# Glial Loss in the Prefrontal Cortex Is Sufficient to Induce Depressive-like Behaviors

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**Background:** Postmortem studies have repeatedly found decreased density and number of glia in cortical regions, including the prefrontal and cingulate areas, from depressed patients. However, it is unclear whether this glial loss plays a direct role in the expression of depressive symptoms.

**Methods:** To address this question, we characterized the effects of pharmacologic glial ablation in the prefrontal cortex (PFC) of adult rats on behavioral tests known to be affected by stress or antidepressant treatments: sucrose preference test (SPT), novelty suppressed feeding test (NSFT), forced swim test (FST), and two-way active avoidance test (AAT). We established the dose and time course for the actions of an astrocyte specific toxin, L-alpha-aminoadipic acid (L-AAA), and compared the behavioral effects of this gliotoxin with the effects of an excitotoxic (ibotenate) lesion and to the effects of chronic stress.

**Results:** The results demonstrate that L-AAA infusions induced anhedonia in SPT, anxiety in NSFT, and helplessness in FST and AAT. These effects of L-AAA were similar to chronic unpredictable stress (CUS)-induced depressive-like behaviors in these tests. However, ibotenate-induced neurotoxic lesion of the PFC had no effect in these behavioral tests.

**Conclusions:** The results demonstrate that glial ablation in the PFC is sufficient to induce depressive-like behaviors similar to chronic stress and support the hypothesis that loss of glia contributes to the core symptoms of depression.

**Key Words:** Anhedonia, glia, gliotoxin, helplessness, prefrontal cortex, stress

Brain imaging studies have reported that both the hippocampus and prefrontal cortex (PFC) undergo selective volume reductions in several stress-related neuropsychiatric illnesses, particularly in major depressive disorder (MDD) (1–3). Growing evidence suggests that glial loss and neuronal atrophy may contribute to these volume reductions and may underlie, in part, the cognitive dysfunction that is a core symptom of depression (4,5). Indeed, one of the most consistent findings in postmortem studies of depressed patients is a decrease in the density and number of glia, as well as a reduction in the size of neuronal cell bodies, in cortical regions, including the prefrontal and cingulate areas (6–10). The decreases in glial density are accompanied by a reduction of astrocytic markers, such as glial fibrillary acidic protein (GFAP) (11) and glutamine synthetase (12). These observations support the hypothesis that cellular alterations in the PFC contribute to the symptoms of depression (5,13–16), but it is unclear whether glial changes are a consequence or cause of illness.

To address this question, we first characterized the effects of chronic unpredictable stress (CUS), a well-documented animal model of depression (17,18), on the density of astrocytes in the rat prelimbic cortex (PLC) and on standard behavioral tests of depression and anxiety. We then investigated the effects of pharmacologic glial ablation in the PFC in the same behavioral tests. For these studies, we used L-alpha-aminoadipic acid (L-AAA), a gliotoxin specific for astrocytes (19,20). L-alpha-aminoadipic acid enters cells via a sodium ($Na^+$)-dependent transporter and blocks essential cellular functions involving glutamate, including protein synthesis and energetic metabolism, thereby inducing glial death (20). L-alpha-aminoadipic acid infusions in vivo induce a transitory ablation of astrocytes (2–4 hours and lasts for ~3 days) (19,21–23). We also compared the effects of L-AAA with a neurotoxic lesion in the PFC. The results demonstrate that CUS reduces glial density in the PFC and that the pharmacologic depletion of cortical astrocytes, but not neurons, produces anhedonia and helplessness, similar to the behavioral consequences of CUS.

## Methods and Materials

### Animals

Male Sprague-Dawley rats (Charles River, Massachusetts) were housed under a 12-hour light/12-hour dark cycle at constant temperature (25°C) with free access to food and water except when animals were subjected to light disturbance or deprivation stressors during the CUS procedure. Animals weighed 250 g to 300 g at the beginning of the experiment. Animal use procedures were in accordance with the Yale University Care and Use of laboratory animals (YACUC) guidelines.

### CUS Procedure

Chronic unpredictable stress is an experimental procedure in which animals are exposed to a variable sequence of 10 mild and unpredictable stressors, 2 per day for 35 days. The stressors were cage rotation, light on, light off, cold stress, isolation, food and water deprivation, stroboscope, cage tilt, and odor. This stress sequence was adapted from our recent study (24).

### Surgery and Toxin Infusions

Cannula guides were implanted into the PLC (coordinates: anteroposterior + 3.2, dorsolateral −.5, depth −4 from bregma) of adult rats anesthetized with xylazine/ketamine (80/6 mg/kg, intramuscular [IM]) using a stereotaxic frame. After 1 week of recovery, drugs and saline were infused bilaterally into the PFC using a 1 mm projection cannula injector once daily for 2 days at a rate of .15 μL/min. The volume of infusion was .5 μL for each

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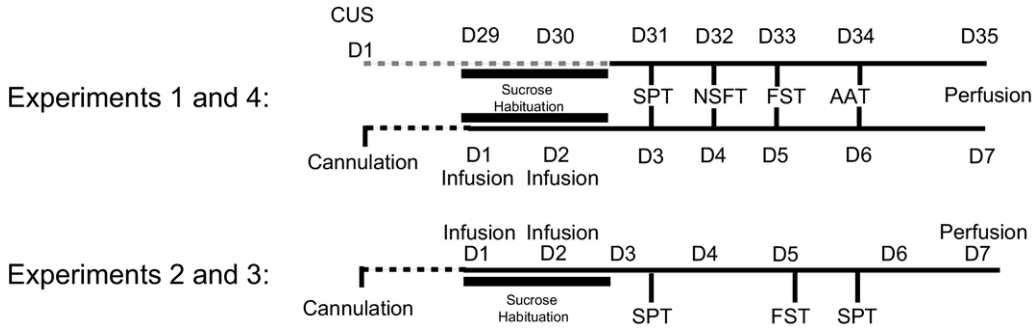


**Figure 1.** Experimental design. Experiment 1, animals were subjected to the chronic unpredictable stress (CUS) procedure and tested in sucrose preference test (SPT), novelty suppressed feeding test (NSFT), forced swim test (FST), and active avoidance test (AAT). Experiment 4, animals were infused with saline, L-AAA, or ibotenate and then subjected to the same four behavioral tests. Experiments 2 and 3, animals were infused with 50 μg/μL and 100 μg/μL of L-AAA into the PFC (experiment 2) or with 100 μg/μL of L-AAA or 5 μg/μL ibotenate (7 days after cannula implantation) and were then subjected to the SPT and FST immediately followed by a second SPT. L-AAA, L-alpha-aminoadipic acid; PFC, prefrontal cortex.

side. The number of animals per group is indicated after exclusion of all animals showing incorrect cannula placement determined by histology.

Two doses of 50 μg/μL and 100 μg/μL of L-AAA (Sigma, St. Louis, Missouri) were used for this study and are within the range used in the literature (19,21,23). Ibotenic acid (Sigma) was infused twice at a dose of 5 μg/μL to obtain a lesion similar to previous reports (25) and to keep the number of infusions consistent with that used for L-AAA.

### Time Course and Experimental Design

Experiment 1 (Figure 1) examined the influence of CUS compared with home cage control animals (HCC, handled daily) on the sucrose preference test (SPT), novelty suppressed feeding test (NSFT), forced swim test (FST), and two-way active avoidance test (AAT) on 4 consecutive days ($n$ = 8 per group). All behavioral tests were performed during the day to avoid bias of dark-cycle locomotor activity changes (26). The order of the tests was chosen to minimize test interactions by introducing the least stressful tasks first (fluid and food deprivation) and the more stressful/invasive tasks last (swimming and foot shock). Animals were perfused on day 35, 24 hours after the AAT, and density of astrocytes in the PLC was quantified.

Experiment 2 examined the dose and time course of L-AAA effects on SPT and FST (Figure 1). Animals were infused with 50 μg/μL or 100 μg/μL L-AAA on day 1 and day 2. On day 3, SPT was measured, and on day 5, animals were tested in FST followed by a second SPT to determine if the gliotoxin lesion was still effective.

Experiment 3 utilized the same protocol to compare the effects of gliotoxic with neurotoxic lesions in the PFC. Animals were infused with L-AAA (100 μg/μL) or ibotenate (5 μg/μL) and tested in SPT and FST, followed by a second SPT.

Experiment 4 utilized the same toxin infusion conditions in another cohort of animals and examined SPT, NSFT, FST, and AAT (same tests as CUS in experiment 1). In all studies, locomotor activity was measured on day 3 and right before the SPT. Animals were perfused on day 7.

### Behavioral Tests

Locomotor activity was measured for 30 min in a cage equipped with automated activity meters (Digiscan animal activity monitor; Omnitech Electronics, Columbus, Ohio) as previously described (27).

Sucrose preference test was conducted as previously described (25,28,29). Briefly, animals were habituated for 48 hours to 1% sucrose (Sigma), and following a 4-hour deprivation period, then preference for sucrose (1%) or water (identical bottles) was determined for 1 hour. The second SPT did not include sucrose habituation.

Novelty suppressed feeding test was performed after 24 hours of food deprivation in an open field, and latency to feed was determined as previously described (27). Home cage food intake was also measured as a control.

Forced swim test is a standard test used as a screen for antidepressant-like compounds (28,29), and immobility was determined in the present study as previously described (27,30).

Active avoidance test was performed in shuttle boxes (Med Associates, St. Albans, Vermont) as previously described (30). Briefly, animals received 30 randomized escapable foot shocks (.65 mA), with the first 5 trials requiring one crossing to terminate the foot shock (FR-1) and the remaining 25 trials requiring two crossings (FR-2) (31–33).

### Immunohistochemistry

Processing and immunohistochemistry were conducted as previously described (24,25). Briefly, postfixed brains were sectioned (40 μm sections, coordinates 4.7 to 1.7 mm from bregma), and every sixth section was mounted on polylysine slides for neuronal nuclei (NeuN) or GFAP immunochemistry using mouse anti-neuron-specific nuclear protein (NeuN; 1:1000, Chemicon, Temecula, California) or rabbit anti-glial fibrillary acidic protein antibody (GFAP; 1:500, DAKO, Glostrup, Denmark) (25,34).

### Quantification

Density of GFAP-positive cells was measured on six to seven sections per animal (at the coordinates indicated for each experiment) using Stereo Investigator software (MicroBrightField, Williston, Vermont) and a 1 × .5 mm square contour placed over the PLC. All GFAP-positive cells within the contour were counted under high magnification. Results are expressed as mean ± SEM number of GFAP-positive cells per $mm^2$.

### Statistical Analysis

Statistical differences were determined by analysis of variance (ANOVA) (Statview 5, SAS Institute Inc., Cary, North Carolina) followed by Scheffe post hoc analysis. The $F$ values and experimental degrees of freedom are included in Results. For experi-