portions of the tree, whereas both DEX- and CORT-treated rats showed a marked reduction of dendritic material at distances greater than 280 μm from the soma when compared with controls (length—CORT: $P = 0.003$,

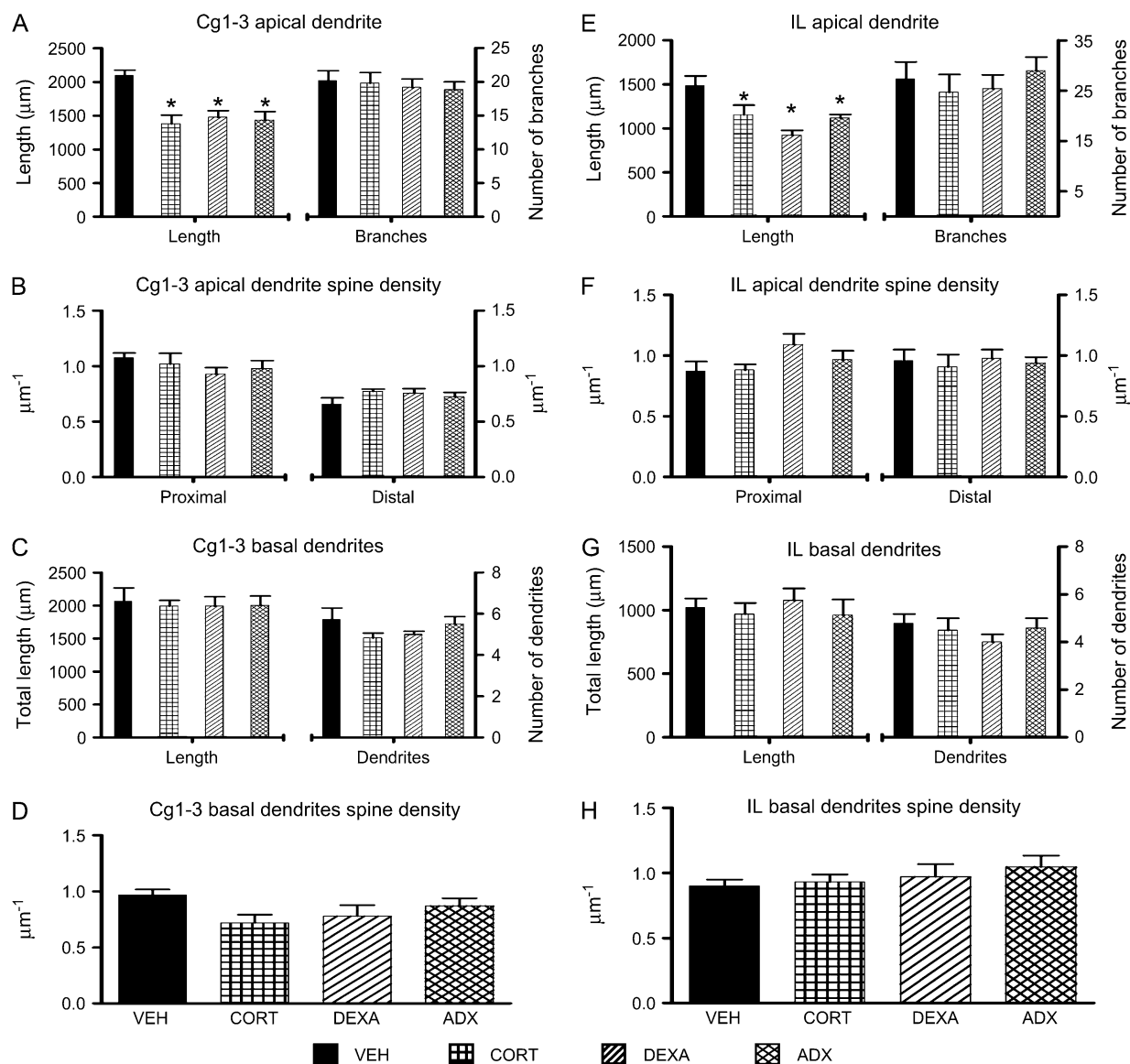


Figure 1. Morphometric analysis of apical dendrites in the mPFC. Note the selective shrinkage of the apical dendrites induced by all treatments. Mean (\pm standard error of mean) total length, branch number, and spine densities in Cg1-3 and IL apical (A, B, E, F) and basal (C, D, G, H) dendrites in layer II/III pyramidal cells. The experiment included 4 treatment groups: VEH—controls (injected with vehicle); CORT—corticosterone-treated group; DEXA—dexamethasone-treated rats; ADX—adrenalectomized animals (injected with vehicle). All treatments lasted 4 weeks and commenced when rats were 8 weeks old. * $P < 0.05$ versus VEH.

DEX: $P = 0.017$; intersections—CORT: $P = 0.045$, DEX: $P = 0.022$). ADX led to a significant reduction of dendritic material at distances between 140 and 220 μm from the soma (length: $P = 0.004$, intersections: $P = 0.030$) but did not affect distal portions of the tree. This reorganization of the dendritic material at the apical dendrites is exemplified in Figures 3 and 4 that illustrate neurons typically seen in each of the 4 treatment groups.

Despite this reorganization, treatments failed to affect the average spine density (proximal— $F_{3,16} = 0.886$, $P = 0.469$, CONT: 1.08 ± 0.04 spines/ μm , CORT: 1.02 ± 0.10 spines/ μm , DEX: 0.93 ± 0.06 spines/ μm , ADX: 0.98 ± 0.07 spines/ μm ; distal— $F_{3,16} = 1.717$, $P = 0.204$, CONT: 0.66 ± 0.05 spines/ μm , CORT: 0.78 ± 0.02 spines/ μm , DEX: 0.76 ± 0.04 spines/ μm , ADX: 0.72 ± 0.04 spines/ μm) (Fig. 1) and the proportion of spines of each morphological category in both proximal and distal segments of the apical dendritic tree (proximal—mushroom: $F_{3,16} = 1.994$, $P = 0.156$; thin: $F_{3,16} = 0.399$, $P = 0.756$; wide: $F_{3,16} = 0.351$,

$P = 0.789$; ramified: $F_{3,16} = 0.661$, $P = 0.588$; distal—mushroom: $F_{3,16} = 0.711$, $P = 0.560$; thin: $F_{3,16} = 0.409$, $P = 0.749$; wide: $F_{3,16} = 0.990$, $P = 0.423$; ramified: $F_{3,16} = 0.569$, $P = 0.644$). (Fig. 5)

Treatment did not exert a significant effect on basal dendritic trees (number: $F_{3,16} = 0.992$, $P = 0.422$, CONT: 5.75 ± 0.53 , CORT: 4.83 ± 0.23 , DEX: 5.00 ± 0.15 , ADX: 5.50 ± 0.36 ; length: $F_{3,16} = 0.416$, $P = 0.744$, CONT: $2071.8 \pm 196.8 \mu\text{m}$, CORT: $1997.3 \pm 81.1 \mu\text{m}$, DEX: $1990.0 \pm 145.0 \mu\text{m}$, ADX: $2001.9 \pm 142.6 \mu\text{m}$; spine density: $F_{3,16} = 2.145$, $P = 0.135$, CONT: $0.97 \pm 0.05 \mu\text{m}$, CORT: $0.72 \pm 0.07 \mu\text{m}$, DEX: $0.78 \pm 0.10 \mu\text{m}$, ADX: $0.87 \pm 0.07 \mu\text{m}$; spine types—mushroom: $F_{3,16} = 0.956$, $P = 0.437$; thin: $F_{3,16} = 2.105$, $P = 0.140$; wide: $F_{3,16} = 1.884$, $P = 0.173$; ramified: $F_{3,16} = 0.980$, $P = 0.427$) (Figs 1 and 5). Sholl analysis of the distribution of basal dendritic material as a function of its distance from the soma did not reveal any between-group differences (length: $F_{3,16} = 1.107$, $P = 0.375$; intersections: $F_{3,16} = 0.738$, $P = 0.545$) (data not shown).

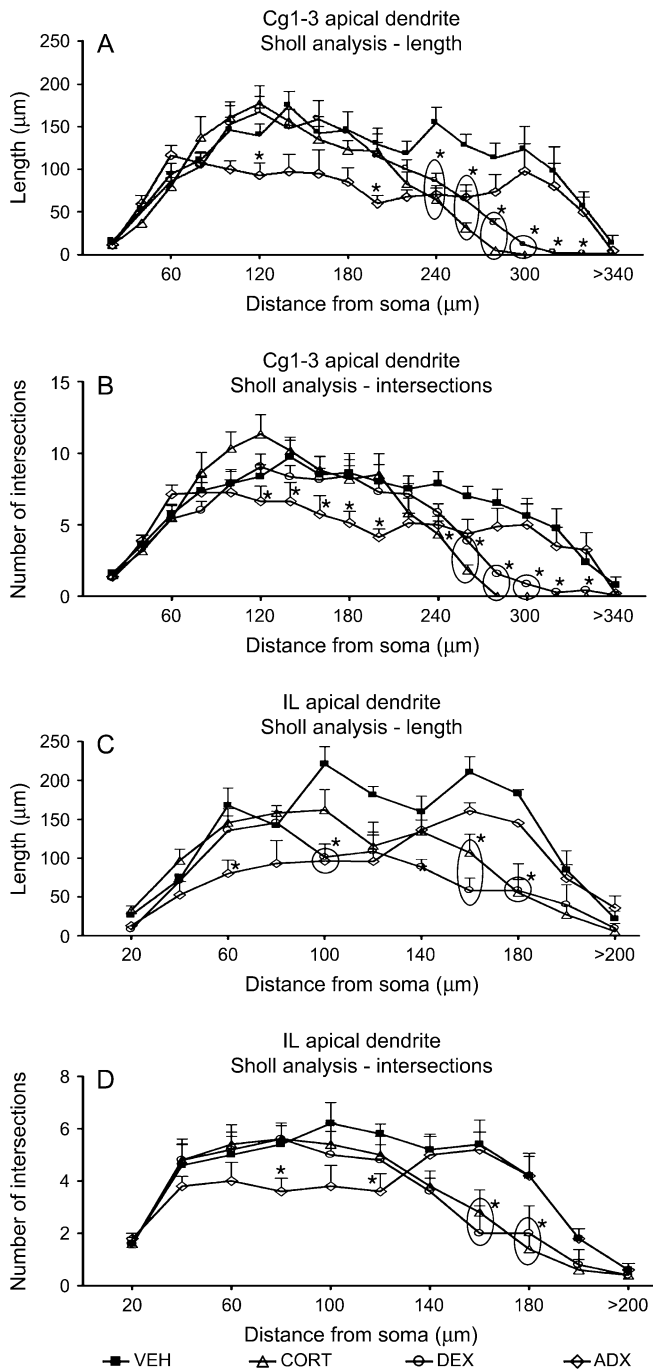


Figure 2. Differential rearrangements of apical dendrites in the mPFC after corticosteroids and ADX. Sholl analysis-derived distribution of mPFC layer II/III pyramidal cell apical dendritic material based on distance from cell body. (A, C) Mean length of apical dendrite branches between consecutive 20- μm -spaced concentric spheres of Cg-prelimbic (Cg1-3) and IL regions. (B, D) Mean number of intersections of apical dendrite branches with consecutive 20- μm -spaced concentric spheres of Cg-prelimbic and IL regions. See Figure 1 for treatment details. * $P < 0.05$ versus VEH. Means and standard error of mean are plotted.

Infralimbic Cortex

The total length of apical dendrites of pyramidal cells of layer III of the IL was significantly reduced by all treatments ($F_{3,16} = 10.725$, $P < 0.001$; CONT: $1492 \pm 105.2 \mu\text{m}$; CORT: $1154 \pm 112.0 \mu\text{m}$ [less 23%], $P = 0.049$; DEX: $825 \pm 53.0 \mu\text{m}$ [less 47%], $P < 0.001$; ADX: $1119 \pm 38.0 \mu\text{m}$ [less 25%], $P = 0.028$); however, the

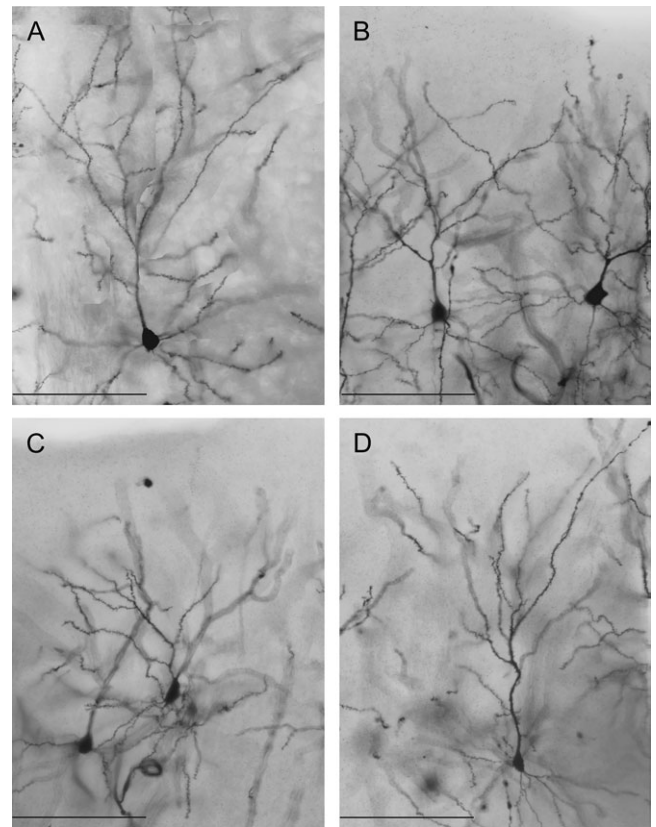


Figure 3. Photomicrographs of neurons of area Cg1-3. Representative examples from VEH-, CORT-, DEX-, and ADX-treated rats (A–D, respectively) are shown. Scale bar represents 200 μm . See Figure 1 for treatment details.

total number of apical branches was not affected by any of the treatments ($F_{3,16} = 0.391$, $P = 0.76$, CONT: 27.40 ± 3.37 , CORT: 24.71 ± 3.54 , DEX: 25.42 ± 2.74 , ADX: 29.00 ± 2.70) (Fig. 1). Sholl analysis of apical dendritic segment lengths and of the number of intersections as a function of their distance from the soma revealed an overall effect of treatment (length: $F_{3,16} = 9.141$, $P = 0.001$; intersections: $F_{3,16} = 4.878$, $P = 0.014$) (Fig. 2). DEX- and CORT-treated rats showed marked reductions of dendritic material at distances greater than 160 μm from the soma when compared with controls (length—CORT: $P = 0.02$, DEX: $P = 0.001$; intersections—CORT: $P = 0.045$, DEX: $P = 0.020$). ADX was accompanied by a reduction of dendritic material between 60 and 120 μm from the soma (length: $P = 0.008$, intersections: $P = 0.033$), but there was no effect on the distal portions of the apical dendritic tree.

Average spine density (proximal— $F_{3,16} = 2.032$, $P = 0.150$, CONT: 0.87 ± 0.08 spines/ μm , CORT: 0.88 ± 0.05 spines/ μm , DEX: 1.09 ± 0.08 spines/ μm , ADX: 0.97 ± 0.07 spines/ μm ; distal— $F_{3,16} = 0.139$, $P = 0.935$, CONT: 0.96 ± 0.09 spines/ μm , CORT: 0.91 ± 0.10 spines/ μm , DEX: 0.98 ± 0.07 spines/ μm , ADX: 0.94 ± 0.04 spines/ μm) (Fig. 1) and the proportion of spine of each morphological category in both proximal and distal segments of the apical dendritic tree were not affected by any treatment (proximal—mushroom: $F_{3,16} = 1.628$, $P = 0.222$; thin: $F_{3,16} = 1.916$, $P = 0.168$; wide: $F_{3,16} = 0.532$, $P = 0.667$; ramified: $F_{3,16} = 0.384$, $P = 0.776$; distal—mushroom: $F_{3,16} = 0.257$, $P = 0.855$; thin: $F_{3,16} = 0.887$, $P = 0.469$; wide: $F_{3,16} = 2.049$, $P = 0.148$; ramified: $F_{3,16} = 1.000$, $P = 0.418$). (Fig. 5)

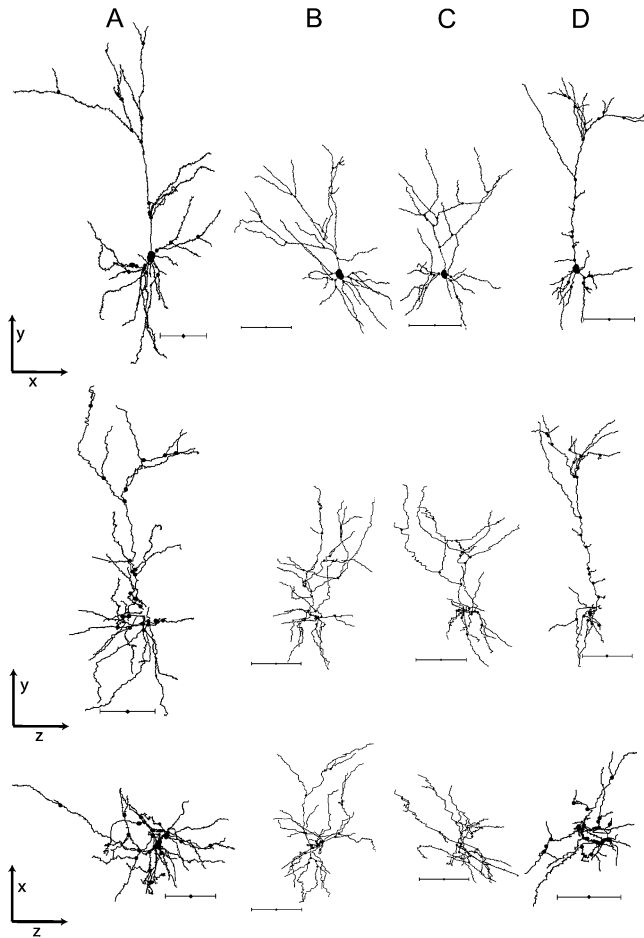


Figure 4. Computer assisted reconstructions of representative neurons. Illustrated are neurons in each of 3 orthogonal planes (XY, YZ, XZ) in the Cg1-3 area from VEH-, CORT-, DEX-, and ADX-treated rats (A–D, respectively). See Figure 1 for treatment details.

Treatment did not exert a significant effect on basal dendritic trees (number: $F_{3,16} = 0.713$, $P = 0.558$, CONT: 4.80 ± 0.37 , CORT: 4.50 ± 0.50 , DEX: 4.00 ± 0.32 , ADX: 4.60 ± 0.40 ; length: $F_{3,16} = 0.329$, $P = 0.804$, CONT: $1026.1 \pm 66.73 \mu\text{m}$, CORT: $971.4 \pm 85.21 \mu\text{m}$, DEX: $1079.1 \pm 92.51 \mu\text{m}$, ADX: $962.7 \pm 123.16 \mu\text{m}$; spine density: $F_{3,16} = 0.708$, $P = 0.561$, CONT: 0.90 ± 0.05 spines/ μm , CORT: 0.93 ± 0.06 spines/ μm , DEX: 0.97 ± 0.10 spines/ μm , ADX: 1.05 ± 0.09 spines/ μm , spine types—mushroom: $F_{3,16} = 0.384$, $P = 0.766$; thin: $F_{3,16} = 1.510$, $P = 0.250$; wide: $F_{3,16} = 1.890$, $P = 0.172$; ramified: $F_{3,16} = 0.616$, $P = 0.614$) (Figs 1 and 5). Sholl analysis of the distribution of basal dendritic material as a function of its distance from the soma failed to reveal any between-group differences (length: $F_{3,16} = 0.070$, $P = 0.975$; intersections: $F_{3,16} = 0.879$, $P = 0.473$) (data not shown).

Discussion

In an attempt to analyze how stress, acting through the mediation of corticosteroids, might induce structural changes in the mPFC, we here carried out a detailed morphometric analysis of dendritic architecture in Golgi-Cox-stained preparations. One principal finding was that chronic administration of corticosteroids selectively induces morphological rearrangements in the apical dendrites of layer II/III pyramidal neurons without affecting the structure of basal dendrites. These

alterations led to an overall remodeling of the pattern of dendritic branching: whereas the number of dendritic branches per neuron was not reduced, corticosteroid treatment led to an increase of dendritic branching in the proximal-to-medial portions of the apical dendrite and, concomitantly, significant reductions in dendritic arborization at the distal portions. With the exception of our present observation that corticosteroids induce significant reductions in the total length of the apical dendrites, our results are consistent with a previous report (Wellman 2001). In an extension of the earlier studies, the experimental design used in this work allowed us to delineate the impact of specific alterations in the corticosteroid milieu in the neuropil of the mPFC.

The selective vulnerability of apical dendrites to manipulations of the corticosteroid milieu, as reported here and by others (see Wellman 2001; Radley et al. 2004; Brown et al. 2005), is particularly interesting. It most likely reflects the topographical distribution of inputs to layer II/III pyramidal cells of the PFC: whereas the soma and basal dendritic tree are innervated by thalamic projections (Shibata 1993), afferents from limbic structures, including the hippocampus, terminate in the superficial layers (Swanson and Cowan 1977), thus making preferential contact with apical dendrites. Furthermore, although both the limbic and thalamic fiber systems are glutamatergic, their post-synaptic actions are primarily mediated by ionotropic N-methyl-D-Aspartate (NMDA) receptors (Rudolf et al. 1996) and metabotropic (α -amino-5-hydroxy-3-methyl-4-isoxazole propionic acid [AMPA]) receptors (Pirot et al. 1994), respectively. Interestingly, a study addressing the expression of NMDA receptors in the rat PFC (Rudolf et al. 1996) has shown that mPFC layer II, where the apical dendrites of pyramidal neurons are located, is more richly endowed with NMDA.R2B-containing receptors than other layers of the same cortex. Importantly, it has been shown that NMDA.R2B plays a crucial role in corticosteroid-induced excitotoxicity (Lu et al. 2003). In contrast, AMPA receptors that transduce thalamic-to-PFC signals are clustered in the basal dendrites and soma and are scarcely localized at the apical dendrite (Vickers et al. 1993); when activated, AMPA receptors can protect neurons against glutamate-induced neurotoxicity (Wu et al. 2004) by stimulating the expression of brain-derived growth factor (Lauterborn et al. 2000).

The pattern of dendritic reorganization observed after sustained exposure to high levels of corticosteroids is comparable with that found in the hippocampal formation (Watanabe et al. 1992; Magarinos et al. 1998; Sousa et al. 1999). Stress-induced apical dendritic atrophy in the hippocampus (CA3 area) was shown to be mediated by glutamate release and subsequent NMDA, but not AMPA receptor, activation (Magarinos and McEwen 1995). It is important to emphasize that the stress response cannot be exclusively attributed to elevated CORT levels, but reports that stress, through the mediatory actions of corticosteroids, can stimulate glutamate release in the mPFC (Takita et al. 2002) strongly suggest that the morphological adjustments observed in the hippocampus and mPFC may be underpinned by similar pathways. Importantly, however, there are other nonexclusive mechanisms putatively implicated in hypercorticalism-induced dendritic atrophy. In fact, it was recently shown that chronic CORT treatment decreases brain-derived neurotrophic factor expression in the PFC (Dwivedi et al. 2006), suggesting that lack of trophic support might also contribute to the herein-observed changes. Another possible mechanism involves corticosteroid-mediated changes in

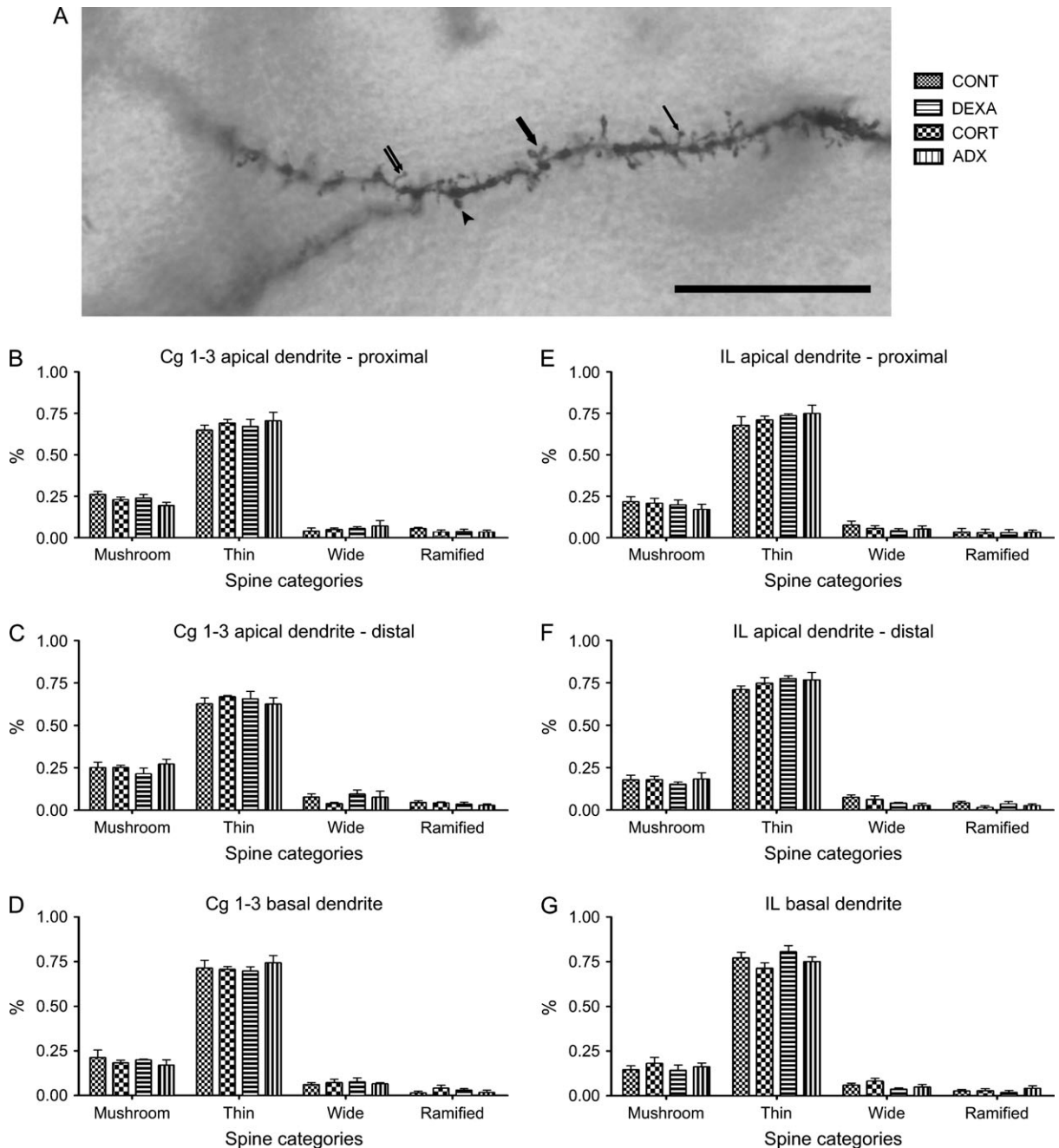


Figure 5. Morphological classification of dendritic spines. (A) High-magnification photomicrograph to exemplify the morphological categories used to classify dendritic spines. Mushroom—single arrow; Thin—double arrow; Wide—arrow head; Ramified—thick arrow. Scale bar represents 10 μ m. (B–G) Mean (\pm standard error of mean) proportion of dendritic spines of each morphological category in Cg1-3 and IL apical (B, C, E, F) and basal (D, G) dendritic trees. See Figure 1 for treatment details.

cytoskeletal proteins because it was previously shown that activation of GR receptors induces changes in the phosphorylation and conformational pattern of the cytoskeletal protein, tau (Green et al. 2006). In addition, altered calcium currents (McEwen 2000), changes in neuronal cell adhesion molecules (Sandi 2004), and serotonergic neuromodulatory activity (Conrad et al. 1996; Stutzmann et al. 1998) are also likely to play a prominent role.

Irrespective of the underlying mechanisms, what might be the functional consequences of stress- or corticosteroid-induced dendritic atrophy in the mPFC? We previously proposed that the structural reorganization that occurs in the

hippocampus following chronic stress may serve an adaptive purpose insofar that it would counteract excessive excitatory inputs that would constrain the eventual regrowth of dendrites and spines upon cessation of the stressor (Sousa and Almeida 2002). A recent report describing the reversibility of stress-induced dendritic plasticity in the mPFC (Radley et al. 2005) suggests that, as in the hippocampus (Sousa et al. 2000), apical dendrite retraction in the mPFC is likely to be of adaptive value.

Atrophy of the PFC and parallel deficits in working memory are commonly found in posttraumatic stress disorder (Rauch et al. 2003), depression (Rajkowska 2000; Rogers et al. 2004), and schizophrenia (Lewis et al. 2004; Shad et al. 2004; Yamasue

et al. 2004); a substantial proportion of patients suffering from these conditions also show dysregulation of endocrine response (corticosteroid secretion) to stress (Altamura et al. 1999; Barden 2004; Ryan et al. 2004). Because the PFC participates in the homeostatic control of corticosteroid secretion (Diorio et al. 1993; Sullivan and Gratton 2002; Mizoguchi et al. 2003) and corticosteroids can, in turn, have profound effects upon PFC-dependent functions (Coburn-Litvak et al. 2003; Roozendaal et al. 2004), it is plausible that corticosteroid-induced atrophy in the PFC feeds into a vicious circle.

Our recent neuroimaging (Cerqueira, Catania, et al. 2005) and histological (Cerqueira, Pego, et al. 2005) studies demonstrated that prolonged administration of the GR agonist DEX causes selective neurodegeneration of the superficial layers of all PFC areas. The results of the present study suggest that those observations of volumetric atrophy in the most superficial layers of the mPFC likely result from the reorganization of apical dendrites of layer II/III pyramidal neurons. One of those earlier studies (Cerqueira, Pego, et al. 2005) also showed that DEX, but not CORT (the natural corticosteroid that displays affinity for both MR and GR), induced neuronal loss in the mPFC and that DEX treatment produced significantly greater cognitive impairment than CORT treatment. As CORT and DEX do not differ significantly in their ability to induce atrophy among the apical dendrites of layer II/III pyramidal neurons, it may be inferred that the herein-observed neuropil changes are mediated by GR; importantly, the further cognitive deterioration produced by DEX should be attributed to the neuronal death-inducing properties of exclusive GR activation, as previously shown in the hippocampus (Sousa et al. 1999).

In this study, we also found that treatment with either CORT or DEX failed to affect the average spine density in 2 distinct regions of the apical dendritic tree of layer II/III pyramidal neurons. Because both treatments resulted in striking reductions of total dendritic length, it is plausible to admit that corticosteroid imbalances lead to decreased total spine number. However, the validity of this assumption must be regarded with care as it might be influenced by 2 factors: 1) because spine densities vary across different portions of the apical dendritic arbor of mPFC pyramidal neurons (Radley et al. 2006), use of randomly selected segments in 2 different regions of the tree might not allow for a generalization of densities in the entire apical dendritic tree; 2) Golgi impregnation techniques assess almost exclusively spines emerging perpendicular to the dendritic shaft and parallel to the plane of the section. As a consequence of the latter, our estimation of spine densities in prefrontal cortical pyramidal neurons is significantly lower (3–4 times) than those obtained with more robust techniques such as 3D reconstruction using confocal (Radley et al. 2006) or serial electron microscopy imaging (Elston et al. 2005); importantly, however, our estimations were of equal magnitude as those obtained by others using a similar approach (Seib and Wellman 2003; Silva-Gomez et al. 2003; Murmu et al. 2006). These technical issues are relevant to understand some of the discrepant observations in the literature regarding spine density in mPFC pyramidal neurons after stress and corticosteroid treatment. Indeed, a previous study using CORT (Seib and Wellman 2003) reported an increased spine density in the proximal region of the dendritic tree (a region endowed with few spines), but not in the distal segments, where we also failed to observe differences. In contrast, in chronic stress models, despite different technical approaches, others (Silva-Gomez et al. 2003;

Radley et al. 2006) found a reduction of spine density in prefrontal pyramidal cells, thus reinforcing the view that, besides technical differences, stress exposure triggers other mechanisms besides elevations of circulating levels of corticosteroids.

This study also sought to identify the structural correlates of impaired performance by corticosteroid-deprived (ADX) animals on tasks (e.g., behavioral flexibility) that depend on the PFC (Mizoguchi et al. 2004; Cerqueira, Pego, et al. 2005). Whereas we did not detect ADX-induced volumetric changes or neuronal losses in our previous work (Cerqueira, Catania, et al. 2005; Cerqueira, Pego, et al. 2005), we here observed that ADX has significant effects on the morphology of apical dendrites in pyramidal layers II/III of the mPFC: ADX rats display decreased branching in the middle third of the dendritic tree (at radial distances, relative to the soma, 120–240 μm in Cg1-3 and 60–120 μm in IL), despite preserved branching patterns at higher radial distances. The topologically different impoverishments observed in ADX versus corticosteroid-treated animals suggest mediation through distinct neurochemical substrates. One important difference between these experimental conditions relies on the glutamatergic system in the PFC: whereas in states of hypercorticalism there is activation of glutamatergic transmission (Takita et al. 2002), ADX is known to reduce glutamate release in the PFC (Moghaddam et al. 1994). Given the putative role of glutamate-mediated excitotoxicity in the neurodegenerative changes herein reported, it is plausible to admit that such differences could contribute for the topographically distinct actions of ADX compared with DEX and CORT treatment.

Altered dopaminergic transmission could also plausibly account for the ADX-induced effects. This hypothesis is based on the following observations: ADX impairs PFC-dependent tasks through a hypodopaminergic status mediated by D1 receptors (Mizoguchi et al. 2000) that show a preferential localization to the proximal portions of the apical dendrite of pyramidal cells in the PFC (Bergson et al. 1995) and whose ablation results in structural changes in the apical dendrites of PFC pyramidal cells (Stanwood et al. 2005). However, because chronic stress can also induce hypodopaminergia (Mizoguchi et al. 2000), the latter alternative should be considered with caution. Another plausible, and not necessarily mutually exclusive, explanation for the difference in neuropil changes between ADX versus CORT-/DEX-treated animals is a lack of trophic support in ADX rats resulting from a process of deafferentation (Mizrahi and Libersat 2002), given the well-known ADX-induced hippocampal degeneration (Sloviter et al. 1989; Sousa et al. 1997).

Without implicating direct clinical relevance, some remarks on the link of the present work to certain disorders may be warranted: 1) schizophrenic patients display a decrease in the thalamo-PFC projections that terminate on the middle layers of the PFC (Lewis et al. 2001), a phenomenon associated with selective atrophy of basal dendritic trees (Shibata 1993) and 2) given the fact that inappropriate adrenocortical activity may be causally related to PFC dysfunction in depressive conditions, we propose that depressed patients most likely display changes in the PFC neuropil that closely resemble those observed in animals treated with corticosteroids.

Notes

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References

- Altamura AC, Boin F, Maes M. 1999. HPA axis and cytokines dysregulation in schizophrenia: potential implications for the antipsychotic treatment. *Eur Neuropsychopharmacol*. 10:1-4.
- Ballmaier M, Toga AW, Blanton RE, Sowell ER, Lavretsky H, Peterson J, Pham D, Kumar A. 2004. Anterior cingulate, gyrus rectus, and orbitofrontal abnormalities in elderly depressed patients: an MRI-based parcellation of the prefrontal cortex. *Am J Psychiatry*. 161:99-108.
- Barden N. 2004. Implication of the hypothalamic-pituitary-adrenal axis in the pathophysiology of depression. *J Psychiatry Neurosci*. 29:185-193.
- Bergson C, Mrzljak L, Smiley JF, Pappy M, Levenson R, Goldman-Rakic PS. 1995. Regional, cellular, and subcellular variations in the distribution of D1 and D5 dopamine receptors in primate brain. *J Neurosci*. 15:7821-7836.
- Brown SM, Henning S, Wellman CL. 2005. Mild, short-term stress alters dendritic morphology in rat medial prefrontal cortex. *Cereb Cortex*. 15:1714-1722.
- Cajal S. 1995. *Histology of the nervous system of man and vertebrates*. New York: Oxford University Press. p. 1672.
- Cerqueira JJ, Catania C, Sotiropoulos I, Schubert M, Kalisch R, Almeida OFX, Auer DP, Sousa N. 2005. Corticosteroid status influences the volume of the rat cingulate cortex—a magnetic resonance imaging study. *J Psychiatr Res*. 39:451-460.
- Cerqueira JJ, Pego JM, Taipa R, Bessa JM, Almeida OFX, Sousa N. 2005. Morphological correlates of corticosteroid-induced changes in prefrontal cortex-dependent behaviours. *J Neurosci*. 25:7792-7800.
- Chao HM, Choo PH, McEwen BS. 1989. Glucocorticoid and mineralocorticoid receptor mRNA expression in rat brain. *Neuroendocrinology*. 50:365-371.
- Coburn-Litvak PS, Pothakos K, Tata DA, McCloskey DP, Anderson BJ. 2003. Chronic administration of CORT impairs spatial reference memory before spatial working memory in rats. *Neurobiol Learn Mem*. 80:11-23.
- Conrad CD, Galea LAM, Kuroda Y, McEwen BS. 1996. Chronic stress impairs rat spatial memory on the Y-maze and this effect is blocked by tianeptine pre-treatment. *Behav Neurosci*. 110:1321-1334.
- Cook SC, Wellman CL. 2004. Chronic stress alters dendritic morphology in rat medial prefrontal cortex. *J Neurobiol*. 60:236-248.
- Cullen TJ, Walker MA, Eastwood SL, Esiri MM, Harrison PJ, Crow TJ. 2006. Anomalies of asymmetry of pyramidal cell density and structure in dorsolateral prefrontal cortex in schizophrenia. *Br J Psychiatry*. 188:26-31.
- Cullinan WE, Herman JP, Battaglia DF, Akil H, Watson SJ. 1995. Pattern and time course of immediate early gene expression in rat brain following acute stress. *Neuroscience*. 64:477-505.
- de Kloet ER, Joels M, Holsboer F. 2005. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci*. 6:463-475.
- Diorio D, Viau V, Meaney MJ. 1993. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J Neurosci*. 13:3839-3847.
- Dwivedi Y, Rizavi HS, Pandey GN. 2006. Antidepressants reverse corticosterone-mediated decrease in brain-derived neurotrophic factor expression: differential regulation of specific exons by antidepressants and corticosterone. *Neuroscience*. 139:1017-1029.
- Elston GN, Benavides-Piccione R, Defelipe J. 2005. A study of pyramidal cell structure in the cingulate cortex of the macaque monkey with comparative notes on inferotemporal and primary visual cortex. *Cereb Cortex*. 15:64-73.
- Feldman ML, Peters A. 1979. A technique for estimating total spine numbers on Golgi-impregnated dendrites. *J Comp Neurol*. 188:527-542.
- Figueiredo HF, Bruetle A, Bodie B, Dolgas CM, Herman JP. 2003. The medial prefrontal cortex differentially regulates stress-induced c-fos expression in the forebrain depending on type of stressor. *Eur J Neurosci*. 18:2357-2364.
- Gibb R, Kolb B. 1998. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *J Neurosci Methods*. 79:1-4.
- Glaser EM, Van der Loos H. 1981. Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. *J Neurosci Methods*. 4:117-125.
- Green KN, Billings LM, Roozendaal B, McGaugh JL, Laferla FM. 2006. Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *J Neurosci*. 26:9047-9056.
- Harris KM, Jensen FE, Tsao B. 1992. Three-dimensional structure of dendritic spines and synapses in rat hippocampus (CA1) at postnatal day 15 and adult ages: implications for the maturation of synaptic physiology and long-term potentiation. *J Neurosci*. 12:2685-2705.
- Herman JP, Ostrander MM, Mueller NK, Figueiredo H. 2005. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. *Prog Neuro-psychopharmacol Biol Psychiatry*. 29:1201-1213.
- Horner CH, Arbuthnott E. 1991. Methods of estimation of spine density—are spines evenly distributed throughout the dendritic field? *J Anat*. 177:179-184.
- Lauterborn JC, Lynch G, Vanderklish P, Arai A, Gall CM. 2000. Positive modulation of AMPA receptors increases neurotrophin expression by hippocampal and cortical neurons. *J Neurosci*. 20:8-21.
- Lewis DA, Cruz D, Eggan S, Erickson S. 2004. Postnatal development of prefrontal inhibitory circuits and the pathophysiology of cognitive dysfunction in schizophrenia. *Ann N Y Acad Sci*. 1021:64-76.
- Lewis DA, Cruz DA, Melchitzky DS, Pierri JN. 2001. Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: evidence for fewer projections from the thalamus. *Am J Psychiatry*. 158:1411-1422.
- Lu J, Goula D, Sousa N, Almeida OF. 2003. Ionotropic and metabotropic glutamate receptor mediation of glucocorticoid-induced apoptosis in hippocampal cells and the neuroprotective role of synaptic N-methyl-D-aspartate receptors. *Neuroscience*. 121:123-131.
- Magarinos 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.
- Magarinos AM, Orchinik M, McEwen BS. 1998. Morphological changes in the hippocampal CA3 region induced by non-invasive glucocorticoid administration: a paradox. *Brain Res*. 809:314-318.
- McEwen BS. 2000. Effects of adverse experiences for brain structure and function. *Biol Psychiatry*. 48:721-731.
- Mizoguchi K, Ishige A, Aburada M, Tabira T. 2003. Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. *Neuroscience*. 119:887-897.
- Mizoguchi K, Ishige A, Takeda S, Aburada M, Tabira T. 2004. Endogenous glucocorticoids are essential for maintaining prefrontal cortical cognitive function. *J Neurosci*. 24:5492-5499.
- Mizoguchi K, Yuzurihara M, Ishige A, Sasaki H, Chui DH, Tabira T. 2000. Chronic stress induces impairment of spatial working memory because of prefrontal dopaminergic dysfunction. *J Neurosci*. 20:1568-1574.
- Mizrahi A, Libersat F. 2002. Afferent input regulates the formation of distal dendritic branches. *J Comp Neurol*. 452:1-10.
- Moghaddam B, Bolinao ML, Stein-Behrens B, Sapolsky R. 1994. Glucocorticoids mediate the stress-induced extracellular accumulation of glutamate. *Brain Res*. 655:251-254.
- Murmu MS, Salomon S, Biala Y, Weinstock M, Braun K, Bock J. 2006. Changes of spine density and dendritic complexity in the prefrontal cortex in offspring of mothers exposed to stress during pregnancy. *Eur J Neurosci*. 24:1477-1487.
- Nugent AC, Milham MP, Bain EE, Mah L, Cannon DM, Marrett S, Zarate CA, Pine DS, Price JL, Drevets WC. 2006. Cortical abnormalities in bipolar disorder investigated with MRI and voxel-based morphometry. *Neuroimage*. 30:485-497.
- Patel PD, Lopez JF, Lyons DM, Burke S, Wallace M, Schatzberg AF. 2000. Glucocorticoid and mineralocorticoid receptor mRNA expression in squirrel monkey brain. *J Psychiatr Res*. 34:383-392.
- Pirou S, Jay TM, Glowinski J, Thierry AM. 1994. Anatomical and electrophysiological evidence for an excitatory amino acid pathway from the thalamic mediodorsal nucleus to the prefrontal cortex in the rat. *Eur J Neurosci*. 6:1225-1234.

- Radley JJ, Rocher AB, Janssen WG, Hof PR, McEwen BS, Morrison JH. 2005. Reversibility of apical dendritic retraction in the rat medial prefrontal cortex following repeated stress. *Exp Neurol*. 196:199-203.
- Radley JJ, Rocher AB, Miller M, Janssen WG, Liston C, Hof PR, McEwen BS, Morrison JH. 2006. Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cereb Cortex*. 16:313-320.
- 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.
- Rajkowska G. 2000. Postmortem studies in mood disorders indicate altered numbers of neurons and glial cells. *Biol Psychiatry*. 48:766-777.
- Rauch SL, Shin LM, Segal E, Pitman RK, Carson MA, McMullin K, Whalen PJ, Makris N. 2003. Selectively reduced regional cortical volumes in post-traumatic stress disorder. *Neuroreport*. 14:913-916.
- Rogers MA, Kasai K, Koji M, Fukuda R, Iwanami A, Nakagome K, Fukuda M, Kato N. 2004. Executive and prefrontal dysfunction in unipolar depression: a review of neuropsychological and imaging evidence. *Neurosci Res*. 50:1-11.
- Roosendaal B, McReynolds JR, McGaugh JL. 2004. The basolateral amygdala interacts with the medial prefrontal cortex in regulating glucocorticoid effects on working memory impairment. *J Neurosci*. 24:1385-1392.
- Rudolf GD, Cronin CA, Landwehrmeyer GB, Standaert DG, Penney JB Jr, Young AB. 1996. Expression of N-methyl-D-aspartate glutamate receptor subunits in the prefrontal cortex of the rat. *Neuroscience*. 73:417-427.
- Ryan MC, Sharifi N, Condren R, Thakore JH. 2004. Evidence of basal pituitary-adrenal overactivity in first episode, drug naive patients with schizophrenia. *Psychoneuroendocrinology*. 29:1065-1070.
- Sandi C. 2004. Stress, cognitive impairment, and cell adhesion molecules. *Nat Rev Neurosci*. 5:917-930.
- Seib LM, Wellman CL. 2003. Daily injections alter spine density in rat medial prefrontal cortex. *Neurosci Lett*. 337:29-32.
- Shad MU, Muddasani S, Prasad K, Sweeney JA, Keshavan MS. 2004. Insight and prefrontal cortex in first-episode schizophrenia. *Neuroimage*. 22:1315-1320.
- Shibata H. 1993. Efferent projections from the anterior thalamic nuclei to the cingulate cortex in the rat. *J Comp Neurol*. 330:533-542.
- Sholl DA. 1956. The measurable parameters of the cerebral cortex and their significance in its organization. *Prog Neurobiol*. 2:324-333.
- Silva-Gomez AB, Rojas D, Juarez I, Flores G. 2003. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Res*. 983:128-136.
- Sloviter RS, Valiquette G, Abrams GM, Ronk EC, Sollas AL, Paul LA, Neubort S. 1989. Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science*. 243:535-538.
- Sousa N, Almeida OF. 2002. Corticosteroids: sculptors of the hippocampal formation. *Rev Neurosci*. 13:59-84.
- Sousa N, Lukoyanov NV, Madeira MD, Almeida OF, 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.
- Sousa N, Madeira MD, Paula-Barbosa MM. 1997. Structural alterations of the hippocampal formation of adrenalectomized rats: an unbiased stereological study. *J Neurocytol*. 26:423-438.
- Sousa N, Paula-Barbosa MM, Almeida OF. 1999. Ligand and subfield specificity of corticoid-induced neuronal loss in the rat hippocampal formation. *Neuroscience*. 89:1079-1087.
- Stanwood GD, Parlaman JP, Levitt P. 2005. Anatomical abnormalities in dopaminergic regions of the cerebral cortex of dopamine D1 receptor mutant mice. *J Comp Neurol*. 487:270-282.
- Stutzmann GE, McEwen BS, LeDoux JE. 1998. Serotonin modulation of sensory inputs to the lateral amygdala: dependency on corticosterone. *J Neurosci*. 18:9529-9538.
- Sullivan RM, Gratton A. 2002. Prefrontal cortical regulation of hypothalamic-pituitary-adrenal function in the rat and implications for psychopathology: side matters. *Psychoneuroendocrinology*. 27:99-114.
- Swanson LW, Cowan WM. 1977. An autoradiographic study of the organization of the efferent connections of the hippocampal formation in the rat. *J Comp Neurol*. 172:49-84.
- Takita M, Kawashima T, Kaneko H, Suzuki SS, Yokoi H. 2002. Sensitization of glutamate release and N-methyl-D-aspartate receptor response by transient dopamine pretreatment in prefrontal cortex of rats. *Neurosci Lett*. 317:97-100.
- Uylings HBM, Groenewegen HJ, Kolb B. 2003. Do rats have a prefrontal cortex? *Behav Brain Res*. 146:3-17.
- Uylings HBM, Ruiz-Marcos A, Van Pelt J. 1986. The metric analysis of three-dimensional dendritic tree patterns: a methodological review. *J Neurosci Methods*. 18:127-151.
- Uylings HBM, van Pelt J. 2002. Measures for quantifying dendritic arborizations. *Network: comput neural syst*. 13:397-414.
- Van Eden CG, Uylings HBM. 1985. Cytoarchitectonic development of the prefrontal cortex in the rat. *J Comp Neurol*. 241:253-267.
- Vickers JC, Huntley GW, Edwards AM, Moran T, Rogers SW, Heinemann SF, Morrison JH. 1993. Quantitative localization of AMPA/kainate and kainate glutamate receptor subunit immunoreactivity in neurochemically identified subpopulations of neurons in the prefrontal cortex of the macaque monkey. *J Neurosci*. 13:2982-2992.
- Watanabe Y, Gould E, Cameron HA, Daniels DC, McEwen BS. 1992. Phenytoin prevents stress- and corticosterone-induced atrophy of CA3 pyramidal neurons. *Hippocampus*. 2:431-435.
- Wellman CL. 2001. Dendritic reorganization in pyramidal neurons in medial prefrontal cortex after chronic corticosterone administration. *J Neurobiol*. 49:245-253.
- Wu X, Zhu D, Jiang X, Okagaki P, Mearow K, Zhu G, McCall S, Banaudha K, Lipsky RH, Marini AM. 2004. AMPA protects cultured neurons against glutamate excitotoxicity through a phosphatidylinositol 3-kinase-dependent activation in extracellular signal-regulated kinase to upregulate BDNF gene expression. *J Neurochem*. 90:807-818.
- Yamasue H, Iwanami A, Hirayasu Y, Yamada H, Abe O, Kuroki N, Fukuda R, Tsujii K, Aoki S, Ohtomo K, et al. 2004. Localized volume reduction in prefrontal, temporolimbic, and paralimbic regions in schizophrenia: an MRI parcellation study. *Psychiatry Res*. 131:195-207.
- Zilles K, Wree A. 1995. Cortex: areal and laminar structure. In: Paxinos G, editor. *The rat nervous system*. 2nd ed. San Diego (CA): Academic Press. p. 649-688.