Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 8537–8541, August 1994
Neurobiology

Morphine induces c-*fos* and *junB* in striatum and nucleus accumbens via D_1 and *N*-methyl-D-aspartate receptors

(immediate early genes/basal ganglia/opioid/drug abuse/dopamine)

JIALING LIU*, JEREMY NICKOLENKO, AND FRANK R. SHARP

Department of Neurology (V127), University of California at San Francisco, and Department of Veterans Affairs Medical Center, San Francisco, CA 94121

Communicated by Ann M. Graybiel†, April 28, 1994 (received for review February 2, 1994)

ABSTRACT **Morphine induced the c-*fos* and *junB* immediate early genes in neurons of the medial and ventral striatum and nucleus accumbens. Induction of c-*fos* and *junB* mRNA and Fos protein was blocked by naloxone, the D_1 dopamine (DA) receptor antagonists SCH23390 and SCH39166, and the *N*-methyl-D-aspartate (NMDA) glutamate receptor antagonist MK801. SCH23390 attenuated morphine induction of AP-1 binding in striatum, suggesting that c-*fos* and *junB* contribute to AP-1 binding. SCH23390 and MK801 did not block morphine induction of c-*fos* and *junB* in septum. Since the morphine induction of c-*fos* and *junB* in striatum and nucleus accumbens (NA) was similar to that observed with cocaine and amphetamine, these data support current concepts that limbic striatum and NA are among the brain regions that mediate drug abuse. Furthermore, since DA and NMDA receptors may mediate opiate reward and opiate induction of c-*fos* and *junB*, the DA/NMDA regulation of c-*fos* and *junB* and their target genes may produce long-term changes in the striatal and NA circuits that contribute to opiate drug abuse.**

Opiates, cocaine, and amphetamine are commonly abused drugs that produce tolerance, dependence, and addiction (1–3). These behaviors involve changes in brain dopamine (DA), norepinephrine, glutamate, and other neurotransmitter systems (1–8) as well as changes in gene expression. Cocaine and amphetamine induce c-*fos* immediate early gene (IEG) expression in medium-sized striatal and nucleus accumbens (NA) neurons (9). Cocaine also induces *junB* (10) and AP-1 binding activity (11) in striatum and NA. Dimers of the *fos* and *jun* families bind to AP-1 sites in promoters of target genes to regulate their expression (12–16). These long-term changes in gene expression could account for some behavioral effects of these drugs.

The present study determined whether morphine produced similar changes in gene expression. Morphine acts at μ opiate receptors (1, 2, 17, 18) to indirectly release DA in striatum (1, 4, 19–22). If there were common DA-mediated mechanisms of addiction for opiates, cocaine, and amphetamine (1, 4), these drugs should induce IEGs in similar brain regions. Moreover, since DA and its receptors are implicated in opiate and cocaine addiction (1–4), and *N*-methyl-D-aspartate (NMDA) receptors are implicated in opiate dependence and tolerance (5, 6), DA and NMDA receptor antagonists might block morphine induction of IEGs in brain regions that mediate behaviors that contribute to opiate abuse.

MATERIALS AND METHODS

Animals and Treatment. Female, albino Sprague–Dawley rats (200–250 g) were anesthetized with ketamine and xylazine (80 and 12 mg/kg i.p., respectively). A catheter was inserted i.p. and exited between the shoulders. The following day a preinjection of either saline (S; 1 ml/kg), MK801 (MK; 0.5, 1.0, or 2 mg/kg), or SCH23390 or SCH39166 (Sch; 0.2 or 1 mg/kg) was infused via the catheters. One hour later, either morphine (M; 10 mg/kg) or isotonic saline (S; 1 ml/kg) was administered every 0.5 h for either 1 h (for *in situ* hybridization) or 2 h (for immunocytochemistry).

Tissue Preparation. Animals processed for immunocytochemistry were anesthetized 3 h after the preinjection and perfused through the aorta with 300 ml of saline followed by 350 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed for 2 days, and 100-μm-thick coronal sections were cut on a Vibratome. Animals processed for *in situ* hybridization were narcotized with CO_2 and decapitated, and the brains were removed 2 h after the preinjection. The brains were frozen and embedded, and 20-μm-thick coronal sections were cut on a cryostat (−18°C) and mounted on ProbeOn slides (Fisher).

Immunocytochemistry was performed using avidin-biotin horseradish peroxidase methods as described (23, 24). Control sections displayed no staining when incubated without primary antibody. The primary antibody was a mouse monoclonal antibody (LA041) to a synthetic peptide derived from amino acid sequences 4–17 of the Fos protein. The antibody produces one band on Western blots (25) and produces patterns of Fos immunostaining that are identical to c-*fos* mRNA induction using *in situ* hybridization (24).

Cell Counting. Fos-immunoreactive nuclei were plotted on camera lucida drawings. The number of stained cells in each structure was counted on six sections from each subject and the values were averaged for each group. Student's *t* tests were performed for each region in the morphine (S-M) compared to saline (S-S) injected subjects and for morphine-treated subjects that received SCH23390 (Sch-M) and MK801 (Mk-M) pretreatments compared to saline (S-M) pretreatment (see Fig. 3).

***In Situ* Hybridization.** Oligonucleotide sequences: 5′-GCAGCGGGAGGATGACGCCTCGTAGTCCGCGTTGAAACCCGAGAA-3′ for c-*fos* is complementary to bases 141–185 (26); 5′-GCCCAGGGACACGTTGGGGGGCGTCACGTGGTTCAT-3′ for *junB* is complementary to bases 756–791 of murine *junB* cDNA (27). These probes and those for c-*jun* (28, 29) and murine *fosB* (30) distinguish between the known *fos* and *jun* family members (10, 31). The oligonucleotides were labeled with deoxyadenosine 5′-[γ-[^{35}S]thio]triphosphate (1500 Ci/mmol; 1 Ci = 37 GBq NEN) using terminal deoxynucleotidyltransferase (BRL) to produce a specific activity of 4–8 × 10^8 cpm per mg of DNA. Sections were incubated overnight at 42°C with 10^7 cpm of labeled probe per ml of hybridization buffer, which consisted of 50% deionized

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: DA, dopamine; EMSA, electrophoretic mobility-shift assay; GABA, γ-aminobutyric acid; IEG, immediate early gene; NA, nucleus accumbens; NMDA, *N*-methyl-D-aspartate.

*To whom reprint requests should be addressed.

†Communication of this paper was initiated by W. J. H. Nauta and, after his death, was completed by Ann M. Graybiel.

formamide, 4× standard saline citrate (SSC), 1× Denhardt's solution, 1% sarcosyl, 10% dextran sulfate, 500 μg of yeast tRNA per ml, 100 μg of salmon sperm DNA per ml, and 60 mM dithiothreitol (DTT). After hybridization, sections were rinsed four times for 15 min each in 1× SSC at 55°C and for 1 h in 1× SSC at room temperature, dried, and autoradiographed.

Preparation of Nuclear Extract. Striata were dissected and homogenized (Dounce) in 4 vol of buffer A [10 mM Tris·HCl, pH 7.9/3 mM $MgCl_2$/1 mM EGTA/0.15 mM spermine/0.5 mM spermidine/0.5 mM DTT/protease inhibitors (0.5 mM phenylmethylsulfonyl fluoride and 1 mg of leupeptin, aprotinin, antipain, chymostatin, and pepstatin A per ml in 0.25 M sucrose)]. The nuclear extract was then prepared as described (32).

Electrophoretic Mobility-Shift Assay (EMSA). Oligonucleotides to the consensus AP-1 site (5'-TCGACGTGACTCAGCGCGCATCGTGACTCAGCGCGC-3') and a mutated AP-1 site (5'-TCGACGCGACTCGGCGCGCATCGCGACTCGGCGCGC-3') were synthesized. Binding sites are underlined. The *in vitro* binding reactions and EMSA were performed as described (32, 33).

RESULTS

Morphine (S-M) induced Fos immunoreactivity in the nuclei of medium-sized striatal neurons (Fig. 1*B*) located in dorsomedial, ventromedial, and ventral lateral striatum (Figs. 1*B*, 2, and 3) in every animal (n = 48). There were few Fos-immunoreactive neurons in anterior striatum (data not shown). In the posterior one-third of striatum adjacent to the globus pallidus, Fos-positive cells were most numerous dorsomedially (data not shown). There were very few Fos-immunoreactive neurons in the striatum of subjects that received saline (S-S) (Figs. 1*A* and 3).

Morphine (S-M) increased the number of Fos-positive neurons in striatum >60-fold, in septum >8-fold, and in NA >4-fold compared to saline (S-S) controls (Fig. 3). Morphine induced Fos in ventrolateral septum at the level of the anterior commissure (Fig. 2). The modest induction of Fos in NA (Fig. 3) was distributed throughout the nucleus (data not shown). Repeated doses of morphine given every 30 min for 2 h were more effective than a single equivalent dose. For example, four 2-mg/kg bolus doses of morphine given i.p. every 30 min for 2 h produced more Fos-immunoreactive neurons than a single 8-mg/kg dose of morphine infused over 2 h (data not shown). This may relate to the 30- to 40-min half-life of morphine in brain (34). Few Fos-immunoreactive neurons were induced in subjects given four repeated 0.1- and 0.5-mg/kg doses of morphine (Fig. 4). Increasing doses increased the numbers of Fos-stained neurons in striatum with the maximal response being obtained with four 10-

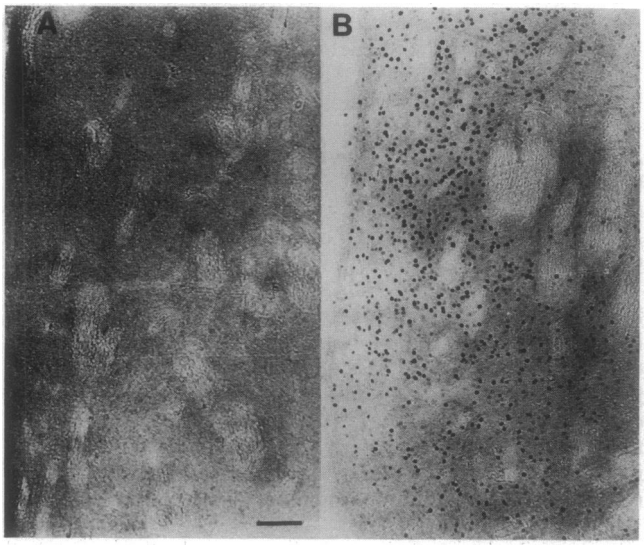

FIG. 1. Photomicrographs of dorsomedial striatum showing no Fos immunostaining in a rat injected with saline (*A*) compared to marked induction of Fos-positive neuronal nuclei in a rat injected i.p. with a single 10-mg/kg morphine dose 2 h previously (*B*). (Bar = 100 μm.)

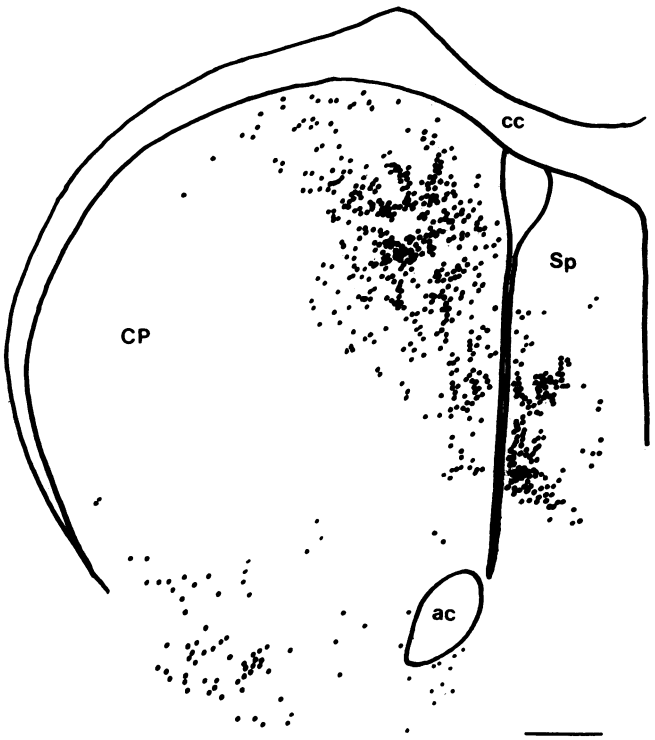

FIG. 2. Camera lucida drawing of rat brain showing the Fos immunostaining induced over 2 h following the administration of morphine (10 mg/kg i.p. given every 30 min) in caudate putamen (CP) and septal nucleus (Sp). Each dot represents one Fos-immunoreactive neuronal nucleus. There were few Fos-immunostained neurons in CP and Sp of saline controls. cc, Corpus callosum; ac, anterior commissure. (Bar = 500 μm.)

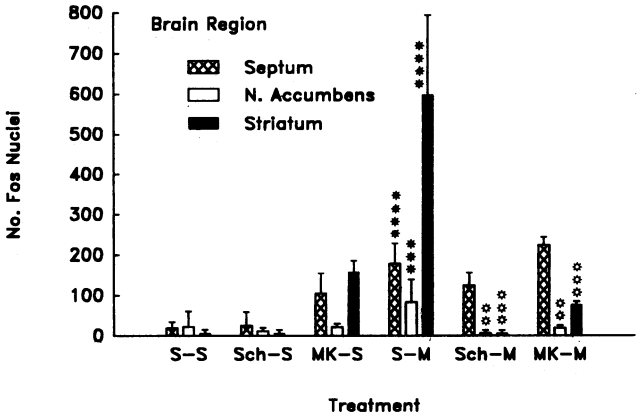

FIG. 3. Counts of the number of Fos-immunoreactive neuronal nuclei in the striatum, NA, and septum. Subjects were pretreated with either saline (S), the D_1 antagonist SCH23390 (Sch; 1.0 mg/kg), or the NMDA antagonist MK801 (MK; 1 mg/kg). After each pretreatment, subjects received either saline (S) or morphine (M; 10 mg/kg four times). Solid asterisks: Morphine (S-M) induced Fos in striatum, NA, and septum compared to saline (S-S) controls (***, $P < 0.005$; ****, $P < 0.0001$). Open asterisks: For morphine subjects, pretreatment with SCH23390 (SCH-M) and MK801 (MK-M) blocked Fos induction in striatum and NA when compared to saline (S-M) pretreatment (**, $P < 0.01$; ***, $P < 0.005$).

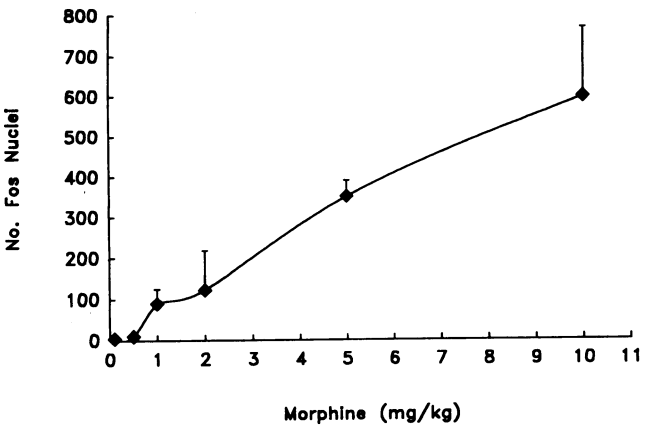

FIG. 4. Number of Fos-immunoreactive neuronal nuclei induced in the rat caudate putamen after bolus administration (given as doses of 0.1, 0.5, 1.0, 2.0, 5.0, or 10.0 mg/kg) of morphine given every 30 min for 2 h. Error bars are SEM (n = 5 for each dose).

mg/kg injections of morphine over 2 h (Fig. 4). Therefore, the four 10-mg/kg doses of morphine were used in most studies. The increasing doses of morphine induced increasing numbers of Fos-immunoreactive neurons in the same anatomical distribution in medial and ventral striatum and NA.

The stress of the injections did not induce Fos in striatum since saline injections alone (S-S) did not induce Fos (Fig. 3). Subjects that received naloxone (2 mg/kg) 1 h prior to morphine (10 mg/kg) had no induction of Fos (data not shown) in brain (n = 6 rats). This suggests that morphine actions at μ opiate receptors (and possibly other opiate receptors blocked by naloxone) were specifically responsible for morphine induction of Fos in rat brain.

Morphine induction of Fos (S-M) in striatum and NA was blocked by prior treatment with the D_1 antagonist SCH23390 (Sch-M) (Fig. 3) at doses of 0.2 and 1.0 mg/kg. Morphine induction of Fos in septum was unaffected by SCH23390 (Fig. 3). Another D_1 antagonist, SCH39166, given in doses of 0.2, 1.0, or 5.0 mg/kg (data not shown), blocked Fos induction in striatum but had no effect on morphine induction of Fos in septum.

Prior administration of MK801 (0.2, 1.0, or 2.0 mg/kg) blocked morphine induction of Fos in NA (Fig. 3) and medial and ventral striatum (data not shown). However, MK801 (MK-S) induced Fos in lateral striatum (Figs. 3 and 5*C*), accounting for the similar numbers of Fos-positive neurons in striatum in subjects that received MK801 and saline (MK-S) compared to those that received MK801 and morphine (MK-M) (Fig. 3). Since MK801 (MK-S) and morphine (S-M) both induce Fos in septum, this accounts for increased numbers of Fos-positive septal neurons (Fig. 3) in animals that received MK801 and morphine (MK-M).

Morphine (S-M) induced c-*fos* (Fig. 5*A*) and *junB* (Fig. 5*D*) mRNAs in medial and ventral striatum. Morphine did not induce *fosB*, c-*jun*, or *junD* (data not shown). Prior administration of SCH23390 to animals that received morphine (Sch-M) blocked induction of c-*fos* (Fig. 5*B*) and *junB* (Fig. 5*E*) in striatum. Prior administration of MK801 to animals that received morphine (MK-M) blocked induction of c-*fos* (Fig. 5*C*) and *junB* (Fig. 5*F*) in medial and ventral striatum. Saline infusions (S-S) had no effect on c-*fos*, *fosB*, c-*jun*, *junB*, and *junD* (data not shown).

MK801 induced c-*fos* mRNA in lateral striatum, cingulate cortex, neocortex, piriform cortex, septum (Fig. 5*C*), and other regions. Similar induction of Fos by MK801 has been previously reported by immunocytochemistry (35, 36). MK801 induces *junB* mRNA (Fig. 5*F*) in a pattern similar to that seen for c-*fos* (Fig. 5*C*).

Morphine induced AP-1 binding >5-fold (S-M) compared to saline (S-S) controls (Fig. 6). Pretreatment with SCH23390 (Sch-M) substantially decreased morphine-induced (S-M) AP-1 binding. AP-1 binding activity was reduced by the presence of excess wild-type unlabeled AP-1 oligonucleotide but not by oligonucleotide containing mutated AP-1 motifs, confirming the specificity of the morphine-induced AP-1 complex.

DISCUSSION

Morphine induction of c-*fos* and *junB* IEGs in striatum and NA required D_1 DA and NMDA glutamate receptor activation, findings similar to those reported for cocaine and amphetamine (9, 10, 37–43). The data support current concepts that many abused drugs activate similar receptors in specific brain regions that mediate behaviors that contribute to drug abuse (1–3). Although morphine has been reported to induce c-*fos* in striatum, the induction occurred throughout the striatum (44). Since these previous studies used polyclonal antibodies that recognized Fos family members (44), the Fos induction in medial and ventral striatum in the present study is accounted for by use of a monoclonal antibody that recognizes only Fos protein (24). Furthermore, our *in situ* hybridization studies confirmed that morphine induced c-*fos* and *junB* mRNAs only in medial and ventral striatum.

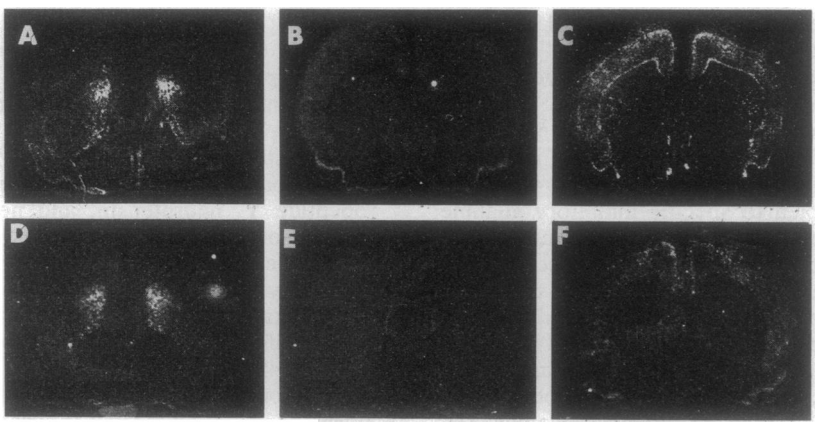

FIG. 5. c-*fos* (*A–C*) and *junB* (*D–F*) *in situ* hybridization of rat brain illustrating induction of c-*fos* (*A*) and *junB* (*D*) mRNA in medial striatum by morphine and the blockade of this gene expression in striatum by SCH23390 (*B* and *E*) and MK801 (*C* and *F*). Rats were injected with morphine (10 mg/kg) (*A* and *D*), SCH23390 (1 mg/kg) followed by morphine (10 mg/kg) (*B* and *E*), or MK801 (2 mg/kg) followed by morphine (10 mg/kg) (*C* and *F*) and the brains were removed 1 h later. MK801 blocked morphine induction of c-*fos* (*C*) and *junB* (*F*) mRNA in medial and ventral striatum. MK801 induced these genes in lateral striatum (*C*) and throughout the cortex (*C* and *F*).

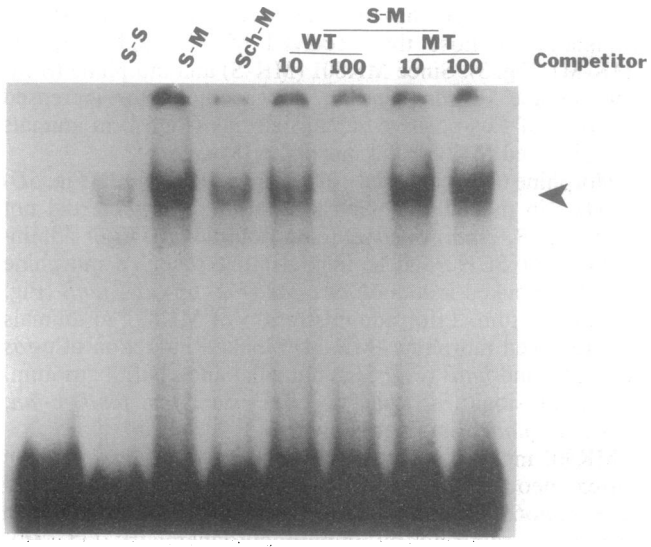

FIG. 6. Nuclear AP-1 binding activity (arrowhead) is markedly induced by morphine (S-M; 10 mg/kg four times) compared to saline (S-S). This morphine induction of AP-1 binding is markedly attenuated by SCH23390 (Sch-M; 1 mg/kg). Striatal nuclear extracts were prepared from rats injected 2 h earlier with saline (S-S), saline and morphine (S-M), or SCH23390 followed by morphine (Sch-M). Extracts were tested by EMSA in the presence or absence of unlabeled wild-type AP-1 (lanes WT) or mutant AP-1 (lanes MT) containing oligonucleotides at 10 and 100 M excess. Unmarked lane represents AP-1 probe alone.

Morphine induction of c-*fos* and *junB* in striatum and NA was blocked by the D_1 antagonist SCH23390 (45–50). SCH23390 also blocks cocaine and amphetamine induction of c-*fos* in striatum (9, 37, 51). Although SCH23390 antagonizes serotonin receptors (52), serotonin antagonists do not block cocaine induction of c-*fos* in striatum (9). Moreover, SCH39166, a D_1 antagonist that does not affect serotonin receptors (47), also blocked morphine induction of c-*fos* in striatum in the present study. Cocaine and amphetamine induce c-*fos* in medium-sized striatal neurons that have D_1 receptors. These neurons are believed to project to substantia nigra, contain a DA- and cAMP-regulated phosphoprotein (DARPP-32), and contain the cotransmitters γ-aminobutyric acid (GABA), substance P, and dynorphin (42, 51, 53–56). It seems likely that morphine induces c-*fos* in the striatal neurons that have D_1 receptors.

MK801 blocks amphetamine induction of Fos in striatum (38) and MK801 blocks morphine induction of c-*fos* and *junB* in striatum in the present study. Since MK801 prevents morphine tolerance and dependence in rats (5, 6), the striatum and NA would be among the many candidate regions for mediating tolerance and dependence to opiates.

Morphine binds to μ opiate receptors (1, 18, 57). Since naloxone blocked morphine induction of Fos, morphine probably induces Fos via μ opiate receptors (1, 2, 4, 5). The μ opiate receptors, coupled to specific inhibitory guanine nucleotide binding proteins (58), inhibit the neurons that morphine acts upon. Morphine activation of μ receptors *per se* probably does not induce c-*fos* and *junB* in the same cell. There is no known G_i coupled mechanism for induction of IEGs. Moreover, μ opiate receptors are distributed in regions such as tectum and substantia nigra where morphine did not induce IEGs. In addition, μ opiate receptors are localized to patches in the striatum (48, 53, 59, 60) called striosomes (53). Since morphine induction of IEGs in medial and ventral striatum involved matrix and striosome compartments—just as amphetamine and cocaine do (1)—the μ opiate receptor distribution (61, 62) does not explain the pattern of Fos induction by morphine.

The highest concentrations of μ receptors in substantia nigra are located around mesial DA neurons (63) in the ventral tegmental area that project to limbic striatum and NA. Morphine could indirectly influence these DA neurons via particular subtypes of μ opiate receptors (18). Alternatively, morphine might act upon other opiate receptor subtypes, including some delta subtypes, that are blocked by naloxone (64), to induce c-*fos* and *junB* in striatum and NA. The explanation for the pattern of Fos induction by morphine is uncertain.

Morphine induction of IEGs may require the serial activation of μ opiate, NMDA, and D_1 receptors. Morphine activation of μ receptors on GABAergic neurons in substantia nigra pars reticulata has been suggested to decrease GABA release onto nigral DA neurons (1) resulting in increased DA neuronal activity (19, 22) and release of DA in striatum (1, 4, 20). The DA release is modulated by NMDA receptors that could be located on presynaptic terminals or cell bodies of DA neurons (65–68). The DA acts on striatal neurons with D_1 receptors to induce c-*fos* and *junB* via mechanisms discussed below. This series circuit is consistent with the presence of μ opiate receptors in substantia nigra and their absence on DAergic terminals in striatum (60). Moreover, corticostriatal fibers synapse on DAergic fibers, and blockade of the NMDA receptor may modulate DA release in striatum with certain stimuli (67, 69).

Activation of μ opiate, NMDA, and D_1 DA receptors on the same striatal neuron might also induce IEGs via parallel intracellular routes. This is unlikely since NMDA receptors act via the serum response element (SRE) and D_1 receptors act via cAMP and the adenylate cyclase response element (CRE) in the c-*fos* promoter (70) to induce c-*fos*. Since stimulation of either the SRE or CRE is sufficient to induce the c-*fos* gene in a single neuron, either a D_1 or an NMDA receptor antagonist alone should not block the c-*fos* response to morphine in a single neuron (70, 71).

Many stimuli induce both c-*fos* and *junB* (29, 31, 72–74) including cocaine and amphetamine (10). The Fos and JunB proteins induced by morphine should form heterodimers. JunB, in the absence of Fos, is a repressor of AP-1 activity by attenuating trans-activation by c-*jun* (75). JunB in the presence of Fos contributes to AP-1 binding (75). Our data show that morphine induces c-*fos* and *junB* and AP-1 binding activity. Since SCH23390 blocks induction of c-*fos* and *junB* by morphine, and it attenuates induction of AP-1 binding activity, Fos and JunB protein heterodimers likely contribute to the AP-1 binding activity induced by morphine.

A target gene of the Fos/JunB complex could be preprodynorphin, which is expressed in striatal neurons with D_1 receptors (54–56) and which has an AP-1 like site in its promoter (13). Although opiates and cocaine affect striatal prodynorphin expression (76–78), it is not known whether the changes are mediated by Fos/JunB AP-1 binding to the prodynorphin promoter. There are a number of other target genes in striatal neurons with AP-1-like sites in their promoters (vasoactive intestinal peptide, cholecystokinin, neuropeptide Y, neurotensin, and nerve growth factor) that could also be regulated by morphine (12, 14–16).

DA release occurs in striatal and mesolimbic pathways with many addictive drugs (1, 4). Lesions of DAergic neurons and blockade of DA receptors impair opiate reward, reduce opiate self-administration, and affect other opiate behaviors (1, 4, 21, 49, 50). NMDA receptor antagonists prevent opiate and benzodiazepine tolerance and dependence (5, 79). Since morphine induction of IEGs in striatum and NA is blocked by D_1 and NMDA receptor antagonists, these structures could mediate reward and other behavioral effects of opiates. Since the morphine induction of IEGs in septum was not blocked by D_1 and NMDA receptor antagonists, the septum must mediate other behavioral effects of opiates. These data

support a growing body of evidence that different brain regions mediate different opiate behaviors since periaqueductal gray neurons appear to play a role in the physical dependence, whereas midbrain DA neurons mediate reward for opiates (1, 80). Furthermore, since DA and NMDA receptors may mediate reward for opiates and mediate opiate induction of c-*fos* and *junB*, the NMDA/DA regulation of these IEGs and their target genes may be involved in long-term changes in the brain circuits that contribute to opiate drug abuse.

We appreciate the assistance of Michael Butman, Mary Pat Hill, Dr. Jari Koistinaho, and Dr. Stephen M. Sagar. This work was supported by National Institute of Neurological Disorders and Stroke Grant RO1 NS28167 and by the Veterans Affairs Merit Review Program.

1. DiChiara, G. & North, R. A. (1992) *Trends Pharmacol. Sci.* **13**, 185–193.
2. Koob, G. F. & Bloom, F. E. (1988) *Science* **242**, 715–723.
3. Nestler, E. J. (1992) *J. Neurosci.* **12**, 2439–2450.
4. DiChiara, G. & Imperato, A. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 5274–5278.
5. Trujillo, K. A. & Akil, H. (1991) *Science* **251**, 85–87.
6. Marek, P., Ben-Eliyahu, S., Gold, M. S. & Liebeskind, J. C. (1991) *Brain Res.* **547**, 77–81.
7. Wise, R. A. & Rompre, P.-P. (1989) *Annu. Rev. Psychol.* **40**, 191–255.
8. Hayward, M. D., Duman, R. S. & Nestler, E. J. (1990) *Brain Res.* **525**, 256–266.
9. Graybiel, A. M., Moratalla, R. & Robertson, H. A. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6912–6916.
10. Moratalla, R., Vickers, E. A., Robertson, H. A., Cochran, B. H. & Graybiel, A. M. (1993) *J. Neurosci.* **13**, 423–433.
11. Hope, B., Kosofsky, B., Hyman, S. E. & Nestler, E. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5764–5768.
12. Morgan, J. I. & Curran, T. (1991) *Annu. Rev. Neurosci.* **14**, 421–451.
13. Naranjo, J. R., Mellstrom, B., Achaval, M. & Sassone-Corsi, P. (1991) *Neuron* **6**, 607–617.
14. Sheng, M. & Greenberg, M. E. (1990) *Neuron* **4**, 477–485.
15. Sonnenberg, J. L., Rauscher, F. J., III, Morgan, J. I. & Curran, T. (1989) *Science* **246**, 1622–1625.
16. Monstein, H.-J. (1993) *NeuroReport* **4**, 195–197.
17. Akil, H., Watson, S. J., Young, E., Lewis, M. E., Khachaturian, H. & Walker, J. M. (1984) *Annu. Rev. Neurosci.* **7**, 223–255.
18. Loh, H. H. & Smith, A. P. (1990) *Annu. Rev. Pharmacol. Toxicol.* **30**, 123–147.
19. Gysling, K. & Wang, R. Y. (1983) *Brain Res.* **277**, 119–127.
20. Spangel, R., Herz, A. & Shippenberg, T. S. (1990) *J. Neurochem.* **55**, 1734–1740.
21. Shippenberg, T. S., Bals-Kubik, R. & Herz, A. (1993) *J. Pharmacol. Exp. Ther.* **265**, 53–59.
22. Matthews, R. T. & German, D. C. (1984) *Neuroscience* **11**, 617–625.
23. Sagar, S. M., Sharp, F. R. & Curran, T. (1988) *Science* **240**, 1328–1331.
24. Sharp, F. R., Sagar, S. M., Marsh, T., Hicks, K., Lowenstein, D. H. & Hisanaga, K. (1991) *J. Neurosci.* **11**, 2321–2331.
25. De Togni, P., Niman, H., Raymond, V., Sawchenko, P. & Verma, I. M. (1988) *Mol. Cell Biol.* **8**, 2251–2256.
26. Curran, T., Gordon, M. B., Rubino, K. L. & Sambucetti, L. C. (1987) *Oncogene* **2**, 79–84.
27. Ryder, K., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1487–1491.
28. Ryseck, R. P., Hirai, S. I. & Bravo, R. (1988) *Nature (London)* **334**, 535–537.
29. Ryseck, R. P. & Bravo, R. (1991) *Oncogene* **6**, 533–542.
30. Zerial, M., Toschi, L., Ryseck, R. P., Schuermann, M., Muller, R. & Bravo, R. (1989) *EMBO J.* **8**, 805–813.
31. Uhl, G. R., Walther, D., Nishimori, T., Buzzi, M. G. & Moskowitz, M. A. (1991) *Mol. Brain Res.* **11**, 133–141.
32. Liu, J., Chiles, T. C., Sen, R. & Rothstein, T. L. (1991) *J. Immunol.* **146**, 1685–1691.
33. Hai, T. & Curran, T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3720–3724.
34. Bhargava, H. N., Villar, V. M., Rahmani, N. H. & Larsen, K. A. (1992) *J. Pharmacol. Exp. Ther.* **261**, 1008–1014.
35. Dragunow, M. & Faull, R. L. M. (1990) *Neurosci. Lett.* **111**, 39–44.
36. Sharp, F. R., Jasper, P., Hall, J., Noble, L. & Sagar, S. M. (1991) *Annu. Neurol.* **30**, 801–809.
37. Young, S. T., Porrino, L. J. & Iadarola, M. J. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1291–1295.
38. Snyder-Keller, A. M. (1991) *Exp. Neurol.* **113**, 155–165.
39. Robertson, H. A., Peterson, M. R., Murphy, K. & Robertson, G. S. (1989) *Brain Res.* **503**, 346–349.
40. Robertson, H. A., Paul, M. L., Moratalla, R. & Graybiel, A. M. (1991) *Can. J. Neurol. Sci.* **18**, 380–383.
41. Robertson, G. S., Vincent, S. R. & Fibiger, H. C. (1990) *Brain Res.* **523**, 288–290.
42. Cenci, M. A., Campbell, K., Wictorin, K. & Bjorklund, A. (1992) *Eur. J. Neurosci.* **4**, 376–380.
43. Dilts, R. P., Helton, T. E. & McGinty, J. F. (1993) *Synapse* **13**, 251–263.
44. Chang, S. L., Squinto, S. P. & Harlan, R. E. (1988) *Biochem. Biophys. Res. Commun.* **157**, 698–704.
45. Tedford, C. E., Ruperto, V. B., Coffin, V. L., Cohen, M., Libonati, M. & Barnett, A. (1992) *Drug Dev. Res.* **26**, 389–403.
46. Nakajima, S. & McKenzie, G. M. (1986) *Pharmacol. Biochem. Behav.* **24**, 919–923.
47. Chipkin, E. R., Irio, L. C., Coffin, V. L., McQuade, J. G., Berger, J. G. & Barnett, A. (1988) *J. Pharmacol. Exp. Ther.* **247**, 1093–1102.
48. Caboche, J., Rogard, M. & Besson, M.-J. (1991) *Dev. Brain Res.* **58**, 111–122.
49. Maldonado, R., Robledo, P., Chover, A. J., Caine, S. B. & Koob, G. F. (1993) *Pharmacol. Biochem. Behav.* **45**, 239–242.
50. Shippenberg, T. S. & Herz, A. (1988) *Eur. J. Pharmacol.* **151**, 233–242.
51. Beretta, S., Robertson, H. A. & Graybiel, A. M. (1992) *J. Neurophysiol.* **67**, 767–777.
52. Bischoff, S., Heinrich, M., Sonntag, J. M. & Krauss, J. (1986) *Eur. J. Pharmacol.* **129**, 367–370.
53. Graybiel, A. M. (1990) *Trends Neurosci.* **13**, 244–254.
54. Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., Jr., & Sibley, D. R. (1990) *Science* **250**, 1429–1432.
55. Gerfen, C. R., McGinty, J. F. & Young, W. S., III (1991) *J. Neurosci.* **11**, 1016–1031.
56. Gerfen, C. R. (1992) *Trends Neurosci.* **15**, 133–139.
57. North, R. A., Williams, J. T., Suprenant, A. & Christie, M. J. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5487–5491.
58. Laugwitz, K.-L., Offermanns, S., Spicher, K. & Schultz, G. (1992) *Neuron* **10**, 233–242.
59. Hurd, Y. L. & Herkenham, M. (1993) *Synapse* **13**, 357–369.
60. Trovero, F., Herve, D., Desban, M., Glowwinski, J. & Tassin, J.-P. (1990) *Neuroscience* **39**, 313–321.
61. McLean, S., Rothman, R. D. & Herkenham, M. (1986) *Brain Res.* **378**, 49–60.
62. Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H. & Watson, S. J. (1987) *J. Neurosci.* **7**, 2445–2464.
63. German, D. C., Speciale, S. G., Manaye, K. F. & Sadeq, M. (1993) *J. Neural Trans.* **91**, 39–52.
64. Miyamoto, Y., Portoghese, P. S. & Takemori, A. E. (1993) *J. Pharmacol. Exp. Ther.* **265**, 1325–1327.
65. Krebs, M. O., Trovero, F., Desban, M., Gauchy, C., Glowinski, J. & Kemel, M. L. (1991) *J. Neurosci.* **11**, 1256–1262.
66. Leviel, V., Gobert, A. & Guibert, B. (1990) *Neuroscience* **39**, 305–312.
67. Martinez-Fong, D., Rosales, M. G., Gongora-Alfaro, J. L., Hernandez, S. & Acebes, J. (1992) *Brain Res.* **595**, 309–315.
68. Wang, J. K. (1991) *J. Neurochem.* **57**, 819–822.
69. Bouyer, J. J., Park, D. H., Joh, T. H. & Pickel, V. M. (1984) *Brain Res.* **302**, 267–275.
70. Bading, H., Ginty, D. D. & Greenberg, M. E. (1993) *Science* **260**, 181–186.
71. Hisanaga, K., Sagar, S. M., Hicks, K. J. & Sharp, F. R. (1992) *J. Neurochem.* **58**, 1836–1844.
72. Kornhauser, J. M., Nelson, D. E., Mayo, K. E. & Takahashi, J. S. (1992) *Science* **255**, 1581–1584.
73. Lanaud, P., Maggio, R., Gale, K. & Grayson, D. R. (1993) *Exp. Neurol.* **119**, 20–31.
74. Naranjo, J. R., Mellstrom, B., Achaval, M., Lucas, J. J., Del Rio, J. & Sassone-Corsi, P. (1991) *Oncogene* **6**, 223–227.
75. Deng, T. & Karin, M. (1993) *Genes Dev.* **7**, 479–490.
76. Sivam, S. P. (1989) *J. Pharmacol. Exp. Ther.* **250**, 818–824.
77. Romualdi, P., Lesa, G. & Ferri, S. (1991) *Brain Res.* **563**, 132–136.
78. Daunais, J. B., Roberts, D. C. S. & McGinty, J. F. (1993) *NeuroReport* **4**, 543–546.
79. Steppuhn, K. G. & Turski, L. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 6889–6896.
80. Bozarth, M. A. & Wise, R. A. (1984) *Science* **24**, 516–517.