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EFFECT OF ELECTRICAL STIMULATION OF THE CEREBRAL CORTEX ON THE EXPRESSION OF THE FOS PROTEIN IN THE BASAL GANGLIA

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Abstract—The protein Fos is a transcription factor which is quickly induced in response to a variety of extracellular signals. Since this protein is expressed in a variety of neuronal systems in response to activation of synaptic afferents, it has been suggested that it might contribute to activity-dependent plasticity in neural networks. The present study investigated the effect of cortical electrical stimulation on the expression of Fos in the basal ganglia in the rat, a group of structures that participate in sensorimotor learning. Results show that the repetitive application of electrical shocks in restricted areas of the cerebral cortex induces an expression of Fos mostly confined to the striatum and the subthalamic nucleus. The induction which can be elicited from different cortical areas (sensorimotor, auditory and limbic areas) does not require particular temporal patterns of stimulation but rather depends on the total number of shocks delivered during a given period of time. Moreover, it appears to be rather independent of the number of spikes discharged by the activated cells.

In the striatum, the distribution of immunoreactive neurons is precisely delineated and conforms to the known topographical organization of stimulated corticostriatal projections. As demonstrated using a variety of double labelling techniques (combination of the immunocytochemical detection of Fos with the autoradiography of μ opioid receptors, calbindin immunocytochemistry, *in situ* hybridization of preproenkephalin and preprotachykinin A messenger RNAs), striatal neurons which express Fos are mostly localized in the matrix compartment and concern equally enkephaline and substance P containing efferent neurons. In the subthalamic nucleus, Fos expression evoked by cortical stimulation is also confined to discrete regions of the nucleus, the localizations corresponding to the primary projection site of the stimulated cortical cells.

These results indicate that in addition to its phasic synaptic influence on the basal ganglia, the cerebral cortex could exert a long-term effect on the functional state of this system via a genomic control. Since the basal ganglia are involved in sensorimotor learning and motor habit formation, it is tempting to speculate that the activity-dependent Fos induction at corticostriatal and subthalamic synapses may contribute to consolidate the functionality of the neuronal networks activated during the completion of given motor tasks. © 1997 IBRO. Published by Elsevier Science Ltd.

Key words: neuronal plasticity, immediate early gene, striatum, subthalamic nucleus, substantia nigra.

The basal ganglia correspond to a major neuronal system of the forebrain which occupies a key position in motor control. Receiving a massive innervation from the entire cerebral cortex, this system acts as an interface between processing in the cerebral cortex and the generation of movement. The synaptic organization of circuits linking the cerebral cortex to the basal ganglia is now well characterized.^{3,10,34,42,55,59,66} Briefly, the striatum which repre-

sents the main input structure of the basal ganglia receives cortical projections from virtually all areas of the cerebral cortex. The cortical efferents impinge on output medium spiny neurons and interneurons of the striatum and exert on these cell populations an excitatory synaptic influence via the release of glutamate. Cortical efferent neurons originating from prefrontal and motor cortical areas also project to the subthalamic nucleus (STN), another main input structure of the basal ganglia. Like the corticostriatal fibres, the corticosubthalamic fibres release glutamate and exert on their target cells a potent synaptic excitatory drive. Activities generated in the striatum and the STN are finally integrated in the output structures of the basal ganglia, i.e. the substantia nigra pars reticulata (SNR) and the entopeduncular nucleus (the rodent equivalent of the primate internal globus pallidus). Striatal spiny neurons which contain GABA inhibit the tonically active cells of the

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Abbreviations: ABC, avidin-biotin-peroxidase complex; CaBP, calcium binding protein; DAB, 3,3'-diaminobenzidine; DEPC, diethyl pyrocarbonate; GP, globus pallidus; LTP, long-term potentiation; MK-801, dizocilpine maleate; NMDA, *N*-methyl-D-aspartate; PPE, preproenkephalin; PPT-A, preprotachykinin A; SNR, substantia nigra pars reticulata; SSC, saline sodium citrate; STN, subthalamic nucleus; TBS, Tris-buffered saline.

SNR and the entopeduncular nucleus. In contrast, STN neurons exert a concurrent excitatory influence on these output neurons via the release of glutamate.

Recently, the demonstration that the electrical stimulation of the cerebral cortex induces an expression of the Fos onco-protein in various structures of the basal ganglia (mainly the striatum and the STN) has raised new issues regarding the function of cortical inputs to this system.^{33,57,83} Since the Fos protein acts as a transcription factor,^{6,21,63} its induction by cortical stimulation has suggested that the cerebral cortex may lead to long-lasting functional changes in the basal ganglia through regulation of gene expression. However, if numerous information concerning the induction of *c-fos* by exogenously applied drugs is available,^{7,30,54,62,65,69} the regulation of this transcription factor by the synaptic activity is not as well documented. In particular, questions still exist in the corticobasal ganglia circuits about the precise modalities of induction and expression of *c-fos* gene by cortical activity. Previous studies have mainly focused on the effect of applications of trains of shocks in the motor cortex.^{33,57,83} However, the relation of Fos induction with the efficacy of different patterns of stimulation, the stimulated area and the electrophysiological activity induced by cortical stimulation, has not been systematically explored. In addition, a considerable variability appears from one study to another in the regional distribution of activated neurons. In the striatum, for instance, while some authors⁸³ reported that the stimulation of the motor cortex induces a Fos expression limited to regions of the projection field of the stimulated area, others^{33,57} described a more generalized expression which transgresses the known topographic arrangement of the corticostriatal projections.

Accordingly, the present study was undertaken to specify the modalities of the control exerted by the cerebral cortex on the transcriptional activation of the *c-fos* gene in the basal ganglia of the rat. For this purpose, electrical stimulations varying in both their temporal pattern and area of application were applied in the cerebral cortex and the resulting expression of Fos in the striatum and related structures of the basal ganglia was analysed. Neurons expressing the protein were characterized by their neurochemical markers.^{8,26,35,37,42} Single-unit recordings were also performed in various regions of the basal ganglia in order to establish possible electrophysiological correlates for Fos activation. Some of the data reported in the present paper have been presented in abstract form.^{1,9}

EXPERIMENTAL PROCEDURES

Surgery and stimulations

Experiments were carried out in 70 male Sprague–Dawley rats (Charles River, France) weighing 250–300 g. Cortical stimulations were applied in animals anaesthetized with pentobarbital sodium (Sanofi, 66 mg/kg i.p.) and placed in a conventional stereotaxic apparatus. Additional intra-

peritoneal injections of pentobarbital (33 mg/kg) were given whenever necessary (as checked by the reflex state of the animal). As Fos protein can be induced by a variety of sensory stimuli, care was taken to minimize uncontrolled sources of stimulation. Animals were handled as little as possible, the skin around the points of stereotaxic contention and areas of incision were infiltrated with xylocaine (R. Bellon Laboratory, 2%) and an opaque strip of paper was placed over the eyes during surgery to prevent activation of visual pathways by the illumination device. Body temperature was monitored throughout the experiment and kept at 37–38°C with an homeothermic blanket. A small craniotomy was made over the stimulated cortical area. Depending on the experiment, stimulation was applied in the motor, somatosensory, visual, auditory, cingulate, prelimbic or orbital area. The stimulation electrodes were implanted according to coordinates given by stereotaxic atlases.^{27,45,67,86} In some experiments an additional craniotomy was made for single-unit recordings in basal ganglia structures.

Cortical stimulations were applied through pairs of wires (Ni-Chrome, 0.2 mm diameter) 1.5 mm apart and inserted at a depth of 1.5 mm from the cortical surface. Electrical stimulations consisted of single shocks (intensity: 200 µA; shock duration: 0.2 ms; applied at a frequency of 4 to 20 Hz) or trains of shocks (intensity: 200 µA; shock duration: 0.2 ms; shock frequency: 100 or 250 Hz; train duration: 26 to 1000 ms) applied at a frequency of 0.1 to 20 Hz for 2.75 to 120 min (see Table 1). The electrode polarity was reversed every 30 s to avoid lesion of cortical tissue by thermocoagulation. At the end of the stimulation, animals were removed from the contention device and kept on the homeothermic blanket under anaesthesia. After a survival time which varied from 30 min to 5 h depending on the experiments, brains were fixed under deep pentobarbital anaesthesia (180 mg/kg) through intracardiac perfusion with 200 ml saline (0.9% NaCl) followed by 500 ml of fixative (4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4). Brains were removed, postfixed in the same fixative for 2 h and then immersed in a solution of 20% sucrose in 0.1 M phosphate buffer, pH 7.4, for one day at 4°C. For *in situ* hybridization experiments, DEPC (diethyl pyrocarbonate, Sigma), an RNAase inhibitor, was added to the sucrose solution.

Control animals

In a first set of experiments (18 rats), we analysed the effect of experimental manipulations on Fos expression. Animals were sham-operated under pentobarbital anaesthesia and placed in the stereotaxic frame for various times corresponding to the different stimulation durations (see Table 1). Then they were kept out of the stereotaxic frame (for 1–5 h) under anaesthesia and body temperature monitoring. Brains were fixed as described above.

In a second set of experiments, the effect of cortical stimulation was examined in awake, freely-moving rats. This control was carried out to evaluate the effect of anaesthesia on Fos expression. The stimulation electrodes were implanted in the jaw area of the motor cortex^{45,67} under pentobarbital anaesthesia and firmly fixed to the skull using acrylic dental cement. The localization of the orofacial motor cortex was precisely identified by the motor responses evoked by an electrical stimulation test (shock intensity: 200 µA; shock duration: 0.2 ms; shock frequency: 250 Hz; train duration: 50 ms). After a period of one week, during which the animal was placed daily in the test cage for habituation to experimental situation, trains of shocks at 4 Hz were applied for 1 h. The intensity of the stimulation was adjusted to obtain the same movement of the jaw as initially observed during the test. One hour after the end of the stimulation, animals were deeply anaesthetized and perfused according to the above described protocol. To ascertain that the observed effect on Fos expression was not

Table 1. Pattern of cortical stimulation and Fos expression in the striatum and the subthalamic nucleus

Pattern	Type	Frequency of application (Hz)	Train duration (ms)	Shock frequency within the train (Hz)	Total duration of stimulation (min)	Total number of shocks	Number of Fos-positive neurons	
							Striatum	Subthalamus
1	single shocks	4			120	28,800	0	0
2		20			120	144,000	1762–3780	205–1607
3	train of shocks	0.1	1000	100	2.75	1500	0	0
4		0.5	30	250	30	5400	0	0
5		0.4	500	250	60	172,800	1014–2010	387–1264
6		4	50	250	60	172,800	1480–3284	208–974
7		4	50	250	30	79,200	304–3500	91–540
8		20	25	250	20	144,000	1002–2294	246–1228
9		4	60	100	105	151,200	1500–3800	300–1170

For each stimulation pattern, Fos-positive neurons were counted in rats allowed 1 h survival time after the end of the cortical stimulation. Cells were counted at two different anteriorities: A: 8.7 mm and A: 9.2 mm anterior to interaural line, in the striatum ipsilateral to the stimulation site. In the STN, cell counts were made ipsilaterally, at one anteriority comprised between 5.2 and 5.4 mm anterior to interaural line. Numbers correspond to the range of Fos-positive neurons observed in each examined structure in two to four rats.

due to metabolic effects resulting from the chronic implantation of the stimulation electrodes, control animals ($n=3$) were implanted with electrodes in the jaw area of the motor cortex and submitted to the same experimental procedures (one week habituation and 1 h of sham stimulation followed by 1 h survival time in the stimulation box).

Fos protein immunocytochemistry

Coronal or sagittal brain sections were cut on a freezing microtome at 30 μ m thickness and rinsed in Tris-buffered saline (TBS) (Tris 0.25 M, NaCl 0.25 M, pH 7.5). The immunocytochemical procedure was adapted from protocols described previously.^{61,64} Briefly, sections were incubated for 5 min in TBS containing 3% H₂O₂ and 10% methanol to inactivate endogenous peroxidases. They were placed in a solution of 0.2% Triton X-100 in TBS for 15 min, then preincubated for 30 min in TBS containing 30% normal rabbit serum. Sections were washed and first incubated for 72 h with the primary Fos antiserum raised in sheep (CRB, Cambridge, U.K.) at a dilution of 1/1000 in 1% normal rabbit serum and 1% normal rat serum. In a second step, sections were incubated for 24 h with biotinylated rabbit antiserum (Lab Vector) at a dilution of 1/500 in TBS containing 1% normal rat serum and 1% normal rabbit serum. After a 90 min incubation in avidin-biotin-peroxidase complex (ABC) (Vector Laboratories, final dilution 1/50), sections were washed (TBS followed by TB (Tris 0.25 M, pH 7.5)) and reacted in 0.1% 3,3'-diaminobenzidine (DAB, 50 mg/100 ml) for 60 min by adding H₂O₂ (0.02%) to the medium. The specificity of the immunostaining was assessed by omission of the primary antibody from the protocol. After processing, tissue sections were mounted onto gelatin-alum-coated slides and dehydrated through graded alcohol to xylene for light microscopic examination. Some of the sections were counterstained by a Nissl staining for identification of structures.

Double immunocytochemical procedure combining Fos and calcium binding protein detection

The calcium binding protein (CaBP) or calbindin 28,000 mol. wt is a marker classically used to visualize the matrix compartment of the striatum.³⁵ When the immunocytochemical detection of CaBP was combined with that of Fos, Fos protein was first visualized using the immunocytochemical procedure described above. After the final reaction step (DAB-H₂O₂), sections were incubated in a primary antibody directed against CaBP prepared in goat (Sigma,

dilution 1/1000 in TBS with 1% normal rabbit serum and 1% normal rat serum for 24 h, Gibco). After three rinses in TBS, sections were placed in biotinylated rabbit anti-goat serum at a dilution of 1/500 in TBS containing 1% normal rabbit serum and 1% normal rat serum for 12 h and finally reacted in a DAB-H₂O₂ solution (same procedure as that used for Fos). While CaBP immunocytochemistry produces a diffuse staining of the striatal neuropil in the matrix compartment, Fos immunostaining was specifically localized to cell nuclei.

Autoradiography of μ opioid receptors

Since the dorsolateral region of the striatum is poorly labelled by CaBP antibodies, the labelling of μ opioid receptors was used as an additional method to visualize the striatal patch/matrix compartments. For this purpose, sections of 30 μ m adjacent to those collected for Fos immunocytochemistry were mounted on gelatin-coated slides and stored at -20°C. After two rinses in Tris-HCl-NaCl buffer (0.1 M; pH 7.4; 0.05 M NaCl), sections were incubated 1 h at 4°C with tritiated Naloxone (Amersham, 50 Ci/mole) at a concentration of 2.5 to 5 nM. Brain sections were rinsed three times for 20 s in phosphate-buffered saline (0.1 M, pH 7.4; 0.05 M NaCl) followed by a quick rinse in distilled water and air-dried. They were exposed for three to four weeks at room temperature on a tritium-sensitive film (³H Hyperfilm, Amersham). After development of films (developer D19, Kodak, fixative Hypam, Ilford), the striatal patches were precisely delineated from autoradiograms by their high μ opioid receptor density.

Double labelling procedure combining Fos immunocytochemistry with in situ hybridization of preproenkephalin or preprotachykinin A messenger RNAs

To identify striatal and subthalamic neuronal populations expressing Fos in response to cortical stimulation, *in situ* hybridization of preproenkephalin (PPE) or preprotachykinin A (PPT-A) mRNAs was utilized. Sections were first processed for Fos immunocytochemistry in RNAase-free conditions. The procedure was similar to that described above except that normal sera were omitted and that DEPC, an RNAase inhibitor (Sigma), was added to incubation media. Sections were mounted on Super-Frost slides (Menzel-Glaser, Germany) in RNAase-free conditions and air-dried before hybridization with specific PPE or PPT-A oligonucleotide probes. The PPE probe

was a 45-mer complementary to nucleotides 103–147 of the rat PPE mRNA.⁴⁹ The PPT-A probe was a 48-mer corresponding to the common sequence 124–171 of the three mRNAs produced by alternative splicing of the rat PPT-A gene.¹⁶ The two probes were obtained from Genset (France). Using a database server (Bisance), all selected sequences were specifically recognized by their corresponding mRNAs and no homology (greater than 83%) was found in other mammalian genes presently cloned. The oligonucleotide probes were labelled with [³⁵S]dATP (1000 Ci/mmol, Amersham) by 3' terminal deoxynucleotide transferase and allowed to precipitate with NaCl and ethanol overnight at –20°C. After centrifugation, radioactive probes were dissolved (specific activity 1–2 × 10⁹ c.p.m./μg) to a final concentration of 2.5 ng/ml in hybridization buffer (50% v/v deionized formamide, saline sodium citrate (SSC) × 4 (trisodium citrate 0.06 M, NaCl 0.6 M), 1 × Denhardt's solution, 1% *N*-lauroyl-sarcosine, 0.5 mg/ml denaturated salmon sperm DNA, 0.25 mg/ml yeast t-RNA, 0.25 mg/ml polyadenosine and 0.1 M dithiothreitol). After three 10 min washes in PBS, Fos-immunostained sections were acetylated for 10 min (SSC × 4, 0.1 M triethanolamine, 0.25% acetic anhydride). The labelled oligonucleotide solution (80 μl/slide) was dropped on sections, which were coverslipped and incubated for 16 h in a humid chamber at 48°C. After hybridization, slides were briefly immersed in SSC × 4 at room temperature to remove the coverslips and then washed at 48°C in successively SSC × 2, SSC × 1 twice and SSC × 0.5. After a quick rinse at room temperature in SSC × 0.5, sections were progressively dehydrated in graded alcohol, air dried and exposed to βmax Hyperfilm (Amersham). Once the films were developed, sections were dipped in K5 emulsion (Ilford), exposed for one to two months, developed and counterstained with Methylene Blue.

Data analysis

Tissue sections were examined under a light microscope. For each structure selected, Fos-immunoreactive cells were plotted at ×25 magnification using a computerized image analyser (Biocom, France). The distribution of immunoreactive cells was examined bilaterally but precise cell counts were only performed (every 600 μm) in the structures ipsilateral to the stimulation site. The sections processed for *in situ* hybridization were digitized at ×25 magnification under bright-field illumination. Cells labelled with PPE or PPT-A probes were visualized by the accumulation of silver grains over the soma. Glial cells were distinguished from neurons by their small, round and very intensely stained nucleus. In these sections (20 μm), single labelled neurons expressing either the prepropeptide mRNA or the Fos protein and double-labelled neurons were counted in the striatum ipsilateral to the cortical stimulation at two different anteroposterior levels (A: 8.7 mm and A: 9.2 mm to interaural) and in three different Fos-positive zones at each level (total examined surface: 2.7 mm² per striatum).

Electrophysiological recordings

In some experiments (four rats) electrophysiological responses evoked by cortical stimulation were recorded in

basal ganglia structures. Single-cell extracellular recordings were made in the striatum, the STN and the SNR using glass micropipettes (5–12 MΩ) filled with 4% Pontamine Sky Blue dissolved in 0.6 M NaCl. Amplified spike potentials were digitized through a voltage discriminator and peristimulus time histograms of unit firing were constructed on-line using the MacLab interface (World Precision Instruments).

RESULTS

Is Fos preferentially induced by a particular pattern of cortical stimulation?

The ability of various patterns of cortical stimulation to induce a Fos expression in the basal ganglia has been tested in 27 anaesthetized rats in which stimulations were applied in the jaw area of the motor cortex. We selected this stimulation site since it was found to produce a restricted movement of the body that allows one to control the correct placement of stimulation electrodes. Moreover, the projections of this cortical area in the basal ganglia have been well characterized in the rat.^{2,24,25,60}

Table 1 details the nine patterns of stimulation tested in this analysis. They consisted of single shocks or trains of shocks of 250 μA intensity. Extracellular recordings were made bilaterally in the lateral part of the striatum, the STN and the SNR to control the efficacy of cortical stimulations. In all cases, the type of responses recorded ipsi- and contralaterally to the stimulation were similar. As illustrated in Fig. 1, the neuronal activity evoked by the cortical stimulation was specific for each structure and varied with the temporal pattern of stimulation applied.

In the striatum, neurons which are mostly silent in resting conditions responded to the repetitive application of single shocks with a single spike discharge. Depending on the recorded cells, spike discharges were observed for 17 to 70% of the shocks applied (see Table 2). In response to an application of trains of shocks (patterns 4 to 9), the temporal pattern of the neuronal discharge changed markedly. The cells emitted one or two spikes during the train application and fired a second time during the interstimulus interval. In this condition, the mean number of spikes emitted by striatal cells was about 20% of the total number of shocks applied in the cortex (see Table 2). When increasing train of shock duration up to 1 s (pattern 3), the most active cells discharged tonically (100 Hz) for a period of time exceeding the end of stimulation by 1 to 2 s.

Abbreviations used in figures

ac	anterior commissure
AUD	auditory area
cc	corpus callosum
cp	cerebral peduncle
CPu	caudate-putamen
GP	globus pallidus
LV	lateral ventricle
MOT	motor area

Mx	matrix compartment
P	patch compartment
SENS	somatosensory area
SN	substantia nigra
st	stria terminalis
VIS	visual area
ZI	zona incerta

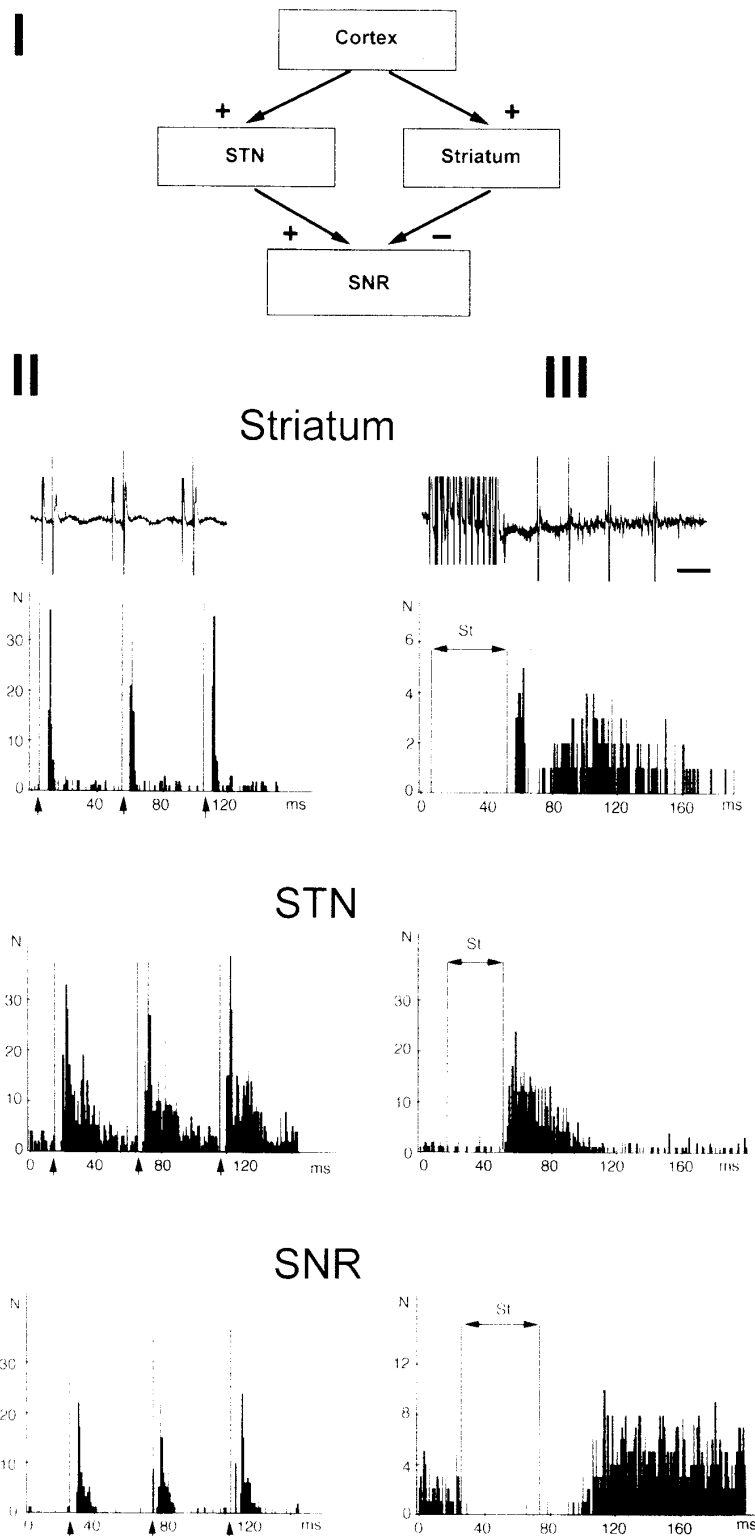


Fig. 1. Electrophysiological activities evoked in the striatum, the STN and the SNR by high frequency stimulation of the orofacial area of the motor cortex. (I) Schematic diagram illustrating the main synaptic circuits linking the motor cortex to the striatum, the STN and the SNR ((+): excitatory, (-): inhibitory connections). (II and III) Extracellular unit recordings and poststimulus time histograms showing the characteristic patterns of discharges evoked in the ipsilateral structures by the cortical stimulation. In the left side (part II), stimulation consisted of single shocks applied at a frequency of 20 Hz (arrow: stimulation artifact). In the right side (part III), stimulation consisted of trains of shocks (250 Hz; 50 ms duration) applied at 4 Hz. The traces and the poststimulus time histograms illustrating the responses of striatal cells are taken from the same neuron. Scale bar=25 ms.

Table 2. Spike discharge evoked by stimulation of the orofacial area of the motor cortex in cells of the striatum, the STN and the SNR

Pattern	Total number of evoked spikes/ total number of delivered shocks		
	Striatum	STN	SNR
2	70%	350%	250%
6	23%	150%	80%
8	17%	160%	35%

For each stimulation pattern, the percentage of evoked spikes was counted, by periods of 1 min every 10 min during the cortical stimulation. Percentages were calculated from the best responsive neurons recorded ipsilaterally to the stimulation site.

The neurons of the STN discharged spontaneously three to 20 spikes/s. In accordance with previous observations,^{55,56} these cells responded to repetitive applications of single shocks or trains of shocks by a complex discharge composed of a short latency spike followed by a burst of four to 10 action potentials. As determined by the number of spikes recorded over periods of 100 stimulations, the number of spikes discharged during the stimulation reached 350% of the total number of shocks for application of single shocks and about 150% for trains of shocks (see Table 2).

In response to cortical stimulation, the SNR cells displayed sequences of excitations and inhibitions. While the inhibitory effect was predominant at the onset of the stimulation, the balance of synaptic influences turned in favour of an excitation with the repetitive application of cortical shocks. The total number of spikes discharged by SNR cells ranged from 35 to 80% of the total number of stimulation shocks for trains of shocks and reached 250% during application of single shocks (see Table 2).

Fos expression was examined under various patterns of cortical stimulation applied in the jaw area of the motor cortex. The total number of electrical shocks delivered per stimulation session varied from 1500 to 172,800. With the exception of the stimulation patterns 1, 3, 4 and 7, which delivered the smallest number of shocks, all the other stimulation protocols produced a marked expression of Fos in the striatum and the STN. Comparison of Fos expression obtained in the striatum and the STN from the different stimulation patterns showed that a minimal number of cortical shocks (about 80,000) was required to activate *c-fos* transcription. Although no precise cell counts were made at different survival time intervals, the qualitative examination of the sections indicated that Fos expression was maximal between 1 and 3 h after the end of the stimulation. Within the striatum and the STN, the distribution of Fos-positive cells was precisely delineated. In the striatum, they occupied the ventrolateral part of the nucleus bilaterally throughout most of its antero-

posterior axis (Fig. 2I). In the STN, the labelled cells were found localized bilaterally in the rostralateral quadrant of this nucleus (Fig. 2II). Ipsilaterally to the stimulation site, where the labelling was always more prominent for both structures, the number of Fos-positive cells per coronal section was consistent throughout the various stimulation patterns. They ranged from 1002 to 3800 for the striatum and 205 to 1607 for the STN (see Table 1). In the SNR, some immunoreactive neurons were observed laterally (Fig. 3), whereas the other basal ganglia structures, i.e. the substantia nigra pars compacta, the entopeduncular nucleus and the globus pallidus, were devoid of Fos-positive cells. In sham animals no immunoreactive cells were ever detected in structures of the basal ganglia.

In addition to the striatum and the STN, the cerebral cortex, the reticularis nucleus and the intralaminar nuclei of the thalamus, the habenula and the amygdala also contained an important number of Fos-immunoreactive cells. At the cortical stimulation site, a high density of labelled neurons was always present throughout the thickness of the cortex. At distance from the stimulation site, the extent of the labelling varied from one case to another, Fos-positive neurons being found in almost the entire ipsilateral cortex in the most extreme cases. Contralaterally, Fos expression was usually restricted to the homologous area of the stimulated cortex. In the reticularis nucleus of the thalamus, Fos expression was bilateral and limited to the cortical projection field. Interestingly, the extent of Fos expression observed in the ipsilateral cerebral cortex was never paralleled by a corresponding spread of Fos-positive cells in the striatum, the STN or the reticularis nucleus. Concerning the other subcortical structures with a Fos response, the immunoreactivity was probably not induced by the cortical stimulation since it was also observed in sham animals.

To assess the effect of barbiturate anaesthesia on Fos expression, the effects of the stimulation of the orofacial area of the motor cortex (pattern 6) were investigated in unanaesthetized animals. Similarly to anaesthetized rats, Fos expression in basal ganglia structures was restricted to the lateral part of the striatum and the STN. In these regions, no Fos expression was found in the corresponding sham awake rats.

Regional distribution of Fos expression

The well delineated localization and the systematic expression of Fos in the striatum and the STN after stimulation of the jaw area of the motor cortex led us to examine the effects of stimulation applied in other cerebral cortical areas. Trains of shocks (pattern 6, Table 1) were applied in the forelimb area of the motor cortex ($n=2$), the somatosensory facial area ($n=6$), the auditory cortex ($n=2$), the visual cortex

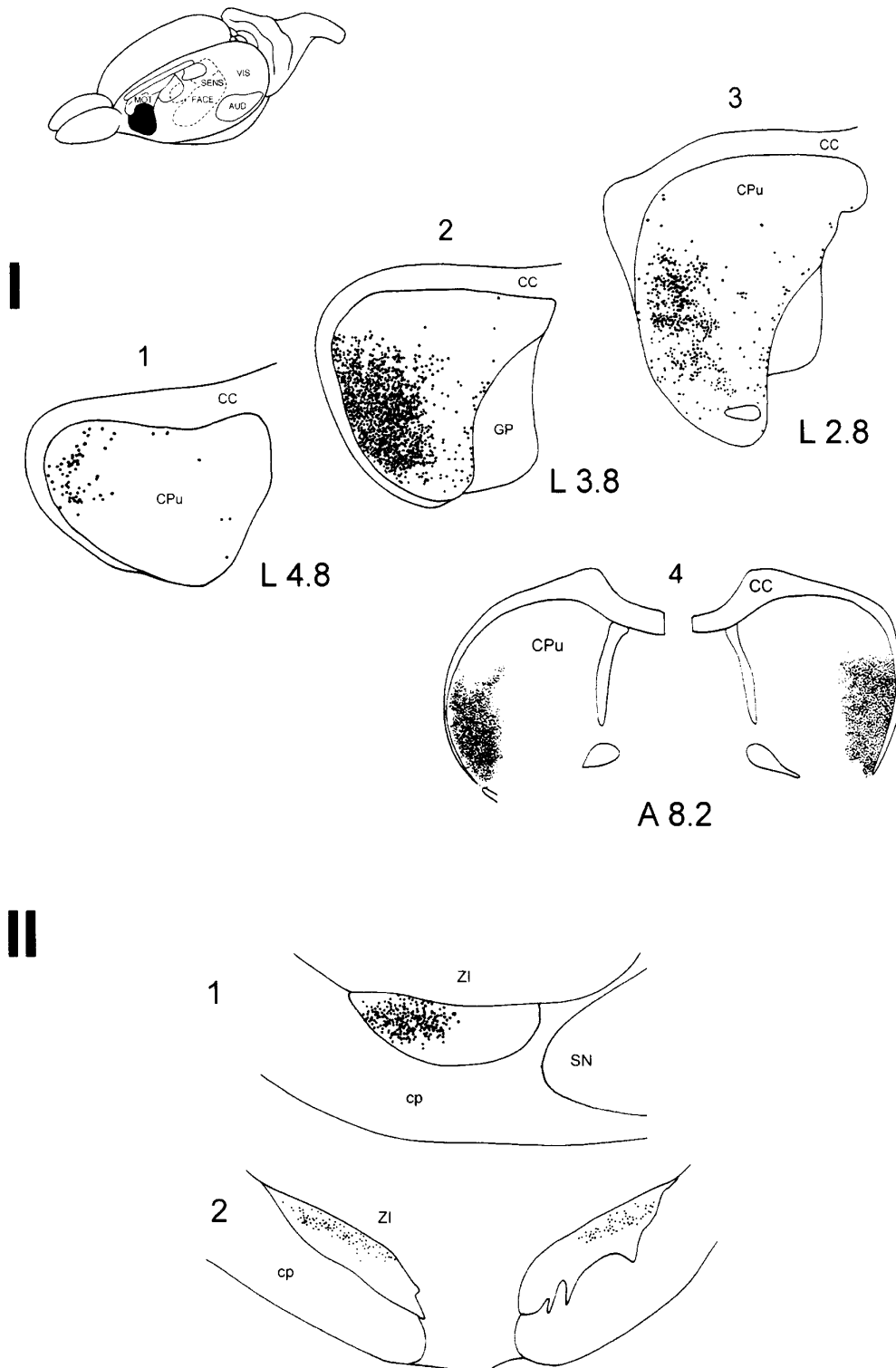


Fig. 2. Regional induction of Fos in the striatum (I) and the subthalamic nucleus (II) following stimulation of the orofacial area of the motor cortex. The localization of the stimulation in the cerebral cortex is shown as a blackened area in a lateral view of the rat brain. The distribution of labelled neurons is shown in three sagittal views of the striatum ipsilateral to the stimulated side (1–3) and in a frontal view (4). The sagittal sections are arranged from lateral (1) to medial (3) parts of the striatum and the laterality values refer to the distance in mm from the midline. The anteriority value indicated under the frontal section (4) refers to the distance in mm from the interaural line. In case II, the distribution of Fos-positive cells in the STN is shown in a sagittal section ipsilateral to the stimulated cortical area (1) and in a coronal brain section (2).

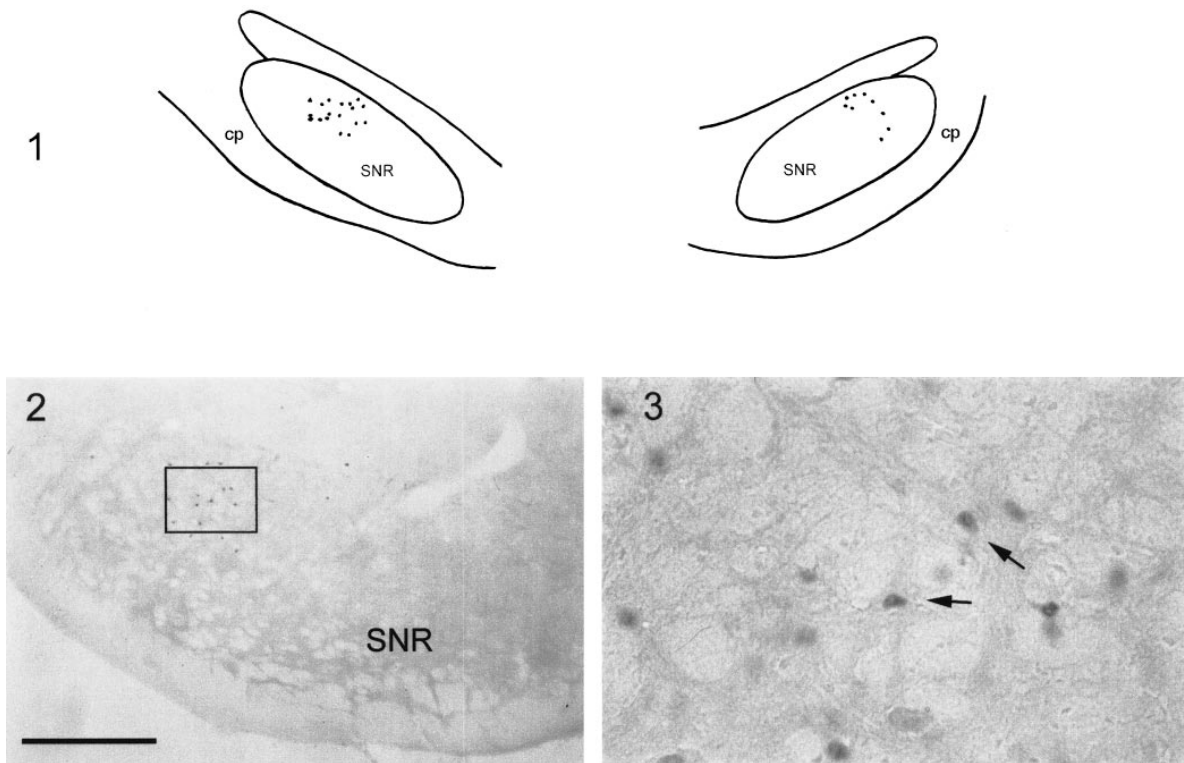


Fig. 3. Bilateral expression of Fos induced in the SNR by stimulation of the orofacial area of the motor cortex. (1) Distribution of labelled cells on coronal sections of the ipsilateral (left) and contralateral (right) SNR. (2,3) Photomicrographs of labelled neurons at low (2) and high magnification (3). The area delimited in picture 2 corresponds to the field shown in 3. Note that the staining is confined to the nuclei of nigral cells. Scale bar=550 μ m for 2 and 91 μ m for 3.

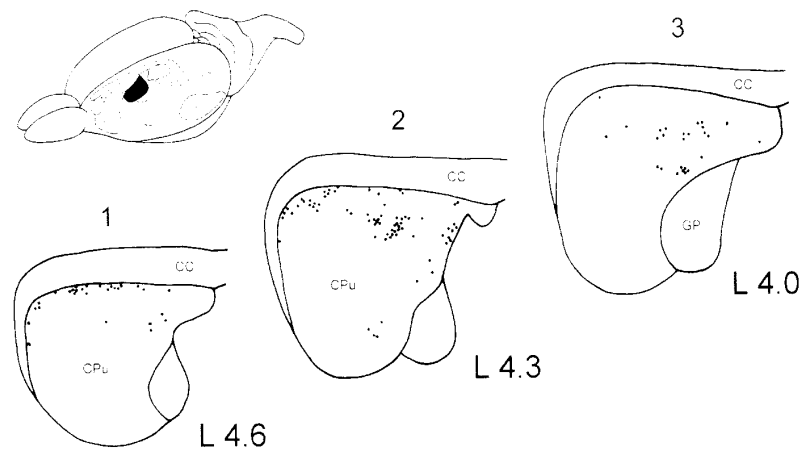


Fig. 4. Regional induction of Fos in the striatum following stimulation of the forelimb area of the motor cortex. The stimulated cortical area is shown in black in a lateral view of the rat brain and the distribution of labelled neurons is represented in three sagittal views of the striatum (1–3) ipsilateral to the stimulated site. The laterality values refer to the distance in mm from the midline.

($n=2$), the dorsal prelimbic cortex ($n=2$) and the rostral part of the orbitary cortex ($n=5$).

In the striatum, the localization of cells expressing Fos varied according to the stimulated cortical area. So, after stimulation of the forelimb sensorimotor area (Fig. 4), Fos-positive neurons were in a region dorsal to that activated by the stimulation of the jaw

area of the motor cortex. Following stimulation of the medial prefrontal cortex, Fos-positive cells were found bilaterally within a narrow band of striatal tissue edging the lateral ventricle (Fig. 5I). After stimulation of the auditory cortex, the expression of Fos was confined to the caudomedial part of the ipsilateral striatum (Fig. 5II). Finally, despite the

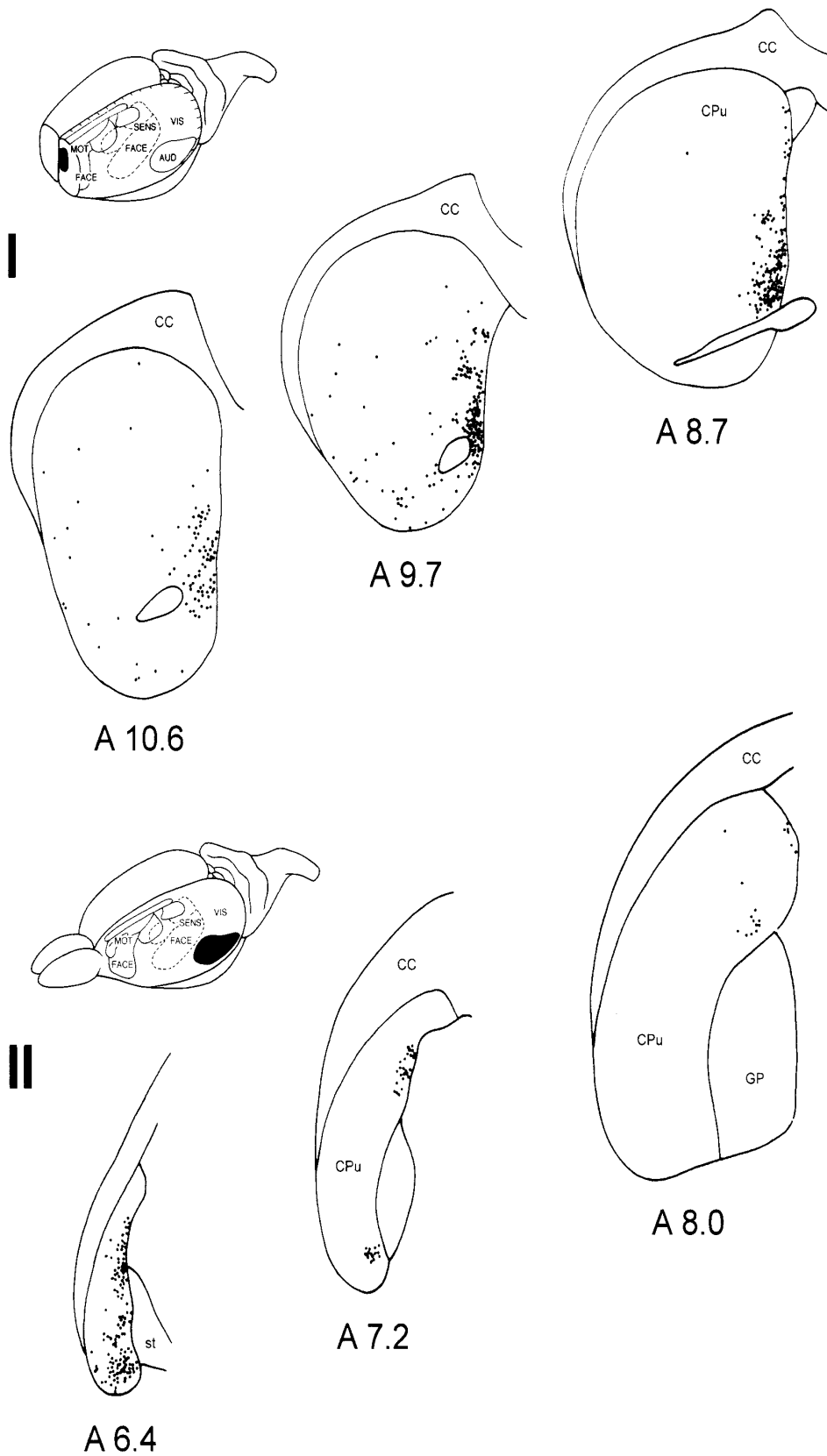


Fig. 5. Distribution of striatal cells expressing the Fos protein following stimulation of the prelimbic (I) or the auditory (II) areas of the cerebral cortex. The stimulated cortical area is shown in black and the Fos-positive cells are represented in three frontal sections of the ipsilateral striatum. The anteriority value indicated under each section refers to the distance in mm from the interaural line.

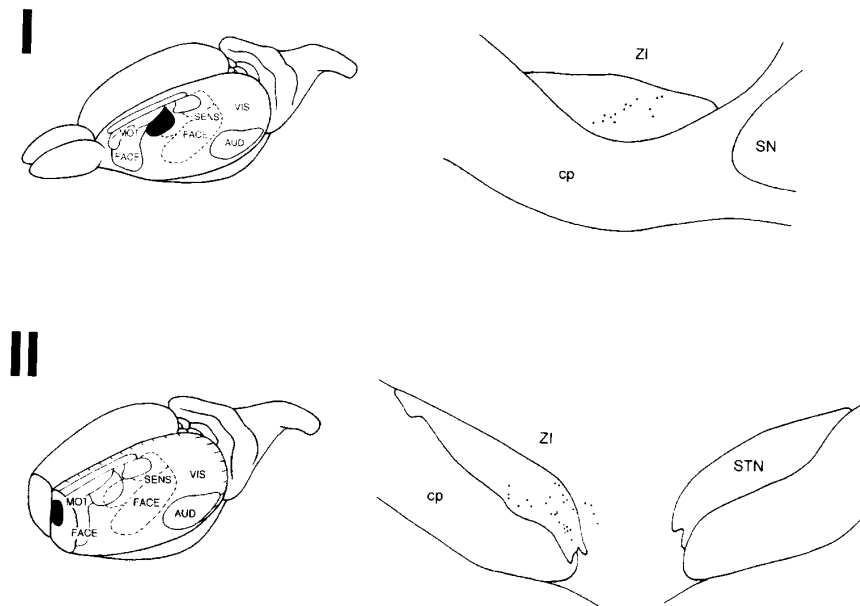


Fig. 6. Regional expression of Fos in the STN following stimulation of the forelimb area of the motor cortex (I) and the prelimbic area of the prefrontal cortex (II). As in previous figures the stimulated cortical area is indicated (blackened area) in lateral views of the rat brain. The distribution of Fos-positive cells in the STN is shown in a sagittal section of the ipsilateral STN in I and in a coronal brain section in II. Note the unilateral Fos expression induced by stimulation of the prelimbic area of the prefrontal cortex (II).

stimulation efficacy attested by the spike discharges recorded in the striatum, no Fos induction could be triggered from the facial somatosensory, visual and orbital cortical areas.

In the STN, the localization of Fos-expressing cells also varied with the stimulated cortical area. After stimulation of the forelimb sensorimotor area (Fig. 6I), Fos-positive neurons were located caudally to those activated by the stimulation of the jaw area (compare to Fig. 2II). Following stimulation of the medial prefrontal cortex, Fos-positive cells were confined to the medial part of the ipsilateral STN (Fig. 6II). No immunoreactive cells were detected in the STN after stimulation of the auditory, somatosensory, visual and orbital cortical areas.

Relation of Fos expression with the patch/matrix compartmentation of the striatum

The Fos immunoreactivity induced in the striatum by stimulation of the cerebral cortex presented an heterogeneous distribution (Fig. 7). Reminiscent of the patch/matrix compartmental organization, Fos-positive neurons were distributed in a matrix-like pattern with islands of unlabelled neurons. The compartment affiliation of Fos-positive neurons has been studied by combining Fos immunocytochemistry with the labelling of the patches (visualized by the binding of [3 H]naloxone to μ opiate receptors) or of the matrix (visualized by CaBP immunostaining). Although CaBP allowed a clear detection of the matrix compartment, the immunostaining presented an important mediolateral gradient. Consequently, if

it was easy to identify patches in the medial part of the striatum, where the projections of the prelimbic cortex terminate, in the lateral sensorimotor part of the striatum, CaBP immunoreactivity was too low to precisely delineate the patch/matrix compartmentation. In this striatal lateral region, only the autoradiographic labelling of μ receptors allowed a clear identification of the patches. As illustrated in Fig. 8, the majority of cells which expressed Fos in response to stimulation of the motor (Fig. 8, parts 1–2) or prelimbic cortex (Fig. 8, parts 3–4) were in the matrix compartment.

Identification of cells expressing Fos

A Nissl counterstaining of sections processed for Fos immunoreactivity revealed that, in the region of Fos expression, striatal neurons (Fig. 7, part 3) and subthalamic neurons (not shown) were not all immunoreactive. In the region with the highest density of Fos-positive neurons, no more than 66% of cells in the striatum and 55% in the STN were found Fos immunoreactive. In the striatum, the cell identification was particularly crucial due to the heterogeneity of neuronal populations. Indeed, by contrast to the STN, which is composed of a rather homogeneous population of neurons projecting to the SNR, the entopeduncular and the globus pallidus (GP) via axon collaterals,^{23,82} the striatum contains at least two categories of output neurons: one innervating the GP and containing enkephalin, the other one innervating the SNR and synthesizing substance P. We have therefore combined the

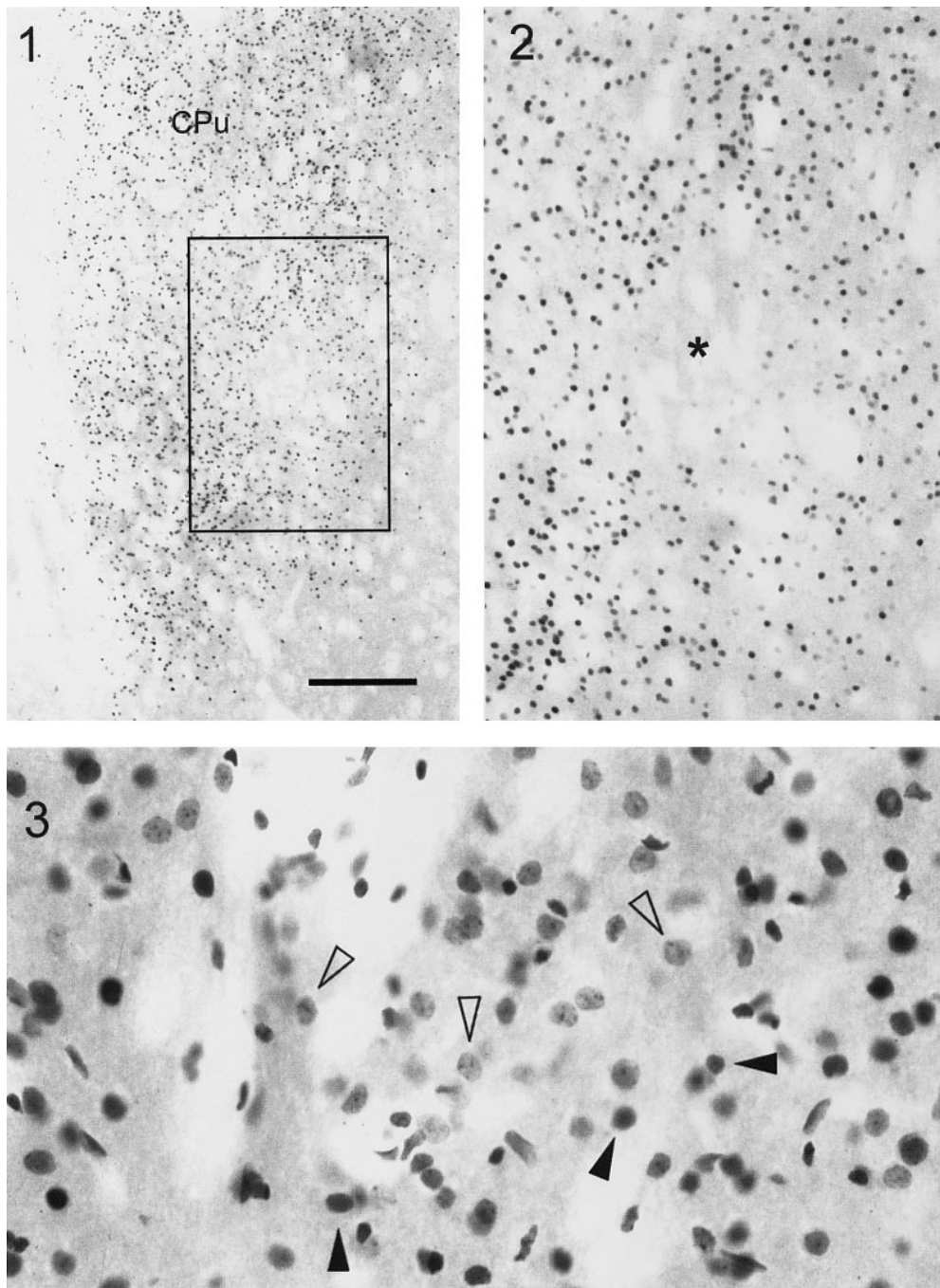


Fig. 7. Photomicrographs of the striatum illustrating the heterogeneous distribution of Fos-positive neurons following stimulation of the orofacial area of the motor cortex. (1,2) Coronal sections at low (1) and high (2) magnification show the presence of islands of non-immunoreactive cells (*). (3) Photomicrograph of such an island area counterstained with Cresyl Violet. Note the presence of unlabelled Fos neurons. Open arrowheads point to non-immunoreactive cells, filled arrowheads point to labelled nuclei of striatal Fos-positive neurons. Scale bar=370 μ m for 1, 160 μ m for 2 and 55 μ m for 3.

immunocytochemical detection of the protein with the *in situ* hybridization of PPE and PPT-A mRNAs expressed in striatopallidal and striatonigral neurons, respectively (Fig. 9). After stimulation of the facial area of the motor cortex ($n=4$, pattern 6), single-labelled cells as well as double-labelled neurons

expressing Fos and PPE mRNA or Fos and PPT-A mRNA were counted on adjacent sections. In sections treated for Fos and PPE mRNA, 678 Fos-positive neurons and 786 PPE mRNA-positive neurons were found on the total surface examined (see Experimental Procedures). In sections treated for

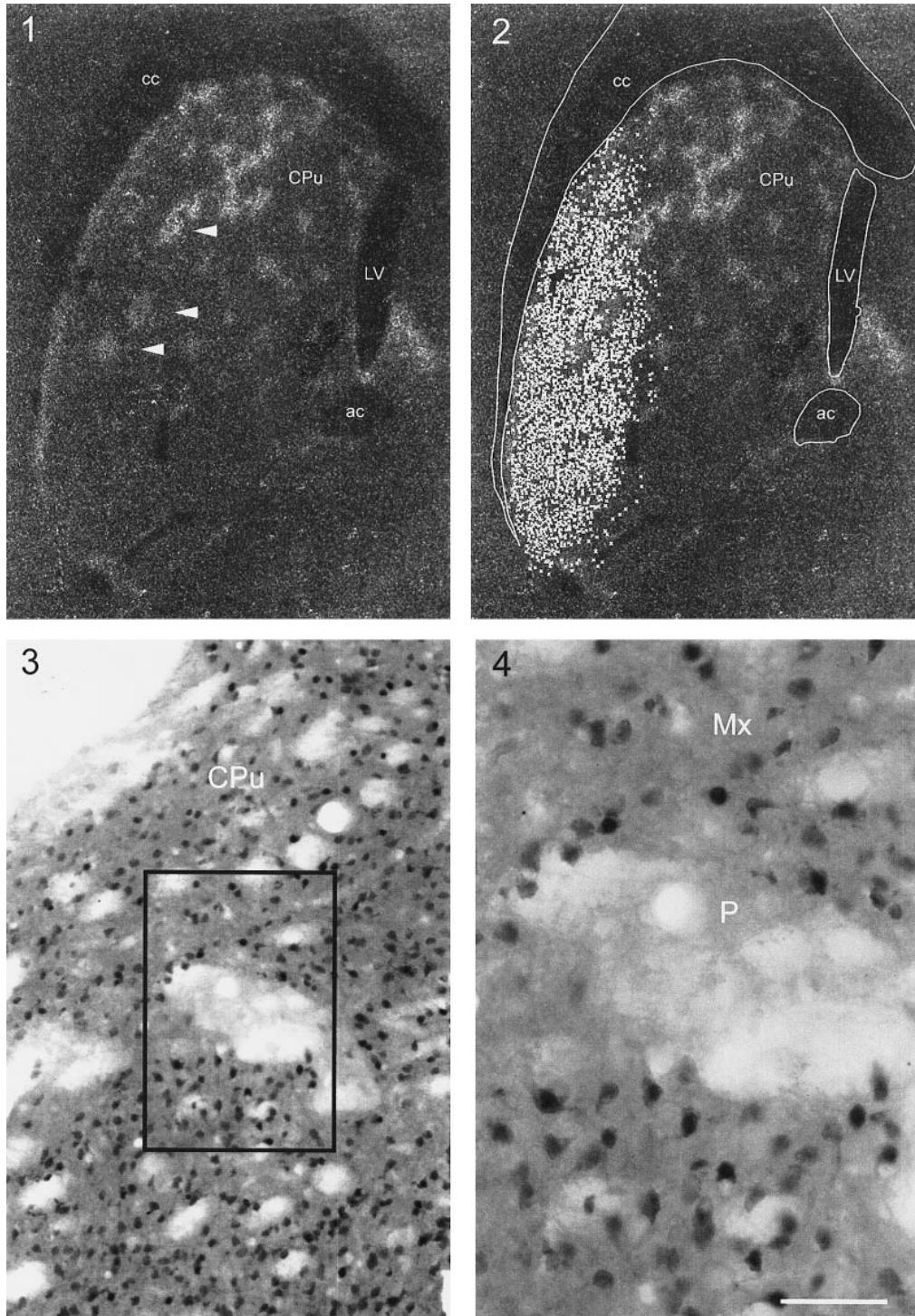


Fig. 8. Compartmental distribution of Fos-positive neurons in the striatum following stimulation of the orofacial area of the motor cortex (1, 2) and the prelimbic cortex (3, 4). (1) Photomicrograph taken from an autoradiogram of a striatal coronal section showing the localization of the patch compartment (white arrowheads) visualized by autoradiography of μ opioid receptors. In (2) the distribution of Fos-positive neurons observed on the adjacent section is superimposed to the photomicrograph. Note that the islands of unlabelled cells correspond to patches of high density of μ opioid receptors. (3,4) Photomicrographs at low and high magnification of the same striatal section treated for Fos and CaBP. Note that Fos-positive neurons are also located in the matrix (Mx) and avoid the patch (P) with a low CaBP immunoreactivity. Scale bar=1400 μ m for 1 and 2, 185 μ m for 3 and 80 μ m for 4.

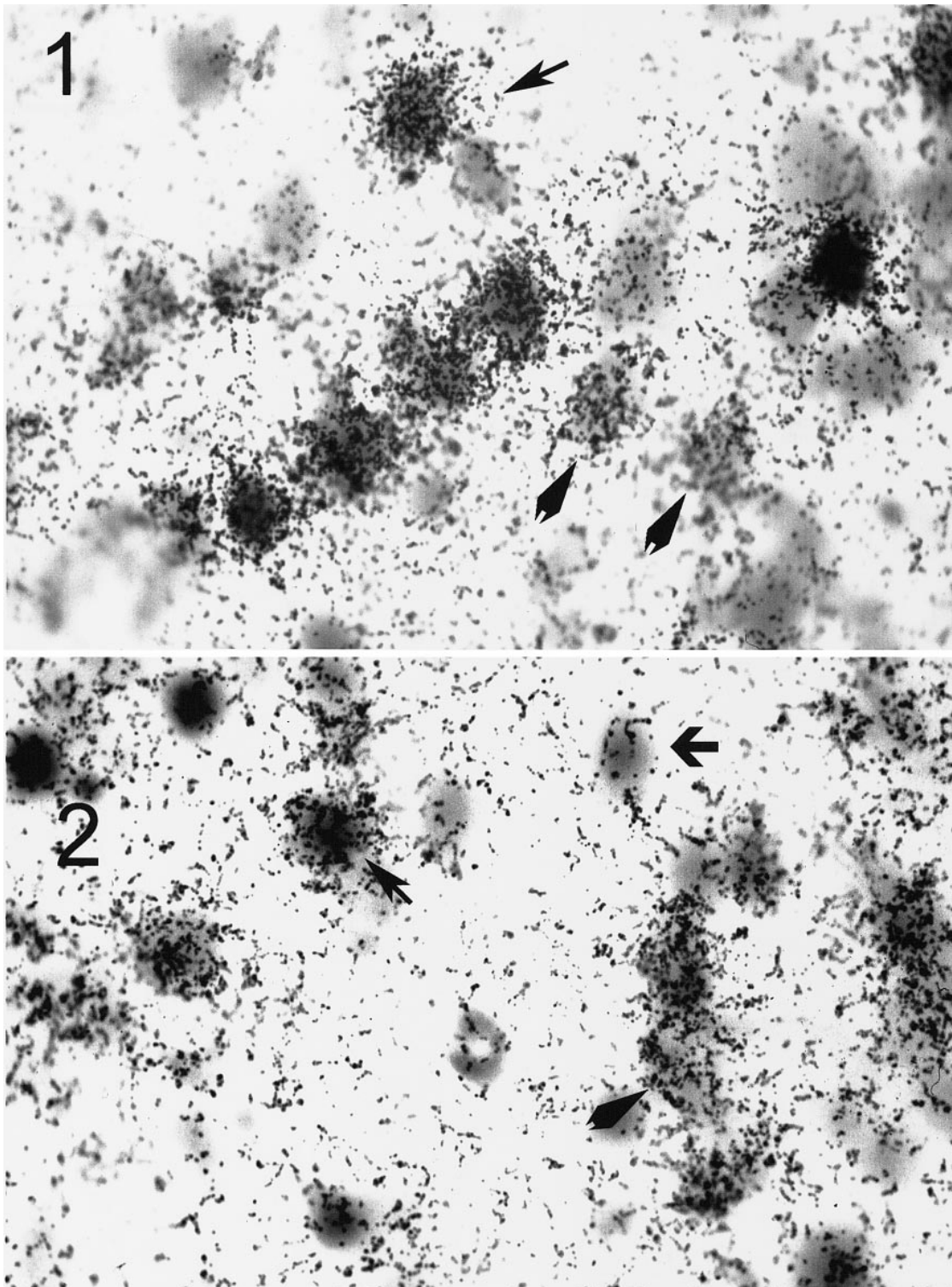


Fig. 9. Characterization of striatal neurons expressing the Fos protein after stimulation of the orofacial motor cortex using *in situ* hybridization of PPE mRNA (1) encoding enkephalin and PPT-A mRNA (2) encoding substance P. Arrowheads point to neurons containing PPE or PPT-A mRNA, large arrows point to Fos-positive neurons (only present in 2), sharp arrows point to double-labelled cells.

Fos and PPT-A mRNA, 830 Fos-positive cells and 638 PPT-A mRNA-positive cells were counted. Among the population of Fos-positive cells, the

average percentage of PPE mRNA-expressing neurons ($71.8\% \pm 4.90$ S.E.M.) was greater than that of PPT-A mRNA-containing neurons ($49.1\% \pm 3.92$

S.E.M.). The difference is statistically significant ($P=0.0112$, Student's *t*-test, unpaired). However, when considering the population of PPE or PPT-A mRNA-containing neurons, the average percentages of cells expressing Fos were equivalent ($59.2\% \pm 7.31$ S.E.M. and $62.1\% \pm 5.61$ S.E.M., respectively).

DISCUSSION

The demonstration by Fu and Beckstead³³ that the electrical stimulation of the cerebral cortex can induce an expression of Fos in striatal neurons has stressed the role of cortical activity on the regulation of genomic expression in the basal ganglia. The present study was aimed at precisely defining the modalities of cortical stimulation required to generate the induction of this protein in basal ganglia and at examining how the extent of the expression is related to the anatomic-functional organization of cortical projections. The results show that focal stimulations of different cortical areas generate a rapid synthesis of Fos localized in precisely delineated regions of the striatum, the STN and occasionally of the SNR. This expression, which can be evoked by different patterns of stimulation, globally conforms to the known topography of cortical projections. Moreover, as indicated by results obtained in the striatum, the induction of Fos respects striatal compartmentation and involves differentially the two efferent striatal cell populations. The large number of electrical shocks required to produce a Fos expression stresses that its triggering requires a high level of synaptic activity. Accordingly, the distribution pattern of Fos immunoreactivity does not provide an exact picture of the electrophysiological activity in neuronal circuits.

Relation of Fos expression with the topographical organization of cortical projections

The existence of a precise topography in the projections from the cerebral cortex to the basal ganglia is well documented.^{3,24,25,31,32,44,60,66,85} In the striatum, which represents the main integrative component of the basal ganglia system, the various functional subdivisions of the cerebral cortex are orderly mapped into a series of longitudinal bands spanning most of the entire extent of the nucleus. In this representation, each cortical area occupies a particular region of the striatum. However, overlaps are commonly observed between projections from anatomically and functionally related cortical areas.^{24,31,32,60} For instance, in the lateral part of the striatum, which receives projections from the sensory and the somatomotor cortices, overlaps exist between projection fields processing sensory and motor information from the same part of the body. Besides the striatum, the STN is the second structure of the basal ganglia that receives a major input from the cerebral cortex. Although the cortical innervation to the

STN is restricted to the frontal cortex, it is as well topographically organized.

In accordance with the topographical organization of cortical projections to the basal ganglia, a clear-cut distribution of Fos-positive neurons was consistently observed in the striatum and the STN following focal stimulations of the cerebral cortex. Briefly, following stimulation of the jaw area of the motor cortex, a delineated Fos expression was observed in the ventrolateral part of the striatum and in the anterolateral quadrant of the STN, two regions receiving projections from the orofacial area of the motor cortex.^{2,14,56} After stimulation in the motor forelimb area, Fos expression was localized in the dorsolateral part of the striatum and in a region of the STN adjacent caudally to that activated by the jaw area. Conforming to previous anatomical descriptions of the projections of the anterior cingulate cortex,^{24,44} stimulation of this cortical region activated neurons localized medially in the striatum and the STN. Finally, stimulations of the auditory cortical areas produced an expected Fos induction in the caudomedial part of the striatum but not in the STN, which is known to be free of afferents from this cortical region.^{2,24,60}

The bilateral expression of Fos observed in the striatum is consistent with the existence of a major crossed component in the corticostriatal pathways.^{15,59,60} By contrast, the finding of a contralateral activation of the STN is more surprising since the cortical afferents to this nucleus are thought to be exclusively ipsilateral.^{2,13,14} Whether this labelling is due to the activation of contralateral corticosubthalamic neurons through commissural cortico-cortical connections or via axon reflexes cannot be determined on the basis of the present data. Anyway, the cell discharges recorded in the ipsilateral and contralateral STN in response to stimulation of the motor cortex support the observed bilateral pattern of Fos expression.

The regional Fos expression, observed in the present study in pentobarbital anaesthetized rats and also found in awake rats, extends previous observations by Wan *et al.*⁸³ Using combination of tracer injection and microstimulation of the motor cortex, these authors reported that cortical activation generates an expression of Fos localized in the projection field of the stimulated cortical area. Such a specific pattern of Fos expression differs strikingly from that described by Fu *et al.*³³ and Liste *et al.*⁵⁷ As shown by these authors, a frontoparietal stimulation involving most of the somatic sensorimotor cortex triggers an expression of Fos in virtually the entire striatum. To account for such a widespread expression, it has been hypothesized that stimulations involved a very large area of the cerebral cortex. However, in view of the topographical organization of cortical projections to the striatum, this hypothesis supposes that virtually all areas of the cerebral cortex have been stimulated. It therefore seems possible that in addition to

activity in corticostriatal synapses, another source of metabolic activation may have contributed to such a diffuse response. In this respect it is noteworthy that mechanisms unrelated to neuronal connections such as spreading depression are currently proposed to explain the extensive expression of immediate early genes observed in the cerebral cortex following various treatments, including KCl application, ischaemia or minimal cortical lesions.^{46,51,84} In some of our experimental cases, we also observed a diffuse expression of Fos involving the entire cerebral cortex homolateral to the stimulated side. Since this widespread expression was never propagated to the striatum, the STN or the contralateral cortical areas, it is likely to involve cellular mechanisms that are not underlain by an important increase in neuronal activity. Therefore, the diffuse Fos expression induced in the striatum following massive stimulations of the cerebral cortex might be related to signalling mechanisms independent of electrical activity in neural circuitry. This possibility requires a critical attention when immediate early genes are used as markers of synaptic activity.

Relation of Fos expression with striatal compartmentation and striatal neuronal populations

One of the most striking aspects of the pattern of Fos expression, induced in the striatum by cortical stimulation, is its mosaic-like organization.^{26,37,42} Conforming to the compartmentation of the striatum into the so-called matrix and striosomes (or patches), Fos-positive neurons delineate unlabelled striatal zones which display the neurochemical characteristics of the patch compartment (low CaBP immunoreactivity and high density of μ opiate receptors). The lack of labelling of striosomal neurons could be due to a specific organization of cortical projections to this compartment or to particular neurochemical properties of these neurons. In favour of a pre-synaptic hypothesis, it has been described that striosomal neurons receive their cortical inputs from deep cortical layers and the matrix neurons from superficial layers.³⁶ However, in view of the localization of stimulation electrodes, it seems unlikely that the cortical stimulations have selectively activated the cortical cells projecting to the matrix. In keeping with a presynaptic hypothesis, the selective labelling of the matrix neurons might reflect a difference in the density of corticostriatal projections, since the density of axon terminals provided by axons is heterogeneous.^{20,47} However, particular neurochemical characteristics of matrix neurons cannot be excluded. There is now increasing evidence that striosomes and matrix neurons are differentially enriched in glutamate receptor subunits.⁵⁸ Finally, cells of the striosomal compartment could also display a high threshold for Fos induction. It can be noted that in the study by Fu *et al.*³³ a conspicuous clustering distribution of Fos-immunoreactive cells reminiscent

of the patchy organization of the striatal mosaic was described despite the diffuse striatal Fos expression produced by the massive applied cortical stimulation.

The matrix itself corresponds to an heterogeneous compartment containing at least two types of efferent neuronal populations defined by their site of projection (SNR vs GP) and their peptide content (substance P vs enkephalin). The present results showed that, after stimulation of the orofacial area of the motor cortex, Fos was equally expressed by these two populations. This result is consistent with anatomical observations that both populations receive afferents from the motor cortex.⁴⁷ When examining the percentage of Fos-positive cells expressing PPE or PPT-A mRNA, a higher proportion of neurons was found in the enkephalinergic population. This result may probably be due to the fact that, in the Fos-positive striatal region, a higher proportion of neurons was found to express the PPE mRNA.

It is noteworthy that when adding the percentages of Fos-positive neurons expressing PPE mRNA and PPT-A mRNA or when adding the percentages of PPE mRNA- and PPT-A mRNA-positive neurons expressing Fos, a value of about 121% was obtained. This result is likely to reflect the existence of a neuronal population expressing both messengers. This is in agreement with anatomical studies indicating the existence of branched neurons innervating both the SNR and GP.⁵² It is also in agreement with immunohistochemical studies^{8,68} showing the existence of a relatively large proportion of neurons expressing both substance P and enkephalin. Interestingly, in a recent study, the proportion of striatal neurons co-expressing PPE and PPT-A mRNAs was evaluated to 17% of medium-sized projection neurons.⁸⁰

Relation of Fos expression with electrophysiological activity

The rapid induction of Fos in neurons submitted to a sustained activation has led to proposals that the expression of this protein could reflect the activity of neurons.^{11,21,50,73} If the Fos-immunoreactive neurons were systematically found in regions of the basal ganglia receiving an excitatory synaptic input driven from the stimulated corticostriatal fibres, it is clear that in the activated striatal area, neurons did not always express the protein. In the striatum, for example, whereas stimulation of the motor, medial prefrontal and auditory cortices readily generated an induction of Fos, the somatosensory facial area, the orbital and the visual cortices were ineffective in producing such an induction. However, the spike discharges of the striatal cells recorded in the projection fields of these cortical areas indicated that the lack of induction was not simply due to an application of subthreshold shocks.

The analysis of Fos expression in the SNR provides another example where high synaptic activity

in afferent pathways does not reliably trigger an induction of Fos. The SNR receives a disynaptic influence from the cerebral cortex via two distinct pathways: the cortico-striato-nigral and the cortico-subthalamo-nigral pathways. The striatal cells are GABAergic whereas the subthalamic neurons are glutamatergic. The convergence of these two inputs in the SNR generates a triphasic electrophysiological response (two excitatory sequences originating from the STN interrupted by an inhibitory synaptic event from the striatum).^{34,55} The prevalence of excitation or inhibition varies with the recorded cells and the modalities of cortical stimulations. Despite the excitatory synaptic input driven by the STN, the SNR cells rarely express Fos protein in response to cortical stimulation. The lack of Fos expression in regions electrophysiologically activated by cortical stimulation stresses that Fos induction does not provide a very accurate picture of neuronal activity. Since striatal and nigral cells are equipped to synthesize Fos,^{5,77,79} the apparent dissociation between the cortically-evoked electrophysiological activity and Fos expression observed in these two structures suggests that a high level of synaptic activity is probably required to trigger the protein induction. The important number of stimulation shocks needed to produce a Fos expression in the postsynaptic target cells is in favour of this view. These observations provide additional evidence that Fos is not representative of neuronal activity *per se* and is likely to reflect the activation of intracellular signalling pathways by synaptic activity. In agreement with this conclusion, the recent study of Deisseroth *et al.*²² shows that the activation of a transcription factor, implicated in the regulation of *c-fos* transcription, is independent of both the stimulation pattern applied (trains of shocks of 50 Hz or single shocks of 5 Hz) and the number of spikes evoked by the activated cells, but is rather related to the synaptic activity. In this respect, the marked differences in the efficacy displayed by the various cortical stimulation sites may reflect the ability to recruit a sufficient number of corticostriatal axons to produce significant intracellular changes in second messengers in target cells. Taking the sensory and the motor facial areas as an example, the marked difference in efficacy between these two cortical areas could be attributed to presynaptic axon recruitment. Due to cortico-cortical connections between somatosensory and motor cortices,²⁸ stimulation applied in the motor cortex can activate not only the axons of motor corticostriatal neurons, but also those of sensory corticostriatal cells via axon reflexes. By contrast, stimulation applied in the somatosensory cortex activates only corticostriatal axons originating from this area. Thus, stimulation of the motor area is expected to produce greater second messenger changes in striatal neurons than stimulation applied in the somatosensory area. Similarly, the inefficacy of visual cortex stimulation to produce a Fos expression could be due

to an insufficient recruitment of corticostriatal axons. Indeed, the primary visual cortex contains the lowest density of neurons projecting to the striatum.⁶⁰

Mechanisms of induction

Among the identified sites of transcriptional regulation on the promoter of the *c-fos* gene, the site calcium and cyclic AMP responsive element has been shown to be particularly relevant to this function. So calcium influx (through either *N*-methyl-D-aspartate (NMDA) receptors or voltage-sensitive calcium channels), or calcium released from internal stores might regulate *c-fos* transcription by multiple signalling pathways that target different regulation sites within *c-fos* promoter.^{38,39,81} Based on the observation that a treatment with dizocilpine maleate (MK-801, a non-competitive antagonist of NMDA receptors) prevents the expression of Fos evoked by cortical stimulation,^{1,57} it has been proposed that glutamate released at the corticostriatal and cortico-subthalamic synapses may mediate *c-fos* transcription via an influx of calcium through activation of NMDA receptors.⁵⁷ Despite the possibility of this scenario, complementary studies are required for a complete demonstration of the participation of NMDA receptors. As a matter of fact, in addition to its action on NMDA receptors, MK-801 has also been reported to exert an inhibitory effect on protein synthesis.¹⁷ Thus, results obtained with this pharmacological agent have to be taken with caution.

Besides the activation of NMDA receptors, there are additional mechanisms by which glutamate released at corticostriatal and corticosubthalamic synapses can produce an increase of calcium in postsynaptic cells. For instance, by acting on α -amino-3-hydroxy-5-methylisoxazole-4-propionate receptors, glutamate can depolarize neurons, leading to an activation of voltage-sensitive calcium channels. Moreover, through activation of metabotropic receptors, glutamate can induce a release of calcium from intracellular stores.⁴⁰ Finally, in addition to the direct postsynaptic effects of glutamate, an indirect influence via the dopaminergic system can also be considered. Stimulation of the cerebral cortex has been reported to produce a release of dopamine in the striatum by acting presynaptically on nigrostriatal terminals.⁴¹ Through activation of D₁ and D₂ receptors expressed by striatal cells, dopamine may secondarily influence the regulatory pathways of *c-fos* transcription via a change in intracellular level of cyclic AMP.^{7,19,30,69-71,74} In the present analysis, however, an increased release of dopamine is unlikely responsible for the induction of Fos evoked by cortical stimulations in the population of enkephalin-positive cells. Indeed, this neuronal population mainly expresses D₂ receptors which are negatively linked to adenylate cyclase.⁷⁸ However, in substance P-positive neurons which express D₁ receptors

positively coupled to adenylate cyclase,⁵³ the role of dopamine on Fos expression cannot be excluded.

Functional aspects of the cortically evoked expression of Fos in the basal ganglia

The results of the present study show that a repetitive stimulation of the cerebral cortex induces a trans-synaptic expression of Fos in the striatum and the STN that conforms to the known anatomical and functional organization of cortical projections. The rapid induction and translocation of the protein into the nuclei of the activated cells is consistent with the idea that it acts as a transcription factor to produce long-term adaptative changes in the functional state of basal ganglia circuits via a genic regulation. What kind of adaptive changes may be produced by the cerebral cortex via Fos expression? A variety of studies using *in vitro* or *in vivo* models have shown that rapid genomic forms to neuronal stimulation have a critical role in long-term changes in synaptic efficacy.^{72,75} In the classical model of long-term potentiation (LTP) evoked at the perforant path–granule cell synapse, the changes in synaptic strength evoked by tetanization of the perforant path are accompanied by a rapid induction of immediate early genes, including *zif/268*, *c-fos*, *c-jun* and *jun-B*.¹⁸ Given that tetanization of cortical fibres also produces changes in efficacy at corticostriatal synapses,¹² an attractive hypothesis would be that Fos may play a role in these forms of activity-dependent synaptic plasticity. However, the relatively moderate stimulations that are used to generate changes in synaptic strength compared to the considerable amount of cortical stimulations required to trigger Fos induction make such an eventuality unlikely. This is in agreement with recent observations in hippocampus indicating that stimulation induced LTP at the perforant–granule cell synapse is not necessarily accompanied by an expression of Fos in the post-synaptic neurons.²⁹ If the experimental conditions of the present analysis provided a reliable indication about the level of synaptic activity required for the induction of Fos in the basal ganglia, it seems unlikely that corticostriatal or corticosubthalamic neurons could reach this threshold in a non-pathological state. However, it is possible that in particular physiological situations, encountered for instance during development or behavioural adaptation to new environmental conditions, a coincidence of synaptic events on striatal or subthalamic cells decreases this threshold via interaction of intracellular signalling pathways. Recordings in behaving

animals^{4,43,48,76} suggest, for instance, that during learning of sensorimotor tasks, striatal neurons are submitted to convergent synaptic influence from the cerebral cortex and the dopaminergic cells of the mesencephalon.

CONCLUSIONS

There is now evidence that the striatal compartmentation provides numerous segregated pathways for processing cortical information through the basal ganglia system. First, the topographic arrangement of the corticostriatal projections imposes a degree of functional regionalization to the striatum. Second, each striatal subregion is further subdivided into a mosaic according to the striosomal and matrix compartments characterized by a distinct cortical connectivity, output pathways and specific neurochemical components. Furthermore, within the striatal matrix, at least two main classes of output neurons are distinguished. The differential expression of Fos induced by cortical stimulation in these matrix cells provides an additional example of the functional heterogeneity in corticobasal ganglia circuits. In the absence of identification of genes regulated by Fos, the consequences of Fos expression in the physiology of basal ganglia remains speculative. It is generally thought that the late response genes targeted by immediate early genes like *c-fos* encode genes of direct physiological significance such as growth factors and their receptors, skeletal microfilaments, enzymes involved in neurotransmitter synthesis and proteins involved in axon outgrowth. Considering the implication of the basal ganglia in sensorimotor learning, one attractive hypothesis would be that activation of novel patterns of gene expression by cortical activity would ultimately modify the physiological state of target cells leading to consolidation of the cortico-basal ganglia circuits in support of certain motor behaviours. The use of the *in vivo* model of cortical stimulation to analyse the activity-dependent regulation of gene transcription in the basal ganglia should contribute to a better understanding of the mechanisms of plasticity underlying motor learning as well as on the long-term adaptative responses observed in the pathological state.

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