Tyrosine hydroxylase (TH), expressed in a population of periglomerular neurons intrinsic to the olfactory bulb, displays dramatic down-regulation in response to odor deprivation. To begin to elucidate the importance of immediate early genes (IEGs) in TH gene regulation, the present study examined expression of IEGs in the olfactory bulb in response to odor deprivation. In addition, the composition of TH AP-1 and CRE binding complexes was investigated in control and odor-deprived mice. Immunocytochemical studies showed that c-Fos, Fos-B, Jun-D, CRE-binding protein (CREB), and phosphorylated CREB (pCREB) are colocalized with TH in the dopaminergic periglomerular neurons. Unilateral naris closure resulted in down-regulation of c-Fos and Fos-B, but not Jun-D, CREB, or pCREB, in the glomerular layer of the ipsilateral olfactory bulb. Gel shift assays demonstrated a significant decrease (32%) in TH AP-1, but not CRE, binding activity in the odor-deprived bulb. Fos-B was found to be the exclusive member of the Fos family present in the TH AP-1 complex. CREB, CRE modulator protein (CREM), Fos-B, and Jun-D, but not c-Fos, all contributed to the CRE DNA-protein complex. These results indicated that Fos-B, acting through both AP-1 and CRE motifs, may be implicated in the regulation of TH expression in the olfactory bulb dopaminergic neurons.

Immediate early genes (IEGs) encode transcription factors that are induced rapidly by extracellular signals and regulate expression of target genes. IEGs have been implicated in many neuronal functions including neuronal plasticity (1, 2). Interactions of these IEG products with specific DNA sequences in gene promoters either activate or suppress the initiation of gene transcription (3). AP-1 (TGACTCA) and CRE (TGACG) motifs, the latter differing from the consensus AP-1 only in the middle base (5, 23–26). The TH CRE was found to be required not only for basal activity but also for cAMP-mediated up-regulation of TH promoter activity in SK-N- BE(2)C and PC12 cells (27, 28). Depolarization-induced up-regulation of TH promoter activity occurred through both the TH CRE and AP-1 sites in PC12 cells (29). The latter study also demonstrated that, whereas the CRE was solely responsible for cAMP-mediated regulation of TH promoter activity, both CRE and AP-1 sites were capable of mediating the effects of calcium influx on TH promoter activity. However, the composition of either AP-1 or CRE DNA-protein complex in any given brain region and cell line remains unclear.

TH expression has been used as a marker to study differentiation and regulation of the large population of dopaminergic periglomerular neurons intrinsic to the olfactory bulb (30–33). These neurons receive innervation from olfactory receptor cells and regulate the activities of mitral and tufted cells (34). In the developing olfactory bulb, TH phenotypic expression begins after precursor cells migrate from the subventricular zone to the glomerular region where they receive sensory afferent stimulation (32, 35). In adult rodents, either olfactory deafferentation or odor deprivation, the latter produced by unilateral naris closure, results in a significant loss of TH expression (30, 36–40). These observations indicate that olfactory input from receptor cells is necessary for both the initiation and maintenance of TH expression in the periglomerular neurons but do

Tyrosine hydroxylase (TH), the first and rate-limiting enzyme in the biosynthesis of catecholamine neurotransmitters, is present in many brain regions including the olfactory bulb. The rodent TH gene promoter contains putative CRE and AP-1 motifs, the latter differing from the consensus AP-1 only in the middle base (5, 23–26). The TH CRE was found to be required not only for basal activity but also for cAMP-mediated up-regulation of TH promoter activity in SK-N- BE(2)C and PC12 cells (27, 28). Depolarization-induced up-regulation of TH promoter activity occurred through both the TH CRE and AP-1 sites in PC12 cells (29). The latter study also demonstrated that, whereas the CRE was solely responsible for cAMP-mediated regulation of TH promoter activity, both CRE and AP-1 sites were capable of mediating the effects of calcium influx on TH promoter activity. However, the composition of either AP-1 or CRE DNA-protein complex in any given brain region and cell line remains unclear.

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not define the molecular mechanisms underlying TH gene regulation.

Lacking is evidence that supports a role for either TH AP-1 or CRE activity in TH gene expression in the dopaminergic periglomerular neurons. Recent studies demonstrated that c-fos message and c-Fos protein are partially colocalized with TH in the glomerular layer (41, 42). In parallel to the decreased TH expression, drastic down-regulation of c-fos expression occurred in periglomerular neurons in the odor-deprived olfactory bulb (42), suggesting a correlation between the c-fos and TH gene regulation. However, a direct interaction between the c-fos gene product and the TH AP-1 motif remains to be demonstrated. Moreover, it is unknown whether the CRE motif on the TH promoter has a role in TH gene regulation in periglomerular neurons of olfactory bulb in response to alterations in either primary afferent innervation or stimulation.

To elucidate some of the cis- and trans-acting elements important in regulating TH expression in the olfactory bulb periglomerular neurons, the current studies employed immunocytochemical, Western blot, and gel shift analyses to investigate the composition of the AP-1 and CRE binding complexes.

**EXPERIMENTAL PROCEDURES**

**Animals**—Adult male CD-1 mice were purchased from Charles River Breeding Laboratory (Kingston, NY) and housed under a 12/12-h light/dark cycle. Unilateral naris closure was produced with a spark-gap electrocautery under pentobarbital anesthesia (30 mg/kg Nembutal). Closure was confirmed visually at the time of sacrifice. Studies were carried out at least 2 months post-closure. All procedures were performed under protocols approved by the Cornell University Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines.

**Materials**—Polyclonal antibodies directed against c-Fos, c-Fos-B, and Jun-D were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Additional polyclonal c-Fos antisera were obtained from GenoSys Biotechnologies (Woodlands, TX) and Oncogene Research Products (Cambridge, MA). Polyclonal antibodies for CREB, pCREB, and CREM were purchased from Upstate Biotechnology (Lake Placid, NY). Polyclonal TH antisera were prepared in the laboratory (43).

**Immunocytochemistry**—Mice were perfused under deep pentobarbital anesthesia with saline containing 0.5% sodium nitrite and 10 units/ml heparin followed by 4% buffered (0.1M sodium phosphate, pH 7.2) formaldehyde. Following 1 h of post-fixation, olfactory bulbs were infiltrated overnight with 30% sucrose. Frozen sections (40 μm) were incubated with antisera overnight at room temperature followed by incubation with the appropriate biotinylated secondary antiserum, the Vector Elite kit (Vector Laboratories; Burlingame, CA), and with 3,3′-diaminobenzidine-HCl (0.05%) and hydrogen peroxide (0.005%) as chromogen (35).

**Extraction of Nuclear Proteins**—Nuclear extracts were isolated from olfactory bulbs according to the method of Roy et al. (44). For each extraction, 0.3–0.5 g was employed, representing about 20 olfactory bulbs. Samples were aliquoted and stored at −70 °C until use. Protein concentrations were determined with a protein assay kit (Bio-Rad).

**Probe Selection and Labeling**—The oligonucleotides containing the AP-1 and CRE motifs (synthesized by Gene Link, Thornwood, NY) were identical to the regions of the TH 5′-apical promoter (38). The sense and antisense oligonucleotides were mixed in a 1:1 molar ratio and end-labeled with 32P using bacteriophage T4 polynucleotide kinase (Boehringer Mannheim) according to Sambrook et al. (47) and electrophoretically transferred onto a nitrocellulose membrane (48). Blots were incubated in blocking solution (BS; 0.1% phosphate-buffered saline, 5% non-fat milk, 0.5% Tween 20) at room temperature for 15 min. Blots were then incubated with polyclonal antibodies (1:2000 dilution in BS) for 2 h. After three 5-min washes in BS, blots were incubated in the peroxidase-conjugated secondary antibody (1:1000 diluted in BS) for 1 h. Blots were washed four times, 5 min each, in 0.1% phosphate-buffered saline containing 0.5% Tween 20 and detected with ECL reagents (Amersham; Arlington Heights, IL) according to manufacturer instructions.

**RESULTS**

**Tissue Localization and Regulation of TH and IEGs**—As previously shown (38), in the control olfactory bulb TH was concentrated in cell bodies and processes of periglomerular neurons located in the glomerular layer of olfactory bulb (Fig. 1A). TH expression was drastically reduced in the olfactory bulb ipsilateral to the naris closure (Fig. 1B). The IEG proteins, c-Fos (Fig. 1, C and D) and Fos-B (Fig. 1, E and F), were localized to nuclei distributed in the glomerular, mitral, and granule cell layers. The number of both c-Fos and Fos-B im-
munopositive neurons showed dramatic reductions in the periglomerular cells of olfactory bulb ipsilateral to the closure. c-Fos expression was absent in the glomerular layer (Fig. 1D). Fos-B expression, which was more widely distributed, showed a 50% reduction in response to odor deprivation (mean number of cells per unit area ± S.E.: contralateral to naris closure 287.3 ± 11.97 versus ipsilateral to naris closure 155.2 ± 6.06; p < 0.001, n = 4). Expression of these early genes also was reduced in the granule, but not the mitral, cell layer. Double label immunofluorescence studies showed that a subpopulation of Fos-B immunoreactive cells in the glomerular layer also expressed TH (data not shown).

In contrast to c-Fos and Fos-B, naris closure did not alter the expression of CREB (Fig. 2, A and B) and Jun-D (Fig. 2, E and F) in the olfactory bulb. Whereas c-Fos, Fos-B, and Jun-D were present primarily in neurons, CREB (Fig. 2, A and B) displayed a broader distribution, that is, this IEG product appeared to be expressed in both neurons and glia. The phosphorylated form of CREB, pCREB, showed a primarily neuronal localization in the olfactory bulb. Levels of pCREB were variably increased by odor deprivation (Fig. 2, C and D).

**TH AP-1 and CRE Binding Activities**—Because naris closure produced down-regulation in expression of some IEG products in parallel to that observed for TH in periglomerular neurons, gel retardation assays were performed to determine whether TH AP-1 and CRE binding activities in olfactory bulb were altered by odor deprivation. TH AP-1 and CRE probes formed distinctive DNA-protein complexes with nuclear extracts isolated from control olfactory bulbs (Fig. 3). The TH AP-1 complex was identified as a single band. Competition assays demonstrated that this binding activity was completely blocked by excess cold AP-1 probe. Also, the AP-1 binding activity was blocked by excess cold TH CRE probe. Compared with the normal olfactory bulb, nuclear extracts from the odor-deprived olfactory bulb showed a 32% decrease (mean optical density in arbitrary units ± S.E.: ipsilateral, 0.094 ± 0.003 versus contralateral, 0.140 ± 0.006; p < 0.003, n = 5) in AP-1 binding activity in parallel with the decline in Fos protein found immunocytochemically.

Interactions of nuclear extracts with the TH CRE probe produced two DNA-protein complexes with distinctive migration rates during gel electrophoresis (Fig. 3). The intensity of the lower band was higher than the upper band. As predicted, excess cold CRE probe abolished the CRE binding activity. Excess cold AP-1 probe only partially blocked the CRE/protein binding. In contrast to the AP-1 binding activity which was down-regulated by odor deprivation and in agreement with the immunocytochemical findings, CRE binding activity remained unchanged with differing olfactory experience.

**Components of TH AP-1 and CRE Complexes**—Supershift assays were performed to identify the nuclear proteins that formed complexes with TH AP-1 and CRE motifs. Polyclonal antibodies for c-Fos, Fos-B, Jun-D, CREB, and CREM were
used to examine the AP-1 complex. Antibodies for Fos-B and Jun-D produced a shift of the AP-1 complex to species with lower migration rates (Fig. 4). Fos-B antibody shifted the whole AP-1 complex, whereas the Jun-D antibody shifted a major portion of the complex. Relative to the control, the Fos-B— as well as the Jun-D-associated binding activities decreased in the olfactory bulb ipsilateral to the naris closure. In contrast, antibodies for c-Fos, CREB, or CREM did not produce a supershift from the AP-1 complex (Fig. 4).

Antibodies for CREB, CREM, Fos-B, and Jun-D, but not c-Fos, supershifted the CRE-protein complexes (Fig. 5). Although the CREB antibody was somewhat more efficient than the CREM antibody, they produced similar supershift patterns retarding all of the upper band and part of the lower band. The antibody for Fos-B supershifted part of the lower band, resulting in a band overlapping the original upper band. The Jun-D antibody supershifted only a small portion of the CRE complexes (Fig. 5). Finally, c-Fos antibodies did not retard the CRE-binding complexes.

**Specificity of Antibodies**—Western blot analyses were performed to demonstrate the specificity of polyclonal antibodies that produced retardation in the supershift studies. Fos-B antibody recognized a protein with a molecular mass of 40–42 kDa (Fig. 6). Consistent with the immunocytochemical findings and gel retardation assays, Fos-B immunoreactivity decreased in the olfactory bulb ipsilateral to the closed naris. The Fos-B antibody also recognized two polypeptides with molecular masses of 27 and 14 kDa. These two polypeptides might represent alternative splicing products of the Fos-B gene and/or proteolytic products of the Fos-B protein. The Fos-B antibody did not recognize c-Fos, which in mouse had a molecular mass of 56 kDa when detected with several c-Fos antibodies (data not shown). The Jun-D antibody recognized a doublet with molecular masses of 39 and 43 kDa. The CREB antibody recognized a 45-kDa polypeptide, and the CREM antibody recognized 44- and 32-kDa polypeptides.

**DISCUSSION**

Regulation of TH gene expression in the rodent olfactory bulb displayed unique activity dependence (30, 36–40, 49, 50). TH expression was rapidly down-regulated following unilateral naris closure, a procedure that prevents odorant access to the olfactory receptor epithelium resulting in reduced stimulation of the olfactory bulb. Previous studies revealed both cell line- (27, 51–53) and CNS region-dependent TH gene regulation (54, 55), suggesting differences in either expression or binding of transcription factors to specific cis-acting elements. The current study investigated the composition and specificity of binding complexes with putative AP-1 and CRE sites in the regulation of TH gene expression in the olfactory bulb.

The present study demonstrated that Fos-B, CREB, pCREB, and Jun-D are present in the periglomerular cells of the glomerular layer and likely colocalize with TH as previously shown for c-Fos (41, 42). Consistent with the idea that Fos expression reflects neuronal activity, both c-Fos and Fos-B immunoreactivities were down-regulated in the olfactory bulb ipsilateral to the naris closure. The parallel reductions in Fos proteins and TH suggested that these two IEGs may be involved in TH gene regulation in an activity-dependent manner. In contrast, naris closure had no effect on immunoreactivities for either Jun-D or CREB and may actually have induced pCREB. The presence of these IEGs may be necessary for the activation of cis-acting elements in the TH gene promoter, but may not directly induce TH gene expression. Thus, the unique expression of the IEGs in the glomerular layer suggested that they may play different roles in regulating gene expression in these dopaminergic neurons.

Gel mobility shift assays in the present study revealed that Fos-B is the only member of the Fos family present in the TH AP-1-protein complex in the olfactory bulb. Fos-B may form heterodimers with both Jun-D and other members of the Jun family because Jun-D antibody only partially supershifts the...
The specificity of the TH AP-1 motif for Fos-B may result from the specific 5’- and 3’-flanking regions of the AP-1 sequence, previously shown to be critical for binding activity (20). Transcription factor affinity with cis-acting elements mediates in part the differential promoter sensitivity of genes to the same extracellular signals. The reductions in the TH AP-1 binding activity and Fos-B immunoreactivity, shown in tissue staining and Western blot analyses in the naris-closed olfactory bulb, suggest a significant role of Fos-B in the TH gene regulation in this brain region. Although c-Fos is present in the periglomerular neurons and down-regulated by naris closure, this IEG product does not appear to contribute to the TH AP-1-protein complex. The Western blot analyses showing that the Fos-B antibody does not cross-react with c-Fos, which has a molecular mass of 56 kDa, further supports the concept that Fos-B, but not c-Fos, may be involved in down-regulation of the TH gene in periglomerular dopaminergic neurons in the naris-closed olfactory bulb.

The absence of c-Fos in the TH AP-1-protein complex in the olfactory bulb may reflect tissue specificity of TH gene regulation and/or differential affinities of the Fos proteins with the TH AP-1 motif. It has been recently reported that the AP-1 motif is involved in the induction of TH gene transcription by reserpine treatment in the rat adrenal medulla but not in the sympathetic ganglia, suggesting tissue-specific regulatory mechanisms for this gene (56). Cell line specificity of TH gene transcription also was shown to involve the AP-1 motif (51, 52).

Alternatively, the inability to detect c-Fos in the DNA-protein complex may be because of low affinity of this IEG product with the TH AP-1 motif. It is possible that c-Fos can bind to the TH AP-1 motif only when it is overexpressed. Both c-Fos and Fos-B expression as well as AP-1 binding activity can be increased by activation of glutamate receptors in the brain (57) and by nerve growth factor treatment in PC12 cells (58). In PC12 cells, c-Fos was identified in the TH AP-1-protein complex shortly after growth factor treatment, but it was replaced gradually by Fos-B which was induced by prolonged treatment (58), indicating a transient effect of c-Fos and a long-lasting effect of Fos-B on TH gene transcription. Indeed, Fos-B and Fos-B-like proteins have been implicated in the long-term biochemical adaptations observed following chronic treatment (59, 60). In sum, the demonstration that the AP-1-protein complex is composed primarily of Fos-B and Jun proteins in the adult mouse olfactory bulb suggests that Fos-B may play an essential role in the TH gene regulation in this brain region.

The present study demonstrated that excess CRE oligonucleotide completely blocked TH AP-1 binding activity. One explanation for this finding is that the TH CRE is also the binding site for the protein complexes in the olfactory bulb extracts that bind to TH AP-1, e.g. Fos/Jun. This competition for transcription factors may occur because the consensus AP-1 and CRE motifs in many genes including TH are very similar in structure, displaying only one nucleotide difference. However, note that the putative TH AP-1 motif differs from that in other genes by one nucleotide located in the middle of the sequence (24). A second possibility is that CRE competes with AP-1 for only certain components that are essential to formation of AP-1-protein complexes. For instance, binding of Jun/CREB to the excess CRE added to the binding reaction might deplete free Jun, thus resulting in a decrease in availability of Fos/Jun complexes. Jun proteins were localized to complexes binding to CRE sites presumably as Jun/Jun homodimers and/or Jun/CREB heterodimers (16, 18). More recently, it was reported that CREB was present in the AP-1-protein complex (19). The finding that AP-1 oligonucleotide partially blocks CRE binding activity suggested that some proteins such as CREB and CREM only bind to the TH CRE. In contrast, the supershift assays show that Fos-B/Jun heterodimers bound both TH AP-1 and CRE sequences. Although the effect of binding of Fos-B/Jun to CRE motif on the TH promoter activity is unknown, the ability of this protein complex to bind to both TH AP-1 and CRE motifs suggests cross-talk between these two cis-acting elements.

The present studies also suggested that the CRE motif may play only a minor role in TH down-regulation in the olfactory bulb ipsilateral to naris closure. The gel shift assays revealed no detectable change in CRE binding activity between the normal and naris-closed olfactory bulbs. Whereas gel supershift assays showed that CREB/CREM proteins are major components of the TH/CRE protein complexes, immunocytochemistry confirmed the binding data that CREB was unchanged in response to naris closure. Although CREB, thought to constitutively bind to the CRE motif, can be activated upon phosphorylation (12, 14, 15), pCREB was, if anything, variably increased in response to naris closure. Although Fos-B/Jun-D are present in the CRE-protein complexes, they represent only minor components. Therefore, it is unlikely that naris closure reduces TH expression in the periglomerular neurons primarily through the CRE motif.

In conclusion, the present study demonstrated unique activity-dependent expression of immediate early genes in the glomerular layer of mouse olfactory bulb. Fos proteins exhibited reduced immunoreactivities in the periglomerular neurons in the olfactory bulb ipsilateral to naris closure. Gel mobility shift assays demonstrated a decrease in the TH AP-1 binding activity. Fos-B was found to be a major component of the TH AP-1-protein complexes and a minor component of the CRE-protein complexes, suggesting that Fos-B may be involved in the activity-dependent regulation of TH expression in the olfactory dopaminergic neurons.

REFERENCES

1. Demmer, J., Dragunow, M., Lawlor, P. A., Mason, S. E., Leah, J. D., Abraham, W. C., and Tate, W. P. (1993) Mol. Brain Res. 17, 279–286.
2. Hughes, P., and Dragunow, M. (1995) Pharmacol. Rev. 47, 133–178.
3. Nakabeppu, Y., and Nathans, D. (1991) Cell 64, 751–759.
4. Rauscher, F. J., III, Sambutetti, L. C., Curran, T., Distel, R. J., and Spiegelman, B. M. (1986) Cell 52, 471–480.
5. Icard-Liepkains, C., Faucon Biguet, N., Vyas, S., Robert, J. J., Sassone-Corsi, P., and Mallet, J. (1992) J. Neurosci. Res. 32, 290–298.
6. Habener, J. F., Meyer, T. K., Yun, Y., Waeger, G., and Hoeffer, J. P. (1990) Metabolism 39, Suppl. 2, 13–16.
7. Curran, T., and Franza, B. R., Jr. (1988) Cell 55, 395–397.
8. Franza, B. R., Jr., Rauscher, F. J., III, Joseph, S. F., and Curran, T. (1988) Science 239, 1150–1155.
9. Sassone-Corsi, P., Ransone, L. J., Lamp, W. W., and Verma, I. M. (1988) Nature 336, 692–696.
10. Yen, J., Wisdom, R. M., Tratner, I., and Verma, I. M. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5077–5081.
11. Halazonetis, T. D., Georgopoulos, K., Greenberg, M. E., and Leder, P. (1988) Cell 55, 917–924.
12. Dey, A., Nebert, D. W., and Ozato, K. (1991) DNA Cell Biol. 10, 537–544.
13. Foulkes, N. S., Borrelli, E., and Sassone-Corsi, P. (1991) Cell 64, 739–749.
14. Gonzalez, G. A., and Montminy, M. R. (1989) Cell 59, 675–680.
15. Lamp, W. W., Dworki, V. J., Ofir, R., Montminy, M., and Verma, I. M. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4320–4324.
16. Hai, T., and Curran, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 3720–3724.
17. Iwashkiw, L. B., Liu, H. C., Kara, C. J., Lamp, W. W., Verma, I. M., and Glimcher, L. H. (1990) Mol. Cell Biol. 10, 1609–1621.
18. Nakabeppu, Y., Ryder, K., and Nathans, D. (1988) Cell 55, 907–915.
19. Pennypacker, K. R., Hudson, P. M., Hong, J. S., and McMillan, M. K. (1995) Dev. Brain Res. 86, 245–249.
20. Ryseck, R. P., and Bravo, R. (1991) Oncogene 6, 533–542.
21. Tinti, C., Conti, B., Cubelli, J. F., Kim, K.-S., Baker, H., and Joh, T. H. (1996) J. Biol. Chem. 271, 25533–25538.
22. Ghee, M., Baker, H., Miller, J. C., and Ziff, E. B. (1998) Mol. Brain Res. 55, 101–114.
23. Rosen, W. J., Vandenbark, G. R., and Hanson, R. W. (1988) J. Biol. Chem. 263, 9063–9066.
24. Harrington, C. A., Lewis, E. J., Krzemien, D., and Chikaraishi, D. M. (1987) Nucleic Acids Res. 15, 2863–2884.
25. Lewis, E. J., Harrington, C. A., and Chikaraishi, D. M. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 3550–3554.
26. Goodman, R., Slater, E., and Hershman, H. R. (1980) J. Cell Biol. 84, 495–506.
27. Kim, K.-S., Lee, M. K., Carr, J., and Joh, T. H. (1993) J. Biol. Chem. 268, 263–268.
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15689–15695

28. Sabban, E. L. (1997) *Semin. Cell Dev. Biol.* 8, 101–111.
29. Nagamoto-Combs, K., Ploch, K. M., Best, J. A., Sun, B., and Tank, A. W. (1997) *J. Biol. Chem.* 272, 6051–6058.
30. Baker, H., Kawano, T., Margolis, F. L., and Joh, T. H. (1983) *J. Neurosci.* 3, 69–78.
31. Baker, H., Kawano, T., Albert, V. R., Joh, T. H., Reis, D. J., and Margolis, F. L. (1984) *Neurosci.* 11, 605–615.
32. McLean, J. H., and Shipley, M. T. (1988) *J. Biol. Chem.* 272, 6051–6058.
33. Baker, H., Kawano, T., Margolis, F. L., and Joh, T. H. (1983) *J. Neurosci.* 3, 69–78.
34. Shepherd, G. M. (1990) *The Synaptic Organization of the Brain*, 3rd Ed., Oxford University Press, New York.
35. Baker, H., and Farbman, A. I. (1993) *Neuroscience* 52, 115–134.
36. Baker, H., Morel, K., Stone, D. M., and Maruniak, J. A. (1993) *Brain Res.* 614, 109–116.
37. Brunjes, P. C., Smith-Crafts, L. K., and McCarty, R. (1985) *Dev. Brain Res.* 22, 1–6.
38. Cho, J. Y., Min, N., Franzen, L., and Baker, H. (1996) *J. Comp. Neurol.* 369, 264–276.
39. Stone, D. M., Wessel, T., Joh, T. H., and Baker, H. (1990) *Mol. Brain Res.* 8, 291–300.
40. Stone, D. M., Grillo, M., Margolis, F. L., Joh, T. H., and Baker, H. (1991) *J. Comp. Neurol.* 311, 223–233.
41. Guthrie, K. M., and Gall, C. M. (1995) *Neuroreport* 6, 2145–2149.
42. Jin, B. K., Franzen, L., and Baker, H. (1996) *Int. J. Dev. Neurosci.* 14, 971–982.
43. Joh, T. H., Geggman, C., and Reis, D. J. (1973) *J. Neurochem.* 39, 342–348.
44. Roy, R. J., Gesselin, P., and Guerin, S. L. (1991) *BioTechniques* 11, 770–777.
45. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
46. Cubells, J. F., Kim, K. S., Baker, H., Volpe, B. T., Chung, Y.-I., Houpt, T. A., Wessel, T. C., and Joh, T. H. (1995) *J. Neurochem.* 65, 502–509.
47. Laemmli, U. K. (1970) *Nature* 227, 680–685.
48. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* 76, 4350–4354.
49. Baker, H. (1990) *Neurosci.* 36, 761–771.
50. Guthrie, K. M., Wilson, D. A., and Leon, M. (1990) *J. Neurosci.* 10, 3402–3412.
51. Lazaroff, M., Matzuk, S., Yoon, S., and Chikaraishi, D. M. (1995) *J. Biol. Chem.* 270, 21579–21589.
52. Yoon, S. O., and Chikaraishi, D. M. (1992) *Neuron* 9, 55–67.
53. Kumer, S. C., and Vrana, K. E. (1996) *J. Neurochem.* 67, 443–462.
54. Weiser, M., Baker, H., Wessel, T. C., and Joh, T. H. (1993) *Mol. Brain Res.* 17, 319–327.
55. Min, N., Joh, T. H., Kim, K. S., Peng, C., and Sun, J. H. (1994) *Mol. Brain Res.* 27, 281–289.
56. Trocme, C., Mallet, J., and Biguet, N. F. (1997) *J. Neurosci. Res.* 48, 489–498.
57. Sonnenberg, J. L., Mitchelmore, C., MacGregor-Leon, P. F., Hempstead, J., Morgan, J. I., and Curran, T. (1989) *J. Neurosci. Res.* 24, 72–80.
58. Ginzburg-Bartosch, E., and Ziff, E. B. (1994) *Mol. Endocrinol.* 8, 249–261.
59. Chen, J., Yee, H. E., Kelz, M. B., Hiroi, N., Nakabeppu, Y., Hope, B. T., and Nestler, E. J. (1995) *Mol. Pharmacol.* 48, 880–889.
60. Hope, B. T., Yee, H. E., Kelz, M. B., Self, D. W., Iadarola, M. J., Nakabeppu, Y., Duman, R. S., and Nestler, E. J. (1994) *Neuron* 13, 1235–1244.