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Regulation of Phosphorylated cAMP Response Element-Binding Protein, Fos-Related Antigen and FosB Expression by Dopamine Agonists in Rat Striatum

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Activation of D1-like dopamine receptors by psychostimulants, such as amphetamine, upregulates the expression of immediate early gene and opioid peptide gene in the striatum. The genomic changes are regulated by phosphorylated transcription factors via complicated intracellular events. To evaluate temporal expression of the transcription factors by dopaminergic stimulation, the D1-like dopamine agonist, amphetamine or SKF82958, was systematically delivered. As intracellular markers in response to the agonist, phosphorylated cAMP response element-binding protein (pCREB), Fos-related antigens (FRA) and FosB immunoreactivity (IR) was compared at 20 and 120 min time points in the selected areas of the striatum. Semi-quantitative immunocytochemistry showed that amphetamine (5 mg/kg, i.p.) significantly increased pCREB-IR at 20 min, sustained up to 60 min and decreased at 120 min after the infusion. Like amphetamine, the full D1 agonist, SKF82958 (0.5 mg/kg, s.c.), also increased pCREB-IR at 20 min, but not at 120 min after the infusion in the dorsal striatum (caudoputaman, CPu) and shell of ventral striatum (nucleus accumbens, NAc). In contrast, FRA- and FosB-IR induced by SKF82958 was significantly increased at 120 min, but not at 20 min after the administration. These data indicate that SKF82958 mimics induction of CREB phosphorylation by amphetamine and differentially regulates temporal induction of pCREB, and FRA and FosB expression in the striatum.

Key Words: Drugs of abuse, CREB, FRA, FosB, Striatum, Transcription factors

INTRODUCTION

Acute administration of amphetamine, an indirect D1-like dopamine receptor agonist, causes direct stimulation of dopamine release from dopaminergic terminals, nigrostriatal and mesoaccumbal neurons (Zetterstrom et al, 1986, 1993; Sharp et al, 1987). An increase in the level of dopamine following acute amphetamine administration leads to genomic changes, such as an increase in mRNA levels of immediate early genes, c-fos (Delfs & Kelley, 1990), and the opioid peptide genes, preprodynorphin (PPD) and preproenkephalin (PPE) in the striatum (Simpson et al, 1995; Wang & McGinty, 1995). The genomic changes have been demonstrated to mediate primarily through D1-like dopamine receptors on postsynaptic striatal medium spiny neurons (Berretta et al, 1992; Cole et al, 1992; Garrett & Holtzman, 1994).

Activation of D1 dopamine receptors, which are positively coupled to adenylate cyclase increases levels of cAMP. Increased levels of cAMP activate either protein kinase A (PKA) (Grewal et al, 2000) and/or mitogen-activated protein kinases (Choe & McGinty, 2000, 2001), resulting in CREB phosphorylation on Ser-133 (Yamamoto et al, 1988; Gonzalez & Montminy, 1989).

Binding of pCREB to calcium and cAMP response elements (CaCREs) located in the promoter regions of c-fos, PPD and PPE results in an increase in

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transcription of these genes (Comb et al, 1988; Goodman, 1990; Ransone & Verma, 1990; Naranjo et al, 1991; Kaynard et al, 1992; McMurray et al, 1992). In addition to CaCREs, the promoter regions of the c-fos, PPD and PPE contain activator protein 1 (AP-1)-like binding sites. The AP-1 sites are hypothesized to bind Fos protein families, Fos/Jun for acute or FRA/Jun heterodimer for chronic stimulation of psychostimulants (Comb et al, 1988; Goodman, 1990; Ransone & Verma, 1990; Naranjo et al, 1991; Kaynard et al, 1992; McMurray et al, 1992), suggesting delayed induction of FRA or FosB in response to acute and/or chronic stimulation of psychostimulants.

The previous study by Simpson et al (1995) demonstrates that pCREB-IR reaches a peak 20 min and decreased 2 hr after a single amphetamine injection in the striatum in vivo. However, the temporal patterns of pCREB-IR between 20~ and 120 min after the administration are not clear.

The present study demonstrates the pattern of pCREB-IR by amphetamine at time points between 20~ and 120 min after the administration. Furthermore, pCREB-, FRA-, and FosB-IR by SKF82958 administration were monitored at 20~ and 120 min time points to test the hypothesis that levels of FRA- and/or FosB-IR, but not pCREB-IR, would be greater following 120 min than 20 min after D1 dopamine receptor activation. Semi-quantitative immunocytochemistry was used to quantify levels of the markers induced by amphetamine or SKF82958 in the striatum.

METHODS

Animals

Adult male Wistar rats (225~250 g) were individually housed in a controlled environment during all experimental treatments. Food and water were provided *ad libitum* and the rats were maintained on a 12 hrs light/dark cycle. On the day of the experiment and drug administration was done in the quiet room to minimize stress. All animal use procedures were approved by the Institutional Animal Care and Use Committee of East Carolina University School of Medicine and were accomplished in accordance with the provisions of the NIH "Guide for the Care and Use of Laboratory Animals".

Experimental design

All doses of D-Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) and R-(+)-SKF-82958 hydrochloride (Research Biochemicals International, Natick, MA) were calculated as the salt and were dissolved in physiological saline. Amphetamine or SKF82958 was injected intraperitoneally (i.p.) or subcutaneously (s.c.), respectively, in a volume of 2 ml/kg. The dose of amphetamine (5 mg/kg) or SKF82958 (0.5 mg/kg) was freshly prepared. Two separate experiments were performed in order to compare alterations of markers at several different time points in different regions of striatum following amphetamine or SKF82958 administration. Rats were euthanized at 20, 40, 60, or 120 min time point following amphetamine administration and at 20 or 120 min time point following SKF82958. Five or six rats were used for saline control and amphetamine or SKF82958 administration.

Immunocytochemistry

At each time point following amphetamine or SKF82958 administration, with the descending aorta clamped, rats were transcardially perfused with 300 ml of 4% paraformaldehyde in 0.1 M PBS at 4°C over 7 min using a Masterflex perfusion pump. Brains were removed and postfixed in 10% sucrose/4% paraformaldehyde for 2 hrs at 4°C and then placed in 20% sucrose/PBS at 4°C overnight. Using a sliding microtome, 50 um frozen sections were cut serially through the striatum and collected in 0.1% sodium azide/PBS. Three sections per antiserum per brain were collected around 1.0 mm anterior to bregma and processed for immunocytochemistry. Phospho-CREB antiserum (New England Biolabs, Beverly, MA, diluted 1 : 1,000) recognizes the CREB phosphoprotein surrounding amino acid Ser133. The FRA antiserum (donated by M Iadarola, NIDR-NIH) recognizes a conserved sequence within the c-fos immediate early gene family proteins, Fos amino acids 127~152 and was used at a dilution of 1 : 25,000. FosB antiserum (Santa Cruz Biotechnology, Santa Cruz, CA, diluted 1 : 5,000) recognizes a peptide corresponding to amino acids 102~117 of the FosB protein. Sections were incubated with each antiserum for 20 hr at 4°C on a shaker. Sections were then incubated in goat anti-rabbit secondary antiserum (Vector Labs, Burlingame, CA) for 1 hr followed by

avidin-biotin-peroxidase reagents (Elite Vectastain kit, Vector Labs) for 1 hr at room temperature. VIP (Vector Labs) was used as the chromagen to yield a purple reaction product. Throughout the immunocytochemical procedure, all the sections were run simultaneously and reacted for precisely the same time. Sections were mounted on chrom-alum/gelatin-coated slides, coverslipped in Permount, and viewed with a Zeiss photomicroscope. CREB antiserum (donated by DD Ginty and ME Greenberg, Department of Microbiology and Molecular Genetics, Harvard Medical School, diluted 1 : 10,000) was used as a control for pCREB-IR because it recognizes CREB regardless of its phosphorylation state and is not inducible. FRA and FosB antisera were neutralized with FRA or blocking peptides FosB (Santa Cruz Biotechnology). Each antiserum was combined with the appropriate blocking peptides in 0.1 M PBS containing 0.1% BSA (0.1 ug/ul) and incubated for 3 hr at room temperature. Striatal sections were incubated with each mixture and the immunocytochemical procedure described above was performed.

Quantitation of immunoreactivity

Levels of CREB-, pCREB-, FRA- and FosB-IR in the dorsal and ventral striatum were semi-quantified using a modification of the procedure of Simpson et al (1995). Briefly, immunoreactive images were captured from a Zeiss photomicroscope using a Paultek Imaging CCD camera and NIH Image 1.62 software. Using a 4× objective and a 1.25× photo lens, the immunoreactivity in three sections per brain stained with one of four antisera was measured in a 600 L× 450 W pixel area in the dorsal striatum; a circle of 478 pixels in diameter around the anterior commissure in the ventral striatum (core region of the nucleus accumbens); and a circle of 120 pixels in diameter in the region of the dorsal shell of the nucleus accumbens. The areas in Fig. 1 represent the regions measured for each antiserum. The density threshold was adjusted so that background staining in white matter regions was eliminated and the number of immunoreactive pixels per selected area was measured above this threshold.

Statistics

Statistical significance of the number of immunoreactive pixels per measured area between groups was determined by a one-way ANOVA on ranked data followed by a Tukey's HSD (honestly significant difference) test using SAS (Cary, NC).

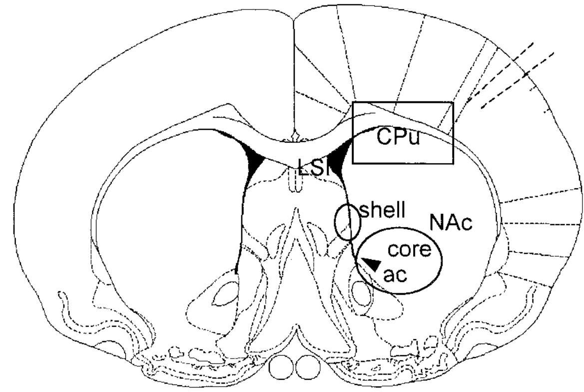

Fig. 1. Diagram depicting areas in which immunoreactivity was measured by semi-quantitative image analysis after administration of amphetamine intraperitoneally or SKF82958 subcutaneously. AC, anterior commissure; Cpu, caudoputamen (dorsal striatum); LSN, lateral septal nuclei; Nac, nucleus accumbens (ventral striatum, core and shell).

RESULTS

Alterations of striatal pCREB-IR by amphetamine

Striatal CREB-IR was constitutively and uniformly expressed throughout the striatum of saline- or amphetamine-administrated rats. Semi-quantitative analysis revealed that there was no change in the number of immunoreactive pixels for CREB 20 min after saline or amphetamine administration in the dorsal striatum and the core region of nucleus accumbens (Fig. 2). Sections from saline-injected controls demonstrated that pCREB-IR in the CPu was relatively lower than that in the core region of the NAc at each time point except for 40 min time point. The intensity of pCREB-IR was significantly increased at 20 min, sustained up to 60 min and decreased at 120 min after amphetamine injection in the CPu, but it did not fully expressed as compared with CREB expression. In the core region of the NAc, pCREB-IR was significantly increased at 20 min, reached a peak at 40 min, and decreased 60 min after amphetamine injection.

Alterations of striatal pCREB-, FRA- and FosB-IR by SKF82958

Immunoneutralization of antisera with FRA and FosB peptides (0.1 ug/ul) blocked any detectable immunostaining, indicating specificity of the antisera for the corresponding FRA and FosB antigens. As shown in Fig. 2, striatal CREB-IR was not changed in the striatum of saline- or SKF82958-injected rats (data not shown).

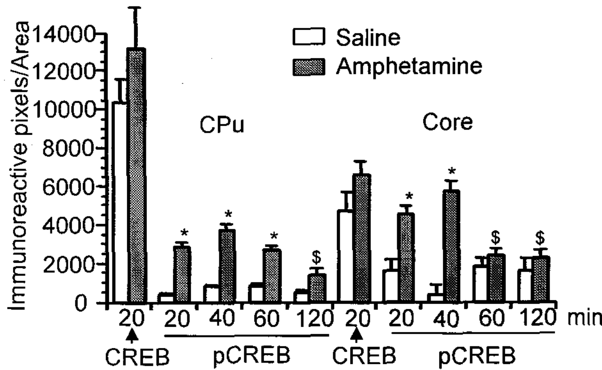

Fig. 2. Striatal pCREB immunoreactivity induced by amphetamine (5 mg/kg, i.p.) at 20, 40, 60 and 120 min after the administration intraperitoneally. *$P<0.01$ as compared with saline control at each time point. $P<0.01 as compared with 20~60 and 20~40 min amphetamine treated groups in the CPu and core, respectively.

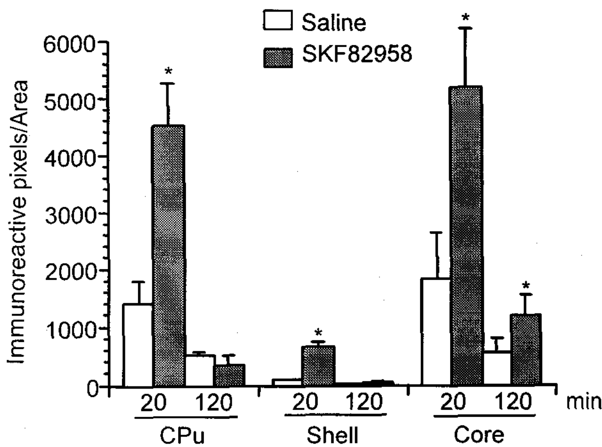

Fig. 3. Striatal pCREB immunoreactivity induced by SKF82958 (0.5 mg/kg, s.c.) at 20 and 120 min after the administration subcutaneously. *$P<0.01$ as compared with saline control at each time point.

Phospho-CREB-IR

In the CPu, sections from 20 min saline controls demonstrated a high basal level of pCREB-IR as compared with 120 min control groups. The intensity of pCREB-IR was profoundly increased 20 min after SKF82958 administration. Two hours after SKF-82958, pCREB-IR seemed to be returning to basal levels. Similarly, in the NAc, sections from 20 min saline controls demonstrated a high basal level of pCREB-IR as compared with 120 min saline control groups. As demonstrated in the CPu, the intensity of pCREB-IR in the NAc was profoundly increased 20 min after SKF82958. P-CREB-IR in the dorsal shell returned to basal levels 120 min after SKF82958 infusion, whereas pCREB in the core remained elevated (Fig. 3).

FRA-IR

In the CPu, sections from both 20~ and 120 min saline controls demonstrated a low basal level of FRA-IR. The intensity of FRA-IR was not altered 20 min after acute SKF82958 administration. FRA-IR was tremendously increased 120 min after SKF82958 administration. In the NAc, sections from 20 min saline controls demonstrated a high basal level of FRA-IR as compared with 120 min control groups. The intensity of FRA-IR by SKF82958 in the shell and the core of the NAC was low at 20 min time point and significantly increased at 120 min time point as compared to the controls (Fig. 4).

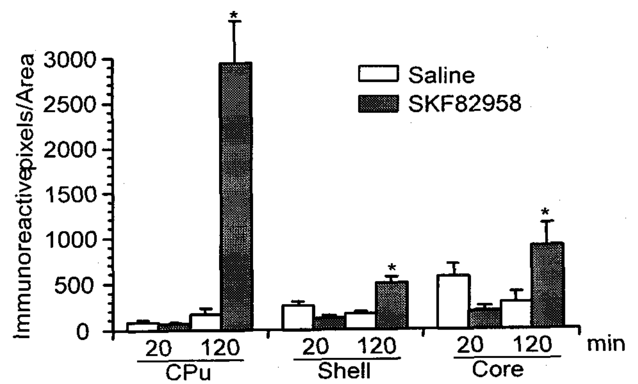

Fig. 4. Striatal FRA immunoreactivity induced by SKF82958 (0.5 mg/kg, s.c.) at 20 and 120 min after the administration subcutaneously. *$P<0.01$ as compared with saline control at each time point.

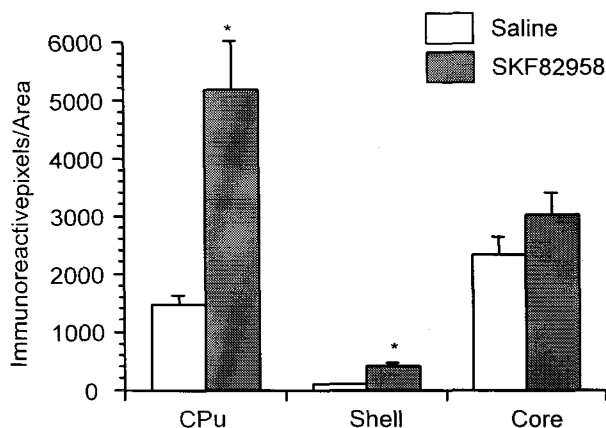

Fig. 5. Striatal FosB immunoreactivity induced by SKF-82958 (0.5 mg/kg, i.p.) at 120 min after the administration subcutaneously. *P<0.01 as compared with saline controls.

FosB-IR

In the CPu, sections from saline controls demonstrated a high basal level of FosB-IR. The intensity of FosB-IR was significantly increased 120 min after acute SKF82958 administration. In the core of the NAc, sections from saline controls demonstrated a high basal level of FosB-IR as compared with the dorsal shell. As shown in Fig. 4, the intensity of FosB-IR was significantly increased in the shell. However, the intensity of FosB-IR was not altered in the core by SKF82958 (Fig. 5).

DISCUSSION

Phosphorylation of CREB has been demonstrated to regulate striatal c-fos, PPD and PPE mRNA induction in response to acute amphetamine (Konradi et al, 1994). Other studies have demonstrated that Fos and FRA protein levels are regulated by cocaine or apomorphine administration in rat striatum (Hope et al, 1992, 1994). In this study, alterations in the induction of pCREB, FRA and FosB were monitored at 20, 40, 60 and 120 min after a single amphetamine administration. Temporal induction of pCREB-, FRA- and FosB-IR by a full D1 agonist, SKF82958, was also compared at 20 and 120 min time points in the striatum. The present results show that amphetamine significantly increased pCREB-IR at 20 min, and decreased at 120 min after the infusion in the dorsal striatum. Phospho-CREB-IR between the two time points was significantly altered and sustained up to 60 min. Similarly, in the ventral striatum, pCREB-IR by amphetamine was increased at 20 min, sustained up to 40 min, then decreased 60 min after the administration. The starting time point, 20 min, was chosen based on the time course of phosphorylation/dephosphorylation in primary striatal cultures (Cole et al, 1995). In response to acute dopamine administration, levels of pCREB are maximal between 15 an 30 min and return to control levels by 3 hrs (Cole et al, 1995). Consistent with our data, pCREB-IR in the dorsal striatum was induced at 20 min and reduced control levels 120 min after acute amphetamine injection (Simpson et al, 1995). These data suggest that levels of pCREB-IR reach a maximum at 20 min, and sustained up to 60 min, and then return back to control levels at 120 min after a single amphetamine administration. However, we do not exclude the possibility that levels of pCREB-IR reach a maximum between 20 and 60 min after amphetamine administration.

Based on the results, pCREB-IR was examined by direct activation of D1-like receptors with SKF82958 at the two different time points, 20 min and 120 min. Temporal induction of pCREB-IR was mimicked with a single administration of amphetamine. In contrast, FRA- and FosB-IR was altered only at 120 min-, but not 20 min time point, except for core region of the ventral striatum. FRA and FRA of 35~37 kDa are induced by chronic electroconvulsive shock or psychostimulant administration (Hope et al, 1992, 1994; Hiroi et al, 1997). The chronic FRA forms AP-1 complexes, which can persist for days following cessation of chronic treatment. In contrast to the previous studies by Hope et al (1992, 1994), levels of FRA and FosB-IR were induced in the dorsal and ventral striatum 120 min after a single SKF82958 infusion. Augmentation of FRA- and FosB-IR by a single administration of SKF82958 supports the fact that these proteins are activated by both acute and chronic stimulation. This assumption may be supported by the finding that augmented levels of FRA-IR in response to repeated amphetamine might be due to an accumulation of chronic FRA (Hope et al, 1994). Levels of FosB-IR in the dorsal core of the nucleus accumbens were not significantly altered at 120 min time point following SKF82958 administration. However, FosB-IR in the shell region of the nucleus accumbens was significantly increased 120

min after the administration. The preferential induction of FosB in the shell, but not the core, region of the nucleus accumbens provides further support for subdividing the nucleus accumbens into a core and shell region based on its functional anatomy.

In conclusion, amphetamine increased CREB phosphorylation at 20 min, sustained up to 60 min, and decreased at 120 min after the administration. SKF82958 mimicked spatiotemporal induction of CREB phosphorylation induced by amphetamine. Furthermore, SKF82958 increased delayed induction of FRA and FosB as compared to pCREB. These data suggest that SKF82958 differently regulates temporal induction of pCREB, and FRA and FosB in the striatum in vivo.

REFERENCES

Berretta S, Robertson HA, Graybiel AM. Dopamine and glutamate agonists stimulate neuron-specific expression of Fos-like protein in the striatum. *J Neurophysiol* 68: 767−777, 1992

Choe ES, McGinty JF. N-methyl-D-aspartate receptors and p38 mitogen-activated protein kinase are required for cyclic AMP-dependent cyclase response element binding protein and Elk-1 phosphorylation in the striatum. *Neuroscience* 101: 607−617, 2000

Choe ES, McGinty JF. Cyclic AMP and mitogen-activated protein kinases are required for glutamate-dependent cyclic AMP response element binding protein and Elk-1 phosphorylation in the dorsal striatum in vivo. *J Neurochem* 76: 401−412, 2001

Cole AJ, Bhat RV, Patt C, Worley PF, Baraban JM. D1 dopamine receptor activation of multiple transcription factor genes in rat striatum. *J Neurochem* 58: 1420−1426, 1992

Cole RL, Konradi C, Douglass J, Hyman SE. Neuronal daptation to amphetamine and dopamine: molecular mechanisms of prodynorphin gene regulation in rat striatum. *Neuron* 14: 813−823, 1995

Comb M, Mermod N, Hyman SE, Pearlberg J, Ross ME, Goodman HM. Proteins bound at adjacent DNA elements act synergistically to regulate human proenkephalin cAMP inducible transcription. *EMBO J* 7: 3793−3805, 1988

Delfs JM, Kelley AE. The role of D1 and D2 dopamine receptors in oral stereotypy induced by dopaminergic stimulation of the ventrolateral striatum. *Neuroscience* 39: 59−67, 1990

Garrett BE, Holtzman SG. D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. *Pharmacol Biochem Beh* 47: 89−94, 1994

Gonzalez GA, Montminy MR. Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133. *Cell* 59: 675−680, 1989

Goodman RH. Regulation of neuropeptide gene expression. *Annu Rev Neurosci* 13: 111−127, 1990

Grewal SS, Horgan AM, York RD, Withers GS, Banker GA, Stork PJS. Neuronal calcium activates a Rap1 and B-Raf signaling pathway via cyclic adenosine monophosphate-dependent protein kinase. *J Biol Chem* 275: 3722−3728, 2000

Hiroi N, Brown JR, Haile CN, Ye H, Greenberg ME, Nestler EJ. FosB mutant mice: loss of chronic cocaine induction of Fos-related proteins and heightened sensitivity to cocaine's psychomotor and rewarding effects. *Proc Natl Acad Sci USA* 94: 10397−10402, 1997

Hope B, Kosofsky B, Hyman SE, Nestler EJ. Regulation of immediate early gene expression and AP-1 binding in the rat nucleus accumbens by chronic cocaine. *Proc Natl Acad Sci USA* 89: 5764−5768, 1992

Hope BT, Nye HE, Kelz M, Self DW, Iadarola MJ, Nakabeppu Y, Duman RS, Nestler EJ. Induction of a long-lasting AP-1 complex composed of altered Fos-like proteins in brain by chronic cocaine and other chronic treatments. *Neuron* 13: 1235−1244, 1994

Hope BT, Kelz MB, Duman RS, Nestler EJ. Chronic electroconvulsive seizure (ECS) treatment results in expression of a long-lasting AP-1 complex in brain with altered composition and characteristics. *J Neurosci* 14: 4318−4328, 1994

Kaynard AH, McMurray CT, Douglass J, Curry TE, Melner MH. Regulation of prodynorphin gene expression in the ovary: distal DNA regulatory elements confer gonadotropin regulation in promoter activity. *Molec Endocr* 6: 2244−2256, 1992

Konradi C, Cole RL, Heckers S, Hyman SE. Amphetamine regulates gene expression in rat striatum via transcription factor CREB. *J Neurosci* 14: 5623−5634, 1994

McMurray CT, Pollock KM, Douglas J. Cellular and molecular analysis of opioid peptide gene expression. *NIDA Res Monograph Ser* 126: 113−131, 1992

Naranjo JR, Mellstrom B, Achaval M, Sassone-Corsi P. Molecular pathways of pain: Fos/Jun-mediated activation of a noncanonical AP-1 site in the prodynorphin gene. *Neuron* 6: 607−617, 1991

Ransone LJ, Verma IM. Nuclear proto-oncogenes FOS and JUN. *Annu Rev Cell Biol* 6: 539−557, 1990

Sharp T, Zetterstrom T, Ljungberg T, Ungerstedt U. A direct comparison of amphetamine-induced behaviors and regional brain dopamine release in the rat using intracerebral dialysis. *Brain Res* 401: 322−330, 1987

Simpson JN, Wang JQ, McGinty JF. Repeated ampheta-

mine administration induces a prolonged augmentation of phosphorylated cyclase response element-binding protein and Fos-related antigen immunoreactivity in rat striatum. *Neuroscience* 69: 441−457, 1995

Wang JQ, McGinty JF. Alterations in striatal zif/268, preprodynorphin and preproenkephalin mRNA expression induced by repeated amphetamine administration in rats. *Brain Res* 673: 262−274, 1995

Yamamoto KK, Gonzalez GA, Biggs WH, Montminy MR. Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. *Nature* 334: 494−498, 1988

Zetterstrom T, Herrera-Marschitz M, Ungerstedt U. Simultaneous measurement of dopamine release and rotational behavior in 6-hydroxydopamine denervated rats using intracerebral dialysis. *Brain Res* 376: 1−7, 1986

Zetterstrom T, Sharp T, Marsden CA, Ungerstedt U. In vivo measurement of dopamine and its metabolites by intracerebral dialysis: changes after D-amphetamine. *J Neurochem* 41: 1769−1773, 1993