Chronic Unpredictable Stress Decreases Cell Proliferation in the Cerebral Cortex of the Adult Rat

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Background: One of the most consistent morphologic findings in postmortem studies of brain tissue from depressed patients is a decrease in the number of glia in the prefrontal cortex. However, little is known about the mechanisms that contribute to this decrease in cell number.

Methods: To address this question, we subjected adult rats to chronic stress, a vulnerability factor for depression, and measured cell proliferation as a potential cellular mechanism that could underlie glial reduction in depression.

Results: We found that exposure to chronic unpredictable stress (CUS) for 15 days significantly decreased cell proliferation in neocortex by approximately 35%. This effect was dependent on the duration, intensity and type of stress, and was region-specific. Analysis of cell phenotype demonstrated that there was a decrease in the number of oligodendrocytes and endothelial cells. Finally, using a CUS paradigm that allows for analysis of anhedonia, we found that chronic antidepressant administration reversed the decrease in cortical cell proliferation, as well as the deficit in sucrose preference.

Conclusion: These findings are consistent with the possibility that decreased cell proliferation could contribute to reductions in glia in prefrontal cortex of depressed subjects and further elucidate the cellular actions of stress and antidepressants.

Key Words: Antidepressant, cell proliferation, chronic stress, depression, endothelial cells, NG2-glia

Depression is a severe neurobiological disorder that affects between 15 and 20 percent of the population at some point in life (1). Despite intensive research on the pathophysiology of depression, the neurobiological substrates underlying the disorder remain poorly characterized. A consistent finding in postmortem studies of brain tissue from depressed patients is a decrease in the density and number of glia in cortical regions, including the prefrontal and cingulate areas (2–7). These studies also report a reduction in the size of neuronal cell bodies but no change in the number of neurons. Such morphologic changes may contribute to the decreased cortical volume observed in brain imaging studies (8–10) and may underlie, in part, the cognitive dysfunction that is a core symptom of depression. Collectively, these observations support the hypothesis that cellular alterations in the cerebral cortex contribute to the clinical symptoms of depression (11–14).

One experimental approach to study the mechanisms and functional consequences of the cellular changes in depression is to use animal models that are based on exposure to repeated stress. A leading model of depression has evolved around the dynamic changes in hippocampal structure and function that are observed after repeated stress, including dendritic atrophy, cell loss, and decreases in cell proliferation and neurogenesis (15). Furthermore, antidepressants can block or reverse the cellular changes induced by chronic stress (12,16,17). Cell proliferation is also observed in the neocortex, although it appears to be restricted largely to nonneuronal populations under physiologic conditions (18–21).

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Received June 22, 2006; revised February 1, 2007; accepted February 5, 2007.

We have recently shown that chronic antidepressant treatment increases cell proliferation in the rat prelimbic cortex and that a substantial fraction of these cells are glia (22,23). Based on this finding and the observed reduction in glial number in depressed patients, we hypothesized that chronic stress would lead to a decrease in cell proliferation within the rodent prefrontal cortex that could be reversed by antidepressant treatment. Evidence is presented using a chronic unpredictable mild stress (CUS) model that supports this hypothesis.

Methods and Materials

Animals and Drug Treatments

Male Sprague–Dawley rats (300–350 g; Charles River, Massachusetts) were pair-housed in wire bottom cages under a 12-hour light–dark cycle at constant temperature (25°C) and humidity with free access to food and water. Animal use procedures were in accordance with the Yale University care and use of laboratory animals guidelines. Animals were administered subcutaneous corticosterone (CORT; 40 mg/kg; Sigma, St. Louis, Missouri) for 15 days (24) or intraperitoneal (IP) fluoxetine (FLX; 5 mg/kg; Lilly, Indianapolis, Indiana) as previously reported (22,25).

To label dividing cells for proliferation studies, bromodeoxyuridine (BrdU; 100 mg/kg IP; Sigma) was administered 4, 10, and 20 hours after the last restraint or CUS stressor, or CORT injection was given as previously reported (26). For a survival study, animals received six injections of BrdU at 12-hour intervals during the last 3 days of stress and were killed 21 days later.

Chronic Stress Paradigms, Behavior, and CORT Levels

For CUS, animals are exposed to a variable sequence of mild, unpredictable stressors, preventing habituation (27,28) and compared with acute or chronic restraint stress (CRS; disposable rodent restrainers, Braintree Scientific, Braintree, Massachusetts). The exact stressors and sequence used for each experiment are shown in Table 1. These stress paradigms increased blood levels of the stress-responsive hormone, corticosterone (Table 2). Control animals were handled every 2 days during weighing. For antidepressant treatment reversal, animals were first exposed to CUS (15 days) and then administered FLX or saline for 21 days

0006-3223/07/$32.00
doi:10.1016/j.biopsych.2007.02.006

Table 1. Daily Schedules for the Chronic Unpredictable Stress (CUS)

Groups	CUS-8	CUS-8/4	CUS-15	CUS-35
Stressors/day	2/day	4/day	2/day	2/day
Duration of CUS	8 days	8 days	15 days	35 days
Cold 4°C 1 hour	D1; (1)	D1, 3, 5; (3)	D1, 13; (2)	D2, 8, 14, 16, 26, 33; (6)
Swim stress 18°C 10 min	D2, 4, 8; (3)	D2, 4, 6, 8; (4)	D2, 4, 8, 11; (4)	D5, 10, 17, 22, 27, 34; (6)
Rotation 1 hour	D2, 3, 4, 7; (4)	D1, 2, 3, 4, 5, 6, 7, 8 (8)	D2, 3, 5, 7, 9, 12, 14, 15; (8)	D1, 3, 13, 20, 28, 30, 32; (7)
Isolation overnight	D6; (1)	D1, 4, 5, 7; (4)	D6, 10, 12; (3)	D4, 6, 13, 18, 21, 31, 33, 35; (8)
Food/water deprivation overnight	D6; (1)	D2, 4, 6; (3)	D6, 9, 13; (3)	D6, 10, 17, 22, 25, 30; (6)
Light on overnight	D1, 4, 8; (3)	D1, 3, 5, 6, 7; (5)	D1, 4, 8, 11, 14; (5)	D1, 8, 15, 27, 32; 34; (6)
Light off 3 hours	D3, 5, 7; (3)	D2, 3, 7, 8; (4)	D3, 5, 7, 10, 15; (5)	D2, 11, 18, 23, 28, 31, 35; (7)
Odor overnight				D7, 12, 20, 24; (4)
Stroboscope overnight				D7, 11, 19, 26, 29; (5)
Wet bedding overnight				D4, 9, 15, 21, 25, 29; (6)
Crowding overnight				D5, 12, 14, 19, 23; (5)
Tilt 45°C overnight				D3, 9, 16, 24; (4)

Shown are the types, duration, and number (in parenthesis) of stressors per day for each procedure.

with continued CUS (total of 35 days; see Table 1). For sucrose consumption in the latter experiment, animals were initially exposed for 48 hours to a palatable sucrose solution (1%) to avoid neophobia. On day 35, 4 hours after water deprivation, sucrose preference was then determined by 1-hour exposure to two identical bottles filled with either sucrose solution or water (adapted from 29). Sucrose preference was defined as the ratio of the volume of sucrose versus water consumed during the 1-hour test. Levels of CORT (ng/mL) were measured in plasma isolated from blood that was collected by intracardiac puncture of anesthetized rats (400 mg/mL chloral hydrate) before perfusion (R&D Systems, Minneapolis, Minnesota).

Immunohistochemistry

For BrdU immunohistochemistry, anesthetized animals (400 mg/kg chloral hydrate) were perfused and brains sectioned (40 μm) as previously reported (22,23). For Ki-67, brains were removed, frozen, and stored at −80°C until sectioning (14 μm). BrdU immunostaining was conducted as previously described [22,23). Briefly, free-floating sections were denatured, incubated with blocking buffer, and then with mouse anti-BrdU antibody (1:100; Becton Dickinson, San Jose, California) in blocking buffer overnight (4°C). Sections were then exposed to biotinylated horse antimouse antibody (1:200) (Vector Laboratories, Burlingame, California; 1 hour), followed by amplification and visualization with diaminobenzidine according to manufacturers specifications (Vector Laboratories).

For double-labeling, sections were incubated with rat anti-BrdU (1:100; Accurate; Harlan Olac, Bicester, United Kingdom; 2 days, 4°C) and one of the following antibodies: rabbit anti-glial acidic fibrillary protein (1:100; GFAP, Dako, Glostrup, Denmark), mouse anti-rat endothelial cell antigen-1 (1:500; RECA-1, Serotec, Oxford, United Kingdom), mouse antirat NG2 (1:200; Chemicon International, Temecula, California), mouse anti-receptor interacting protein (1:1000; Developmental Studies Hybridoma Bank, University of Iowa, Iowa) or rabbit anti-ionized calcium binding adaptor-1 (1:100; Wako Pure Chemical Industries, Richmond, Virginia) as previously reported (22, 23). Sections were then exposed (1 hour) to the antibodies Alexa Fluor 546 goat anti-mouse (1:200), Alexa Fluor 488 goat antirat (1:200), and Alexa Fluor 546 goat antirabbit (1:200; Molecular Probes, Eugene, Oregon).

For Ki-67-immunostaining, fresh-frozen sections were fixed with 4% paraformaldehyde for 20 min, washed in phosphate buffered saline (PBS), incubated for 30 min in blocking buffer (10% normal goat serum in .3% Triton X-100, PBS) and exposed overnight (4°C) to rabbit polyclonal anti-Ki-67 antibody (1:100; Novocastra Laboratory, Newcastle, United Kingdom) in blocking buffer. After washing, sections were incubated in biotinylated goat antirabbit (1:200; Vector Laboratories) in blocking buffer for 1 hour, followed by amplification and visualization with diaminobenzidine (Vector Laboratories).

Table 2. Levels of Plasma Corticosterone

Group	Corticosterone Plasma Concentration ng/mlL
CTR	84.1 ± 10.4
CRS-15	214.2 ± 23.4[a]
CUS-15	265.9 ± 59.4[a]
CTR	130 ± 13
CORT-15	265.7 ± 56.1[a]
CTR	64 ± 12.4
CUS-35	353.3 ± 58.4[a]
CUS-35+FLX	223.3 ± 28.3[b]

CTR, control animals; CUS-15- and 35, animals that underwent 15- and 35-day chronic unpredictable stress, respectively.

The results are expressed as ng/mL of plasma and are the mean ± SEM.

[a] $p < .01$ compared with the respective CTR.

[b] $p < .05$ compared with CUS-35 (analysis of variance and Newman–Keuls' post hoc test).

Quantification of Labeled Cells

The number of BrdU- or Ki-67-labeled cells was determined in every eighth section throughout the region of interest as described previously (22). Using this spacing ensures that the same cell will not be counted in two sections. The number of sections analyzed for each region was as follows: prelimbic and primary motor cortices, 8 sections (coordinates: 4.20–2.20 mm from bregma (30); cingulate cortex, 6 sections (2.7–1.2 mm from bregma); and the dorsal striatum, 4 sections (1.70–1.0 mm from bregma). Using Stereo Investigator software (MicroBrightField, Williston, Vermont), a 1 × 1 mm^2 contour was placed over the region of interest (Figure 1A) and all BrdU-positive cells within the contour were counted under high-power magnification (400×). For the dentate gyrus, every 10th section throughout the entire brain region was analyzed as previously reported (22,25).