scaled ornamentation have been described from Upper Cambrian to Middle Ordovician rocks, it has yet to be conclusively shown that any of them are vertebrate.
5. R. H. Denison, *Fieldiana Geol.* **16** (no. 6), 131 (1967).
6. K. A. Lehtola, *J. Paleontol.* **57**, 605 (1983).
7. L. B. H. Tarlo, *Acta Paleontol. Pol.* **7**, 294 (1962).
8. N. Spjeldnaes, *Colloq. Int. C.N.R.S.* **163**, 11 (1967).
9. D. G. Darby, *J. Paleontol.* **56**, 1187 (1982).
10. E. R. Lankester, *Paleontol. Soc.* **33**, 1 (1869).
11. T. Ørvig, *U.S. Nat. Mus. Proc.* **108**, 1 (1958).

12. I thank A. Blieck, P. Janvier, L. Montgomery, and Y. H. Liu for helpful comments and discussion, and L. Grande for permission to prepare the specimen. T. Melis and T. Mueller prepared the illustrations.

19 March 1987; accepted 3 June 1987

Mapping Patterns of c-*fos* Expression in the Central Nervous System After Seizure

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A dramatic and specific induction of c-*fos* was observed in identifiable neuronal populations in vivo after administration of the convulsant Metrazole. This effect was time- and dose-dependent and was abolished by prior treatment with the anticonvulsant drugs diazepam or pentobarbital. About 60 minutes after administration of Metrazole, c-*fos* messenger RNA reached a maximum and declined to basal levels after 180 minutes. A further decrease below that in normal brain was observed before a return to basal levels after 16 hours. While Metrazole still elicited seizures during this period, reinduction of c-*fos* was largely refractory. At 90 minutes, c-*fos* protein was observed in the nuclei of neurons in the dentate gyrus, and in the pyriform and cingulate cortices. Subsequently, c-*fos* protein appeared throughout the cortex, hippocampus, and limbic system. Thus, seizure activity results in increased c-*fos* gene expression in particular subsets of neurons.

THE C-*fos* GENE IS THE CELLULAR homolog of the oncogene (v-*fos*) carried by the FBJ and FBR murine osteogenic sarcoma viruses (*1*). It encodes a nuclear protein (Fos) that is associated with chromatin and exhibits a DNA-binding activity in vitro (*2, 3*). In most cell types, the basal level of c-*fos* expression is relatively low; however, it can be induced rapidly and transiently by a diverse range of extracellular stimuli (*4*). Although initial observations suggested that Fos was associated with mitogenesis, this viewpoint has been challenged, since induction of c-*fos* has been observed where promotion of differentiation rather than stimulation of cell division occurs. Furthermore, in studying the mechanisms that couple membrane events to c-*fos* activation in PC12 cells, we found that agents or conditions that provoke a voltage-dependent calcium influx were potent inducers of c-*fos* (*5*). Similarly, others have found that occupation of the nicotinic acetylcholine receptor on PC12 cells also elicited c-*fos* induction (*6*). These studies led us to speculate that c-*fos* expression might be regulated by neuromodulators in vivo. Therefore, we studied c-*fos* expression in brains of mice that had been treated with a convulsant. The c-*fos* messenger RNA (mRNA) was monitored by Northern transfer and hybridization, while the subcellular and brain regional localization of the c-*fos* protein were followed by immunocytochemistry.

We have used a procedure, Metrazole

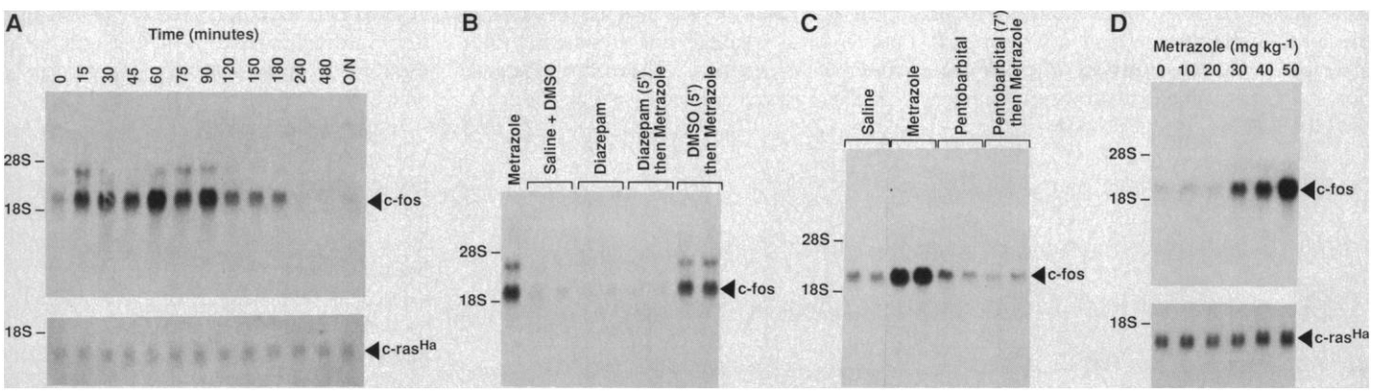

Fig. 1. RNA analysis of Metrazole-induced c-*fos* expression. BALB/c mice were injected intraperitoneally with Metrazole [50 mg/kg in (A) and (B) at the doses indicated in (C)]; the animals were killed by decapitation [at times given in (A) and (B), or at 60 minutes in (C)]. The whole brain was removed and immediately frozen in liquid nitrogen, and frozen tissues were homogenized in a 3*M* LiCl plus 6*M* urea solution. The homogenates were held overnight on ice, and RNA was then extracted by a modification of the LiCl and urea procedure (*20, 21*). After gel electrophoresis and transfer onto nitrocellulose filters, RNA's were hybridized with *fos* and *ras*^{Ha} probes (*22*). (**A**) Time course of c-*fos* expression. Each animal was injected intraperitoneally with Metrazole (50 mg/kg) and killed at the time indicated following treatment. Identical filters were probed either for c-*fos* or for c-*ras*^{Ha} mRNA. The position of 28*S* and 18*S* RNA's are indicated (c-*fos* filter exposure 4 hours, c-*ras*^{Ha} filter exposure 17 hours). (**B**) Effect of diazepam on Metrazole-induced c-*fos* expression. Pairs of animals were injected with Metrazole alone (in saline) (50 mg/kg), diazepam alone [in dimethyl sulfoxide (DMSO)] (10 mg/kg), or diazepam (10 mg/kg) 5 minutes before administration of Metrazole (50 mg/kg). Pairs of control animals injected with either a saline plus DMSO solution or with DMSO alone 5 minutes before administration of Metrazole (50 mg/kg) were treated in parallel. Animals were killed 60 minutes after treatment and total RNA was isolated from whole brain (see A). Northern analyses for the presence of c-*fos* mRNA was as described for (A) (4-hour film exposure). (**C**) Effect of pentobarbital on Metrazole-induced c-*fos* expression. Pairs of BALB/c mice were injected with saline, Metrazole (50 mg/kg), sodium pentobarbital (80 mg/kg), or sodium pentobarbital (80 mg/kg) 7 minutes prior to Metrazole (50 mg/kg). Animals were killed 60 minutes after treatment with Metrazole, and total RNA was isolated from whole brain as described in (A). Northern analyses for the presence of c-*fos* mRNA was performed as in (A). (**D**) Dose response of Metrazole-induced c-*fos* expression. Animals were injected with Metrazole at 0, 10, 20, 30, 40, or 50 mg/kg and killed after 60 minutes. Animals given higher doses did not survive the treatment. Identical Northern filters were probed either for c-*fos* or for c-*ras*^{Ha}, filter was exposed for 17 hours.

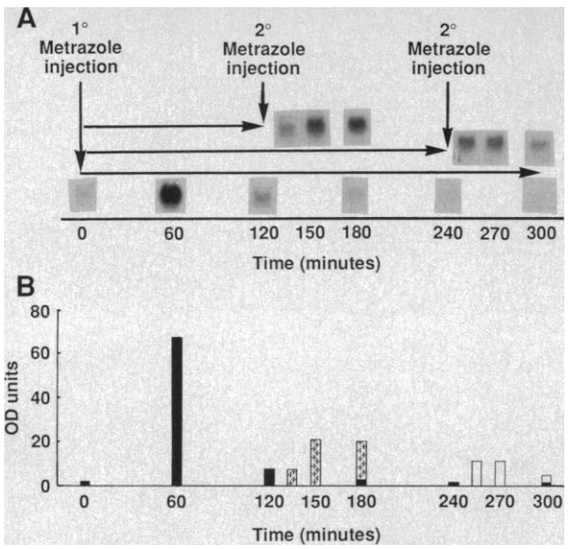

Fig. 2. Refractory period of c-*fos* mRNA induction (**A**) Northern analysis of c-*fos* mRNA under conditions indicated. (**B**) Quantitative densitometry of data shown in (A). Twelve animals were injected with a primary dose (50 mg/kg) of Metrazole at 0 minutes; and each was subsequently killed at the time indicated (0, 60, 120, 180, 240, and 300 minutes) as a control time course (solid bars on diagram showing relative RNA levels). At 120 minutes three animals were given a secondary injection of Metrazole (50 mg/kg) and these animals were killed 15, 30, and 60 minutes after this second dose (135, 150, and 180 minutes after the primary doses; shaded bars on diagram). A similar secondary time course was performed on the three remaining animals 240 minutes after the primary injection (open bars on diagram) such that these animals were killed 255, 270, and 300 minutes after the primary dose.

(pentylenetetrazole)-induced seizures, that has been used as a screening method for detecting anticonvulsant drugs that are effective in the treatment of epilepsy (*7*). While the precise mechanism of action of Metrazole is unclear the available evidence indicates that it interacts with the γ-aminobutyric acid (GABA) receptor–benzodiazepine receptor–chloride ionophore complex (*8*). Inhibition of the GABA receptor complex would eliminate the suppressive action of GABA and result in increased activation of neurons. Approximately 60 minutes after administration of Metrazole (50 mg/kg), we observed a substantial increase in c-*fos* mRNA in whole brain (by a factor of about 20) (Fig. 1A). The increase was detected within 15 minutes and persisted as long as 180 minutes but had declined to below the original values by 240 minutes (Fig. 1A). These subbasal levels persisted for at least another 4 hours, but returned to normal resident values by 16 hours after treatment. The time course reported here for c-*fos* induction in vivo is protracted compared to that in serum-stimulated fibroblasts (*9*) and in PC12 cells treated with nerve growth factor (*10*). Alternatively, this could represent successive waves of c-*fos* mRNA induction in distinct neuronal populations with peak expression at 30, 60, and 90 minutes after treatment, respectively (Fig. 1A).

The benzodiazepines are neuropharmacological agents that have anticonvulsant properties mediated via their agonistic interaction with the GABA receptor–chloride ionophore complex (*11*). Although administration of a benzodiazepine (diazepam) at an anticonvulsant dose had no effect on c-*fos* expression, it abolished completely the induction by Metrazole (Fig. 1B). The various carrier solvents used in our study had no effect on either basal or stimulated c-*fos* expression (Fig. 1B). Similarly, administration of another distinct anticonvulsant drug, sodium pentobarbital (Nembutal), (*12*) did not induce c-*fos* while it blocked completely the effects of Metrazole on c-*fos* expression (Fig. 1C). Taken together, these data imply that the induction of c-*fos* by Metrazole is attributable to the seizure-causing activity of the drug.

With a dose of Metrazole at 50 mg/kg, mice had seizures within 1 to 2 minutes and then sporadically up to 30 minutes after treatment. Subsequently, the mice recovered and exhibited no apparent ill effects after 120 minutes. The maximal effect was obtained with 50 mg/kg (Fig. 1D). Animals did not survive increased doses. Elevation of c-*fos* mRNA was seen with 30 mg/kg, although at this dose mice did not usually have overt convulsions. At no time after administration of any dose of Metrazole were alterations in the levels of c-ras^{Ha} (Fig. 1, A and D), 18*S* ribosomal RNA, and c-*myc* transcripts observed (*13*).

To determine whether the period of reduced c-*fos* expression represented a refractory phase for c-*fos* induction, we examined the effect of Metrazole on reinduction of c-*fos* mRNA at various times after the initial dose of the convulsant. When Metrazole was given at either 120 or 240 minutes after a primary dose of the drug, the induction was markedly attenuated (Fig. 2). The reduction in response was more pronounced at 240 minutes when mRNA levels were below basal values, than at 120 minutes when they were still above control levels (Fig. 2). However, all mice receiving a second dose of Metrazole exhibited severe seizures despite the differences in c-*fos* induction. Therefore, induction of c-*fos* mRNA is not invariably a consequence of seizure. However, during the refractory period examined here, c-*fos* protein levels were elevated throughout the brain (see below). At 16 hours after an initial dose of Metrazole, when the c-*fos* protein had returned to basal

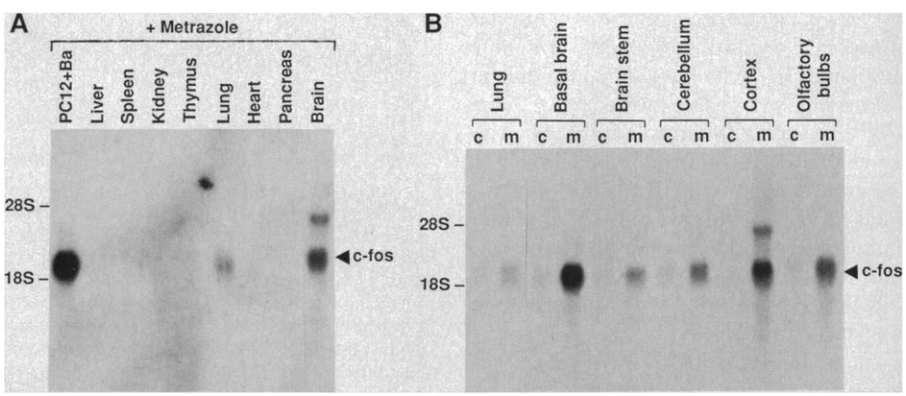

Fig. 3. Distribution of c-*fos* expression in Metrazole-treated mice. (**A**) Animals were injected intraperitoneally with Metrazole (50 mg/kg) and killed 60 minutes later. Liver, spleen, kidney, thymus, lung, heart, pancreas, and whole brain tissues were removed immediately and frozen in liquid nitrogen. RNA was prepared and analyzed by gel electrophoresis, Northern transfer, and hybridization with a *fos* probe as described in Fig. 1. The PC12 plus Ba^{2+} sample (PC12 pheochromocytoma cells induced with Ba^{2+}) provides, for comparison, an RNA sample prepared from a population of cells all expressing c-*fos* mRNA (17-hour exposure). (**B**) Four animals were injected intraperitoneally with Metrazole (50 mg/kg) and killed at 60 minutes. Four control animals were treated in parallel with saline. Lung and brain tissues were removed from each animal and the brains were dissected into basal brain, brainstem, cerebellum, cortex and olfactory bulb regions (*23*). Tissue samples from the brain regions were pooled for RNA isolation. RNA preparation and Northern analyses were performed as in Fig. 1 (4-hour film exposure). c, control; m, Metrazole.

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levels, a second dose induced c-*fos* as efficiently as a single administration (*14*). Thus, the induction of c-*fos* is regulated, perhaps, by a negative feedback mechanism involving the c-*fos* protein.

In animals treated with Metrazole for 60 minutes, c-*fos* expression was detected only in brain and lung (Fig. 3A). This finding suggested that the induction of c-*fos* in brain was not attributable to a general stress response perhaps mediated by glucocorticosteroids. In addition, it was found that brain had a readily detectable basal level of c-*fos* expression (Fig. 3B) implying that c-*fos* may play a role in the normal physiology of the nervous system. The unexpected finding of c-*fos* induction in lung is intriguing; however, its precise cellular origin and significance are unknown.

Comparison of c-*fos* mRNA levels from distinct brain regions of control and Metrazole-treated mice (Fig. 3B) showed that, although c-*fos* mRNA rose after administration of Metrazole in all brain areas, the major increases at 60 minutes occurred in basal brain and cortex. This suggested that Metrazole not only had differential effects upon c-*fos* expression throughout the brain but also that regions known to be implicated in seizures (for example, hippocampus) might be targets for c-*fos* induction.

To localize the c-*fos* protein in brain we performed an immunocytochemical analysis. Consistent with the observation of c-*fos* mRNA in untreated mice (Fig. 1), an antiserum to the c-*fos* protein (Fos antiserum) stained certain areas in untreated mouse brain. In the control mice, Fos-positive neurons were observed throughout the brain, but particularly in the pyriform cortex, anterior olfactory nucleus, and the bed nucleus of the stria terminalis. Sporadic cells were also stained in the limbic system including in the dentate gyrus and hippocampus (Fig. 4A), and other regions of the cerebral cortex. However, some variability was observed among control animals in the levels and distribution of Fos immunostaining. In Metrazole-treated mice, both the intensity and the number of cells labeled in these regions increased dramatically (Fig. 4, A to D).

The immunostaining for Fos was specific by several criteria. (i) The same staining pattern was observed with three different antisera to Fos but not with an irrelevant antiserum. (ii) Prior absorption of the Fos antiserum with the synthetic peptide used as immunogen completely blocked staining. (iii) At higher magnification the Fos antiserum stains nuclei (Fig. 4E), which is consistent with the known nuclear location of the Fos protein (*2*). Thus, the immunoreactive substance in brain is the c-*fos* protein.

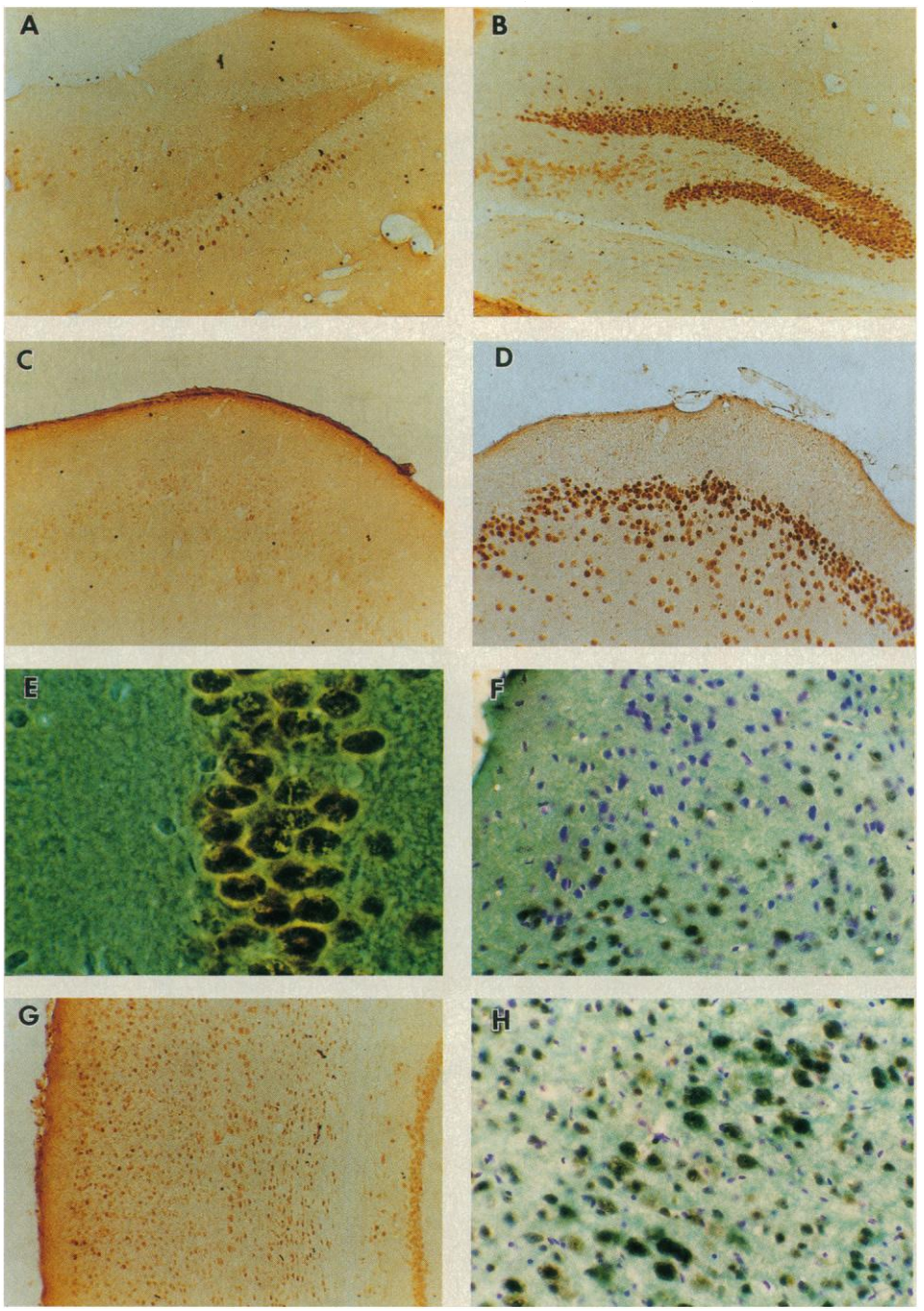

Fig. 4. Localization of c-*fos* in mouse brain. (A through D) A comparison of immunoperoxidase staining of c-*fos* in control (A and C) and Metrazole-treated (B and D) mice. (**A**) Hippocampal region of control mouse showing sporadic immunoreactivity in dentate gyrus (×125). (**B**) Equivalent region from a mouse receiving Metrazole (50 mg per kilogram of body weight) 3 hours before they were killed. All granule cells of the dentate gyrus were Fos-positive at this time (×125). (**C**) Pyriform cortex of a control mouse showing low-level staining (×125). (**D**) An equivalent, heavily labeled, region from a mouse treated 90 minutes earlier with Metrazole at 50 mg/kg (×125). There is an absence of staining in layer 1 and the gradation of immunoreactivity from layers 2 through 6. (**E**) A high magnification (×800), of the dentate gyrus from a mouse receiving Metrazole (50 mg/kg) 90 minutes before being killed. Note specific staining of nuclei. (**F**) A cerebral cortical region of a mouse receiving Metrazole (50 mg/kg) 4 hours before they were killed. The section has been immunoperoxidase-stained for c-*fos* and counterstained with cresyl violet and fast green (×125). Both Fos-positive and Fos-negative nuclei are present. (**G**) A cerebral cortical region and a portion of the CA1 layer of hippocampus immunostained for Fos from the same mouse reported in (F). Large Fos-positive neurons are apparent at this time. (**H**) A higher power view (×310) of the large neurons seen in (G) after immunostaining for c-*fos* and counterstaining with cresyl violet and fast green. Details of perfusion (*24*) and cryomicrotomy and immunostaining (*25*) have been described. The antiserum to Fos (*26*) was diluted 1:20 for these experiments and bound antibody was detected with an avidin-biotin-peroxidase complex (Vectastain Inc., Burlingame, California).

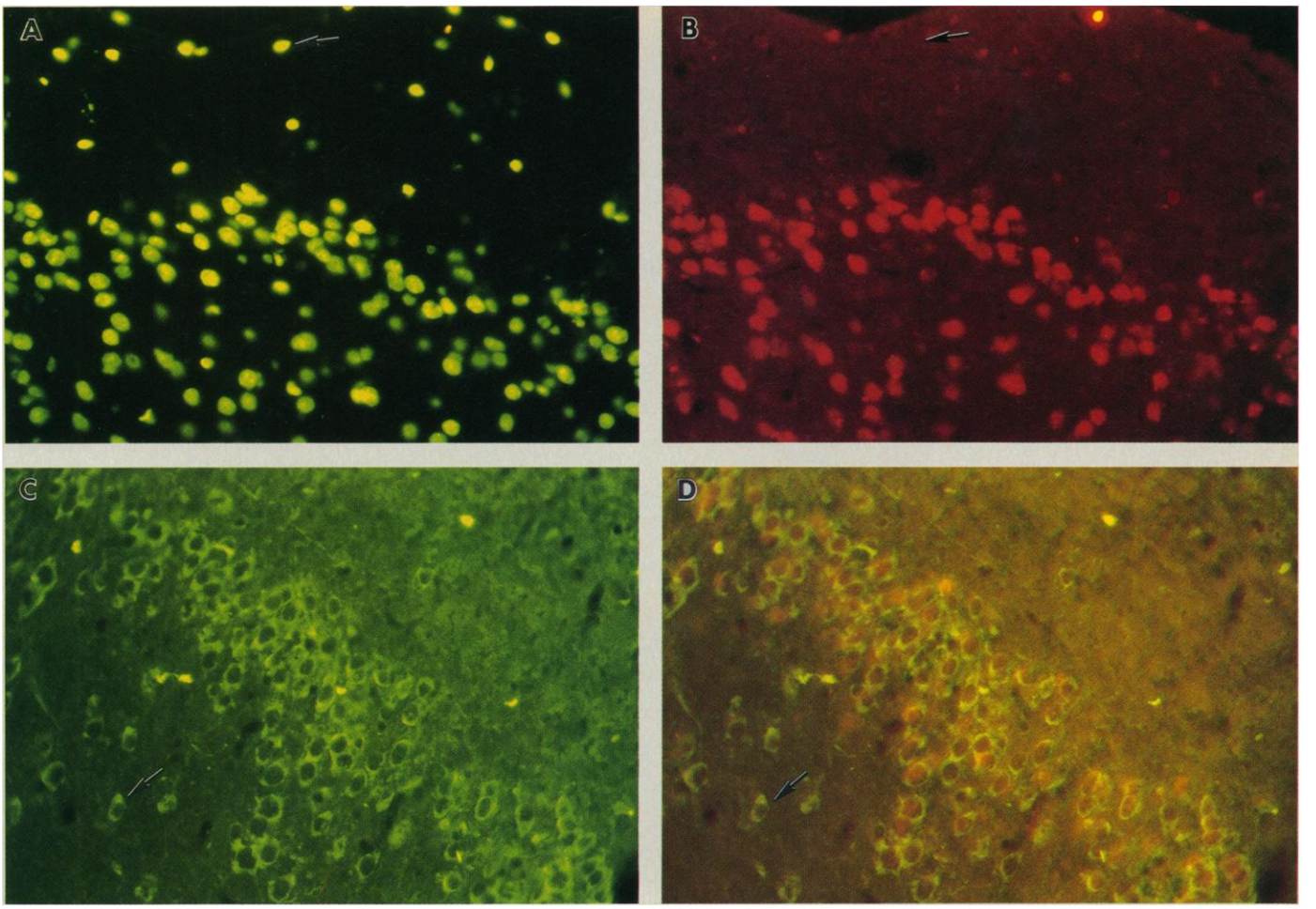

Fig. 5. Identification of c-*fos*–positive cells as neurons by double immunofluorescence. To determine the phenotype and incidence of Fos-positive cells a double immunofluorescence analysis was performed. (**A**) Shows all nuclei in the pyriform cortex of a mouse treated for 90 minutes with Metrazole (50 mg/kg) labeled with a monoclonal antibody (NUC-1) to nuclei (*15*). (**B**) The same section stained for Fos. Antiserum to Fos stains only nuclei and, although some nuclei are heavily labeled, others are weak or negative. (**C**) Pyriform cortex from the same animal stained with a monoclonal antibody to the neuron-specific intermediate filaments, neurofilament (Labsystems, Chicago). (**D**) The same section double exposed for c-*fos* and neurofilaments. Again, c-*fos* labels only nuclei that are always rimmed by staining for neurofilaments. However, some neurofilament-positive cells are Fos-negative. Details of fixation, processing and double immunofluorescence are as described (*24, 25*). Fluorescent secondary antibodies were purchased from Cappel Inc., Cochranville, Pennsylvania (×310).

Immunoperoxidase staining for Fos and a cresyl violet counterstain for nuclei indicated that not all brain cells react with the antiserum to Fos (compare brown peroxidase–stained and violet-stained nuclei in Fig. 4F). At later times (4 hours) after seizure, several additional layers of the cortex are positive (Fig. 4G), including the large pyramids of Betz (Fig. 4H). Although the number and position of the stained bodies would indicate them to be neuronal cell nuclei, this was confirmed by double-labeling experiments (Fig. 5). Analysis of a cortical section from a Metrazole-treated mouse using an antibody reactive with all nuclei (Fig. 5A) (*15*) and Fos antiserum (Fig. 5B) reveals that the Fos antiserum only stains a subset of nuclei. Interestingly, several nuclei, particularly those in cortical layer one, do not contain c-*fos* protein (indicated by arrows in Fig. 5, A and B). Staining of another region of cortex with an antibody to the neuron-specific neurofilament protein (Fig. 5C) or antibody to the neurofilament protein plus Fos antiserum (Fig. 5D) shows that all Fos-containing cells are neurons. Several neurofilament-positive cells do not stain with Fos antiserum (indicated by arrows in Fig. 5, C and D).

The time course of appearance of Fos immunoreactivity after administration of Metrazole was investigated (Fig. 6). Increased staining of the dentate gyrus was first evident after 30 minutes and was maximal after 60 minutes (Fig. 6). As noted above there was an increase in the number and intensity of stained neurons in defined cortical regions between 60 and 90 minutes. Subsequently, staining of further cortical layers and regions became evident (Fig. 4, G and H, and Fig. 6). By 4 hours after treatment, essentially all neurons in the cortex and limbic system were labeled. Although occasional neurons were labeled in the hippocampus up to 90 minutes, the entire structure became Fos-positive by 3 hours. At 90 minutes after drug administration, immunoreactivity was also observed in the amygdala, septum, and olfactory bulb. Fos was conspicuously absent from some areas of brain at all times examined, notably the superior and inferior colliculi, the geniculate bodies, central gray, substantia nigra, and cerebellar cortex. Thus, after seizure, there ensues a successive recruitment of specific cohorts of neurons expressing c-*fos* throughout the cerebral cortex and limbic system.

The c-*fos* protein may play a part in the normal function of the neuron since Fos-positive cells are present in untreated mouse brain. This study establishes that Metrazole-induced seizure activity in vivo results in a dramatic and rapid alteration of c-*fos* expression. Presently, it is not clear whether this stimulation involves membrane depolarization and enhanced firing rates, analogous to

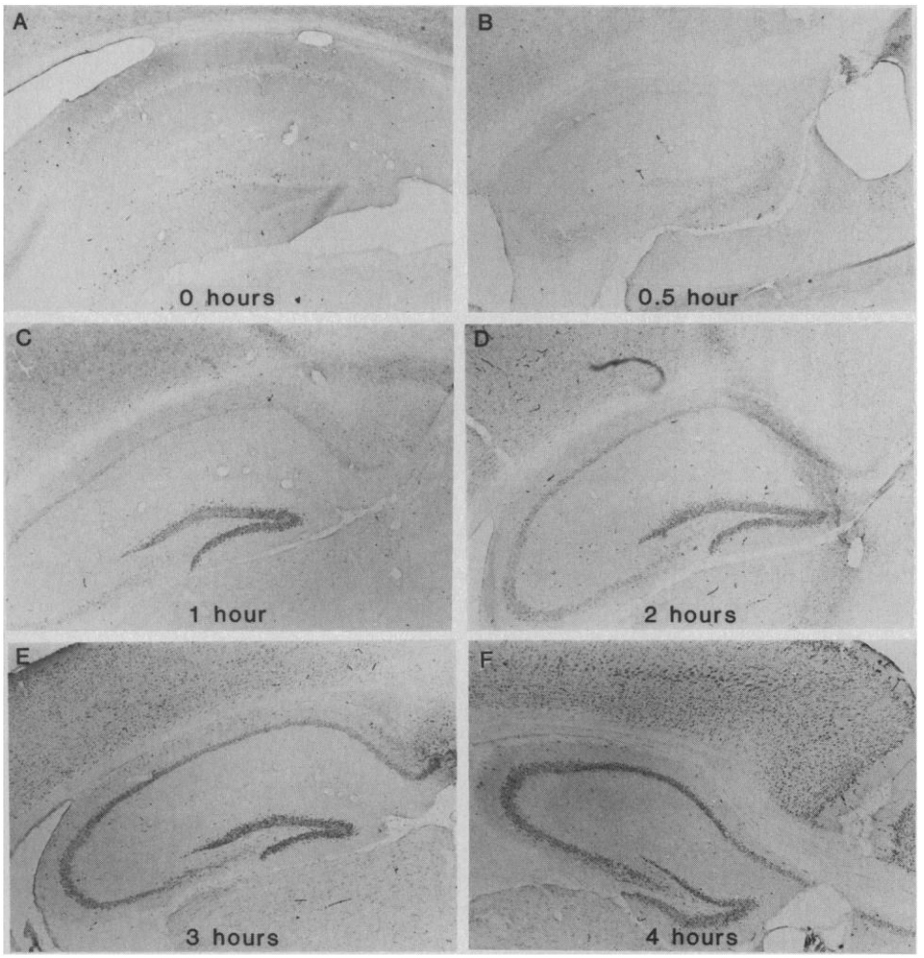

Fig. 6. Time course of Fos appearance in Metrazole-treated mouse brain. Mice received Metrazole (50 mg/kg, intraperitoneally) at time 0 and then at the indicated time each was killed and the tissues were processed for Fos-immunoperoxidase staining as described in the legend to Fig. 4 and in (*24, 25*). The data are representative coronal sections at approximately the same position in brain to show the complete hippocampal formation. Fos-immunostaining first appears in the dentate gyrus at 30 minutes after seizure and is maximal between 1 and 2 hours. The hippocampus proper becomes stained with a slower onset and is maximal between 3 and 4 hours. During this time course, the cerebral cortical layers become Fos-positive at different times. In addition, after 4 hours the caudate becomes positive (×50).

the situation in PC12 cells in vitro (*5*), or whether it is an indirect effect occurring as a result of secondary stimulation of neurons by neurotransmitters or neuromodulators. Interestingly, the distribution of Fos-positive neurons after Metrazole-induced seizures parallels the density of binding sites of the anticonvulsant MK-801 (*16*). This compound is an antagonist of the excitatory amino acid transmitter *N*-methyl-D-aspartate (*17*), which is implicated in seizures (*18*).

Because Fos appears after seizure, it cannot be involved in the generation of convulsion as emphasized by the fact that reinduction of Fos is semirefractory, whereas seizure is not. It is possible that Fos, a nuclear protein that can bind to DNA (*2, 3*), is involved in the long-term adaptive response to convulsions. This would include the alterations in receptor numbers reported to occur in the dentate gyrus following seizures (*19*); a structure shown here to be an early target for Fos induction. Thus, it may be that the expression of Fos in nerve cells contributes to neuronal plasticity.

We have demonstrated that expression of c-*fos* can be used as a marker, with single-cell specificity, to follow the temporal and spatial involvement of certain subsets of neurons during and following seizure. The demonstration of these areas of c-*fos*–positive neurons should provide an impetus for the electrophysiological analysis of these same cells in this, and other, paradigms of seizure. Potentially, the strategy employed here can also be used to delineate targets and interactions of other neuropharmacological agents or diverse sensory inputs.

REFERENCES AND NOTES

1. T. Curran and N. M. Teich, *J. Virol.* **42**, 114 (1982); T. Curran, G. Peters, C. Van Beveren, N. M. Teich, I. M. Verma, *ibid.* **44**, 674 (1982); T. Curran and I. M. Verma, *Virology* **135**, 218 (1984).
2. T. Curran, A. D. Miller, L. Zokas, I. M. Verma, *Cell* **36**, 259 (1984).
3. L. C. Sambucetti and T. Curran, *Science* **234**, 1417 (1986).
4. T. Curran, in *The Oncogene Handbook*, E. P. Reddy, A. M. Skalka, T. Curran, Eds. (Elsevier, Amsterdam, in press).
5. J. I. Morgan and T. Curran, *Nature (London)* **322**, 552 (1986).
6. M. E. Greenberg, E. B. Ziff, L. A. Greene, *Science* **234**, 80 (1986).
7. E. A. Swinyard and J. H. Woodhead, in *Antiepileptic Drugs*, D. M. Woodbury, J. K. Penry, C. E. Pippenger, Eds. (Raven, New York, ed. 2, 1982), pp. 111–126.
8. R. F. Squires, E. Saederup, J. N. Crawley, P. Skolnick, S. M. Paul, *Life Sci.* **35**, 1439 (1984).
9. R. Muller, R. Bravo, J. Burckhardt, T. Curran, *Nature (London)* **312**, 716 (1984).
10. T. Curran and J. I. Morgan, *Science* **229**, 1265 (1985).
11. R. H. Mattson, in *Antiepileptic Drugs*, D. Woodbury, J. K. Penry, R. P. Schmidt, Eds. (Raven, New York, 1972), pp. 497–516.
12. J. W. Prichard, in *Antiepileptic Drugs*, D. M. Woodbury, J. K. Penry, C. E. Pippenger, Eds. (Raven, New York, 1982), pp. 365–376.
13. For all experiments, the concentration and intensity of RNA were monitored by ethidium bromide staining of ribosomal RNA in each sample.
14. J. I. Morgan and T. Curran, unpublished observations.
15. A. Farbman, J. L. Hempstead, J. I. Morgan, unpublished observations.
16. E. H. F. Wong *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7104 (1986).
17. J. C. Watkins and R. H. Evans, *Annu. Rev. Pharmacol. Toxicol.* **21**, 165 (1981).
18. I. Mody and U. Heinemann, *Nature (London)* **326**, 701 (1987).
19. C. Shin, H. B. Pedersen, J. O. McNamara, *J. Neurosci.* **5**, 2696 (1985); F. Valdes, R. M. Dashieff, F. Birmingham, K. A. Crutcher, J. O. McNamara, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 193 (1982).
20. C. Auffrey and F. Rougeon, *Eur. J. Biochem.* **107**, 303 (1975).
21. All solutions contained 0.1% diethylpyrocarbonate (DEPC) to inhibit RNA degradation by ribonucleases during the extraction procedure. The RNA was recovered by precipitation with ethanol and centrifugation; it was redissolved in tris-EDTA buffer (10 m*M* tris-HCl, *p*H 7.4, 1 m*M* EDTA) containing 0.1% DEPC and stored at −70°C. Portions (10 μg) of total RNA were removed, lyophilized, and resuspended in RNA sample buffer containing 1× gel running buffer (1× = 200 m*M* MOPS, *p*H 7.0, 50 m*M* sodium acetate, 10 m*M* EDTA), 50% formamide, and 2.2*M* formaldehyde. The RNA samples were denatured in this buffer at 65°C for 5 minutes and separated on horizontal 0.8% agarose gels containing 1× gel running buffer and 2.2*M* formaldehyde, usually at 75 V for 3 hours. After electrophoresis, gels were stained in 1× gel running buffer containing ethidium bromide (10 μg/ml) for 30 minutes, then destained twice for 20 minutes in 1× gel running buffer and blotted onto nitrocellulose paper with 20× SSC (1× SSC buffer contains 0.015*M* trisodium citrate and 0.15*M* NaCl) overnight. The filters were then rinsed in 2× SSC and baked at 80°C for 2 hours in a vacuum oven. Baked filters were immersed briefly in 2× SSC and then hybridized at 42°C for 1 to 4 hours in a solution containing 0.75*M* NaCl, 50 m*M* sodium phosphate, *p*H 7.0, 5 m*M* EDTA, 0.2% SDS, 1% glycine, 5× Denhardt's solution (1× contains 0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin) and 50% formamide. The Eco RI–Sal I 1.35-kbp fragment of the chimeric *fos* gene and the 500-bp v-*ras*^{Ha} insert fragment of pBS9 (*22*) were used in the generation of nick-translated probes (specific activity 1×10^9 to 2×10^9 cpm/μg). Hybridizations were carried out at 42°C for 15 hours in a solution containing 0.75*M* NaCl, 50 m*M* NaH_2PO_4, *p*H 7.0, 25 m*M* EDTA, 0.2% SDS, 1% glycine, 1× Denhardt's solution, 50% formamide, and nick-translated probe (1×10^6 to 2×10^6 cpm/ml). Final washings of the filters were in 0.2× SSC plus 0.1% SDS at 55°C for filters probed for c-*fos* and 1× SSC plus 0.1% SDS at 55°C for filters probed for c-*ras*^{Ha}.
22. R. W. Ellis *et al.*, *Nature (London)* **292**, 506 (1981); L. C. Sambucetti *et al.*, *Gene* **43**, 69 (1986).

23. M. Palkovits and M. J. Brownstein, in *Brain Microdissection Techniques*, A. C. Cuello, Ed. (Wiley, New York, 1983), pp. 1–36.
24. BALB/c mice (Charles River) were anesthetized at the relevant times with Nembutal and perfused via the aorta sequentially with buffered (*p*H 7.4) sodium nitrite–heparin sulfate followed by 4% buffered (*p*H 7.4) paraformaldehyde.
25. J. R. Slemmon, W. Danho, J. Hempstead, J. I. Morgan, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7145 (1985); J. Hempstead and J. I. Morgan, *J. Neurosci.* **5**, 438 (1985).
26. T. Curran, C. Van Beveren, N. Ling, I. M. Verma, *Mol. Cell. Biol.* **5**, 167 (1985).

27. We thank H. Baker, S. Korn, J. McNamara, E. Mugnaini, S. Paul, E. Roberts, S. Spector, and F. Sulzer for helpful discussion and R. Horn and F. Margolis for critical reading of the manuscript.

26 March 1987; accepted 25 May 1987