Archival Report

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Chronic Stress Increases Prefrontal Inhibition: A Mechanism for Stress-Induced Prefrontal Dysfunction

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ABSTRACT

BACKGROUND: Multiple neuropsychiatric disorders, e.g., depression, are linked to imbalances in excitatory and inhibitory neurotransmission and prefrontal cortical dysfunction, and are concomitant with chronic stress.

METHODS: We used electrophysiologic (n = 5–6 animals, 21–25 cells/group), neuroanatomic (n = 6–8/group), and behavioral (n = 12/group) techniques to test the hypothesis that chronic stress increases inhibition of medial prefrontal cortex (mPFC) glutamatergic output neurons.

RESULTS: Using patch clamp recordings from infralimbic mPFC pyramidal neurons, we found that chronic stress selectively increases the frequency of miniature inhibitory postsynaptic currents with no effect on amplitude, which suggests that chronic stress increases presynaptic gamma-aminobutyric acid release. Elevated gamma-aminobutyric acid release under chronic stress is accompanied by increased inhibitory appositions and terminals onto glutamatergic cells, as assessed by both immunohistochemistry and electron microscopy. Furthermore, chronic stress decreases glucocorticoid receptor immunoreactivity specifically in a subset of inhibitory neurons, which suggests that increased inhibitory tone in the mPFC after chronic stress may be caused by loss of a glucocorticoid receptor–mediated brake on interneuron activity. These neuroanatomic and functional changes are associated with impairment of a prefrontal-mediated behavior. During chronic stress, rats initially make significantly more errors in the delayed spatial win-shift task, an mPFC-mediated behavior, which suggests a diminished impact of the mPFC on decision making.

CONCLUSIONS: Taken together, the data suggest that chronic stress increases synaptic inhibition onto prefrontal glutamatergic output neurons, limiting the influence of the prefrontal cortex in control of stress reactivity and behavior. Thus, these data provide a mechanistic link among chronic stress, prefrontal cortical hypofunction, and behavioral dysfunction.

Keywords: Chronic variable stress, GABA, Glucocorticoid receptor, mIPSC, Prefrontal cortex, Stress

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An imbalance between excitatory and inhibitory neurotransmission is proposed to underlie multiple neuropsychiatric disorders, including major depressive disorder (MDD), schizophrenia, epilepsy, anxiety, Parkinson's disease, and bipolar disorder (1–4). Many of these disorders are comorbid with chronic stress and hypercortisolemia (5–10), which suggests that impaired neurotransmission occurs in the context of altered stress hormone signaling. Importantly, these disorders are accompanied by prefrontal cortical dysfunction (11–14). Moreover, studies in rodents indicate that the ventromedial prefrontal cortex is critical for control of neuroendocrine stress responses, and the infralimbic subdivision is particularly important for chronic stress regulation (15–19). In combination, these lines of evidence suggest that chronic stress may lead to prefrontal cortical dysfunction and stress-related physiologic and emotional pathologies.

Morphologic analysis of the medial prefrontal cortex (mPFC) after chronic stress suggests altered neuronal excitability associated with decreases in the dendritic complexity of pyramidal neurons (20–25) and increases in interneuron dendritic arborization (26). Stress-induced decreases in glutamatergic neuronal complexity in layer V of the mPFC correlate with reduced excitatory neural responses to serotonin, which suggests that chronic stress may affect prefrontal-mediated behaviors (27,28). Morphologic studies are supported by behavioral data suggesting that chronic stress disrupts multiple aspects of mPFC-dependent behaviors, including behavioral flexibility, strategic planning, rule learning, behavioral inhibition, and spatial memory (22,28–36). Collectively, the data suggest that chronic stress may inhibit mPFC output, compromising the functionality of the mPFC.

In this study, we employ an interdisciplinary approach to test the hypothesis that chronic stress shifts the balance between excitatory and inhibitory neurotransmission toward greater inhibition of infralimbic prefrontal glutamatergic output neurons, resulting in impaired prefrontal-mediated behavior. Our data indicate that chronic stress increases inhibition of

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infralimbic cortex (ilPFC) glutamatergic output neurons via an increase in gamma-aminobutyric acid (GABA) release, likely owing to increased GABAergic innervation of glutamatergic output neurons. Stress-induced inhibition of infralimbic pyramidal cells is accompanied by impaired acquisition of a delayed spatial win-shift (DSWS) contingency, which provides evidence of impaired prefrontal cortical control of behavior.

METHODS AND MATERIALS

Ex Vivo

Subjects. Male Sprague Dawley rats (Harlan, Indianapolis, IN), aged 54–57 days or 200–225 g upon arrival, were doubly housed throughout the experiment in a temperature- and humidity-controlled room on a 14- and 10-hour light/dark cycle. Food (Teklad, Harlan) and water were available ad libitum. All experimental procedures described subsequently were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Animals and were approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Chronic Variable Stress. Half of the animals were unhandled (naive; $n = 5–6$) or underwent chronic variable stress (CVS) for 14 days ($n = 5–6$) beginning at about postnatal day 60 (to obviate developmental confounds). The CVS was composed of twice-daily (AM and PM) repeated and unpredictable stressors, including cold swims (10 minutes, 16–18°C), warm swims (20 minutes, 30–32°C), cold room exposure (1 hour, 4°C), shaker stress (1 hour, 100 rpm), open field (5 minutes), and hypoxia (30 minutes, 8% oxygen) (19). The same combination and sequence of stressors were used for all CVS experiments throughout the current study and has consistently led to attenuated body weight gain and adrenal hypertrophy, markers of a successful CVS regimen (19).

Electrophysiology

Patch Clamp. Whole-cell patch clamp recordings were obtained from layer V pyramidal neurons in the ilPFC, which were easily identifiable in the slice on the basis of somal morphology and the presence of a prominent apical dendrite. Miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) were recorded in the presence of tetrodotoxin (500 nmol/L). We

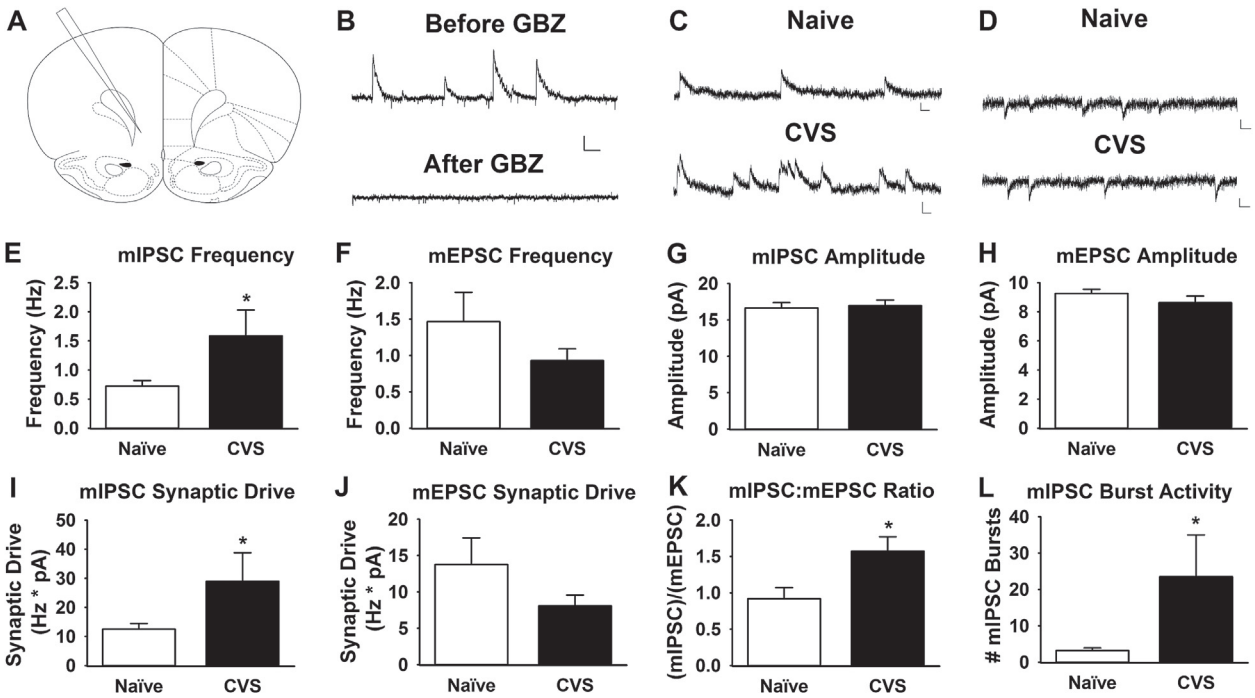

Figure 1. Chronic stress increases inhibitory neurotransmission in the infralimbic cortex. **(A)** Large pyramidal (>100 picofarad [pF] on average) neurons were recorded from layer V of the infralimbic prefrontal cortex near bregma +3.2 to +2.2 (37). **(B)** The mIPSCs recorded at a holding potential of 0 mV before and after 10 μmol/L gabazine (GBZ) (gamma-aminobutyric acid type A receptor antagonist) bath application. Scale bars = 20 pA (vertical scaling) and 125 ms (horizontal scaling). **(C, D)** Representative miniature inhibitory postsynaptic current (mIPSC) and miniature excitatory postsynaptic current (mEPSC) traces, respectively, from naive and chronically stressed animals. Scale bars = 10 pA (vertical scaling) and 25 ms (horizontal scaling). **(E, F)** The mIPSC and mEPSC frequencies in slices from naive and chronically stressed animals (CVS). Chronic stress significantly increases mIPSC frequency ($p < .05$), with no effect on mEPSC frequency ($p > .05$). **(G, H)** The mIPSC and mEPSC amplitudes in slices from naive and chronically stressed animals. There are no statistical differences in mIPSC ($p > .05$) or mEPSC ($p > .05$) amplitudes in chronically stressed vs. naive animals. **(I, J)** Chronic stress increased mIPSC synaptic drive ($p < .05$), with no effect of stress on excitatory synaptic drive in slices from chronically stressed animals ($p > .05$). **(K)** The ratio of mIPSC to mEPSC frequency in each cell was significantly shifted toward more mIPSCs after chronic stress ($p < .05$). **(L)** Bursting, defined as a cluster of three or more mIPSCs separated by less than 150 ms, was also significantly higher in chronically stressed animals ($p < .05$) ($n = 21–25$ cells/group, 5 animals/group). *$p < .05$.