branes was also displaced by unlabeled Leu^{15}–G2-17–Gly, but not by G17 (Fig. 5B). Unlabeled G17 and both G-CCK$_B$ receptor antagonists completely inhibited the binding of ^{125}I-labeled Leu^{15}-G17 to AR4-2J cells, but G2-17–Gly, in concentrations as high as 1 μM, had no effect on ^{125}I-labeled Leu^{15}-G17 binding (Fig. 5C).

Our results indicate that amidated gastrin and its glycine-extended posttranslational processing intermediates induce AR4-2J cell proliferation. In contrast to the difference in the potencies of amidated gastrin and G-Gly in stimulating gastric acid secretion (*11*), the two peptides appear to be equally potent in inducing cell proliferation. Moreover, selective inhibition by L365,260 and PD-134308 of the effect induced by amidated G17, but not by G17-Gly, implies that there are two different receptors that mediate the proliferative actions of the peptides. In view of the observations that both plasma and tissue concentrations of G-Gly are higher than those of amidated gastrin, growth-related receptors for G-Gly may mediate physiological or pathophysiological effects. Our data indicate that the precursor and the product of peptide α amidation may have different biological actions mediated through separate receptors.

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Effect of the Nigrostriatal Dopamine System on Acquired Neural Responses in the Striatum of Behaving Monkeys

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Dysfunction of the nigrostriatal dopamine system results in marked disorders of movement such as occur in Parkinson's disease. Functions of this dopamine-containing projection system were examined in monkeys trained in a classical conditioning task, and the effects of striatal dopamine depletion were tested. Unilateral dopamine loss substantially reduced the acquired sensory responsiveness of striatal neurons monitored electrophysiologically. This effect was ipsilateral and selective, and could be reversed by apomorphine. These results suggest that the primate nigrostriatal system modulates expression of neuronal response plasticity in the striatum during sensorimotor learning.

Understanding the neural mechanisms underlying sensorimotor learning is a cardinal goal of neurobiology. To approach this problem, we investigated neurons of the basal ganglia, central structures in the motor system. We recorded from a clearly identifiable class of neurons in the striatum (the tonically active neurons, or TANs) while monkeys underwent training in a Pavlovian conditioning task. We found that the TANs acquire responsiveness to the sensory conditioning stimuli during behavioral learning (*1*). This systematic learning-dependent plasticity of TANs opened the possibility of determining whether dopamine, a major catecholamine neurotransmitter in the striatum, affects such behaviorally contingent neural plasticity. We therefore recorded from TANs before, during, and after monkeys were trained in behavioral conditioning, and tested the effects of manipulating their dopaminergic inputs (*2*).

Before conditioning, we confirmed that only a small fraction of TANs responded to the clicks used as conditioning stimuli (51 of 305 cells, or 16.7%) (*3*). The TANs were readily identified by their characteristic 2- to 8-Hz spontaneous discharge rates, action potential waveforms, and sparse distribution at 0.5- to 1.0-mm intervals. During training, many TANs became responsive to the conditioned stimuli (Fig. 1). In all, 71.4% of cells (95 of 133) recorded in the caudate nucleus and 52.0% of cells (91 of 175) recorded in the putamen responded to the conditioned stimuli after behavioral conditioning. The responses consisted of a brief pause in tonic firing (Fig. 1D), which began about 60 ms after the conditioned stimulus, lasted about 300 ms, and was often flanked by initial and rebound excitation periods (*2*).

After the conditioned behavior was acquired, we infused 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a dopaminergic neurotoxin, into the caudate-putamen complex of one hemisphere in each monkey (*4*). Unilateral dopamine deficits were evident in the home-cage behavior of the monkeys and were confirmed histologically by immunostaining for tyrosine hydroxylase (TH) after completion of the experiments (*5*). Histology showed dose-related partial (monkey R) to massive (monkey D) loss of TH-like immunoreactivity in the caudate nucleus and putamen, with near total loss near the injection site and graded depletion beyond (Fig. 2A). Postinfusion recordings were made within 5 mm of the injection sites, in the regions of maximum depletion. TH immunostaining was also regionally reduced in the substan-

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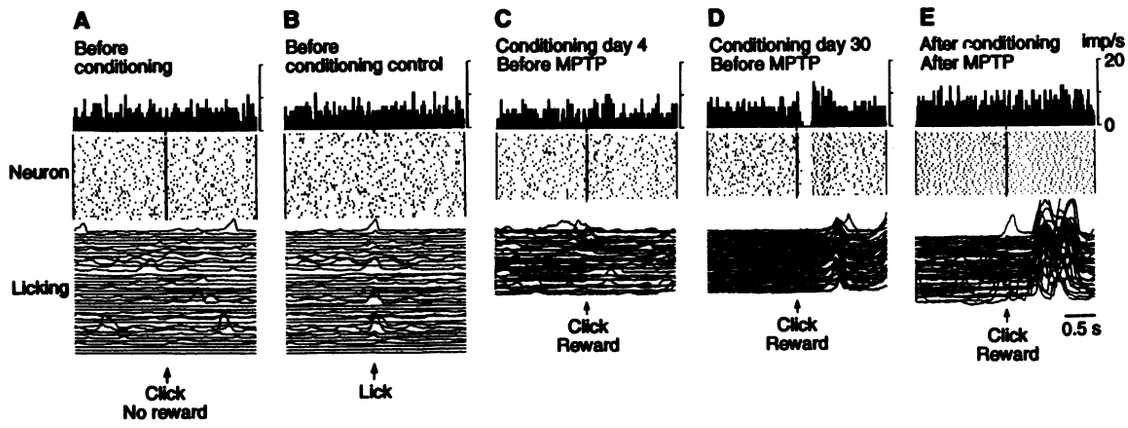
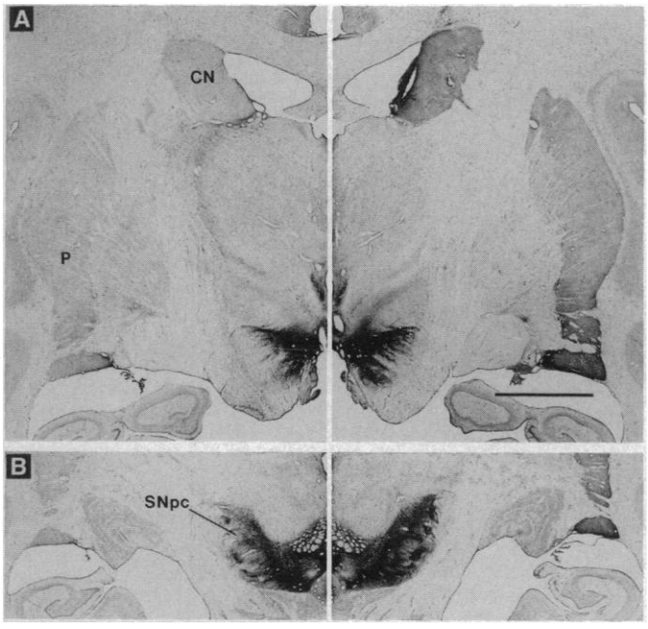


Fig. 1. Acquisition of sensory responsiveness by striatal neurons during learning and loss of responsiveness after dopamine depletion. Activity profiles of tonically active neurons (TANs) recorded in the right striatum before (**A** and **B**), during (**C**), and after (**D**) behavioral conditioning are shown for monkey R. (**E**) Activity recorded in the left striatum after infusion of MPTP on this side. Neuronal activity is aligned on the time of presentation of the clicks (A and C to E) or, as a control, on licking movements (B), and is expressed as raster displays and (at top) in average histograms. Records of licking movements are shown below.

tia nigra pars compacta (Fig. 2B).

Dopamine depletion resulted in a sharp reduction in the acquired sensory responsiveness of TANs recorded on the side of the MPTP injection. Summed neural activity histograms (Fig. 3) showed an almost complete abolition of the robust pause response on the side of MPTP administration. The spontaneous activity of the TANs appeared normal, however, and there was a small activation response to the clicks. Thus, the TANs on the MPTP-treated side had physiologic activity characteristic of normal TANs, but were unresponsive. The incidence of responsive TANs ipsilateral to MPTP infusion (32 of 211) was similar to that before conditioning (35 of 183), as though the conditioning had not occurred. By contrast, the acquired TAN responses to the conditioned stimuli remained on the uninjected side. There were, taking caudate nucleus and putamen together, 16.7% responsive TANs (51 of 305 cells) before conditioning, 60.4% (186 of 308 cells) after conditioning, and 59.8% (58 of 97 cells) after contralateral MPTP infusion.

Results of two further experiments supported this evidence for modulation of acquired responsiveness of striatal TAN activity by dopamine (Fig. 4). First, for eight TANs in the MPTP-treated striatum, it was possible to record for long enough to test the effects of systemic application of the dopamine receptor agonist apomorphine (6). In four cells, the sensory responses emerged within 16 to 30 min of apomorphine injection. Effects of dopamine receptor blockade in the intact hemisphere were studied by local application of haloperidol, a dopamine receptor antagonist at the recording sites (7). In each of three TANs successfully held for prolonged recording (~30 to 60 min), 100 μM haloperidol



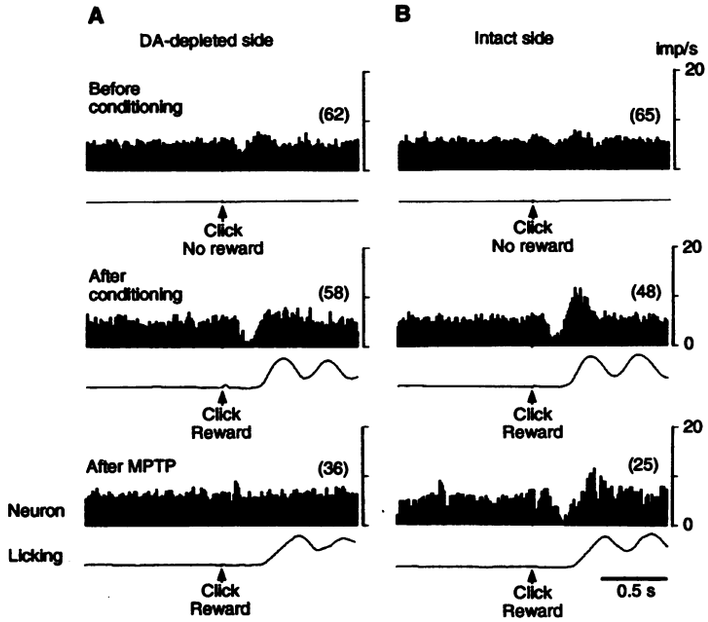


Fig. 2 (left). Unilateral depletion of tyrosine hydroxylase (TH)–like immunostaining in the brain of monkey R after infusion of MPTP into the left striatum. Sections illustrated show the caudal extent of the recording sites (**A**) and mid-levels through the substantia nigra pars compacta (**B**). CN, caudate nucleus; P, putamen; SNpc, substantia nigra pars compacta. Bar, 5 mm. **Fig. 3 (right).** Population activity of TANs recorded in the striatum of monkey D before and after MPTP on the side of the infusion (**A**) and on the intact side (**B**). Population histograms are centered on the time of the presentation of the conditioning clicks. Averaged licking movements are shown below. Numbers of neurons included for each histogram are shown in parentheses. The acquired neural responses to the conditioned stimuli largely disappeared after MPTP treatment on the side of infusion (A), but remained on the uninjected side (B).

diminished neuronal responses to the conditioned stimuli but did not diminish the cells' spontaneous activity.

To test whether conditioned responses of striatal TANs could be established with further behavioral conditioning, we continued to train the monkeys for 2 to 4 months after MPTP infusion. Despite almost daily conditioning sessions, the TANs recorded on the side of prior MPTP infusion failed to express the conditional responses (18.7% responded, compared to 15.4% before conditioning), whereas TANs on the control side continued to show strong responses to the conditioned stimuli (59.8% responded, compared to 60.4% before contralateral MPTP treatment).

These findings suggest that the nigrostriatal dopamine system is essential for the expression of response profiles acquired by striatal neurons during behavioral learning in the primate. The blockade of the conditioned TAN responses by local haloperidol injection, though observed in a small sample of cells, reinforces this view. The reinstatement of responses on the MPTP-treated side after application of apomorphine suggests that dopaminergic input functions not to convey specific input for conditioned responses, but to act as an enabling system (*8*) gating the expression of behaviorally relevant neuroplasticity in the striatum.

The concept that the nigrostriatal dopamine system is important in the expression of neural plasticity accompanying sensorimotor learning is concordant with clinical and behavioral observations indicating that disabilities accompanying nigrostriatal dopamine depletion extend beyond purely sensory or motor effects (*9*). Our results may also be important in interpreting evidence that in parkinsonian monkeys or humans, destruction of the sensorimotor outflow of the basal ganglia or of its subthalamic modulatory loop restores many apparently normal movements (*10*).

Schultz *et al.* (*11*) have found that dopamine-containing neurons in the primate substantia nigra discharge in response to alerting environmental stimuli, including primary rewards and stimuli with conditioned motivational significance. If such neurons project directly or indirectly to striatal TANs, they could influence the acquisition of TAN responses to behaviorally significant stimuli, which in turn could modulate striatal activity during and after learning. These dopaminergic midbrain neurons are reported to lose their responsiveness to conditioned stimuli even after prolonged training. Thus, the nigrostriatal dopamine system may exert not only phasic modulation of striatal neuron activity during sensorimotor learning, but also tonic modulation of the acquired activity after learning (*12*). This function may represent a sensorimotor parallel to the acknowledged importance of the mesolimbic-ventral striatal dopamine system in reward learning (*13*).

It will clearly be necessary to identify the phenotype of the TANs in the primate striatum in order to assess the ultimate behavioral consequences of this modulation of TAN activity by nigrostriatal dopamine. The available evidence suggests that TANs may be interneurons in the matrix and at striosome-matrix borders (*14*), and their properties resemble those of striatal cholinergic interneurons identified in the rat (*15*). Thus, they may integrate dopaminergic and cholinergic influences in the striatum and act on its projection neurons. This suggestion is in accord with in vitro evidence (*16*) that synaptic modifiability of striatal projection neurons depends on both dopamine receptors and muscarinic cholinergic receptors.

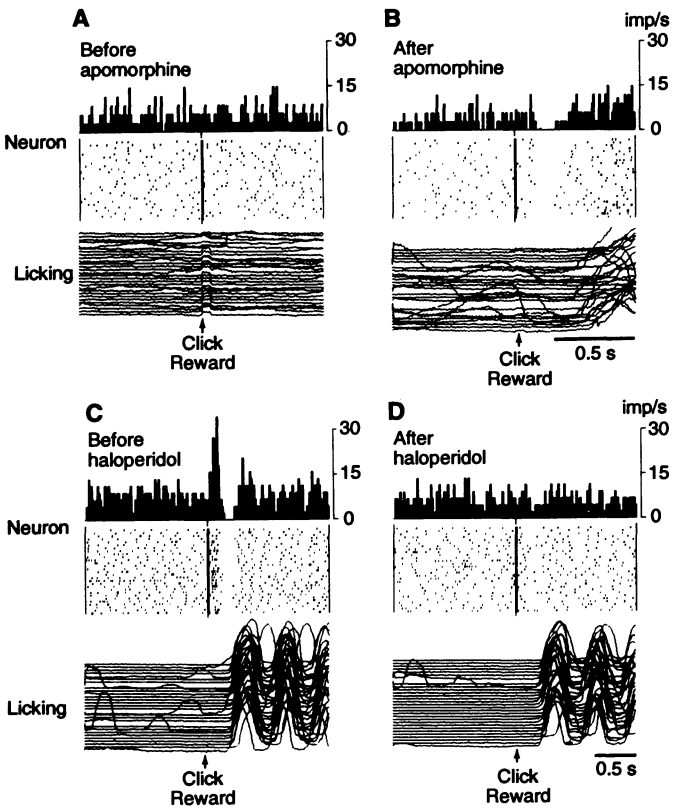


Fig. 4. Effects of dopamine receptor agonists and antagonists on the conditioned activity of striatal TANs. Activity of a TAN in the MPTP-treated striatum is shown before (**A**) and after (**B**) systemic injection of apomorphine. Activity of a TAN in the intact striatum is shown before (**C**) and after (**D**) haloperidol. Traces of licking movements are shown below the raster plots.

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2. Three male monkeys (*Macaca fuscata*) were trained in a soundproof room to associate the presentation of clicks with a simultaneously presented liquid reward delivered on a spoon in front of their mouths but outside of their visual fields. The heads of the monkeys were fixed, and licking movements were monitored by a strain gauge attached to the spoon and by electromyographic electrodes in the tongue. The activity of single neurons was recorded extracellularly by conventional techniques with glass-insulated Elgiloy microelectrodes having uninsulated tips of 15 to 35 μm and impedances of 0.5 to 1.5 megohm.
3. Statistical evaluation of changes in neuronal firing rate was made with a two-tailed Wilcoxon test for matched pairs. Response onset and offset were defined, respectively, by the first of three or more consecutive 15-ms bins of the peristimulus time histograms in which activity deviated from the average value during 750 ms preceding stimulation, or returned to control levels. Differences were considered significant at $P < 0.01$.
4. MPTP (4 mg in one monkey and 6 mg each in two monkeys) dissolved in 200 μl of 0.15 M NaCl was infused into the left hemisphere of each animal by means of an Alzet osmotic minipump (average rate, 0.5 μl/hour). The infusion needle (0.8 mm outer diameter, 0.5 mm inner diameter) was implanted under pentobarbital sodium anesthesia 11 to 12 mm deep at atlas coordinates A18-A20, a site between the putamen and caudate nucleus [H. Imai, T. Nakamura, K. Endo, H. Narabayashi, *Brain Res.* **474**, 327 (1988); J. W. Tetrud and J. W. Langston, *Science* **245**, 519 (1989)].
5. Two weeks after MPTP infusion, the monkeys exhibited weak ipsiversive turning (about three turns per minute) in their home cages, reluctance to use the contralateral limbs actively, and pronounced contraversive turning (about 15 turns per minute) after systematic injection of the nonselective dopamine receptor agonist apomorphine [0.1 mg/kg, intramuscular (i.m.)]. Immunohistochemistry was performed as described [A. M. Graybiel, E. G. Hirsch, Y. A. Agid, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 303 (1987)].
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TECHNICAL COMMENTS

Rates of *p16* (*MTS1*) Mutations in Primary Tumors with 9p Loss

A critical area of chromosomal loss at region 9p21-22 has been implicated in the genesis of different types of primary tumors. Initial observations defined deletions of this region in leukemia, gliomas, and cell lines derived from a wide spectrum of human tumors (*1*). In addition, linkage studies pointed to a gene in this area responsible for familial and sporadic melanoma (*2*). Subsequent analysis in human tumors demonstrated loss of heterozygosity (LOH) or homozygous deletion of this region (*3, 4*). Moreover, loss of chromosome 9 was found to occur early in the progression of several of these cancers (*5*).

Recently $p16^{INK4}$, identified as an inhibitor of activated cyclin D–cdk4 complexes (*6*), emerged as a candidate tumor suppressor gene when it was localized to 9p21 and found to be within the critical deleted region (*7, 8*). The demonstration by Skolnick and co-workers (*7*) of point mutations in melanoma cell lines prompted us to examine the role of Multiple Tumor Suppressor gene (*MTS1*) (which encodes *p16*, an inhibitor of cyclin-dependent kinase 4) in a variety of primary tumors. After excluding tumors (*4*) that demonstrated homozygous deletions of the region, we selected 75 primary tumors, all previously mapped by microsatellite markers, that displayed allelic loss of the region on chromosome 9p (Table 1). We then amplified exons 1 and 2 independently from the *p16* gene, cloned the amplified products into a plasmid vector, and sequenced p16 from pooled clones (*9*).

According to Knudson's hypothesis (*10*), if *p16* was the target of the deletion, tumors with a 9p loss should have intragenic mutations of *p16*. This argument was used in the initial observation that *p53* was the critical tumor suppressor gene on chromosome 17. Subsequently, *p53* mutations were found in the majority of carcinomas with 17p loss (*11*). In contrast, we identified only two polymorphisms and two mutations of *p16* among all 75 primary tumors. One prevalent polymorphism, a G→A transition at 436 nucleotides (codon 140), was found in four tumors and resulted in an amino acid change from alanine to threonine. The second polymorphism occurred in one tumor at 172 nucleotides (codon 52) and resulted in the same amino acid change. Both of these changes were present in germline DNA, and the codon 140 polymorphism had been described previously in one melanoma cell line (*7*). Two tumors (one bladder, one head and neck) contained mutations, a CGA→TGA (Arg→stop) at codon 50 (Fig. 1) and a GAG→TAG (GLU→stop) at codon 25, respectively. Both represented new somatic mutations, and both occurred in neoplasms of advanced stage. We tested all 75 primary tumors by comparative multiplex polymerase chain reaction (PCR) (*4*) with exons 1 and 2 of the *p16* gene and found no *p16* deletions. To further exclude small deletions extending into the *p16* gene, we then tested all bladder and kidney tumor and normal DNAs by Southern blot analysis (*12*) and found no homozygous deletions or rearrangements of *p16*.

We sequenced 97% (excluding four codons in exon 3) of the coding region of *p16* and found only two somatic mutations in 75 tumors. We excluded tumors with homozygous deletion of the region containing *p16*, and therefore it is unlikely that we amplified and sequenced normal tissue DNA. Although it is possible that unusual mutations in the promoter or noncoding regions of this gene could be involved in inactivation of *p16*, this would be unlikely as the predominant mechanism of mutation.

So, what is the role of *p16* in primary tumors? The demonstration of mutations

Table 1. Chromosome 9 loss in primary tumors. Primary neoplasms were microdissected to remove nonneoplastic tissue and were scored for allelic loss by microsatellite analysis (*4*). Tumors with partial loss (including the region 9p21-22) or monosomy of chromosome 9 are listed. Lung, nonsmall cell; bladder, transitional cell; kidney, clear cell; head and neck, squamous cell carcinomas; brain, gliomas. *p16/MTS1* was sequenced in all 75 tumors.

Tumor	9p loss	Monosomy	Total
Lung	9	6	15
Bladder	15*	10	25
Kidney	2	9	11
Head and neck	15*	0	15
Brain	4	5	9
Total	45	30	75

*Only one tumor in each of these groups contained a *p16* mutation.

Fig. 1. Autoradiograph of a sequencing gel, demonstrating a mutation in codon 50 of *p16* (CGA→TGA) in a primary bladder tumor (arrow). The C→A transition (lane 1), was confirmed by reamplification, recloning, and resequencing (lane 2). Sequencing primers for exon 2 and other methods are described in (*9*). A, adenine; C, cytosine; G, guanine; T, thymine.