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Convulsant-induced increase in transcription factor messenger RNAs in rat brain

(*jun*/transcription factor AP-1/"zinc finger" proteins/hippocampus)

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ABSTRACT **Administration of the convulsants pentylenetetrazole (Metrazole) or picrotoxin to rats caused a dramatic increase in mRNAs of four putative transcription factor genes, *zif/268*, c-*jun*, *jun-B*, and c-*fos*, in neurons of the hippocampus and dentate gyrus, as well as other areas of the cerebral cortex, including pyriform cortex and cingulate cortex. The increase in these mRNAs was rapid and transient: amounts peaked within 1 hr and returned to baseline within 2 hr. These results extend the observation made by Morgan *et al.* [Morgan, J. I., Cohen, D. R., Hempstead, J. L. & Curran, T. (1987) *Science* 237, 192–197] that c-*fos* mRNA and protein are induced in rat brain after seizures. We hypothesize that the increase of these putative transcription factor mRNAs in the brain is part of a programmed genomic response of neurons to intense stimulation, which is analogous to the genomic response of nonneuronal cells to growth factors.**

Many extracellular ligands, such as neurotransmitters and protein growth factors, modify target cell properties through receptor-mediated changes in gene expression. Indeed, it has been proposed that long-term neuronal effects of neurotransmitters and neuromodulators that underlie learning and memory occur by means of the activation of genes that encode one or more transcription factors, which then regulate the activity of other genes (1, 2). This postulated pattern of gene activation and control is similar to the emerging picture of growth factor-induced gene expression in nonneuronal cells in culture, in which there is sequential activation of sets of genes, beginning with "immediate early" or "competence" genes (3–7) that encode transcription factors.

An example of an immediate early gene that may play a role in cell growth and in neuronal response to stimulation is the protooncogene c-*fos*, which is transcriptionally activated in quiescent fibroblasts by growth factors (8) and is expressed in neurons in response to a variety of stimuli (9). The synthesis of fos protein is rapidly induced in neurons in the dorsal horn of the spinal cord after sensory stimulation of the appropriate dermatome (10) and in specific regions of rat brain after pharmacologically induced seizures (11) and electrically induced seizures associated with the kindling paradigm (12, 13). The c-*fos* gene is also transcriptionally activated in PC12 pheochromocytoma cells after stimulation of nicotinic acetylcholine receptors (14) and depolarization of the cell membrane (15). In light of these findings for c-*fos*, we examined the response of other growth factor-regulated genes in rat brain after pharmacologically induced seizures.

The genes we examined were identified in BALB/c 3T3 mouse cells as immediate early, growth factor-activated, genes and encode known or probable transcription factors: c-*jun* encodes the mammalian transcription factor AP-1 (16–20); *jun-B* (21) shares extensive sequence homology with c-*jun*; and *zif/268* (22), the murine homolog of the rat nerve growth factor inducible (NGFI)-A gene (23), encodes a protein with three "zinc finger" sequences characteristic of a class of transcription factors (24). Immediate early genes, which include c-*fos*, are distinguished from other genes modulated by growth factors by their rapid transcriptional activation even in the absence of protein synthesis (3–7). Transcription of these genes increases dramatically, generally within minutes after addition of growth factor or serum, and returns to preinduced levels after a few hours. Moreover, immediate early mRNAs decay rapidly, resulting in transient synthesis of their encoded proteins. It is thought that some of these immediate early proteins regulate the subsequent genomic response to growth factors (3–7).

To determine if the expression of immediate early genes identified in the 3T3 fibroblast line can be modulated in neurons, we used electrophoretic blot hybridization analysis and *in situ* hybridization to look for changes in amounts of the mRNAs corresponding to these genes in rat brains after seizures induced by the administration of the convulsants pentylenetetrazole (Metrazole) or picrotoxin. Pentylenetetrazole-induced seizures were previously shown by Morgan and coworkers (11) to cause a dramatic and rapid increase in c-*fos* mRNA and c-fos protein in distinct areas of the brain. We report here the observation that pentylenetetrazole and picrotoxin also cause a rapid increase in brain mRNAs for the murine immediate early genes c-*jun*, *jun-B*, and *zif/268*. The anatomical and temporal distributions of these mRNAs (as well as c-*fos* mRNA) after convulsions are similar to those observed for c-fos protein (11). Our observations thus point to a complex change in the regulation of gene expression induced by pharmacological agents that cause extensive neuronal stimulation.

MATERIALS AND METHODS

RNA Analysis. Total cellular RNA was isolated by using the procedure of Chirgwin *et al.* (25). Blot hybridization analysis was conducted as described by Linzer and Nathans (26). Murine *jun-B* cDNA (21) and murine c-*fos* (27) genomic DNA sequences were cloned in pGEM-2 (Promega Biotec, Madison, WI), and *zif/268* cDNA (22) was cloned in Bluescript M13+ (Stratagene, LaJolla, CA). The murine c-*jun* cDNA was isolated from a BALB/c 3T3 DNA library previously described (4, 5) by using *jun-B* (16) as a probe and was inserted into the *Eco*RI site of pGEM-2 (K.R. and D. Nathans, unpublished results).

DNA and RNA Probes. ^{32}P-labeled DNA probes were prepared by nick-translation (28). ^{35}S-labeled probes were prepared by using uridine 5′-[α-[^{35}S]thio]triphosphate (New England Nuclear) and T7 or SP6 RNA polymerase (Promega Biotec) to synthesize RNA sense and antisense transcripts of

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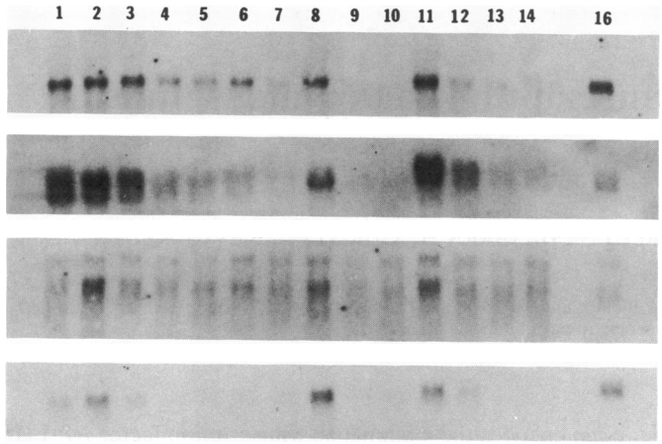

FIG. 1. Blot hybridization analysis of *zif/268*, *jun-B*, c-*jun*, and c-*fos* mRNAs in different regions of the brain 30 min after the administration of saline (control) or pentylenetetrazole (PTZ). Each lane contains 15 μg of total RNA isolated from the indicated regions of rat brain, which was resolved by electrophoresis in formaldehyde/agarose gels, transferred to a nitrocellulose filter, and hybridized to ^{32}P-labeled DNA probes. Lanes 1–3, forebrain (including hippocampus) (PTZ); lanes 4–6, forebrain (control); lanes 7 and 8, cerebellum (PTZ); lanes 9 and 10, cerebellum (control); lanes 11 and 12, hippocampus (PTZ); lanes 13 and 14, hippocampus (control); lane 16, BALB/c 3T3 RNA (0.2 μg) isolated 3 hr after stimulation of quiescent cells with serum in the presence of cycloheximide at 10 μg/ml. The RNA in each lane was isolated from different animals except for the hippocampus lanes, where RNA from two animals was pooled for each lane. Exposure time: 4 days.

cDNA sequences cloned in pGEM-2 or Bluescript essentially as described in ref. 29. RNA antisense probes for the 3′ nontranslated regions for *zif/268*, *jun-B*, and c-*jun* were prepared by linearizing the appropriate plasmid with a restriction enzyme that cuts 3′ to coding sequences.

Tissue Preparation. Seizures were induced in male Sprague–Dawley rats (200–300 g) by intraperitoneal injections of pentylenetetrazole (Metrazole) or picrotoxin in sterile water or saline at dosages of 50 mg/kg or 5 mg/kg, respectively. Control animals received injections of similar volumes of sterile water or saline (0.25 ml). To control for nonspecific neuronal activation, another group of animals was treated with apomorphine, a nonspecific dopamine agonist (2–5 mg/kg), or *p*-chloroamphetamine (5 mg/kg), a serotonin-releasing agent. At appropriate times, the animals were sacrificed by decapitation and the brains were rapidly dissected.

***In Situ* Hybridization.** Frozen 10-μm-thick sections of whole brains or half-brain pairs from experimental and control animals were mounted on glass slides and prepared for hybridization to RNA probes as described by Largent *et al.* (30). *In situ* hybridization was performed as described by Segal and Shilo (31), with the following modifications: ^{35}S-labeled RNA probes were not denatured with alkali; prehybridization was usually omitted; hybridization was performed in a moist chamber under unsealed, silane-treated coverslips at 46°C, using 5×10^5 to 1×10^6 cpm of probe for each section; slides were washed twice in 2× SSC (1× SSC is 150 mM NaCl/15 mM sodium citrate, pH 7), incubated for 15 min at 37°C in 2× SSC containing RNase A (Worthington) at 10 μg/ml, and washed three times in 2× SSC; after washing overnight as described, sections were dehydrated by sequential immersion in 30%, 50%, 70%, 95%, and 100%, (vol/vol) ethanol, air dried, and exposed to Hyperfilm β-max (Amersham) or Ultrofilm (LKB) at 4°C for 5–15 days. For resolution of hybridization at the cellular level, sections were exposed to Kodak autoradiography emulsion (NTB3) for 3 weeks at 4°C, developed, and stained with toluidine blue.

RESULTS

Blot Hybridization Analysis of Brain RNA. Tissues from different regions of the brain were surveyed for mRNAs of a group of genes that are known to be activated rapidly in 3T3 fibroblasts after growth factor stimulation. These genes (*zif/268*, *jun-B*, c-*jun*, and c-*fos*) encode proteins thought to be involved in regulating the genomic response of 3T3 cells to growth factors (3–7, 21). As shown in Fig. 1, 30 min after intraperitoneal injection of pentylenetetrazole, *zif/268*, *jun-B*, c-*jun*, and c-*fos* mRNAs in the forebrain were increased compared to animals that received saline injections. These mRNAs were also increased in the cerebellum and hippocampus of treated animals; however, there was considerable interanimal variability.

Localization of mRNAs by *in Situ* Hybridization. To obtain a more detailed localization of the mRNAs detected by blot hybridization, we prepared tissue sections of brains from untreated and pentylenetetrazole-treated rats and performed *in situ* hybridization using probes for *zif/268*, *jun-B*, c-*jun*, and c-*fos*. Representative results are presented in Figs. 2–4 and 6. In most animals, increased hybridization was noted in the hippocampus and dentate gyrus, as well as pyriform cortex, cingulate cortex, and neocortex, after pentylenetetrazole administration. In several animals, increased hybridization was observed only in cortical areas. A similar

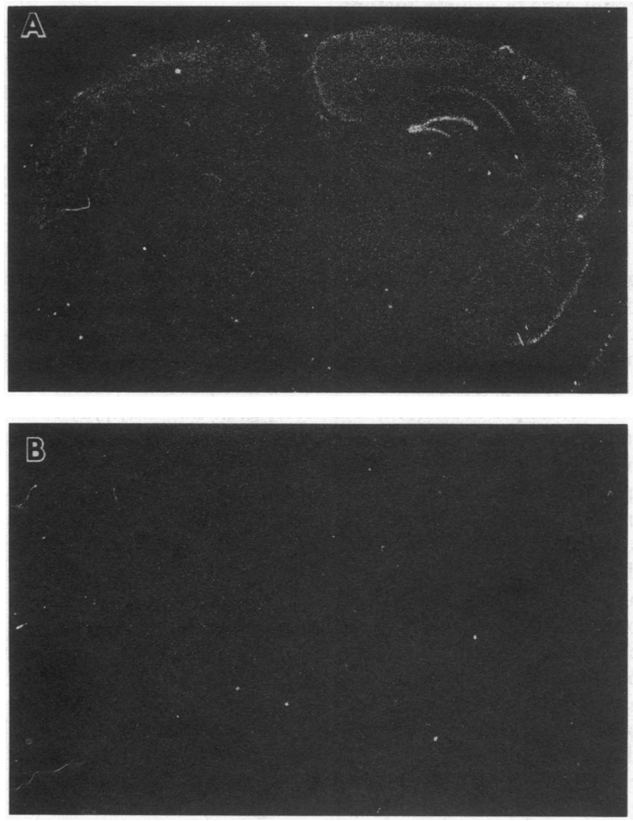

FIG. 2. Response of *zif/268* mRNA in rat brain 30 min after the administration of pentylenetetrazole. Autoradiographs of 10-μm frozen sections after *in situ* hybridization with ^{35}S-labeled antisense *zif/268* RNA probe. (*A*) Coronal sections of paired hemibrains from rats that received saline (left) or pentylenetetrazole (right) injections. (*B*) Adjacent section hybridized to sense-strand *zif/268* RNA probe. Exposure time: 1 week. (×6.)

pattern of hybridization was obtained after administration of the convulsant picrotoxin (Fig. 5). For each of the genes examined, hybridization was observed only for the antisense-strand probe (i.e., the probe complementary to the mRNA), and not the sense-strand probe under identical conditions.

Fig. 2 shows typical results obtained with the *zif/268* probe (19 of 21 animals tested). Increased *zif/268* mRNA was particularly evident in regions that contain dense concentrations of neurons such as the dentate gyrus and the CA1–CA4 layer of the hippocampus and layer II of the pyriform cortex and cingulate cortex. Increases in *zif/268* mRNA were not observed in the midbrain, brain stem, or cerebellum, even in regions with densely packed neurons such as the cerebellar granule cell layer (data not shown). The same pattern of hybridization was obtained when antisense RNA probes containing exclusively 3′ untranslated sequences of *zif/268* were used (3 of 4 animals). No hybridization was observed with the sense-strand *zif/268* probe (10 of 10 animals) (Fig. 2*B*). After picrotoxin-induced seizures, the patterns of hybridization were essentially the same as those observed after pentylenetetrazole administration (2 of 2 animals).

To localize *zif/268* mRNA more precisely, the tissue sections were exposed to photographic emulsion for 3 weeks and examined by dark- and light-field microscopy. In the dentate gyrus, the majority of the silver grains were found over the densely packed granule cell neurons and over isolated cells in the hilus (Fig. 3 *A* and *B*). In the pyriform cortex, there were dense concentrations of grains over the neuron-rich layer II of the cortex and sparse grains over cortical layer I, which contains few neuronal cell bodies (Fig. 3 *C* and *D*).

As shown in Fig. 4, mRNAs for c-*jun* (8 of 10 animals) and *jun-B* (13 of 13 animals) also increased dramatically in specific regions of the brain after the administration of pentylenetetrazole, and they showed a spatial distribution similar to that obtained for *zif/268*. Although c-*jun* and *jun-B* encode closely related proteins, it is unlikely that there is sufficient homology at the level of DNA sequence to permit cross-hybridization at the level of stringency used in this experiment. (Also, cross-hybridization was not observed in blot hybridization analysis.) In addition, antisense RNA probes prepared from the 3′ untranslated sequences of c-*jun* (2 of 2 animals) and *jun-B* (2 of 2 animals) cDNAs each yielded the same patterns of hybridization as the full-length RNA probes. Since there is no significant sequence homology

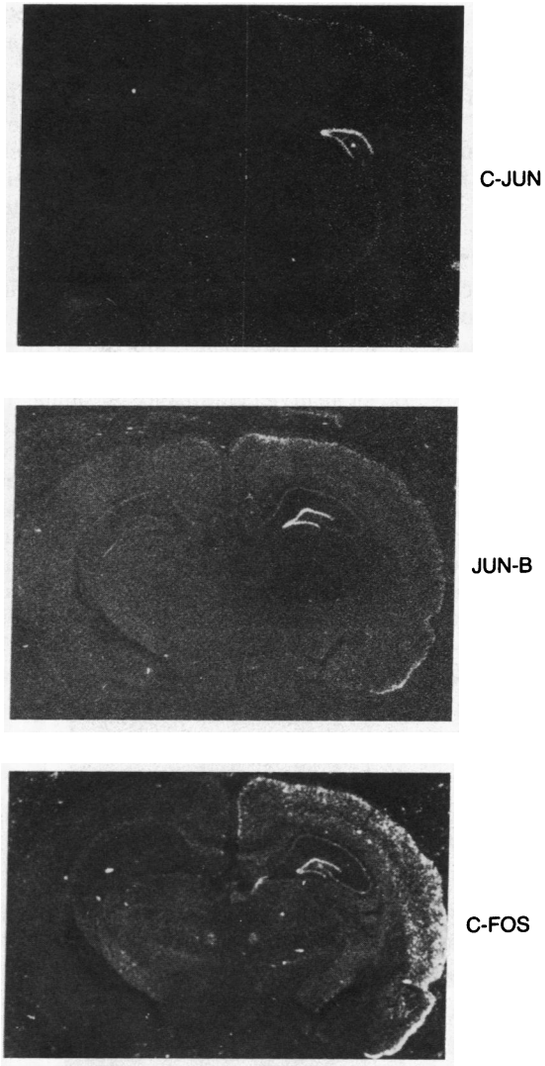

FIG. 4. Response of c-*jun*, *jun-B*, and c-*fos* mRNAs in rat brain 60 min after administration of pentylenetetrazole. Autoradiographs of coronal tissue sections of hemibrains from rats that received saline (left) or pentylenetetrazole (right) injections after *in situ* hybridization with ^{35}S-labeled antisense probes. Exposure time: 1 week. (×4.5.)

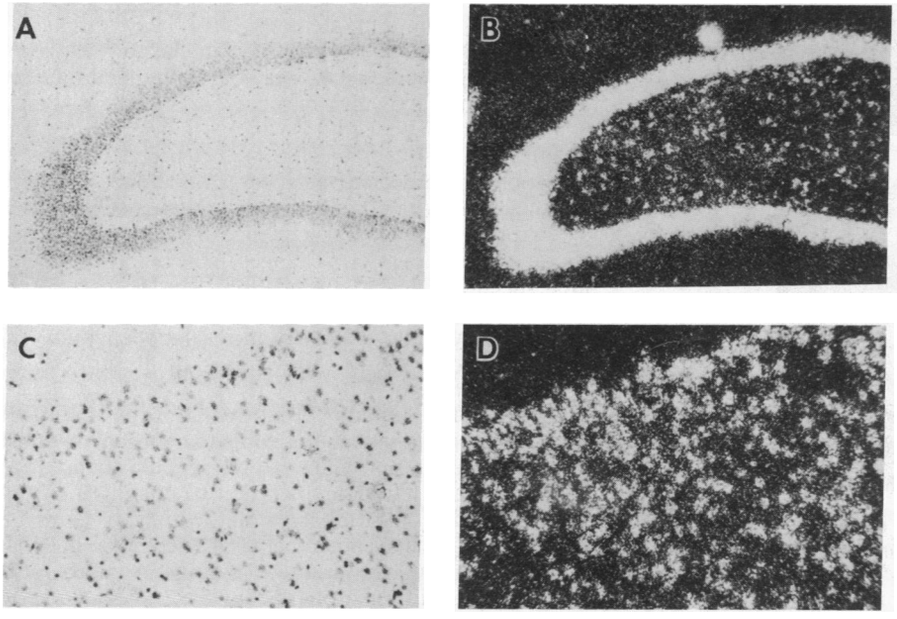

FIG. 3. Autoradiographic localization of *zif/268* mRNA to neurons in the dentate gyrus and neocortex of rat brain 60 min after administration of pentylenetetrazole. Photomicrographs of dentate gyrus (*A* and *B*) and temporal cortex adjacent to the rhinal fissure (*C* and *D*) in coronal tissue sections stained with toluidine blue after development of photographic emulsion. Light-field (*A* and *C*) and dark-field (*B* and *D*). Exposure time: 3 weeks. (*A* and *B*: ×25; *C* and *D*: ×60.)

between the 3′ untranslated regions of these cDNAs (K.R., unpublished data), it is probable that the c-*jun* and *jun-B* probes detected distinct mRNAs.

The pattern of hybridization obtained with the c-*fos* RNA probes (9 of 9 animals) is also shown in Fig. 4. As expected from the results of Morgan *et al.* (11), c-*fos* mRNAs increased in both the hippocampus and other cortical areas after the induction of seizures with pentylenetetrazole. As depicted in Fig. 5, patterns of increased hybridization similar to those observed after pentylenetetrazole administration were observed for c-*jun*, *jun-B* and c-*fos* after picrotoxin-induced seizures (2 of 2 animals).

Time Course of mRNA Inductions. Morgan *et al.* (11) reported that fos protein appears rapidly in rat hippocampus after administration of pentylenetetrazole. A similar rapid response is found for the appearance of *zif/268*, c-*jun*, *jun-B*, and c-*fos* mRNAs. In the dentate gyrus, these mRNAs were detected as early as 15 min after administration of pentylenetetrazole or picrotoxin, reached maximal levels within 60 min, and were barely detectable at 120 min (Fig. 6). The rapid appearance and disappearance of these mRNAs is similar to the pattern of mRNA expression previously observed for immediate early genes in 3T3 cells after stimulation with serum or platelet-derived growth factor (ref. 5; K.R. and D. Nathans, unpublished observations).

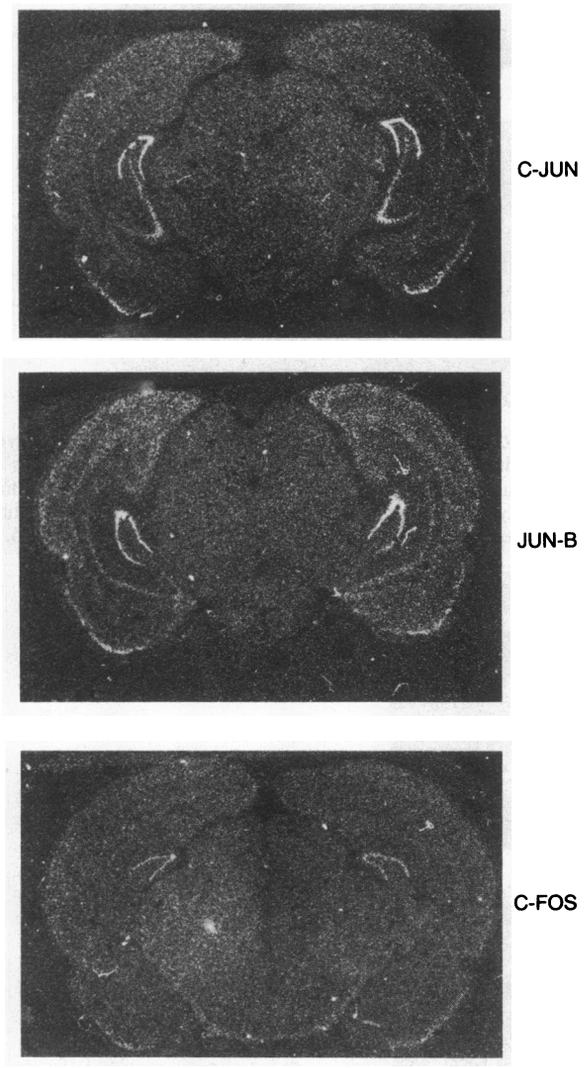

FIG. 5. Response of c-*jun*, *jun-B*, and c-*fos* mRNA in rat brain 60 min after administration of picrotoxin. Autoradiographs of coronal tissue sections of whole brain after *in situ* hybridization with ^{35}S-labeled antisense probes. Exposure time: 1 week. (×4.5.)

DISCUSSION

The principal finding of this study is that specific neuronal populations in the central nervous system respond to intense electrical activity by rapidly increasing *zif/268*, c-*jun*, and *jun-B* mRNAs, which encode putative transcription factors. Although only c-*jun* has been proven to encode a transcription factor, AP-1 (17–20), *jun-B* encodes a protein closely related to AP-1 (21), and *zif/268* encodes a protein with zinc finger sequences that are related to those present in the known transcription factors TFIII-A (32) and Sp1 (33). Our observations extend the findings of Morgan *et al.* (11), who showed that c-*fos* mRNA and protein are rapidly and transiently increased in rat brain after pentylenetetrazole-induced seizures. Recently, Sukhatme *et al.* (34) also described the rapid rise of *egr-1* (identical to *zif/268*) mRNA in the brains of mice after pentylenetetrazole-induced seizures. The increased mRNA levels are induced by intense stimulation, not simply by the administration of centrally active drugs, since neither apomorphine or *p*-chloroamphetamine [nonconvulsant drugs that stimulate dopamine receptors (35) and release serotonin (36), respectively] increased mRNA levels for any of the genes tested (data not shown).

Considerable variability in the intensity and duration of the behavioral response was observed after pentylenetetrazole administration. As might be expected, therefore, we noted considerable interanimal variation in mRNA elevations. Nevertheless, the anatomical pattern observed by *in situ* hybridization was remarkably similar for *zif/268*, c-*jun*, *jun-B*, and c-*fos* mRNAs, suggesting that these genes may be coordinately expressed in the same set of neurons. In a few experiments performed on the cerebellum, mRNA increases were observed by blot hybridization analysis but not by *in situ* hybridization. This discrepancy may stem from a particularly large interanimal variability in response in this area or may reflect difficulty in detecting a diffuse increase in mRNA by the *in situ* technique.

The induction of transcription factor mRNAs after seizures suggests that another wave of gene expression follows, implying a programmed genetic response to intense neuronal activity. Possible similarities between the mechanism by which cells respond to growth factors and the mechanism by which neurons may respond to electrical and chemical stimuli to establish long-term memory have been discussed by Goelet *et al.* (1) and Berridge (2). In the former case, the interaction of specific growth factors with membrane receptors initiates the activation of second messenger systems that ultimately induce genes whose expression is required for cell growth. An intermediate step in this process is the rapid activation of immediate early or competence (3–7) genes encoding transcription factors that initiate a second wave of gene expression. A similar sequence of events is proposed for the induction of long-term changes in the behavior of neurons after specific types of stimulation. The observations that protein and RNA synthesis inhibitors block long-term changes in synaptic efficiency in the hippocampus (37, 38) and in the invertebrate *Aplysia* (39) lend support to this model.

Although it is difficult to extrapolate from the effects of pharmacologically induced seizures to neuronal responses to natural stimuli, it is possible that the specific changes in gene expression after the administration of convulsants that we and others have observed are amplifications of normal brain processes. If so, one should be able to detect similar changes in gene expression after more natural or more localized stimuli that modify neuronal behavior.

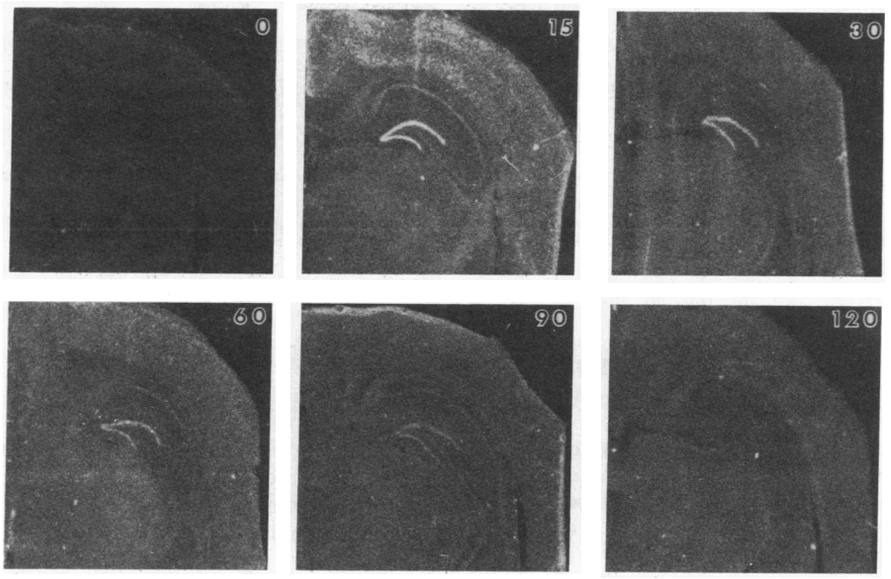

FIG. 6. Response of *jun-B* mRNA in dentate gyrus at 0, 15, 30, 60, 90, and 120 min after administration of pentylenetetrazole. Autoradiographs of coronal tissue sections after *in situ* hybridization with ^{35}S-labeled antisense probe. Exposure time: 1 week. (×5.5.)

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1. Goelet, P., Castellucci, V. F., Schacher, S. & Kandel, E. R. (1986) *Nature (London)* **322,** 419–422.
2. Berridge, M. (1986) *Nature (London)* **323,** 294–295.
3. Cochran, B., Reffel, A. C. & Stiles, C. D. (1983) *Cell* **33,** 939–947.
4. Lau, L. F. & Nathans, D. (1985) *EMBO J.* **4,** 3145–3151.
5. Lau, L. F. & Nathans, D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 1182–1186.
6. Sukhatme, V. P., Kartha, S., Toback, F. G., Taub, R., Hoover, R. G. & Tsai-Morris, C.-H. (1987) *Oncogene Res.* **1,** 343–355.
7. Chavrier, P., Zerial, M., Lemaire, P., Almendral, J., Bravo, R. & Charnay, P. (1988) *EMBO J.* **7,** 29–35.
8. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311,** 433–438.
9. Verma, I. M. & Graham, W. R. (1987) *Adv. Cancer Res.* **49,** 29–52.
10. Hunt, S. P., Pini, A. & Evan, G. (1987) *Nature (London)* **328,** 632–634.
11. Morgan, J. I., Cohen, D. R., Hempstead, J. L. & Curran, T. (1987) *Science* **237,** 192–197.
12. White, J. D. & Gall, M. G. (1987) *Mol. Brain Res.* **3,** 21–29.
13. Dragunow, M. & Robertson, H. A. (1987) *Nature (London)* **329,** 441–443.
14. Greenberg, M. E., Ziff, E. B. & Greene, L. A. (1986) *Science* **234,** 80–83.
15. Morgan, J. I. & Curran, T. (1986) *Nature (London)* **322,** 552–555.
16. Maki, Y., Bos, T. J., Davis, C., Starbuck, M. & Vogt, P. K. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2848–2852.
17. Vogt, P. K., Bos, T. J. & Doolittle, R. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3316–3319.
18. Bohmann, D., Bos, T. J., Admon, A., Nishimura, T., Vogt, P. K. & Tjian, R. (1987) *Science* **238,** 1386–1392.
19. Angel, P., Imagawa, M., Chiu, R., Stein, B., Imbra, R. J., Rahmsdorf, H. J., Jonat, C., Herrlich, P. & Karin, M. (1987) *Cell* **49,** 729–739.
20. Angel, P., Allegretto, E. A., Okino, S. T., Hattori, K., Boyle, W. J., Hunter, T. & Karin, M. (1988) *Nature (London)* **332,** 166–171.
21. Ryder, K., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 1487–1491.
22. Christy, B. A., Lau, L. F. & Nathans, D. (1988) *Proc. Natl. Acad. Sci. USA* **85,** in press.
23. Milbrandt, J. (1987) *Science* **238,** 797–799.
24. Evans, R. M. & Hollenberg, S. M. (1988) *Cell* **52,** 1–3.
25. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
26. Linzer, D. I. H. & Nathans, D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 4271–4275.
27. Miller, A. D., Curran, T. & Verma, I. M. (1984) *Cell* **36,** 51–60.
28. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
29. Melton, D. A., Krieg, P. A., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12,** 7035–7056.
30. Largent, B. L., Jones, D. T., Reed, R. R., Pearson, R. C. A. & Snyder, S. H. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 2864–2868.
31. Segal, D. & Shilo, B.-Z. (1986) *Mol. Cell Biol.* **6,** 2241–2248.
32. Miller, J., McLachlan, A. D. & Klug, A. (1985) *EMBO J.* **4,** 1609–1614.
33. Kodanaga, J. T., Carner, K. R., Masiarz, F. R. & Tjian, R. (1987) *Cell* **51,** 1079–1090.
34. Sukhatme, V. P., Cao, X., Chang, L. C., Tsai-Morris, C.-H., Stamenkovich, D., Ferreira, P. C., Cohen, D. R., Edwards, S. A., Shows, T. B., Curran, T., Le Beau, M. M. & Adamson, E. D. (1985) *EMBO J.* **4,** 1609–1614.
35. Leff, S. E. & Creese, I. (1983) *Trends Pharmacol. Sci.* **4,** 463–467.
36. Sanders-Bush, E. & Massari, V. J. (1977) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **36,** 2149–2153.
37. Stanton, P. K. & Sarvey, J. M. (1985) *Brain Res.* **361,** 276–283.
38. Stanton, P. K. & Sarvey, J. M. (1984) *J. Neurosci.* **4,** 3080–3088.
39. Montarolo, P. G., Goelet, P., Castellucci, V. F., Morgan, J., Kandel, E. R. & Schacher, S. (1986) *Science* **234,** 1249–1254.