

Daily injections alter spine density in rat medial prefrontal cortex

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Abstract

Apical dendrites of pyramidal neurons in medial prefrontal cortex are reorganized after chronic corticosterone treatment. In this study, we assessed the effects of chronic corticosterone administration on dendritic spines. Adult male rats received sc injections of either corticosterone or vehicle daily for 3 weeks or were left untreated. Layer II–III neurons in medial prefrontal cortex were stained using a Golgi–Cox procedure and spines were counted on the portions of apical dendrites where maximal corticosterone-induced dendritic changes occur. In vehicle- and corticosterone-treated rats, spine density proximal to the soma was increased relative to untreated rats, while spine density in the distal portion of the apical arbor was unchanged, suggesting that daily handling alone altered spine density. This stress-induced change in spine density likely reflects important functional changes in excitatory synaptic inputs in prefrontal cortex.

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Prefrontal cortex is a target for glucocorticoids involved in the stress response [9], shows neurochemical changes in response to stress (e.g. Ref. [10]), and mediates many of the behaviors that are altered by chronic corticosterone administration (e.g. Refs. [2,17]). Previously, we showed that apical dendrites of pyramidal neurons in medial prefrontal cortex are reorganized after chronic corticosterone treatment: dendritic material proximal to the cell body is increased, while material distal to the soma is decreased [16]. This corticosterone-induced reorganization of apical dendrites likely reflects important functional changes in medial prefrontal cortex. For instance, pyramidal neurons in medial prefrontal cortex tend to segregate inputs, with extracortical afferents (for instance, from the mediodorsal nucleus of the thalamus and hippocampal area CA3) tending to cluster on distal dendrites (i.e. in layer I; [4,15]) and synapses of local cortical circuits tending to cluster on proximal portions of the apical and basilar arbor [13]. Thus, the corticosterone-induced reorganization of the apical dendrites of layer II–III pyramidal neurons in medial prefrontal cortex likely results in a shift in emphasis from subcortical to intracortical information.

Dendritic spines are the principal site of excitatory input

in the neocortex [7], and thus potential stress- or corticosterone-induced changes in spine density could have even more direct and profound effects on cortical function than does dendritic reorganization. Furthermore, alterations in dendritic spines could either amplify the dendritic reorganization or counteract the effects of the reorganization. Therefore, to determine if glucocorticoid-induced dendritic changes in medial prefrontal cortex are paralleled by changes in synaptic connectivity, we assessed the effect of chronic corticosterone administration on the density of spines on apical dendrites of layer II–III pyramidal cells in medial prefrontal cortex.

Adult male Sprague–Dawley rats (175–200 g, approximately 50-days-old at the initiation of the experiment; Harlan Sprague–Dawley, Indianapolis, IN) received sc injections of either corticosterone (RBI, Natick, MA; 10 mg in 250 μ l sesame oil; $N = 8$) or vehicle (250 μ l; $N = 8$) daily for 3 weeks. This dose, based on that of Woolley et al. [18], results in peak plasma corticosterone levels comparable to stress levels, which then fall to non-stress levels within 24 h [5], and is sufficient to produce both pronounced atrophy of apical dendrites of pyramidal neurons in hippocampal area CA3 [18] and reorganization of apical dendrites in medial prefrontal cortex [16]. A third group of rats served as untreated controls ($N = 4$). Rats were group housed (four per cage, each treatment group in

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different cages) 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 2:00 p.m., were approved by the Bloomington Institutional Animal Care and Use Committee, and were carried out in accordance with NIH guidelines.

Approximately 24 h after the final injection, tissue was processed using Glaser and Van der Loos' modified Golgi stain [3], which allows visualization of whole neurons including processes. Animals were overdosed with urethane and perfused with 0.9% saline. Brains were removed and immersed for 14 days in Golgi–Cox solution (a 1:1 solution

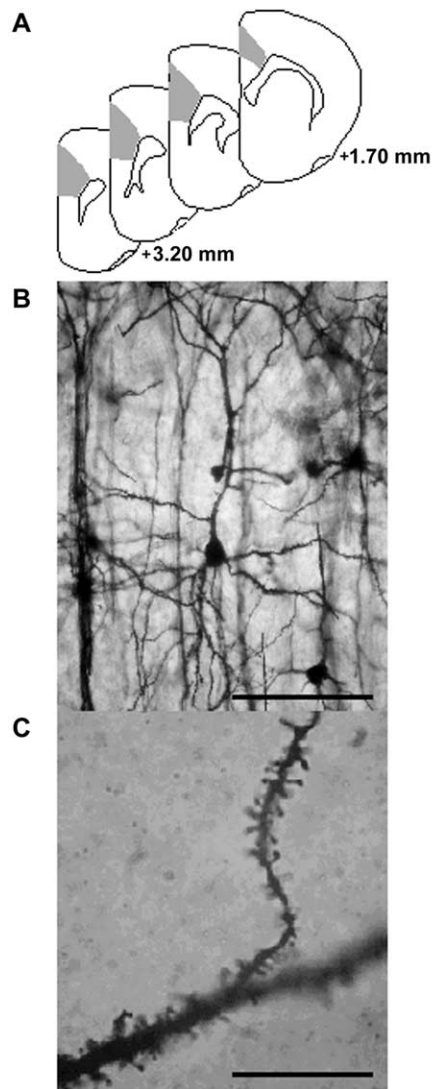


Fig. 1. (A) Schematic diagram of coronal sections through rat prefrontal cortex. Shaded areas indicate portions of area Cg1-3 from which neurons were sampled. Coordinates indicate position relative to bregma [11]. (B) Photomicrograph of Golgi-stained neuron in layer II–III of medial prefrontal cortex in an untreated rat. Scale bar = 100 μm . (C) Photomicrograph showing spines on apical dendrite of a layer II–III pyramidal cell in medial prefrontal cortex. Scale bar = 20 μm .

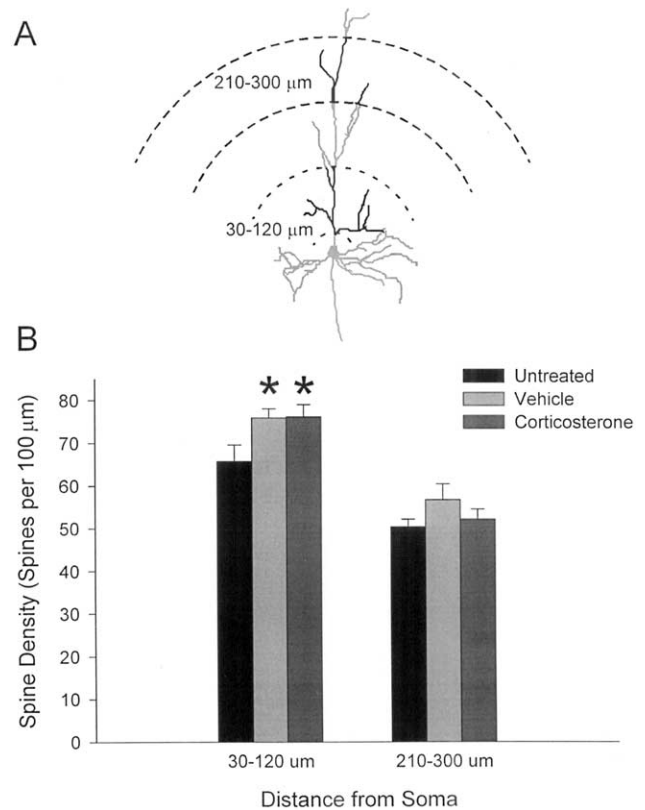


Fig. 2. (A) Segments of apical dendrites at either 30–120 μm or 210–300 μm from the soma (shown in black) were drawn. (B) Mean spine density on the proximal (30–120 μm) and distal (210–300 μm) portions of apical dendrites for untreated, vehicle-, and corticosterone-treated rats. Vertical bars represent SEM values. Asterisks indicate significant difference relative to untreated controls.

of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with 5% potassium chromate). Brains were then dehydrated and embedded in celloidin; coronal sections were cut at 145 μm on a sliding microtome (American Optical 860). Free-floating sections were alkalinized in ammonia, developed in Dektol (Kodak), fixed in Kodak Rapid Fix, dehydrated, cleared, mounted, and coverslipped [3].

Spines were counted on pyramidal neurons in layer II–III of medial prefrontal cortex (Zilles and Wree's Cg1 and Cg3; [19]; Fig. 1A). Both the Cg1-3 area and layer II–III are readily identified in Golgi-stained material based on location and cytoarchitecture [1,19]. Pyramidal neurons were defined by the presence of a basilar dendritic tree, a distinct, single apical dendrite, and dendritic spines (Fig. 1B).

For each neuron, a template consisting of two concentric circles with radii of either 30 and 120 μm or 210 and 300 μm was centered on the soma, and the segments of apical dendrites falling between these two concentric spheres were drawn (see Fig. 2A). These distances were chosen because they include the portions of the arbor where maximal corticosterone-induced dendritic changes occur [16]. Spines

were then counted on these portions of the apical dendrite at $600\times$ using a computer-based neuron tracing system (NeuroLucida, MicroBrightfield) with the experimenter blind to condition. Spines were identified based on the morphological criteria for ‘mushroom’ and ‘thin’ spines described by Peters and Kaiserman-Abramof [12]: only protrusions perpendicular to the dendritic shaft and possessing a clear neck and bulbous head were counted (Fig. 1C). These two spine types make up approximately 81% of the spine population in rat neocortex. For each animal, spines were counted on 15–20 neurons; this number yielded a mean within-animal error of $6 \pm 1\%$, and thus was considered to provide a valid and representative sample of spine density in layer II–III pyramidal neurons in medial prefrontal cortex. Length of apical dendrites proximal (between 30 and 120 μm) and distal to the soma (between 210 and 300 μm) was recorded, and spine density (spines per 100 μm) was then calculated for each neuron.

In all treatment groups, complete impregnation of numerous cortical pyramidal neurons was apparent (Fig. 1B), and layer II–III was readily identifiable. In addition, spines were readily identifiable at a magnification of $600\times$ (Fig. 1C).

To assess changes in dendritic spines, average spine density proximal and distal to the soma were compared in untreated, vehicle-, and corticosterone-treated rats. Two-way ANOVA (treatment \times location in arbor) revealed a significant effect of treatment [$F(2, 1) = 4.51$, $P < 0.05$; Fig. 2]. Post-hoc analyses (Fisher’s PLSD) indicated that spine density proximal to the soma was increased by approximately 16% in both vehicle- and corticosterone-treated rats relative to untreated rats, [$t(13) = 10.19$ and 10.84 , respectively, P ’s < 0.05], while spine density in the distal portion of the apical arbor was not significantly altered in either vehicle- or corticosterone-treated rats [$t(13) = 9.38$ and 5.45 , respectively, ns]. To confirm that differences in spine density were not due to differential sampling across branch orders, mean dendritic length across branch orders was compared among groups. Two-way ANOVAs (treatment \times branch order) indicated that the distribution of branch orders of dendritic segments sampled were not significantly different across treatment groups [for proximal dendrites, $F(2, 6) = 0.44$, ns; for distal dendrites, $F(2, 6) = 0.49$, ns].

Our results demonstrate alterations in spine density on the apical dendrites of layer II–III neurons in medial prefrontal cortex resulting from both chronic corticosterone administration and injections of vehicle alone. In both groups, spine density proximal to the soma was increased relative to untreated rats, while spine density in the distal portion of the apical arbor was unchanged. Thus, the stress of daily subcutaneous injections appears to alter spine density on apical dendrites of layer II–III pyramidal neurons, and this effect is not amplified by supplemental injections of corticosterone. This effect contrasts with our previous study, in which alterations in apical dendritic material in vehicle-treated animals were intermediate to those of corticoster-

one-treated animals [16], suggesting that spine morphology may be more sensitive to stress than is dendritic morphology. This possibility is consistent with studies showing restraint stress-induced increases in spine density on area CA3 apical dendrites [14] as well as increases in spine density on area CA1 neurons resulting from daily intraperitoneal saline injections [6]. Given that both vehicle and corticosterone were administered via injection, the present study does not allow us to discriminate between the effects of corticosterone versus the effects of injection alone. However, a study using a noninvasive method of administration, which will allow the dissociation of corticosterone from stress effects, is currently under way.

Previously, we demonstrated that 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 [16]. Our current data indicate that changes in dendritic spine density on the proximal portion of the apical arbor parallel proximal dendritic changes, while dendritic atrophy distal to the soma is not accompanied by significant alterations in spine density. Given that spines are the principal site of excitatory input in the neocortex [7], the stress-induced increase in spine density documented here combined with the previously-documented corticosterone-induced increases in apical dendritic material likely results in a massive increase in total number of excitatory synapses on the proximal portions of the apical dendrites. On the other hand, the previously-documented decrease in dendritic material distal to the soma [16] without a change in spine density could result in a net loss of excitatory synapses. This reorganization of excitatory connectivity likely results in important functional changes in medial prefrontal cortex, and may contribute to stress-induced changes in cognition.

Finally, changes in spine density proximal but not distal to the soma suggest different mechanisms for dendritic changes in these two locations. For instance, given that changes in synaptic activity have been shown to alter spine density [8], stress-induced increases in spine density proximal to the soma may be mediated by increases in the activation of local cortical afferents, which tend to cluster on proximal portions of the apical arbor [13].

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