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Cocaine Self-Administration and Extinction Leads to Reduced Glial Fibrillary Acidic Protein Expression and Morphometric Features of Astrocytes in the Nucleus Accumbens Core

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ABSTRACT

BACKGROUND: As a more detailed picture of nervous system function emerges, diversity of astrocyte function becomes more widely appreciated. While it has been shown that cocaine experience impairs astroglial glutamate uptake and release in the nucleus accumbens (NAc), few studies have explored effects of self-administration on the structure and physiology of astrocytes. We investigated the effects of extinction from daily cocaine self-administration on astrocyte characteristics including glial fibrillary acidic protein (GFAP) expression, surface area, volume, and colocalization with a synaptic marker.

METHODS: Cocaine or saline self-administration and extinction were paired with GFAP Westerns, immunohistochemistry, and fluorescent imaging of NAc core astrocytes (30 saline-administering and 36 cocaine-administering male Sprague Dawley rats were employed). Imaging was performed using a membrane-tagged lymphocyte protein tyrosine kinase-green fluorescent protein (Lck-GFP) driven by the GFAP promoter, coupled with synapsin I immunohistochemistry.

RESULTS: GFAP expression was significantly reduced in the NAc core following cocaine self-administration and extinction. Similarly, we observed an overall smaller surface area and volume of astrocytes, as well as reduced colocalization with synapsin I, in cocaine-administering animals. Cocaine-mediated reductions in synaptic contact were reversed by the β-lactam antibiotic ceftriaxone.

CONCLUSIONS: Multiple lines of investigation indicate that NAc core astrocytes exist in a hyporeactive state following cocaine self-administration and extinction. Decreased association with synaptic elements may be particularly meaningful, as cessation of chronic cocaine use is associated with changes in synaptic strength and resistance to the induction of synaptic plasticity. We hypothesize that the reduced synaptic colocalization of astrocytes represents an important maladaptive cellular response to cocaine and the mechanisms underlying relapse vulnerability.

Keywords: Astrocyte, Cocaine, Colocalization, GFAP, Nucleus accumbens, Self-administration

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Astrocytes are critically involved in a wide range of physiological processes in the nervous system, including synapse formation, synaptic transmission, neuronal energy metabolism, extracellular ion homeostasis, blood flow, and sleep (1–3). In addition, disruption in astrocyte-mediated modulation of neuronal function has been implicated in a wide range of disease processes, including schizophrenia, depression, and addiction (4). Supporting a role for astrocytes in plasticity associated with drug seeking, Bull *et al.* (5) have shown that activating Gq-coupled signaling via designer receptors exclusively activated by designer drugs (DREADD) receptors selectively expressed in nucleus accumbens (NAc) core astrocytes inhibits motivation to seek ethanol. Similarly, activating glial Gq-DREADD receptors in the NAc core decreases cued cocaine seeking, an effect mediated by group II metabotropic glutamate receptors (6). In agreement with the proposed role for astrocytes in addiction-related processes, Turner *et al.* (7) reported that transgenic overexpression of a dominant negative component of vesicular release machinery in astrocytes leads to deficits in cocaine reinstatement and conditioned place preference. Collectively, these and other studies support a role for astrocytes in the cellular mechanisms of addiction [for review, see (8)].

A seminal feature of cocaine-induced adaptations in astrocytes is decreased expression and activity of the high-affinity glial glutamate transporter (GLT-1) (9). In general, decreased expression of GLT-1 is associated with an increase in reactive astrogliosis following injury, ischemia, and neural degeneration

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(10–12), raising the hypothesis that NAc astrocytes may exist in a state of reactive astrogliosis following self-administration and extinction. Indeed, astrocyte activation has been reported following noncontingent administration of cocaine, methamphetamine, and opiates (13–16) and is associated with methamphetamine-induced neurotoxicity and opiate-induced hypersensitivity to pain (17,18). Further, astrocyte activation is generally characterized by increased expression of the intermediate filament protein, glial fibrillary acidic protein (GFAP) (19). Thus, we hypothesized that operant cocaine self-administration and extinction would similarly impact GFAP expression as well as structural aspects of astrocyte physiology.

However, results from a series of complementary approaches indicated opposite effects of cocaine self-administration. Cocaine self-administration and extinction training rendered NAc astrocytes in a state characterized by decreased GFAP expression, surface area, volume, and decreased colocalization with synapsin I. The cocaine-mediated decrease in synaptic colocalization was reversed by administration of ceftriaxone, a compound previously shown to restore expression of GLT-1, normalize extrasynaptic glutamate levels, and impair cocaine reinstatement (9,20). These data expand the spectrum of the adaptations that occur in response to chronic cocaine exposure in the central nervous system and infer broad implications toward a more complete understanding of the cocaine-induced adaptations in synaptic communication and plasticity responsible for relapse vulnerability.

METHODS AND MATERIALS

Animals and Surgical Procedures

Male Sprague Dawley rats (200–250 g) were purchased from both Harlan (Boston, MA) and Charles River (Indianapolis, IN). They were housed individually in a temperature-controlled environment on a 12-hour reverse light cycle. Following approximately 1 week of environmental acclimation, animals were anesthetized with ketamine (100 mg/kg) and xylazine (7 mg/kg), together with ketorolac analgesic (0.28–0.32 mg/kg). A 13-cm Bio-sil Silastic catheter (0.02 inner diameter, 0.047 outer diameter [Dow Corning, Midland, MI]) was implanted into the right jugular vein, exiting the back attached to a 22-gauge cannula (Plastics One, Wallingford, CT). Prophylactic antibiotic (Timentin 10 mg/0.1 mL, intravenous; GlaxoSmithKline, Research Triangle Park, NC) was administered during surgery and 4 days postoperatively. Catheters were flushed daily with heparin (0.1 mL, 100 U/mL) until the end of self-administration.

For imaging of green fluorescent protein (GFP)-labeled astrocytes, lymphocyte protein tyrosine kinase (Lck)-GFP expressed under the control of the GfaABC1D promoter in adeno-associated virus type 5 (21) was microinjected immediately following catheterization into the nucleus accumbens (1.0 μL per side, 7.3×10^{12} particles/mL at 0.1 μL/min) followed by a >15-minute diffusion time, and microinjectors were slowly removed over a period of 1 to 2 minutes. Virus was microinjected into the NAc core with 26-gauge injectors at a 6° angle at the following coordinate (mm): +1.5 anterior/posterior, +2.6 medial/lateral, −7.2 dorsal/ventral (22). Lck plasmid provided by Baljit Khakh (University of California, Los Angeles) was packaged into adeno-associated virus type 5 by the University of North Carolina at Chapel Hill Viral Vector core.

Behavioral Training

All operant training (2 hours/session) was performed in standard rat modular test chambers (Med Associates, St. Albans, VT) at the same time daily. Before onset of cocaine self-administration, animals received a food training session (10 to 12 hours) in which an active lever press resulted in the administration of a single 45 mg food pellet (Bio-Serv, Flemington, NJ). Following food training, animals were maintained on ~20 g chow per day. Self-administration of cocaine was performed on a fixed-ratio 1 schedule, 0.2 mg per infusion paired with a light and tone (70–72 dB). Each infusion was followed by a 20-second time-out period. Infusions were capped to 40 for the first 2 days of self-administration and unrestricted thereafter. Criteria for self-administration was 10 days of at least 10 cocaine infusions received, followed by 14 to 16 sessions of extinction training. Cocaine was provided by the National Institute on Drug Abuse Drug Supply Program. Ceftriaxone (100 mg/kg, intraperitoneal) (Hospira Worldwide, Inc., Lake Forest, IL) or vehicle (sterile saline) was administered 30 minutes before the last 10 extinction sessions.

Western Blotting and Immunohistochemistry

For Western blotting, animals were rapidly decapitated 24 hours after the last extinction training session. Tissue was homogenized in 0.32 mol/L sucrose, 10 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.4) and protease/phosphatase inhibitor cocktail (Thermo Scientific, Waltham MA). Following centrifugation at 1000 *g* for 10 minutes, the supernatant was separated into a crude membrane fraction and a crude cytosolic fraction by centrifugation at 12,000 *g* for 20 minutes. Protein content in the S2 cytosolic fraction was determined by the bicinchoninic acid method (Thermo Scientific) and 10 μg was separated on BisTris gels (Bio-Rad, Hercules, CA) and transferred to polyvinylidene fluoride membranes, then probed using anti-GFAP (ab7779, 1:1000; Abcam, Cambridge, MA) and anti-glyceraldehyde 3-phosphate dehydrogenase (1:2000; Cell Signaling, Danvers, MA).

For GFAP immunohistochemistry, animals were deeply anesthetized with pentobarbital 24 hours after the last extinction session and perfused with 150 mL 1× phosphate buffer (PB) followed by 200 mL 4% paraformaldehyde in 1× PB. Brains were postfixed overnight in 4% paraformaldehyde in 1× PB. Brains were then transferred to 30% sucrose in 1× PB containing 0.1% sodium azide for 1 to 2 days at 4°C, then transferred to 1× phosphate buffered saline (PBS) and sliced 45 μm on a cryostat. Serial sections (every six sections, approximately 2.6 to 1.0 mm anterior to bregma) were stained for GFAP and visualized using vasoactive intestinal peptide peroxidase substrate as follows: slices were blocked with 10% normal goat serum (NGS) in PBS with Tween (PBST) for an hour and probed at room temperature overnight in anti-GFAP (ab7779, 1:1000; Abcam) in PBST containing 3% NGS. Slices were washed three times (10 minutes each) in PBST containing 3% NGS and