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Amphetamine-Induced *c-fos* mRNA Expression Is Altered in Rats With Neonatal Ventral Hippocampal Damage

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ABSTRACT To further characterize the mechanisms underlying enhanced dopamine-related behaviors expressed during adulthood in rats with neonatal excitotoxic ventral hippocampal (VH) damage, we studied the expression of *c-fos* mRNA in these rats after a single saline or amphetamine (AMPH) (10 mg/kg, i.p.) injection using in situ hybridization. The VH of rat pups was lesioned with ibotenic acid on postnatal day 7 (PD7). At the age of 90 days, rats were challenged with AMPH or saline, and the expression of *c-fos* mRNA using an oligonucleotide probe was assessed 30, 90, and 180 min later. AMPH significantly increased *c-fos* mRNA expression in medial prefrontal cortex, piriform cortex, cingulate cortex, septal region, and dorsolateral and ventromedial striatum in control and lesioned rats. However, this response to AMPH was attenuated 30 min after AMPH injection in all of these regions in the lesioned as compared to the sham-operated rats. No significant changes were seen at other time points. These results indicate that the neonatal VH lesion alters time-dependent intracellular signal transduction mechanisms measured by AMPH-induced *c-fos* mRNA expression in cortical and subcortical brain regions. Changes in *c-fos* mRNA expression in this putative animal model of schizophrenia may have implications for long-term alterations in cellular phenotype because of altered regulation of certain target genes.

INTRODUCTION

Immediate early genes (IEG), such as *c-fos* and *c-jun,* are important elements in intracellular signal transduction. They encode transcription factors that help mediate neuronal responses to extracellular stimuli. *C-fos* encodes the nuclear phosphoprotein Fos, which together with the protein product of *c-jun,* Jun, binds cooperatively to the activator protein (AP-1) DNA regulatory site of certain target genes to promote gene expression (Gentz et al., 1989; Morgan and Curran, 1989; Sheng and Greenberg, 1990). Fos is present in most neurons at relatively low levels, but its expression can be induced dramatically by a variety of physiological and pharmacological stimuli. Depolarizing stimuli as well as intracellular metabolic events induce Fos (Sagar et al., 1988). *C-fos* mapping has thus been proposed as a useful tool for characterizing transsynaptically activated neurons in different regions of the brain (Morgan and Curran, 1991; Sagar et al., 1988). Experimental manipulation of dopamine (DA) neurotransmission has been shown to alter gene regulation in neurons in the striatum (Gerfen, 1992). For example, dopamine D_2 receptor antagonists and D_1 receptor agonists induce *c-fos* mRNA expression in the rat (Dragunow et al., 1990; Graybiel et al., 1990). Acute amphetamine (AMPH) administration is known to induce *c-fos* mRNA in striatal patch neurons (Graybiel et al., 1990) as well as in neocortical layers (Merchant et al., 1994), probably by activating dopamine D_1 receptors.

In an earlier series of experiments, we have shown that rats with neonatal excitotoxic damage of the ventral hippocampus (VH) express a variety of abnormal behaviors that emerge postpubertally (Lillrank et al., 1995; Lipska et al., 1993). These behavioral changes include novelty- and AMPH-induced hyperlocomotion, increased responsiveness to stress, reduced haloperidol-induced catalepsy, and potentiated stereotypic re-

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sponses to apomorphine as well as reduced prepulse inhibition of startle (Lipska and Weinberger, 1993; Lipska et al., 1993, 1995). These behavioral abnormalities are thought to be linked primarily to dysfunction of mesolimbic and nigrostriatal dopaminergic areas where the ventral hippocampus makes direct connections. Rats with neonatal VH lesions also show some behavioral similarities to rats with adult lesions of the medial prefrontal cortex (MPFC) (Jaskiw and Weinberger, 1992; Jaskiw et al., 1990), particularly in respect to their abnormal hyperresponsiveness to stressful stimuli. This suggests that the developmental lesion has important upstream implications for development and function of prefrontal cortex and prefrontal-limbic connectivity, some of which may not involve direct hippocampal projections.

In order to elucidate whether neonatal VH excitotoxic damage alters neural function at the level of intracellular signal transduction mechanisms, we studied the expression of *c-fos* mRNA in response to an AMPH challenge. Although AMPH challenges primarily the dopaminergic system, other neuronal pathways are also likely to be affected, either directly or indirectly. While *c-fos* mapping per se cannot clarify the precise neurochemistry affected, it can provide an illustration of the anatomical distribution of changes in intracellular signal transduction. We, thus, assumed that changes in AMPH-induced *c-fos* expression associated with the lesion would reflect alterations in integrative cellular events in widespread brain areas and not necessarily only in those directly connected with the hippocampus. We hypothesized that the expression of *c-fos* mRNA would be altered in both cortical and striatal areas in lesioned rats because the profound behavioral changes suggest dysfunction in these regions.

MATERIALS AND METHODS

Subjects and drugs

Rats were lesioned as described previously (Lipska et al., 1993). Briefly, pregnant Sprague-Dawley rats obtained at 12–15 days of gestation (Zivic-Miller Labs, Zelienople, PA) were housed individually in breeding cages with a 12 h light/dark cycle and fed ad libitum. Litters of four to eight male pups were formed. On the seventh day of age (PD7, weight 15–18 g), pups were randomly assigned to lesion or sham status and anesthetized by hypothermia (placed on ice for 10–20 min). The pups were taped to a platform fixed to a stereotaxic Kopf instrument. An incision was made in the skin overlying the skull. Ibotenic acid (0.3 μl) (Sigma, St. Louis, MO; 10 μg/μl) (in lesion rats) or artificial cerebrospinal fluid (in sham) was infused bilaterally through a Hamilton needle using an infusion pump into the ventral hippocampal formation (AP − 3.0 mm, ML ± 3.5 mm, VD − 5.0 mm, relative to Bregma) at a rate of 0.15 μl/min. On the twenty-fifth day of age rats were weaned and separated by lesion status and grouped two to three to a cage. At the age of 35 and 56 days, locomotor activity was tested in response to novelty and to AMPH challenge (1.5 mg/kg, i.p.) (Lipska and Weinberger, 1995). After testing, rats were returned to their home cages.

Experiment 1

At the age of 90 days sham and lesion rats (440–720 g) were randomly assigned to receive either saline (sal) or AMPH (10 mg/kg, i.p.) and were sacrificed 30 or 90 min after the injection. There were thus eight groups of animals (sham/sal 30, n = 4; sham/sal 90, n = 3; sham/AMPH 30, n = 5; sham/AMPH 90, n = 3; lesion/sal 30, n = 4; lesion/sal 90, n = 3; lesion/AMPH 30, n = 5; lesion/AMPH 90, n = 4). Because the results from experiment 1 indicated that in response to AMPH *c-fos* mRNA expression is still greatly increased 90 min after the injection, we performed experiment 2 to test for longer time effects.

Experiment 2

A new cohort of lesioned and sham-operated rats was treated as described before, except that the rats were sacrificed 30 and 180 min after AMPH injection. There were thus four groups of animals in experiment 2 (sham/AMPH 30, n = 7; sham/AMPH 180, n = 5; lesion/AMPH 30, n = 8; lesion/AMPH 180, n = 7). Rats were injected and then killed in separate rooms. The total number of animals used in both experiments was 58.

Tissue section preparation

All rats were killed by decapitation, and the brains were quickly removed, frozen for 15 s in isopentane, and then cooled on dry ice and stored at −80°C until cryostat sectioning. Coronal sections (20 μm) were thaw-mounted onto gelatin subbed RNAse free glass slides and then allowed to dry for 10 min before being frozen at −80°C. For processing for in situ hybridization histochemistry (ISHH), the slides were first warmed at room temperature and fixed in a phosphate-buffered saline (PBS) with 4% formaldehyde for 5 min, treated with 0.25% acetic anhydride in 0.1 M triethanolamine hydrochloride (pH 8.0), dehydrated in a series of ascending concentrations of ethanol, defatted for 5 min in chloroform, rehydrated, and air-dried.

Oligonucleotide probes

An antisense oligonucleotide probe for rat *c-fos* mRNA was used (5′GGG-ATA-AAG-TTG-GCA-CTA-GAG-ACG-GAC-AGA-TCT-GCG-CAA-AAG-TCC-TGT-GTG 3′) (Oligo Etc. Inc., Wilsonville, OR). The probe was labeled on the 3′ end using [^{35}S]-dATP and terminal transferase by the method described by Young et al. (1986). Control sections incubated with a sense probe did not show any labeling associated with cellular elements (data not shown).

Hybridization

ISHH was performed according to Young et al. (1986). A saturating concentration of labeled probe ($0.5–1 \times 10^6$ dpm/25 μl) was added to hybridization buffer (80 mM Tris-HCl, pH 7.5, 50% Formamide, 600 mM NaCl, 4 mM EDTA, 0.1% NaPyrophosphate, 0.2 mg/1 ml sodium Heparin, 10% Dextran SO_4, 0.2% SDS, 100 mM DTT), which was then added to each brain section. The sections were coverslipped with parafilm and incubated overnight at 37°C. After incubation, the coverslips were floated off in 1 × saline–sodium citrate (SSC), and the slide-mounted sections were rinsed quickly in four washes of 1 × SSC. The slides were then washed four times for 15 min in 1 × SSC at 60°C followed by two 30 min washes in 1 × SSC at room temperature and a brief water and 70% ethanol rinse before being air-dried. The slides and the ^{14}C standard were then apposed to β-Max Hyperfilm (Amersham, Arlington Heights, IL) for 2 weeks. After the films were developed, the slides were dipped into NTB3 autoradiography emulsion (Kodak, Rochester, NY) (diluted 1:1 with water), exposed for 8 weeks, and developed.

Analysis of autoradiograms

Hybridization signal was analyzed at a prefrontal cortical and a striatal level (3.2 mm from Bregma and 1.7 mm from Bregma, respectively [Paxinos and Watson, 1986] (Fig. 1). The regions chosen for analysis were medial prefrontal cortex (MPFC), cingulate cortex (CG), piriform cortex (PIR), septum (SEPT), dorsolateral striatum (DLS), and ventromedial striatum (VMS). Densitometric analysis of sections was done blind to the status of a rat from film using Macintosh-based image analysis software (NIH; Image). For each region of interest, mean densities were assessed in both hemispheres and averaged. Densities interpolated along a ^{14}C standard curve were converted to disintegrations per minute/millimeters squared (dpm/mm^2) according to Miller (1991).

Statistics

Data are presented as the mean ± S.E.M. In experiment 1 differences between means were analyzed using three-way analysis of variance (ANOVA) to determine the effects of lesion status, drug treatment, and time. In experiment 2, in which only AMPH was used, two-way ANOVA was used to determine the effects of lesion status and time. If significant effects or interactions were found, the data were then further analyzed with the Newman-Keuls post-hoc test for critical values. For the graphic presentation, the data from both experiments were converted to a percent of values of the sham-operated AMPH-injected rats decapitated 30 min after injection (sham/AMPH 30).

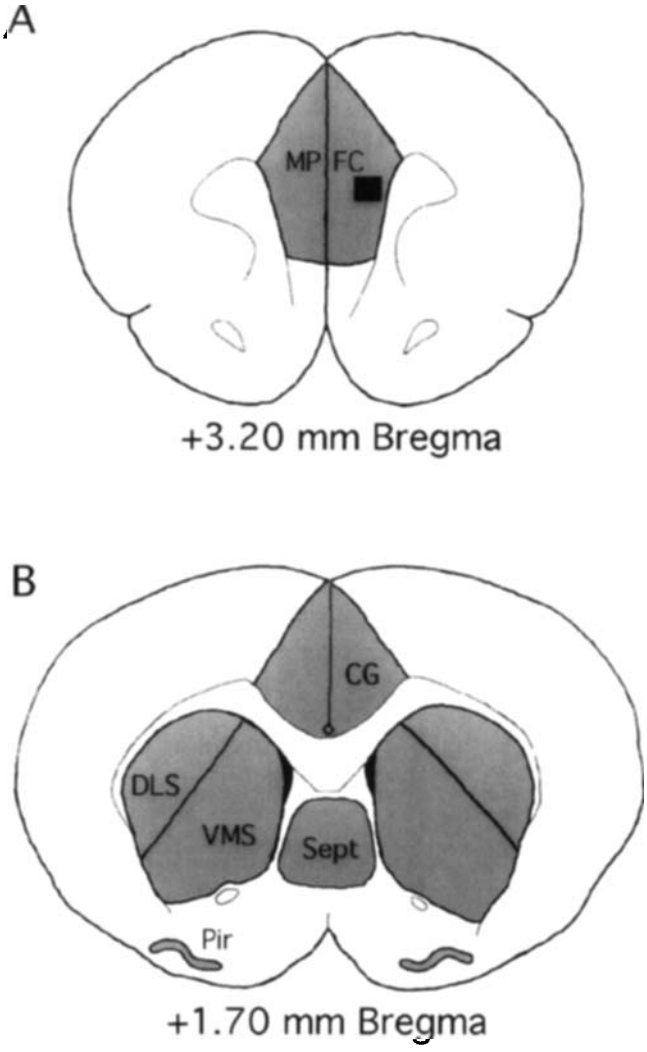

Fig. 1. The regions in which expression of *c-fos* mRNA was analyzed at the **(A)** frontal and **(B)** striatal level. Regions chosen for analysis were medial prefrontal cortex (MPFC), cingulate cortex (CG), septum (Sep), piriform cortex (PIR), dorsolateral striatum (DLS), and ventromedial striatum (VMS). A dark square indicates the area in the prelimbic cortex shown in Fig. 4.

RESULTS

Verification of the lesion

All brains were examined under light microscopy for location of the ibotenic acid lesion. Nissl stained sections showed that neuronal loss and gliosis were confined to the VH as has been reported previously (Lipska et al., 1993). In all rats the cytoarchitectural divisions in the ventral aspects of the hippocampus (CA1-CA4) as well as parts of the subiculum were affected. As reported previously (Lipska and Weinberger, 1993; Lipska et al., 1993), in approximately 50% of rats the most temporal portions of the hippocampal formation were spared. One subject was deleted from further analysis (experiment 1) due to improper location of the lesion.

Behavioral testing

Rats used in these experiments were tested for locomotor activity in response to novelty and AMPH challenge (1.5 mg/kg) at the age of 35 and 56 days. Locomotor activity was similar in control and lesioned rats at the age of 35 days, but in early adulthood, at 56 days of age, animals with the neonatal VH lesion were significantly more active in response to both novelty and AMPH. The behavioral results are reported in detail elsewhere (Lipska and Weinberger, 1995).

Effects of amphetamine on *c-fos* mRNA expression

In experiment 1, ANOVA showed a significant effect of AMPH treatment as compared to saline injection in all regions studied (MPFC: $F_{1,23} = 73.67$, $P < 0.0001$; CG: $F_{1,24} = 69.04$, $P < 0.0001$; PIR: $F_{1,23} = 29.99$, $P < 0.0001$; SEPT: $F_{1,23} = 72.15$, $P < 0.0001$; DLS: $F_{1,23} = 55.10$, $P < 0.0001$; VMS: $F_{1,23} = 84.38$, $P < 0.0001$). Post-hoc analysis showed that in all regions AMPH, as compared to saline, increased *c-fos* mRNA expression in both lesioned and sham-operated rats (Fig. 2). There were no significant differences between the lesioned and sham-operated animals in *c-fos* mRNA expression at any time point (30 and 90 min) in any region after saline injection. Because lesion and time effects differed between the regions, they will be presented for each region separately.

Medial prefrontal cortex

In experiment 1, there was a significant main effect of treatment but no main effect of lesion or time or significant interactions. Post-hoc analysis revealed that the expression of *c-fos* mRNA was significantly decreased in the lesioned AMPH-injected rats as compared to the sham-operated AMPH-injected rats 30 min after the injection (lesion/AMPH 30 vs. sham/AMPH 30, $P < 0.05$). There was no difference between these groups at 90 min. However, in experiment 2 there were significant effects of lesion ($F_{1,23} = 10.7$, $P < 0.05$) and time ($F_{1,23} = 20.76$, $P < 0.0001$) (Fig. 3). This difference in statistical significance between experiment 1 and experiment 2 is most likely due to a larger number of animals in the latter experiment and to the fact that different time points were studied in experiments 1 and 2 (30 and 90 min or 30 and 180 min, respectively). Post-hoc analysis in experiment 2 revealed that, similarly to experiment 1, expression of *c-fos* mRNA was significantly decreased in the lesion/AMPH rats 30 min after the AMPH injection as compared to the sham/AMPH rats (lesion/AMPH vs. sham/AMPH, $P < 0.01$). This effect did not persist at 180 min after AMPH injection. The labeling of *c-fos* mRNA was evenly distributed in all cortical layers. Microscopic evaluation in the prelimbic part of the prefrontal cortex showed that the silver grains were located on or next to the cells (Fig. 4).

Cingulate cortex

Apart from the drug effect, there were no significant time ($F_{1,23} = 0.06, P < 0.8$) or lesion ($F_{1,23} = 2.1, P < 0.16$) effects or significant interactions in cingulate cortex in experiment 1 (Fig. 2). Further post-hoc analysis indicated that there were no significant differences between lesioned and sham-operated animals. However, in experiment 2 there was a significant time ($F_{1,23} = 33.96$, $P < 0.0001$) and lesion ($F_{1,23} = 9.41$, $P < 0.01$) effect but no significant interaction. Post-hoc analysis showed decreased *c-fos* expression in lesion/AMPH as compared to sham/AMPH rats 30 min after AMPH injection ($P < 0.05$) (Fig. 5).

Piriform cortex

In experiment 1, there was a significant drug x time interaction ($F_{1,22} = 5.25, P < 0.05$) but no time or lesion effects. Post-hoc analysis revealed decreased expression of *c-fos* mRNA in the lesion/AMPH as compared to the sham/AMPH rats at 30 min after the injection ($P < 0.05$). In experiment 2, there was a significant effect of lesion ($F_{1,23} = 12.78, P < 0.01$) but no significant interaction. There was a trend for decreased expression of *c-fos* mRNA in the lesion/AMPH as compared to the sham/AMPH rats at 30 min after AMPH ($P < 0.1$), whereas there was a significant reduction in expression of *c-fos* in the lesion/AMPH as compared to sham/AMPH at 180 min ($P < 0.05$) (Figs. 2, 6).

Septum

The septal region was analyzed as a block including both lateral and medial septal nuclei. In experiment 1, there was a significant effect of lesion ($F_{1,22} = 4.53$, $P < 0.05$) as well as a lesion x drug interaction ($F_{1,22} = 5.20$, $P < 0.05$) but no significant effect of time. The post-hoc analysis revealed that the expression of *c-fos* mRNA was decreased in the lesion/AMPH rats as compared to sham/AMPH rats 30 min after AMPH injection ($P < 0.05$). In experiment 2, there was a significant effect of lesion ($F_{1,23} = 14.75, P < 0.001$). Lesion/AMPH rats showed significantly reduced *c-fos* mRNA expression as compared to the sham/AMPH rats at 30 min after the injection ($P < 0.01$) (Fig. 7).

Striatum

The distribution of AMPH induced *c-fos* mRNA expression in the striatum in both sham and lesioned rats was similar to that in previous reports (i.e., it showed a patchy pattern) (Graybiel et al., 1990; Merchant et al., 1994). The labeling of nucleus accumbens was barely detectable and was thus not analyzed in this study (Fig. 2). In addition to the significant drug effect in both DLS and VMS, there was a significant effect of time in the DLS ($F_{1,23} = 4.69$, $P < 0.05$) but not in the VMS ($F_{1,23} = 2.81$, $P < 0.1$). There were no significant differences between lesion/AMPH and sham/AMPH rats at

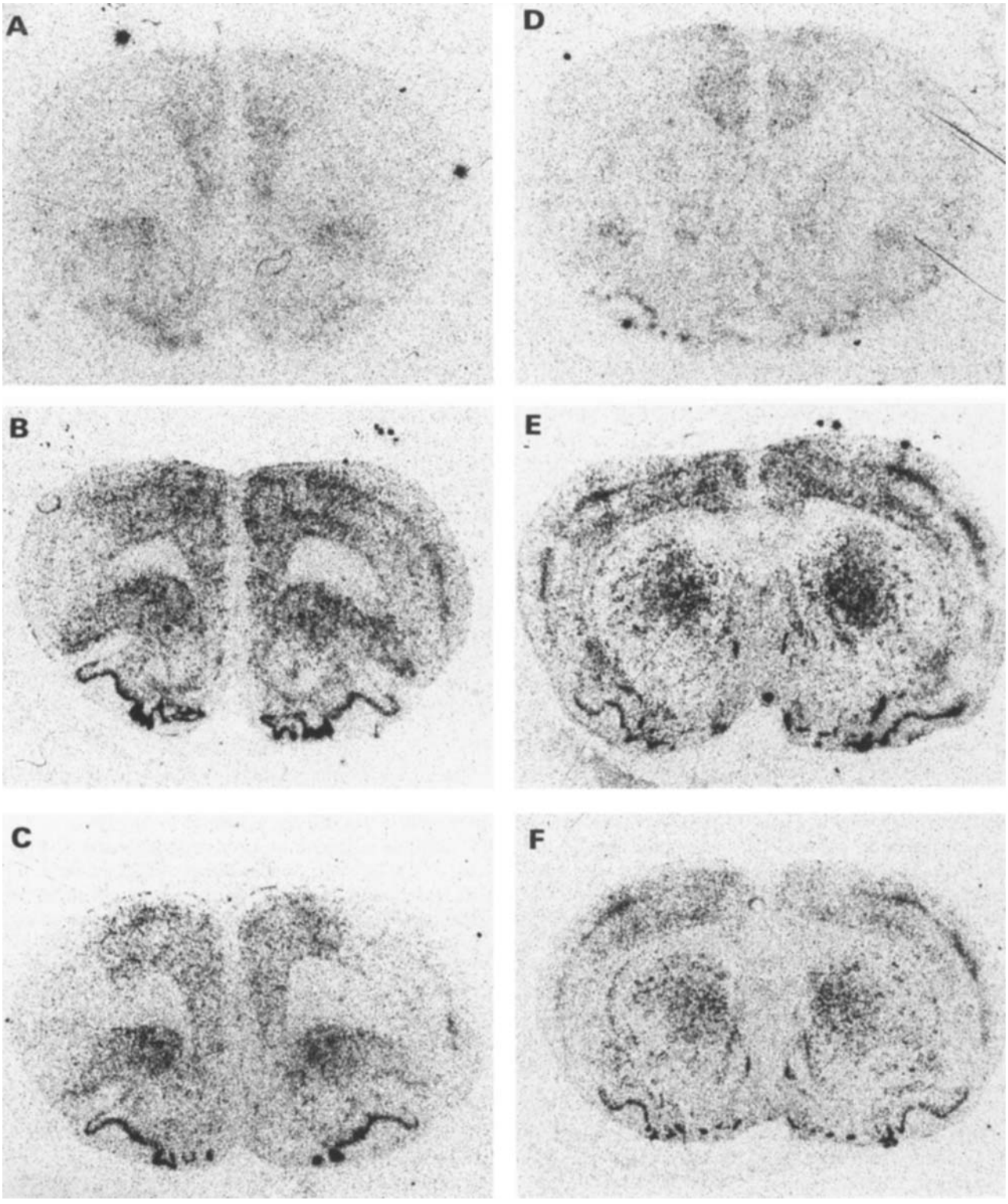

Fig. 2. In situ hybridization autoradiograms showing the distribution of *c-fos* mRNA expression in coronal brain sections from the frontal (left side) and the striatal (right side) level. *C-fos* mRNA expression was measured 30 min after saline or amphetamine (AMPH) (10 mg/kg, i.p.) challenge. **A,D:** Sham-operated saline-injected rats. **B,E:** Sham-operated AMPH-injected rats. **C,F:** Lesioned AMPH-injected rats.

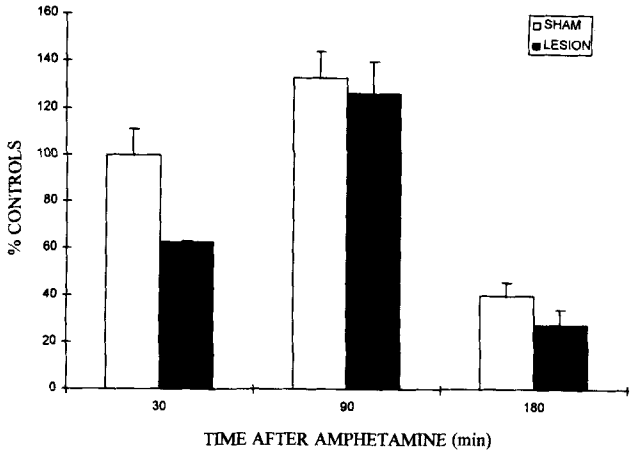

Fig. 3. Quantification of *c-fos* mRNA hybridization signal in the MPFC of sham-operated (SHAM) and VH lesioned (LESION) rats 30, 90, and 180 min after amphetamine (10 mg/kg) injection. Data are presented as percent of medial prefrontal cortex (MPFC) expression in sham-operated rats 30 minutes after amphetamine injection (2.04 ± 0.23 dpm/mm^2).

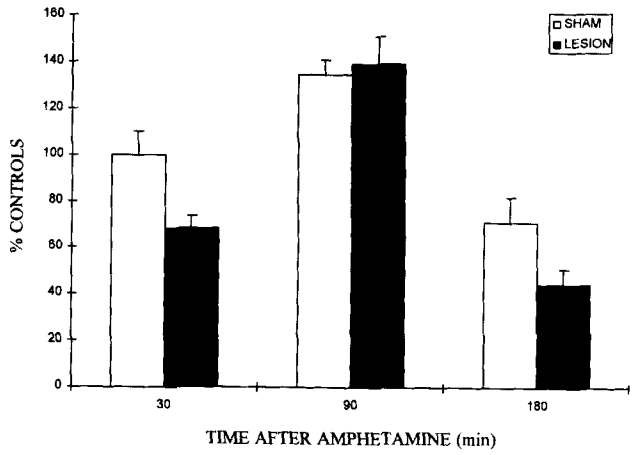

Fig. 5. Quantification of *c-fos* mRNA hybridization signal in the piriform cortex of sham-operated (SHAM) and VH lesioned (LESION) rats 30, 90, and 180 min after amphetamine injection (10 mg/kg, i.p.). Data are presented as percent of PIR expression in sham-operated rats 30 min after amphetamine injection (1.81 ± 0.18 dpm/mm^2).

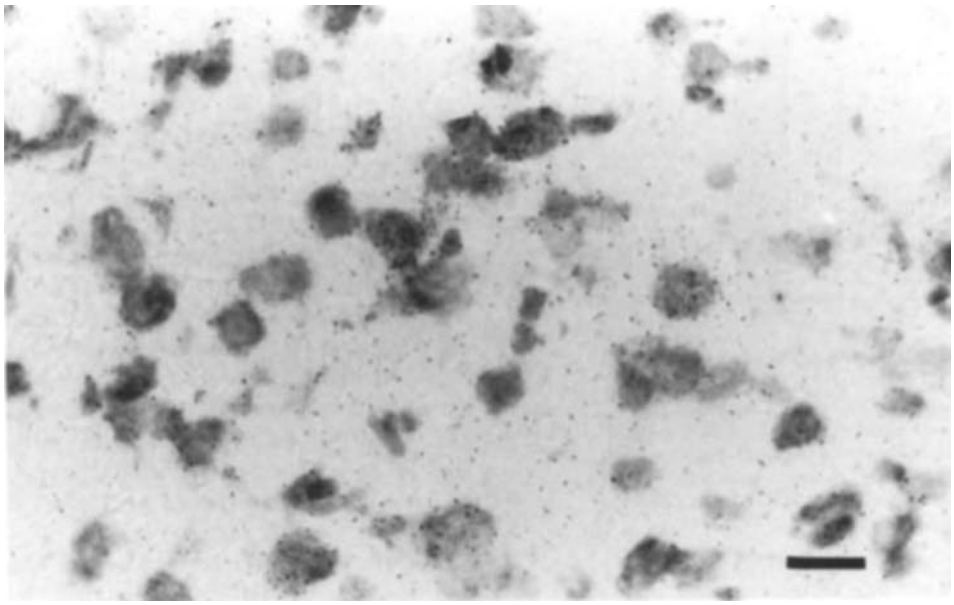

Fig. 4. A high-magnification, brightfield photomicrograph of cells expressing *c-fos* mRNA in the prelimbic part of medial prefrontal cortex of a sham-operated rat 30 min after amphetamine (10 mg/kg, i.p.) injection. Emulsion-coated and Nissl stained slides were viewed using a ×40 objective. Dark grains over stained neurons indicate hybridization-positive cells. Calibration bar = 40 μm.

30 or 90 min. However, in experiment 2, where the number of animals per group was larger, there were significant time ($F_{1,23}$ = 81.0, $P < 0.0001$) and lesion ($F_{1,23}$ = 5.4, $P < 0.05$) effects in the DLS as well as a trend for a lesion x time interaction ($F_{1,23}$ = 2.96, $P = 0.099$). Likewise, in the VMS there were significant time ($F_{1,23}$ = 142.82, $P < 0.0001$) and lesion ($F_{1,23}$ = 16.21, $P < 0.001$) effects and a lesion x time interaction ($F_{1,23}$ = 8.28, $P < 0.01$). Further analysis of the data revealed that lesion/AMPH rats showed decreased *c-fos* mRNA expression as compared to sham/AMPH rats in the DLS ($P < 0.01$) and VMS ($P < 0.001$) at 30 min but not at 180 min after AMPH injection (Fig. 8).

DISCUSSION

The major finding of this study is that our neonatal VH lesion leads to altered intracellular signal transduc-

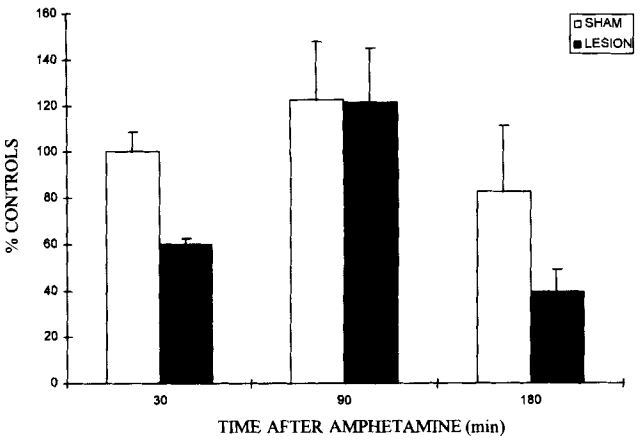

Fig. 6. Quantification of *c-fos* mRNA hybridization signal in the septum of sham-operated (SHAM) and VH lesioned (LESION) rats 30, 90, and 180 min after amphetamine injection (10 mg/kg). Data are presented as percent of SEPT expression in sham-operated rats 30 min after amphetamine injection (1.57 ± 0.13 dpm/mm^2).

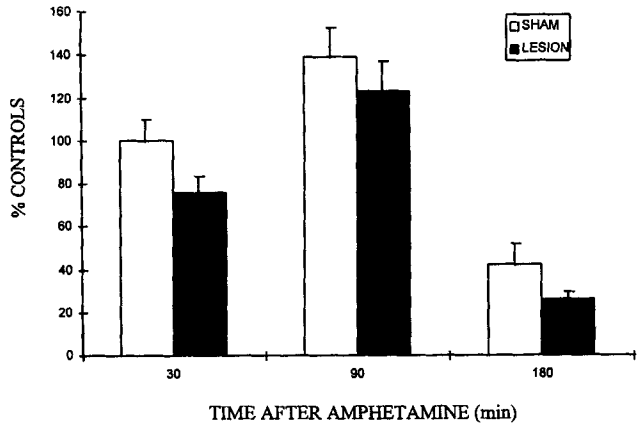

Fig. 7. Quantification of *c-fos* mRNA hybridization signal in the cingulate cortex of sham-operated (SHAM) and VH lesioned (LESION) rats 30, 90, and 180 min after amphetamine injection (10 mg/kg). Data are presented as percent of CG expression in sham-operated rats 30 min after amphetamine injection (1.49 ± 0.14 dpm/mm^2).

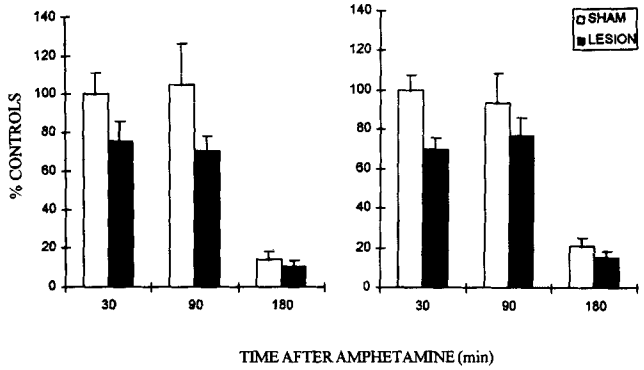

Fig. 8. Quantification of *c-fos* mRNA hybridization signal in the dorsolateral striatum **(left)** and ventromedial (VMS) **(right)** striatum of sham-operated and VH lesioned rats 30, 90, and 180 min after amphetamine injection (10 mg/kg, i.p.). Data are presented as percent of DLS and VMS expression in sham-operated rats 30 min after amphetamine injection (DLS: 1.46 ± 0.16 dpm/mm^2; VMS: 1.72 ± 0.12 dpm/mm^2).

tion in distributed brain regions during adult life. We have demonstrated in two independent experiments decreased expression of *c-fos* mRNA in the medial prefrontal cortex, piriform cortex, cingulate cortex, septum, dorsolateral striatum, and ventromedial striatum in the lesioned rats 30 min after AMPH administration. The distribution of labeling of *c-fos* mRNA after AMPH challenge reported in this study is in agreement with previously published findings from in situ hybridization studies (Graybiel et al., 1990; Johansson et al., 1994; Merchant et al., 1994), localizing the effects predominantly to the striatum but also to cortical regions. After AMPH administration, *c-fos* mRNA was expressed in the caudate-putamen in a patchy pattern, similar to that observed with immunohistochemistry of Fos (Graybiel et al., 1990) and with in situ hybridization for *c-fos* mRNA under similar conditions (Merchant et al., 1994).

Induction of *c-fos* mRNA is transient, with transcriptional activation occurring within 15 min of stimulation and continuing for more than 60 min (Greenberg et al., 1985). *C-fos* mRNA accumulates and reaches peak values at 30–40 min poststimulation (Müller et al., 1984), decreasing thereafter. As expected from earlier work, we found that *c-fos* expression was apparent 30 min after AMPH challenge but was still elevated 90 min later in MPFC, PIR, CG, DLS, VMS, and SEPT, declining thereafter. Moreover, our data suggest that peak expression occurs at 30 min in the striatal regions but at 90 min in the SEPT and cortical regions. This may suggest that the peak expression of *c-fos* mRNA in the latter regions occurs as a consequence of transsynaptic activation perhaps in response to changes in the striatum. As compared with animals sacrificed 30 and 90 min after AMPH injection, rats sacrificed 180 min after AMPH showed decreased labeling of *c-fos* mRNA in all regions.

Significantly reduced *c-fos* mRNA expression in neonatally VH lesioned rats as compared with the sham-operated rats 30 min after AMPH injection was seen in all regions studied. Although a high level of *c-fos* expression was seen at 90 min in MPFC, PIR, CG, SEPT, and DLS, no significant differences were found at this later time point. This suggests that decreased expression of *c-fos* at 30 min in rats with the neonatal VH lesion reflects a deficiency in time-dependent mechanisms of IEG expression rather than an absolute inability to express *c-fos*. A deviation at the molecular level to adjust to sudden changes in the environment with an appropriate change in target genes and cellular phenotype might correspond to the behavioral abnormalities observed in these rats after stressful stimuli. Although the molecular events that are triggered by the

changes in IEGs are complex, it is conceivable that delayed or sluggish expression of *c-fos* could affect expression of later-response genes that regulate long-term changes in neuronal activity within cortex and the striatum.

Some of the abnormal behaviors expressed in adulthood after the neonatal VH lesion have been related to abnormal DA transmission in the mesolimbic/nigrostriatal system.

Previously reported increases in novelty-, amphetamine-, or apomorphine-induced locomotion are in general assumed to be associated with enhanced mesolimbic DA transmission (Radhakishun and Van Ree, 1987; Robbins et al., 1990). Accentuated stereotypies after apomorphine treatment presumably reflect enhanced postsynaptic DA transmission within the dorsal striatum (Lipska and Weinberger, 1993). Consistent with the assumption of increased DA tone in rats with VH lesions is the finding that locomotor hyperactivity can be blocked in these animals by antidopaminergic drugs (Lipska and Weinberger, 1994; Rupniak et al., 1985). Because our behavioral studies strongly suggest involvement of cortical and striatal regions in abnormalities associated with the neonatal lesion, these areas were of particular interest to us in our analysis of *c-fos* expression induced by AMPH injection.

Activation of receptors associated with increased cAMP and/or intracellular Ca^{2+} is known to induce *c-fos* expression. Thus dopamine D_1 but not D_2 receptor agonists increase *c-fos* mRNA expression in the striatum (Paul et al., 1992; Robertson and Fibiger, 1992; Robertson et al., 1989). Induction of *c-fos* in the striatum by AMPH is partly mediated through activation of dopamine D_1 receptors, which are positively coupled to adenylate cyclase (Graybiel et al., 1990; Stoof and Kebabian, 1981). This induction can be abolished by pretreatment with selective D_1 receptor antagonists (Graybiel et al., 1990). If, indeed, AMPH induces *c-fos* expression by activating D_1 receptors, at least in the striatum, then decreased *c-fos* expression in the lesioned vs. sham-operated rats would suggest decreased dopaminergic D_1 activity in this region. Our binding studies using [^{3}H]SCH-23390 as a D_1 ligand in prefrontal cortex, nucleus accumbens, and dorsolateral and ventromedial striatum do not support this possibility (Knable et al., 1994). Another possibility is that D_2 receptors, the activation of which is known to inhibit adenylate cyclase (Vallar and Meldolesi, 1989) and possibly reduce the expression of *c-fos,* may be upregulated in rats with VH lesions. Again, our binding studies using [^{3}H]-raclopride and [^{3}H]YM-09151-2 do not support this explanation (Knable et al., 1994). Alternatively, AMPH may release less DA in the lesioned vs. sham-operated rats. Such a possibility, while difficult to reconcile with our behavioral data, may be consistent with the results of other experiments with this lesion. Striatal DA release, as measured by 3-MT accumulation, is attenuated at baseline and after chronic mild stress in the lesioned vs. control rats (Lipska et al., 1995). A possibility that has yet to be explored is that alterations in second messenger levels are responsible for the changes in mRNA expression.

Despite the behavioral data implicating enhanced dopamine function in rats with VH lesions, our present data show at least transiently reduced *c-fos* mRNA expression following AMPH stimulation. It is plausible, therefore, the neurotransmitter systems other than DA account for this *c-fos* finding. For instance, agonists of glutamate receptor subtypes (N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid [AMPA] are known to be potent inducers of *c-fos* (Berretta et al., 1992; Cole et al., 1989; Lerea et al., 1992). Moreover, stimulation of glutamate receptors may be involved in mediating effects of central stimulants such as amphetamine. This might suggest that the initial decrease in *c-fos* mRNA expression found in this study could be a result of decreased glutamatergic tone. The ibotenic acid lesion destroys intrinsinc VH neurons, many of which are glutamatergic projection neurons bound for some of the regions we analyzed (e.g., MPFC). Previous studies from this laboratory have suggested that early hippocampal deefferentation affects the development of other brain regions, such as the MPFC, that are also involved in the regulation of striatal DA function (Lipska and Weinberger, 1993). Rats with a neonatal VH lesion show some similarities to rats with adult lesions of the MPFC, particularly in respect to their abnormal hyperresponsiveness to stressful stimuli (Jaskiw et al., 1990). VH sends glutamatergic projections to the nucleus accumbens (NA) and prefrontal cortex (Sesack and Pickel, 1990); prelimbic prefrontal cortex sends glutamatergic projections also to the NA (Jay and Witter, 1991), and these projections are in close apposition to DA terminals ascending from VTA. It is possible that due to the neonatal VH lesion less glutamate is released in target areas (MPFC, NA) that could lead to lesser stimulation of postsynaptic glutamate receptors and subsequently to reduced expression of *c-fos* and possibly to transsynaptic effects in other brain regions. Consistent with this possibility are recent data from our laboratory showing that adult rats with neonatal VH lesion are hypersensitive to the stimulatory effects of MK-801, a glutamate receptor antagonist (Lipska, Wood, and Weinberger, unpublished observations).

Another possible explanation for decreased *c-fos* expression is that *c-fos* mRNA might be degraded faster in the lesioned as compared to control rats (Dragunow and Faull, 1989). It has recently been shown that at least two destabilizing domains are present in *c-fos* mRNA that target the transcript for rapid degradation (Schiavi et al., 1994). Changes in mRNA and protein stability probably contribute to neurotransmitter-induced changes in protein levels. As the total amounts

of proteins can be regulated in a neuron transcriptionally, posttranscriptionally, and posttranslationally, it is plausible that even slight changes in the mechanisms regulating these actions can lead to disruption of the sensitive homeostatic balance (Hyman and Nestler, 1993). Nevertheless, while this potential explanation for our finding cannot be ruled out, it seems unlikely that increased degradation would affect *c-fos* expression only within the 30 min after AMPH.

A potential complication that should be considered is that our oligonucleotide probe was not specific enough to detect small changes in *c-fos* expression. The use of a ribonucleotide probe in future experiments would enhance specificity and the limit of detection. Furthermore, dose-response curves would provide additional information about how this lesion changes *c-fos* expression after AMPH challenge. Finally, the possibility that diminished *c-fos* expression in widespread brain areas occurs as a direct result of the neurotoxic action of ibotenic acid merits consideration. Although our gross histological analysis of Nissl stained sections did not reveal any abnormalities in brain regions outside the hippocampus, it cannot be precluded that more subtle anatomical abnormalities occur during development as a result of this lesion in regions directly or indirectly connected with the hippocampus.

Whatever the mechanisms of reduced *c-fos* mRNA expression in the neonatally lesioned rat, the finding itself may have implications for regulation of second order target genes. It is known that stimulation of neuronal receptors brings about changes in second messenger levels leading to an induction of *c-fos* as well as other IEGs and, after translation in the cytoplasm, to the protein products of the respective IEGs. An important step in regulation of transcriptional activity of the next order target genes is dimerization of IEG protein products before high affinity binding to the AP-1 DNA-binding site on the target gene can occur. Variations in the heterodimer composition or affinity for the AP-1 binding site probably lead to different consequences for regulation of the target gene by either activating or inhibiting its transcription (Morgan and Curran, 1991). Thus, it is possible that a decrease in *c-fos* mRNA expression could lead either to decreased expression of an AP-1–induced target gene or to increased expression of a particular target gene through decreased inhibition at an AP-1 site. So far studies on identifying neuronal target genes for cellular IEGs have suggested a relationship between drug-induced *c-fos* induction and expression of dynorphin (Steiner and Gerfen, 1993), proenkephalin (Nguyen et al., 1990), and neurotensin (Merchant et al., 1994) as well as tyrosine hydroxylase (Hyman and Nestler, 1993). These are candidates for further study in our neonatal VH lesion model.

We have studied the effects of this neonatal VH lesion as a potential animal model for certain aspects of schizophrenia, a disorder in which developmental structural pathology in the hippocampal region and postpubertal onset of symptoms are implicated (Lillrank et al., 1995; Weinberger and Lipska, 1995). In order to elucidate the mechanisms underlying the postpubertally emerging behavioral abnormalities in this animal model that may shed slight on the pathophysiological mechanisms involved in schizophrenia, we have previously investigated extracellular events associated with the early lesion (i.e., release of dopamine) as well as the events on the neural surface (i.e., binding of certain ligands to specific dopamine receptors) (Knable et al., 1994; Lipska et al., 1995). Interestingly, only modest disturbances in these indices of neural function have been found. In contrast, the current study, involving intracellular processes, provides evidence that the developmental VH lesion leads to changes in AMPH-induced gene expression and, by inference, to changes in functional properties of a relatively widespread population of neurons. Although it remains unknown which particular neurons are affected, how they play a critical role in the behavioral outcome, or what the implications of these changes are for the behavioral abnormalities, the results of this study suggest that transcription factors and probably their target genes may be important candidates responsible for behavioral disturbances associated with the developmental VH lesion. If, indeed, this animal model reproduces critical mechanisms involved in schizophrenia, these data may in turn suggest that the transcription factors and/or their target genes may be important targets for therapeutic action.

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