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d-LSD-induced c-Fos expression occurs in a population of oligodendrocytes in rat prefrontal cortex ☆

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Abstract

Induction of mRNA or protein for immediate–early genes, such as c-*fos*, is used to identify brain areas, specific cell types, and neuronal circuits that become activated in response to various stimuli including psychoactive drugs. The objective of the present study was to identify the cell types in the prefrontal cortex in which lysergic acid diethylamide (d-LSD) induces c-Fos expression. Systemic administration of d-LSD resulted in a dose-dependent increase in c-Fos immunoreactivity. Although c-Fos-positive cells were found in all cortical layers, they were most numerous in layers III, IV, and V. d-LSD-induced c-Fos immunoreactivity was found in cells co-labeled with anti-neuron-specific enolase or anti-oligodendrocyte Oligo1. The Oligo1-labeled cells had small, round bodies and nuclear diameters characteristic of oligodendrocytes. Studies using confocal microscopy confirmed colocalization of c-Fos-labeled nuclei in NeuN-labeled neurons. Astrocytes and microglia labeled with glial fibrillary acidic protein antibody and OX-42 antibody, respectively, did not display LSD-induced c-Fos expression. Pyramidal neurons labeled with anti-neurofilament antibody also did not show induction of c-Fos immunoreactivity after systemic d-LSD administration. The present study demonstrates that d-LSD induced expression of c-Fos in the prefrontal cortex occurs in subpopulations of neurons and in oligodendrocytes, but not in pyramidal neurons, astrocytes, and microglia.

Keywords: d-LSD; c-Fos; Oligodendrocytes; Neurons; Prefrontal cortex; Immunocytochemistry

1. Introduction

Since the serendipitous discovery of lysergic acid diethylamide (d-LSD) by Albert Hoffman more than 60 years ago, hallucinogens have emerged as major drugs of abuse with special appeal for adolescents and young adults. In humans, administration of d-LSD results in alterations in consciousness, perception, cognition, and mood (Hofmann 1959; Abraham et al., 1996; Halpern and Pope 2003; Nichols 2004). The mechanism by which d-LSD elicits these responses as well as the brain regions involved is unclear. Elucidating these issues would be beneficial with regard to treating the abuse liability of d-LSD and also may provide insight into various forms of mental illness including anxiety, depression and psychosis.

The serotonin 5-HT_{2A} (5-hydroxytryptamine) receptor subtype plays a prominent role in the behavioral effects of d-LSD and related hallucinogens such as the phenethylamines 2,5-dimethoxy-4-methylamphetamine (DOM) and 1-(2,5-dimethoxy-4-iodophenyl)-aminopropane (DOI). The relevance of the serotonergic system in the subjective effects of these compounds was shown initially by the ability of serotonergic antagonists to block the discriminative stimulus effects of d-LSD and DOM (Winter, 1978). Later, the specific involvement of the 5-HT_2 receptor family was implicated based on the high correlation between agonist affinity at this receptor

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subtype and both potency in substituting for DOM-induced stimulus control as well as hallucinogenic potency in man (Glennon et al., 1983, 1984). Using antagonist correlational analysis as well as selective serotonergic receptor antagonists, subsequent studies demonstrated that activation of the 5-HT_{2A} receptor subtype is essential for the stimulus effects of d-LSD and related hallucinogens in rodents (Schreiber et al., 1994; Fiorella et al., 1995; Eckler et al., 2003; Winter et al., 2004). Furthermore, in humans the subjective effects of the indoleamine hallucinogen, psilocybin, were blocked by a 5-HT_{2A} receptor antagonist (Vollenweider et al., 1998).

Immediate–early genes, such as c-*fos*, are involved in various CNS functions including learning and memory as well as sensory processing (Morgan and Curran, 1991; Kovács, 1998; Guzowski, 2002). In addition, induction of immediate–early gene mRNA and protein also have been used to identify brain areas, specific cell types, and neuronal circuits that become activated in response to various stimuli including psychoactive drugs (Kovács, 1998; Guzowski et al., 2005). d-LSD and the hallucinogen DOI have been shown to induce expression of c-*fos* mRNA and to increase levels of c-Fos protein in various cortical and limbic areas (Abi-Saab et al., 1999; Scruggs et al., 2000; Gresch et al., 2002; Frankel and Cunningham, 2002; Zhai et al., 2003; Nichols et al., 2003). The hallucinogenic effects of d-LSD would appear to involve the cerebral cortex as a positive correlation was found between the subjective effects of psilocybin and its ability to increase cerebral metabolic rates of glucose in the prefrontal and temporomedial cortices (Vollenweider et al., 1997). In addition, the anterior cingulate cortex, a component of the prefrontal cortex, appears to be involved in mediating the discriminative stimulus effects of d-LSD (Gresch et al., 2007). Similarly, induction of c-Fos expression was reported in the prefrontal, frontoparietal, and somatosensory cortices after administration of d-LSD and DOI (Abi-Saab et al., 1999; Scruggs et al., 2000; Gresch et al., 2002; Zhai et al., 2003). However, injection of a low dose of d-LSD increased the number of c-Fos-positive cells in the anterior cingulate cortex, but not in the frontal or parietal cortices (Frankel and Cunningham, 2002).

The induction of c-Fos mRNA and protein by d-LSD and DOI are blocked by 5-HT_{2A} receptor antagonists but not by 5-HT_{1A} or 5-HT_{2C} receptor antagonists (Scruggs et al., 2000; Gresch et al., 2002; Nichols et al., 2003). Thus, similar to the behavioral effects of these hallucinogens, induction of c-Fos expression also requires activation of 5-HT_{2A} receptors. 5-HT_{2A} receptors are widely distributed throughout the CNS and are expressed in high density in the neocortex (Pazos et al., 1985; López-Giménez et al., 1997; Cornea-Hébert et al., 1999). Double labeling studies, however, show that the increase in c-Fos immunoreactivity does not occur in cells expressing 5-HT_{2A} receptors (Maćkowiak et al., 1999; Scruggs et al., 2000; Gresch et al., 2002). The objective of the present study was to identify the cell types in the prefrontal cortex in which d-LSD induces c-Fos expression. These studies demonstrate that in addition to increasing c-Fos immunoreactivity in neurons, d-LSD also induced c-Fos expression in oligodendrocytes.

2. Materials and methods

2.1. Animal model

For the experiments in this study, 35 male Fischer 344 rats (Harlan Sprague–Dawley Inc., Indianapolis, IN), were used at 12 weeks of age when they weighed approximately 300 g. Rats were housed in pairs with free access to food and water and were exposed to a 12-h light–dark cycle. For the eight days prior to the d-LSD treatment, rats were handled 15 min/day to reduce stress-induced c-Fos expression incurred by handling and immobilization (Ceccatelli et al., 1989; de Medeiros et al., 2005). Rats received an intraperitoneal injection of either saline (control) or (+)-d-LSD (+)-tartrate (2:1) [0.1, 0.25, 0.5, 1.0 mg/kg] which was generously provided by the National Institute on Drug Abuse (Rockville, MD). d-LSD was diluted in 0.9% bacteriostatic saline (w/v) to a total volume of 0.25 ml.

All animal treatment in this study was in accordance with US Public Health Service Policy on Humane Care and Use of Laboratory Animals as amended August 2002. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo.

2.2. Perfusion and tissue preparation

Ninety minutes after the injection, rats were anesthetized with 100 mg/kg sodium pentobarbital and perfused through the aorta with an 80 ml bolus of physiological saline to which 5 ml of heparin was added followed by 300 ml of 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The 90 min pretreatment time was previously shown to result in maximal expression of c-Fos (Kovács, 1998; Chaudhuri et al., 2000). Brains were removed immediately after perfusion and postfixed for 24 h. Forebrains were cryoprotected in 30% (w/v) sucrose in 0.1 M phosphate buffer (pH 7.4) and coded to prevent possible investigator bias.

To remove the entire rat prefrontal cortex, the forebrain was grossly dissected by making a coronal incision immediately anterior to the optic chiasm. The rat prefrontal cortex is located anterior to the motor region of the frontal lobe (Lewis, 2004) and includes frontal cortex area 2 and the medial prefrontal cortex. The medial prefrontal cortex is further subdivided into the anterior cingulate cortex, the prelimbic cortex, and the infralimbic cortex (Uylings and van Eden, 1990; Mogensen and Holm, 1994; Uylings et al., 2003). Isolation of the prefrontal cortex was confirmed in preliminary studies by demonstrating the absence of granule neurons in layer IV cortical areas using toluidine blue staining (Van Eden and Uylings, 1985).

2.3. Immunocytochemistry

Forebrains were completely sectioned in the coronal plane at 40 μm on a rotary microtome with a freezing stage (Hacker Industries, Fairfield, NJ). Every 10th section was retained yielding about 100 sections from each (~4 mm) forebrain for

analysis. Of these, 20 free-floating sections (every fifth section) were selected for staining. The immunocytochemical staining procedure was as follows. Sections were washed thrice in phosphate-buffered saline (PBS) and incubated in 0.3% (v/v) hydrogen peroxide in PBS for 30 min to eliminate endogenous peroxidase activity. Sections were blocked at room temperature for 2 h in PBS containing 2% (w/v) bovine serum albumin (BSA) and 0.25% (v/v) Triton X-100 and then incubated for 12 h at 3 °C in PBS containing 1% (w/v) BSA, 0.25% (w/v) Triton X-100, and the appropriate primary antibodies.

For the d-LSD-dose–responses studies, sections were incubated with an antibody to c-Fos (1:10,000; Calbiochem, San Diego, CA), washed three times in PBS, and then secondarily labeled with a Vectastain Elite ABC horseradish peroxidase kit (Vector Laboratories, Burlingame, CA). Labeling was visualized with the chromagen 3,3'-diaminobenzidine (DAB) (Vector Laboratories Burlingame, CA). Stained sections were rinsed with distilled water, mounted on slides, dried, and coverslipped with Permount (Fisher, Fair Lawn, NJ).

For the cellular localization studies, sections were incubated simultaneously with the polyclonal antibody for c-Fos and a monoclonal antibody that was specific for each of the cell types. Neurofilament 160 (1:400; Sigma-Aldrich, St. Louis, MO), neuron-specific enolase (1:40; Serotec Inc., UK), and NeuN (1:500; Chemicon, UK) antibodies were used to identify neurons. Olig1 (1:50; Sigma-Aldrich, St. Louis, MO) and anti-2',3'-cyclic nucleotide-3'-phosphodiesterase (1:500; Sigma-Aldrich, St. Louis, MO) antibodies were used to identify oligodendrocytes. Glial fibrillary acidic protein antibody (1:400; Sigma-Aldrich, St. Louis, MO) was used to identify astrocytes. OX-42 antibody (1:100; Serotec Inc., UK) was used to identify microglia. Sections were washed five times and incubated for 1 h at room temperature in PBS containing both an Alexa Fluor® 568 secondary antibody (1:200; Invitrogen, Carlsbad, CA) to visualize c-Fos positive cells and an Alexa Fluor® 488 secondary antibody (1:200; Invitrogen, Carlsbad, CA) to react with the monoclonal antibody. Sections then were washed, dried, and mounted in MOWIOL® (Calbiochem, San Diego, CA).

2.4. Quantification of c-Fos positive cells

For the d-LSD dose–response studies, light microscopic analysis was performed on 10 c-Fos labeled sections (every other section) with a 20× objective as previously described (Dlugos and Pentney, 2002). The prefrontal cortex, including the anterior cingulate region of the medial prefrontal cortex and frontal area 2, were delineated on each slide from 4.70–0.7 mm anterior to the bregma (Fig. 1; Paxinos and Watson, 1986). An automatic stage control (Biopoint Keypad, Ludl Electronics Ltd, Hawthorne, NY) moved each mounted tissue section in a raster pattern through the delineated cortex. Approximately 75 microscopic fields/slide were quantified. Each field was projected onto a Commodore 1084S monitor that displayed a fixed counting frame representing 23,460 μm^2 (797× = total magnification). Standard rules for inclusion and exclusion of particles within the counting frame were used (Gundersen, 1977). Cells positive for c-Fos were counted only if their nuclei contained dark brown or black particles. The areal density of c-Fos-postive cells/slide was obtained by dividing the total number of cells/slide by the area of the counting frame. Values from the 10 slides were averaged to determine the mean areal density of c-Fos-positive cells in each rat.

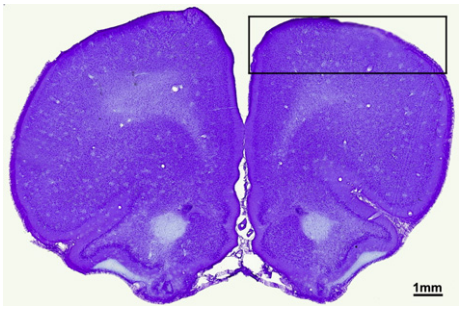

Fig. 1. Representative cresyl-violet stained coronal section of the rat cerebrum. The prefrontal cortex (boxed area) was sampled on both sides from 4.7–0.7 mm anterior to the bregma.

2.5. Cell identification

Colocalization of c-Fos with the different cell markers was carried out with a Nikon Eclipse E600 microscope equipped with an Expo-X-Cite™ 120 fluorescence illumination system (Nikon Inc., Melville, NY). Ten fields in each section were chosen in a random, systematic fashion (West, 1993); field size was 147,400 μm^2 (29,674× = total magnification). The stage was moved in a raster pattern throughout the prefrontal cortex as previously described. Individual fields were captured with a digital camera (Diagnostic Instruments Inc., Sterling Heights, MI) using two different emission filters (603 nm and 519 nm) and SPOT software for Windows© (Diagnostic Instruments Inc. V. 4.0.5, Sterling Heights, MI). Single fields labeled with one antibody were viewed using Adobe Photoshop 5.0 and quantified individually to determine the number of cells labeled for c-Fos, Olig1 or neuron-specific enolase. The individual fields, one labeled with a monoclonal and one with a polyclonal antibody, were then overlaid to determine the percentage of c-Fos-positive cells colocalized with the oligodendrocyte marker, Olig1 or the marker for neurons, neuron-specific enolase. The criterion for cellular colocalization was that the red c-Fos-labeled nucleus was clearly within the boundaries of the green neuron-specific enolase- or Olig1-stained cytoplasm.

2.6. Confocal microscopy

Confocal microscopy was use to confirm localization of c-Fos in NeuN-labeled neurons. As both NeuN and c-Fos antibodies label nuclear proteins, the number of pixels simultaneously labeled for c-Fos and NeuN could be quantified. Confocal microscopy was performed using a Zeiss LSM 510 Meta NLO microscope mounted on a Axioimager Z1 upright microscope stand. Images were analyzed using Zeiss LSM Image Examiner 4.0.09.1.

2.7. Statistical analysis

Means (±S.E.M.) for each dose in the d-LSD dose–response studies were determined by averaging the values from the 10 slides in each animal. One-way ANOVA with the Student–Newman–Keuls post-hoc test was used to detect differences in d-LSD-induced c-Fos expression. An alpha level of 0.05 was used to determine whether differences were statistically significant. All analyses were conducted using SigmaStat 2.03 for Windows (Jandel Scientific Software, San Rafael, CA).

3. Results

Systemic injection of d-LSD resulted in the appearance of c-Fos labeled cells in the prefrontal cortex and several other cortical areas. Although observed in all cortical layers, c-Fos-positive cells were most numerous in layers III, IV and V (Fig. 2). The density of c-Fos-positive cells after systemic d-LSD administration was dose-dependent; statistically significant increases in the number of c-Fos-positive cells was observed with d-LSD doses of 0.5 and 1.0 mg/kg [$F(4,24)=12.315$; $P<0.001$] (Fig. 3).

Morphologically, the c-Fos-positive cells were small and round. As a prelude to identifying these c-Fos labeled cells, neurons, astrocytes, oligodendrocytes, and microglia in the prefrontal cortex were labeled with immunocytochemical markers. In the present study, neurofilament antibody (NF 160) labeled soma and processes of a widely dispersed population of neurons, while neuron-specific enolase antibody labeled neurons with small round soma, but no visible processes. Astrocytes that were labeled with anti-glial fibrillary acidic protein had oval nuclei and extensive, radiating processes. Microglia that were labeled with anti-OX-42 had very small oval nuclei and spider-like processes. Oligodendrocytes that were labeled with 2',3'-cyclic-3'-phosphodiesterase and oligodendrocyte Olig1 antibodies had round nuclei and less extensive processes. As Olig1 labeling produced more robust staining, it was used in subsequent studies.

Cells labeled with glial fibrillary acid protein, OX-42 or neurofilament antibodies did not display an induction of c-Fos

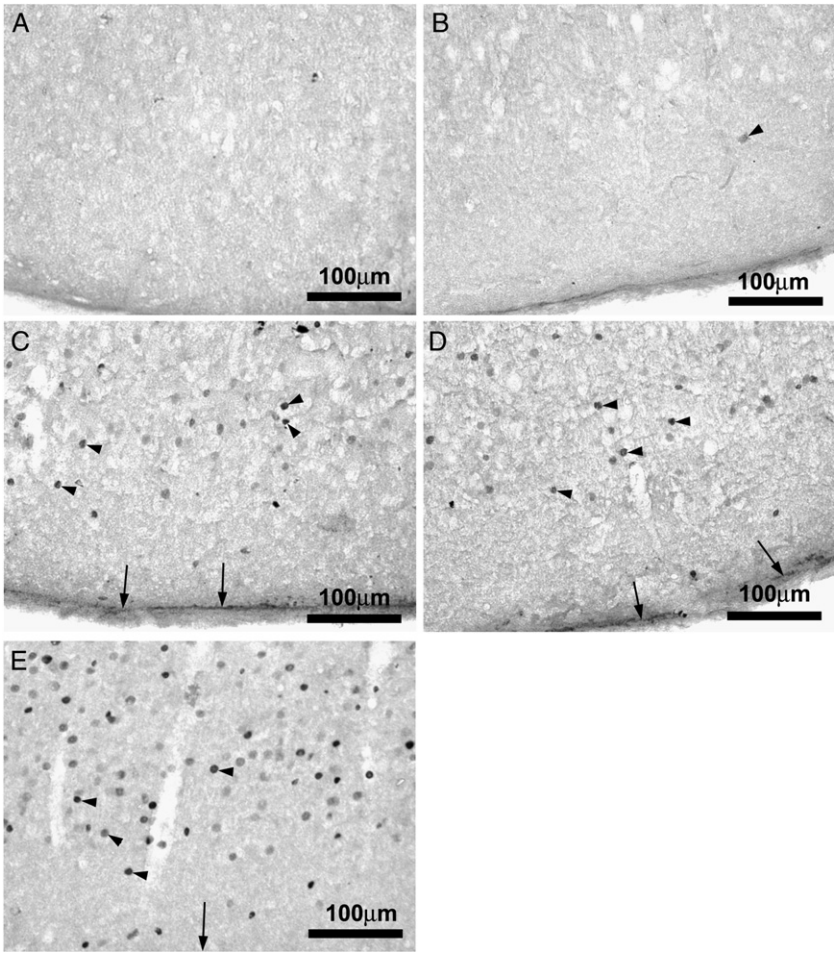

Fig. 2. c-Fos expression in nuclei of the prefrontal cortex 90 min after systemic injection of various doses of d-LSD or vehicle. A. c-Fos positive cells are absent with injection of vehicle. B. Injection of 0.1 mg/kg of d-LSD showed one cell expressing c-Fos in the field (arrowhead). C, D, E. Injection of 0.25 (C), 0.5 (D), and 1.0 (E) mg/kg d-LSD showed an increasing number of dark, c-Fos-positive nuclei (arrowheads) within the deeper layers (e.g., III, IV, and V) of the cortex. Pial surfaces are indicated with arrows.

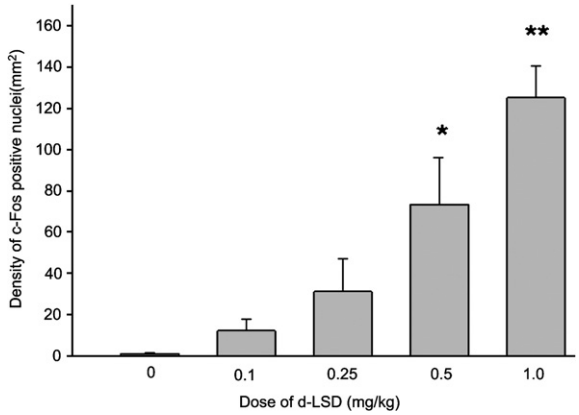

Fig. 3. Dose–response relationship for d-LSD-induced increase in the density of c-Fos-positive nuclei in rat prefrontal cortex. Each dose represents the mean (+S.E.M.) of 5 animals. Rats were sacrificed 90 min after injection of either d-LSD or vehicle. *$P<0.001$, increase in c-Fos positive nuclei compared with vehicle and 0.1 mg/kg d-LSD. **$P<0.001$ increase in c-Fos positive nuclei compared to all other doses.

immunoreactivity after systemic d-LSD administration (Fig. 4). Of the total cells labeled with neuron-specific enolase, 22.3±4.01% ($N=5$ rats) were found to be labeled with c-Fos antibody; the mean density of cells co-expressing c-Fos and neuron-specific enolase was 25.20±7.0 cells/5 mm^2 (Fig. 4). For the Olig1-labeled oligodendrocyte, 14.2±4.48% ($N=5$ rats) displayed c-Fos immunoreactivity; the mean density of cells expressing both c-Fos and Olig1 was 4.34±1.0 cells/5 mm^2 (Fig. 4). To confirm the expression of c-Fos in oligodendrocytes, nuclear measurements were performed on 100 c-Fos-positive cells. The nuclear diameter of these cells was 9.1±0.132 μm (mean±S.E.M.) which is comparable to the maximum diameter of oligodendrocyte nuclei (6.8±0.8 μm) reported in a previous study (Wolswijk, 2000). Confocal microscopy was used to confirm colocalization of c-Fos-labeled nuclei in NeuN-labeled neurons. Image analysis (Fig. 5) produced a weighted coefficient of colocalization of 0.923, indicating a significant overlap of green NeuN-labeled cells and red c-Fos-labeled nuclei. By contrast, analysis of the adjacent

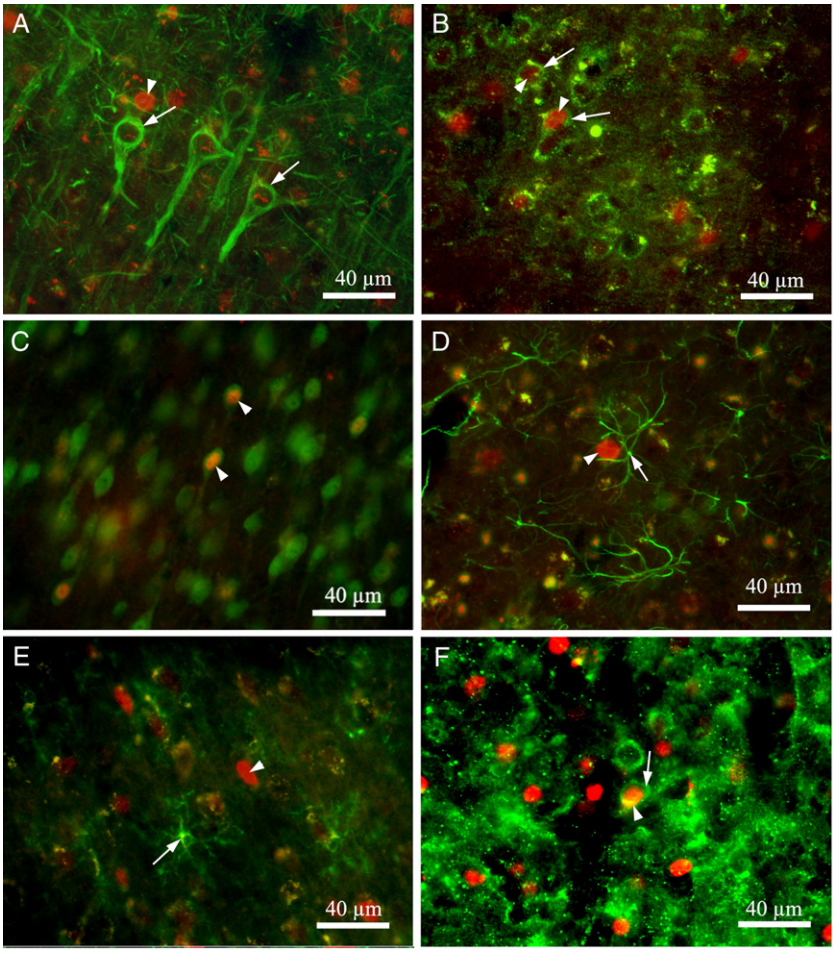

Fig. 4. Double labeling of c-Fos expressing cells (green; Alexa Fluor 488) in the rat prefrontal cortex and markers for neurons and glia (red; Alexa Fluor 568). A. Neurofilament containing pyramidal cells labeled with the neurofilament NF160 antibody (arrows) did not correspond with c-Fos-positive nuclei (arrowhead). B. A subpopulation of neurons labeled with neuron-specific enolase enolase (arrows) did express c-Fos (arrowheads). C. A subpopulation of neurons also colocalized NeuN and c-Fos within the nucleus (arrowheads). D, E. c-Fos-labeled nuclei (arrowhead) did not occur in astrocytes with glial fibrillary acidic protein-labeled cytoplasm (D; arrow) or in microglia with OX-42-labeled cytoplasm (E; arrow). F. Some oligodendrocytes positive for the cytoplasmic Olig1 marker (arrow) also contained c-Fos-positive (arrowhead) nuclei. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

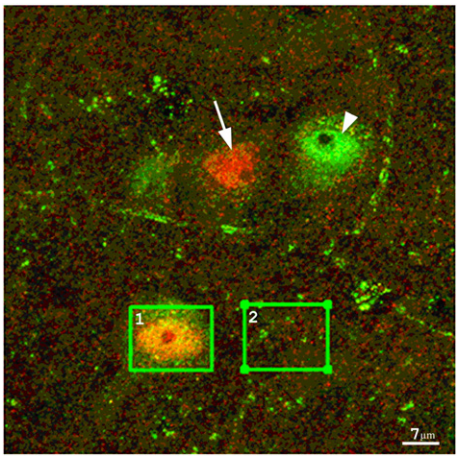

Fig. 5. Confocal microscopy of sections double labeled with c-Fos (red; Alexa 568) and Neu N (green; 488) antibody. The nucleus at top left is labeled with c-Fos (arrow), but not with Neu N. The nucleus at the top right is labeled with Neu N (arrowhead), but not c-Fos. Box 1, at the lower left, contains a neuronal nucleus in which c-Fos and NeuN are colocalized (weighted correlation coefficient=0.923). Box 2, at the lower right, represents background area in which c-Fos and NeuN colocalization did not occur (weighted correlation coefficient=0.169). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

background area produced a weighted colocalization coefficient of 0.169. Colocalization of c-Fos-labeled nuclei in Olig1-labeled oligodendrocytes could not be confirmed using this technique, because Olig1 is a cytoplasmic marker and would not be expected to overlap with nuclear c-Fos.

4. Discussion

Systemic administration of d-LSD resulted in a dose-dependent increase in c-Fos immunoreactivity in the prefrontal cortex. Although c-Fos-positive cells were found in all cortical layers, they were most numerous in layers III, IV, and V. In the present study d-LSD induced expression of c-Fos in the prefrontal cortex occurred both in neurons and in oligodendrocytes, but not in astrocytes or microglia. Thus, colocalization of c-Fos immunoreactivity was not observed in astrocytes labeled with glial fibrillary acidic protein antibody or in microglia labeled with OX-42 antibody. Rather, cells displaying c-Fos immunoreactivity were also labeled with neuron-specific enolase antibody or with the oligodendrocyte Olig1 antibody. The identification of some c-Fos-positive cells as neurons was confirmed by confocal microscopic studies in which a significant overlap of c-Fos- and NeuN-labeled nuclei was found. The colocalization of c-Fos with anti-Neu-N and not with the neurofilament antibody, NF160, strongly suggests that d-LSD-induced c-Fos expression does not occur in pyramidal cells of the prefrontal cortex as these cells display robust immunoreactivity to neurofilament proteins (Hof and Nimchinsky, 1992; Shetty and Turner, 1995). Furthermore, pyramidal neurons in the medial prefrontal cortex also express 5-HT_{2A} receptors (Willins et al., 1997; Jakab and Goldman-Rakic, 1998; Jansson et al., 2001), and hallucinogen-induced expression of c-Fos does not occur in cells expressing this serotonergic receptor subtype (Maćkowiak et al., 1999; Scruggs et al., 2000; Gresch et al., 2002). Some of the c-Fos expressing neurons may be GABAergic interneurons as these neurons lack neurofilament immunoreactivity (Hof and Nimchinsky, 1992; Shetty and Turner, 1995), and DOI administration was shown to increase c-Fos immunoreactivity in cells expressing GAD_{67} in the prefrontal cortex (Abi-Saab et al., 1999).

Identification of some of the c-Fos-positive cells as oligodendrocytes is indicated by the double-labeling of cells with c-Fos and Olig1 antibodies. In addition, these cells have the small, round bodies and nuclear diameters similar to those of oligodendrocytes, (Wolswijk, 2000). Previous studies have reported that c-Fos protein and mRNA can be induced in non-neuronal cells (Pyykönen and Koistinaho 1991; Simpson and Morris 1994; Herkenham et al., 1998). In particular, in oligodendrocytes in vitro, c-*fos* mRNA can be induced by stimulation of muscarinic acetylcholine receptors (Cohen et al., 1996), and oxidative stress elicited by hydrogen peroxide (Richter-Landsberg and Vollgraf, 1998). Furthermore, an increase in c-Fos expression was observed in Schwann cells, which produce the myelin sheaths in the peripheral nervous system, after transection of the rat sciatic nerve (Pyykönen and Koistinaho, 1991).

The possible role of oligodendrocytes in the effects of hallucinogens is unclear. Oligodendrocytes are responsible for enveloping axons with the myelin sheaths that facilitate electrical signal conduction. In addition, oligodendrocytes can serve as neuronal satellites providing trophic support (Uranova et al., 2004) and also are involved in energy metabolism (Sánchez-Abarca et al., 2001). Oligodendrocyte dysfunction and abnormalities have been implicated in psychiatric disorders such as schizophrenia, bipolar disorder, and major depression (Tkachev et al., 2003; Christensen et al., 2004; Uranova et al., 2004). Because processing and integration of information requires proper synchrony of neuronal activity among brain areas, schizophrenia has been suggested to involve focal disruption of the myelin thereby altering the rate of information transmission. The resulting de-synchronization of networks would result in altered perception, thought, and actions (Miller, 2000, Christensen et al., 2004). Although speculative, similar focal disruptions of oligodendrocytes could be, at least partly, responsible for the psychotropic effects of LSD.

In response to d-LSD administration only 14% of Olig1-labeled oligodendrocytes and 22% of neuron-specific enolase-labeled neurons show co-labeling with the c-Fos antibody. This finding most likely reflects the heterogeneity of these two cell populations and the inability of the antibodies used to label all the neurons or oligodendrocytes. For example, the Olig 1 antibody used in the present study is one of five recognized markers for mature oligodendrocytes (Lu et al., 2001). It is likely that each oligodendrocyte does not express every marker, so for example, some oligodendrocyte precursors, expressing Olig 2 and Olig 4 markers, would not be labeled in the present study. Similarly, the diversity and numbers of subpopulations of neurons may preclude the identification of neuronal subpopulations with a single marker. This diversity has been

recognized in the monkey prefrontal cortex where morphologic characterization of interneurons revealed bouquet, bipolar, basket, chandelier, Martinotti and neuroglial form neurons (Gabbott and Bacon, 1996). In rat prefrontal cortex the cellular diversity may not be as great, but different neuronal populations respond differently to various neuronal markers, and many cells are not labeled with classical neuronal or glial markers (Dayer et al., 2005). Furthermore, Neu N immunoreactivity does not occur in inner nuclear layer retinal cells, Cajal–Retzius cells, Purkinje cells, inferior olivary dentate nucleus neurons, and sympathetic ganglion cells (Mullen et al., 1992; Wolf et al., 1996).

The induction of c-Fos expression by LSD and DOI involves activation of 5-HT_{2A} receptors (Scruggs et al., 2000; Gresch et al., 2002; Nichols et al., 2003), and the observed LSD-induced c-Fos immunoreactivity occurs in the same cortical layers that have been reported to display this serotonergic receptor subtype (Willins et al., 1997; Jakab and Goldman-Rakic, 1998; Jansson et al., 2001). However, as noted above the increase in c-Fos immunoreactivity does not occur in cells expressing 5-HT_{2A} receptors (Maćkowiak et al., 1999; Scruggs et al., 2000; Gresch et al., 2002). Thus, the increase in c-Fos expression is not a direct intracellular response to LSD stimulation of 5-HT_{2A} receptors, but rather involves at least one additional intercellular connection. We would suggest that glutamatergic neurotransmission is a component of this circuit and most likely is situated downstream of the 5-HT_{2A} receptor. In the prefrontal cortex 5-HT_{2A} receptors are found on glutamatergic neurons (Jakab and Goldman-Rakic, 1998; Santana et al., 2004), and LSD increases extracellular glutamate levels in this brain area through stimulation of 5-HT_{2A} receptors (Muschamp et al., 2004). Release of glutamate also appears to be necessary for the discriminative stimulus effects of LSD (Winter et al., 2004). In addition, the mGlu2/3 receptor agonists LY379268, which would be expected to decrease glutamate release (Conn and Pin, 1997; Lorrain et al., 2003), reduced the ability of DOI to induce c-*fos* mRNA levels in the prefrontal cortex (Zhai et al., 2003). It should be noted that while neurons contain AMPA/kaiante and NMDA receptors, these ionotropic glutamate receptors also have been found on oligodendrocytes (Butt, 2006; Matute, 2006).

In summary, systemic administration of LSD causes a dose-dependent induction in c-Fos expression in the medial prefrontal cortex. This increase in c-Fos immunoreactivity occurs in both neurons and oligodendrocytes.

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