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Paradoxical increase in striatal neuropeptide gene expression following ischemic lesions of the cerebral cortex

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ABSTRACT **Ischemic lesions of the cerebral cortex occur frequently in humans as a result of stroke. One major consequence of the death of cortical neurons is the loss of excitatory cortical projections to subcortical regions. Little is known, however, about the transsynaptic effect of such lesions on neurotransmitter expression in subcortical structures. We have examined the effects of ischemic cortical lesions on the peptidergic neurotransmitters enkephalin and tachykinins in the striatum, a brain region massively innervated by glutamatergic cortical inputs. The levels of enkephalin and tachykinin mRNAs increased in the striatum of adult rats after thermocoagulation of pial vessels. The effects were more pronounced in the striatal region most heavily innervated by the lesioned cortex but were also observed in other striatal regions and on the contralateral side. Increased gene expression was accompanied by increased immunoreactivity for the two peptides. Elevated levels of enkephalin mRNA were observed up to 3 months after surgery in the ipsilateral striatum. Whereas results of previous studies of acute cortical ablations suggested that excitatory corticostriatal neurons were necessary to maintain normal peptide levels in striatal efferent neurons, the present data indicate that lesions of the same corticostriatal neurons secondary to local ischemia result in a paradoxical transsynaptic activation of neuropeptide synthesis in subcortical structures. This effect may play a role in the functional consequences of cortical strokes and progressive cortical atrophy in humans and may have critical bearing for their treatment and prognosis.**

The cerebral cortex sends excitatory inputs to many subcortical regions, including the caudate putamen (striatum) (1–3). Corticostriatal neurons innervate medium-sized spiny neurons, which constitute the large majority of efferent neurons in the caudate putamen (4, 5). These neurons contain either enkephalin or tachykinins (substances P and K). The former project to the globus pallidus (external pallidum); the latter project to the internal pallidum (entopeduncular nucleus in rats) and the substantia nigra (6, 7).

Acute cortical lesions by ablation in adult rats decreased the level of expression of enkephalin and tachykinins in striatal efferent neurons, suggesting that corticostriatal inputs play a critical role in maintaining normal levels of peptide synthesis in striatum (8, 9). There is, however, limited information on the effect of loss of cortical tissue secondary to local ischemia on these striatal neurotransmitters. In the present study, extensive cortical ischemia was induced by thermocoagulation of pial vessels in the adult rat. This procedure results in a gradual loss of the cerebral cortex underlying the area thermocoagulated, with minimal involvement of the subjacent corpus callosum and subcortical structures (10, 11). The levels of mRNAs encoding enkephalin and tachykinins, and the level of the corresponding peptides, were measured in the striatum, pallidum, and substantia nigra with *in situ* hybridization histochemistry and radioimmunohistochemistry, respectively. Histochemical methods were used to obtain information on the topography of the effects. The results show that, in contrast to acute cortical ablations, progressive loss of cerebral cortex secondary to local ischemia results in long-lasting increases in neuropeptide expression in striatal output neurons.

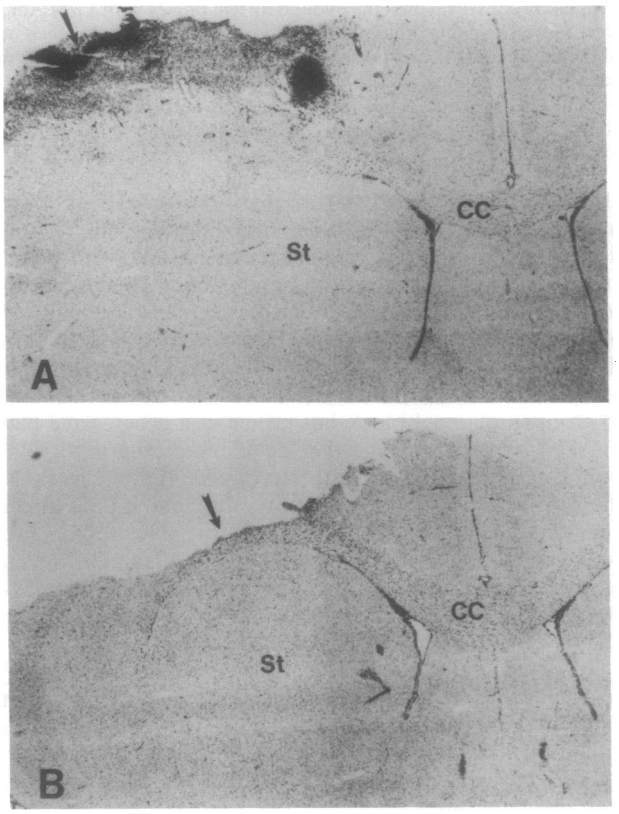

FIG. 1. Photomicrographs of Nissl-stained sections of rat brains showing the cortical lesion (arrow) 5 (*A*) and 21 (*B*) days after thermocoagulation of pial vessels. CC, corpus callosum; St, striatum. (×10.)

MATERIALS AND METHODS

Adult Sprague–Dawley rats (300 g) were subjected to a superficial coagulation of the frontoparietal cortex under deep anesthesia with equithesin as described (10). The lesion involved the whole rostrocaudal extent of the surface of the frontal and parietal cortices. Control rats were anesthetized, but, to avoid inducing any nonspecific cortical damage, no surgery was performed. Sets of animals that underwent surgery on the same day were randomly assigned to one of two groups, one sacrificed by decapitation (for *in situ* hybridization histochemistry) and the other by perfusion with 4% paraformaldehyde through the heart under anesthesia (for

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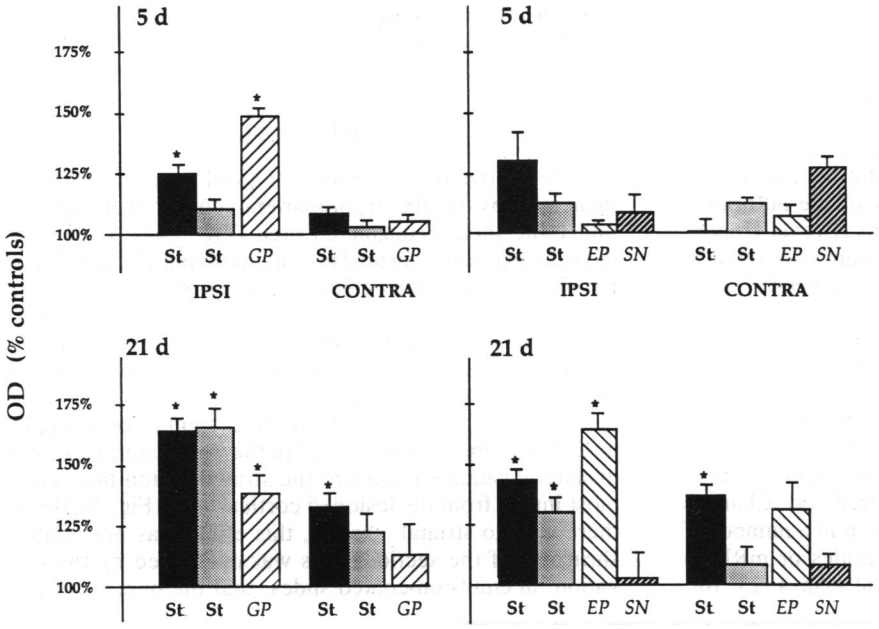

FIG. 2. Effects of unilateral thermocoagulatory lesion of frontoparietal cortex on the level of expression of enkephalin (*Left*) and tachykinins (*Right*) and their corresponding mRNAs in structures ipsilateral (IPSI) and contralateral (CONTRA) to the cortical lesion. Data are means ± SEM of optical densities of film autoradiograms of *in situ* hybridization (solid bars) and radioimmunohistochemistry (other bars) of sections from animals sacrificed 5 (5 d) and 21 (21 d) days after surgery. Measurements made in two or three sections per rat were averaged in each animal. Absolute values were used for statistical analysis and were subsequently expressed as percentage of the corresponding control values. *, $P < 0.05$ with the two-tailed Mann–Whitney U test (n = 3–6). St, striatum (dorsolateral); GP, globus pallidum (dorsolateral); EP, entopeduncular nucleus; SN, substantia nigra.

immunohistochemistry). The brains were removed and frozen on dry ice; sections (10 μm) were cut on a cryostat and mounted on gelatin-coated slides.

In situ hybridization histochemistry was performed as described (12) with ^{35}S-radiolabeled RNA probes complementary to preproenkephalin and preprotachykinin mRNAs. The corresponding cDNAs were provided by S. Sabol (13) and H. U. Affolter (Lofstrand Laboratories, Gaithersburg, MD), respectively. Sections were fixed in paraformaldehyde, acetylated, and dehydrated before hybridization with 3–8 ng of radiolabeled probe (≈4 × 10^5 dpm/ng) in humid chambers at 50°C. Posthybridization treatments included washes at 52°C in 50% formamide and 2× SSC (0.3 M NaCl/0.03 M sodium citrate) and incubation with RNase A (100 μg/ml) in 2× SSC at 37°C.

Sections for immunohistochemistry were processed with primary antibodies for enkephalin (1:1000; Incstar) and tachykinins (1:1000; Incstar) and ^{125}I-radiolabeled secondary antibodies (8). Briefly, sections, surrounded with rubber cement, were incubated 36 hr at 4°C in humid chambers with primary antibody diluted in 0.1 M phosphate-buffered saline (PBS) (pH 7.4) containing 1% normal goat serum and 0.3% Triton X-100. After incubation with the primary antibody, the sections were rinsed in PBS containing 1% normal goat serum and 0.3% Triton X-100 and incubated 30 min at 37°C with 120 μCi of ^{125}I-labeled goat anti-rabbit IgG per ml (2–10 μCi/μg; 1 Ci = 37 GBq) in PBS containing 3% normal goat serum and 0.3% Triton X-100 under mild agitation. After incubation in secondary antibody, sections were rinsed in PBS, quickly dipped in distilled water, and dried under a stream of cold air. Specificity of the *in situ* hybridization signal was confirmed by the absence of specific labeling in sections processed with sense RNA probes identical to cellular mRNAs and by verification of the discrete anatomical localization of neu-

Table 1. Labeling for enkephalin mRNA in striatum after unilateral cortical lesion

	Labeled neurons per mm²		Pixels per neuron	
	Ventromedial	Dorsolateral	Ventromedial	Dorsolateral
5 days				
Control	624.3 ± 22.6	627.3 ± 26.6	17.2 ± 0.8	18.8 ± 0.4
Lesion				
Ipsi	619.2 ± 31.0	633.6 ± 31.7	17.2 ± 1.0	28.7 ± 2.5*
Contra	608.4 ± 42.5	611.2 ± 26.1	17.3 ± 0.1	22.4 ± 0.8*
21 days				
Control	606.7 ± 23.6	611.1 ± 23.3	14.5 ± 0.6	14.6 ± 0.5
Lesion				
Ipsi	615.8 ± 28.0	685.0 ± 30.7	22.6 ± 0.7*	28.5 ± 0.9*
Contra	609.2 ± 25.0	603.5 ± 24.7	17.7 ± 0.8	19.9 ± 0.5*
90 days				
Control	584.1 ± 9.7	558.5 ± 13.9	9.6 ± 0.1	9.4 ± 0.2
Lesion				
Ipsi	569.1 ± 27.8	538.6 ± 14.1	9.8 ± 0.5	14.0 ± 0.2*
Contra	501.9 ± 21.2	414.1 ± 11*	8.8 ± 0.3	11.2 ± 0.3*

Effects of unilateral thermocoagulation of frontoparietal cortex on number of neurons labeled for enkephalin mRNA and level of labeling (pixels occupied per silver grain) per neuron in ventrolateral and dorsolateral striata ipsilateral (Ipsi) and contralateral (Contra) to the cortical lesion and in unlesioned animals (Controls). Number of days indicates survival time. Data are means ± SEM of values obtained in three to five animals per group. Sections from each group and corresponding controls were processed in parallel. Note that absolute values vary among groups because sections were processed in separate experiments.

*$P < 0.05$ when compared to controls with the two-tailed Mann–Whitney U test.

ronal labeling in each experiment. Controls for immunohistochemistry included blockade of the antibodies with the corresponding peptide and omission of either the primary or the secondary antibody. No specific labeling was observed in control sections.

For both techniques, sections were apposed to Kodak X-Omat films for 3 days to 3 weeks in light-tight cassettes. After development, the relative intensity of the autoradiographic signal was measured with a Dumas (Drexel University) image analysis system. Gray levels were converted to optical densities by using standard curves generated from Kodak autoradiographic standards. After exposure to film, sections processed for *in situ* hybridization histochemistry were coated with Kodak NTB3 autoradiographic emulsion, exposed at 4°C for 4–10 days, developed, and analyzed with the Morphon image analysis system (14). No attempt was made to convert the values obtained into absolute levels of mRNA or peptides. Therefore, only data obtained in sets of sections processed in parallel were compared (14). Changes in autoradiographic signals have been shown in a number of studies to reflect changes in levels of peptides or mRNAs similar to those measured with biochemical assays (15, 16). Statistical analyses were performed with the nonparametric two-tailed Mann–Whitney U test (17), with $P < 0.05$ considered significant.

RESULTS

Five days after surgery, morphological alterations with minimal atrophy of the frontoparietal cortex were observed under the thermocoagulated area (Fig. 1*A*). At 21 and 90 days, an extensive cortical lesion, involving all cortical layers but sparing the corpus callosum, was present on the ipsilateral side (Fig. 1*B*). No differences in surface areas of the head of the caudate putamen were observed in sections taken at the level of the cortical lesion at any time point examined (data not shown).

At 5 days, the autoradiographic signal for enkephalin mRNA on films was increased in the dorsolateral part of the ipsilateral caudate putamen, the striatal region that receives most inputs from the lesioned cortical area (Fig. 2). Because there was no striatal atrophy, this effect was not related to shrinkage of the section. This was confirmed by the observation, in emulsion-coated slides, that the number of grains

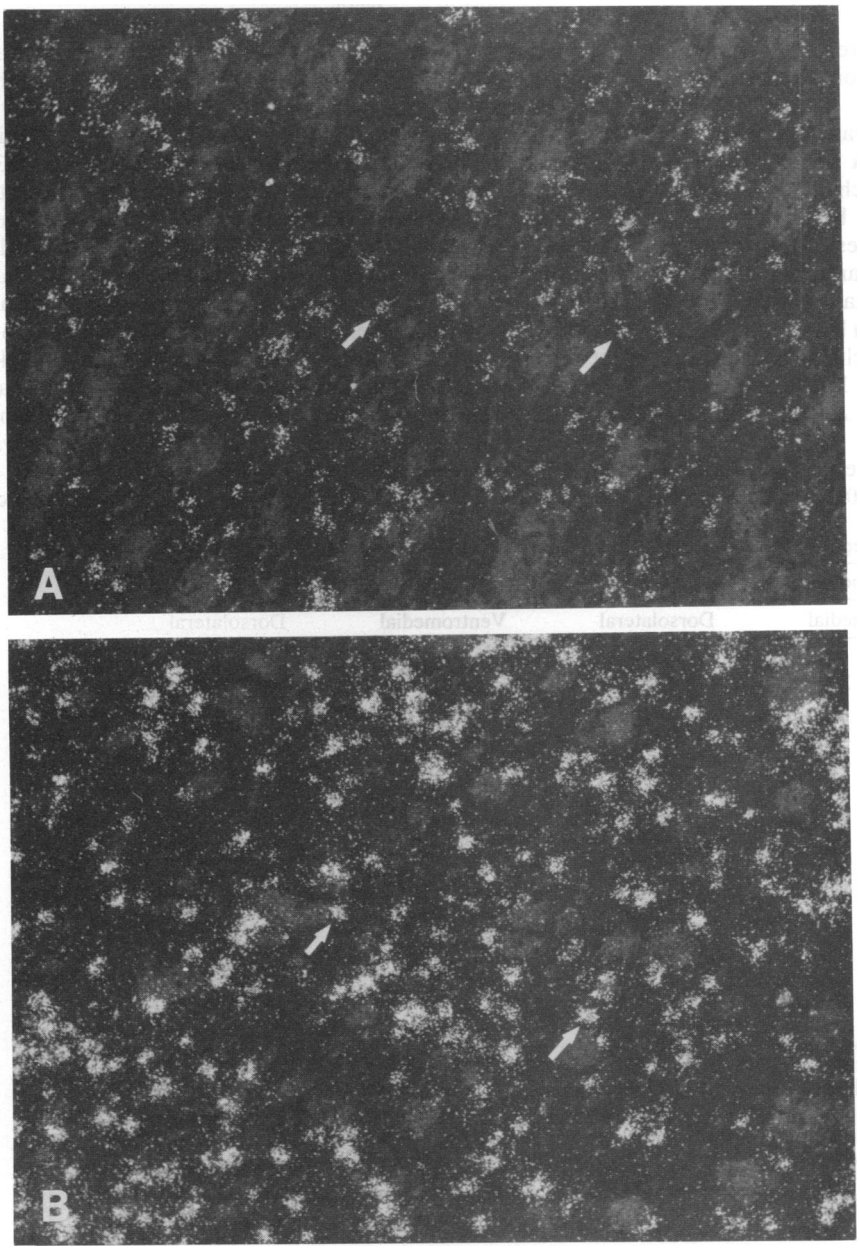

FIG. 3. Dark-field photomicrographs of two striatal sections processed in parallel for *in situ* hybridization histochemistry with a radiolabeled probe for enkephalin mRNA. Arrows point to labeled cells in dorsolateral striatum from a control rat (*A*) and from an animal sacrificed 21 days after thermocoagulatory lesion of the ipsilateral frontoparietal cortex (*B*). (×100.)

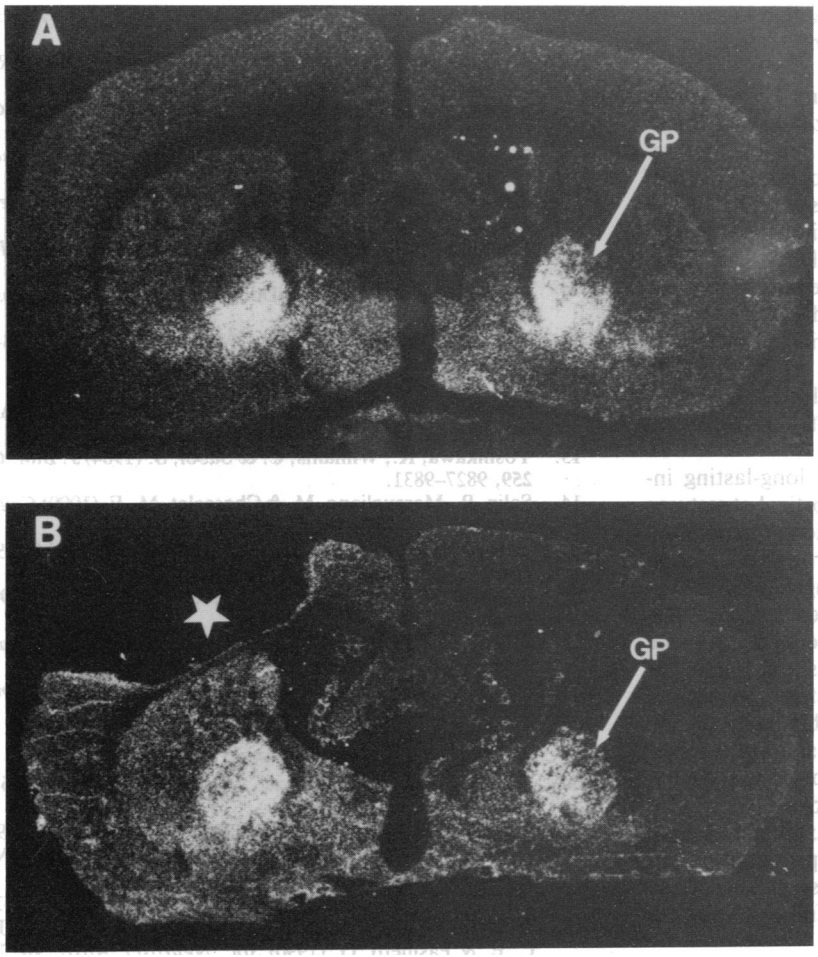

FIG. 4. Photomicrographs of film autoradiograms of sections processed for enkephalin radioimmunohistochemistry. Labeled regions appear white. (*A*) Section from a control rat. (*B*) Section from a rat with a unilateral cortical lesion by thermocoagulation (star) sacrificed 21 days after surgery. GP, globus pallidus. (×7.6.)

per cell was increased in cells labeled for enkephalin mRNA without significant changes in the density of labeled neurons (Table 1). At this time point, the increase in enkephalin mRNA in dorsolateral striatum was accompanied by a marked increase in enkephalin immunoreactivity in the dorsolateral part of the ipsilateral globus pallidus. Changes in tachykinin mRNA in dorsolateral striatum were not significant at this time point, and there were no changes in tachykinin immunoreactivity (Fig. 2).

The increase in enkephalin mRNA (Fig. 3) was more pronounced at day 21 and then also present in the ventral part of the ipsilateral caudate putamen as well as in the dorsolateral part of the contralateral striatum (Fig. 2; Table 1). An increase in tachykinin mRNA labeling was also observed in both striata at this time point (Fig. 2). Both immunoreactive peptides were increased in the striatum on the side ipsilateral to the lesion. In addition, enkephalin and tachykinin immunoreactivities were increased in the ipsilateral globus pallidus (Figs. 2 and 4) and entopeduncular nucleus, respectively (Fig. 2). No changes in tachykinin immunoreactivity were detected in the area of the substantia nigra examined, but a localized change could have been missed in this region, which is highly heterogeneous with regard to the distribution of its striatal inputs (18).

One set of animals was kept alive up to 3 months after surgery and examined for mRNA expression only. A smaller but significant increase in labeling for enkephalin (Table 1) but not tachykinin mRNA (data not shown) was still observed in the dorsolateral striatum on both sides of the brain at this time. The small increase in level of labeling per neuron on the contralateral side, however, was accompanied by a significant decrease in the number of labeled neurons in the dorsolateral striatum.

DISCUSSION

Enkephalin and tachykinin immunoreactivities in the pallidum are largely confined to axons originating from the striatum (6, 7). Therefore, the increase in immunoreactivity observed in these regions most likely reflects an increased level of the corresponding peptides in striatal efferent neurons. Together with the increased levels of mRNA encoding these peptides in the striatum, the results suggest that enkephalin and tachykinin synthesis increased in striatal efferent neurons after lesions of the cerebral cortex resulting from local ischemia.

This observation was unexpected because uni- or bilateral cortical lesions by excision or aspiration in rats resulted in a decreased expression of enkephalin, tachykinins, and their mRNAs in striatal efferent neurons (8, 9). One possible explanation for the difference in the effects of cortical ablation and those observed in the present study is that excitatory amino acids may be massively released from degenerating corticostriatal neurons shortly after alteration of cortical blood supply, as observed in response to ischemia in other brain regions (19). Supporting this hypothesis, striatal injections of glutamatergic receptor agonists increased enkephalin levels in the striatum and the globus pallidus (20).

A salient feature of the present data is that increased neurotransmitter expression persisted well beyond the initial postoperative period. Decreased glutamate uptake (10) and supersensitivity of glutamatergic receptors may contribute to the long-term effects observed after cortical lesion. It should be noted, however, that similar unilateral thermocoagulatory lesions of the cortex did not increase binding to several subtypes of glutamatergic receptors 2–3 weeks after surgery (21, 22). Alternatively, postlesion synaptic plasticity and/or

sprouting, which occur in the striatum after unilateral cortical lesions (23, 24), could play a role in lasting alterations of neurotransmitter expression.

The topography of changes in mRNA and neuropeptide expression reflected the anatomical organization of corticostriatal neurons in rats. Ipsilateral effects were either restricted or more pronounced in the dorsolateral parts of striatum and globus pallidus, areas corresponding to the regions receiving most afferents from the lesioned cortex and the corresponding striatal area, respectively (2, 25). Similarly, the contralateral changes in mRNA levels were more pronounced in the dorsolateral striatum and may have been elicited by the lesion of crossed corticostriatal projections (26). An activation of corticostriatal neurons originating from the sensorimotor cortex contralateral to the lesion, however, cannot be excluded in view of the loss of corticocortical connections induced by the lesion.

The present results provide evidence of long-lasting increases in peptide gene expression in subcortical structures after cortical lesions resulting from interruption of local blood flow. Enkephalin and tachykinins are neurotransmitters in separate striatal output pathways, and changes in their synthesis are likely to have profound effects on basal ganglia function (27). Whereas the experimental model used in the present study does not strictly model strokes in humans, the progressive loss of cortical tissue and, therefore, the remote consequences are likely to be the same. This bears on the study and clinical management of vascular lesions of cerebral cortex in humans. It has been recently reported that tachykinin immunoreactivity increases in the basal ganglia of patients with cortical atrophy related to Alzheimer disease (28). Therefore, increased neuropeptide expression in subcortical structures may be a common consequence of the progressive loss of cortical neurons in a number of distinct pathological conditions.

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