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16. The proto-oncogene cDNA was isolated from an oligo(dT)-primed library prepared by standard methods with polyadenylated $[poly(A)^+]$ RNA from the *Xiphophorus* embryonal epithelial cell line A2 (*8*). The *D* locus and X*mrk* oncogene promoter clones were isolated from genomic sublibraries (*X. maculatus* stock Rio Jamapa $X^{Tu\text{-}Sd}/Y^{Tu\text{-}Sr}$) constructed as described (*3*), except that DNA fragments of 1.5 to 2.5 kb (*D* locus clone) and 4 to 6 kb (oncogene) were used for ligation. Probes were labeled by random priming (*14*). The A2 cDNA library was screened with probe 3-2E (*3*), and the X*mrk* oncogene sublibrary was screened with a 544-bp Eco RI–Eco RV fragment of 3-2E (5′ end of the cDNA). The *D* locus sublibrary was screened with a PCR fragment comprising nucleotides 1 to 431 of the X*mrk* oncogene. The primers used for the amplification were DA10 (5′-GAATTCCCCAGACCTGTTTGTGTTG-GAGG-3′, position 1 to 29 of the oncogene sequence) and DA11 (5′-CCTTTCTGTCCGGGTCT-GTGCTGCAGCAG-3′, position 402 to 431 of the oncogene sequence). The PCR reaction contained 200 μM deoxynucleotide triphosphate, 100 pmol of each primer, 1 ng of template DNA, and 0.5 U of Taq DNA polymerase (Amersham) in 50 μl of 1× reaction buffer (Amersham). Amplifications were carried out for 35 cycles (92°C for 60 s, 60°C for 60 s, and 72°C for 90 s). Hybridizations were done at 42°C in 50% formamide, 5× standard saline citrate (SSC), and 1% SDS, and washes were done at 68°C, 0.1× SSC, and 1% SDS. The BESTFIT, GAP, and WORDSEARCH alignments of the GCG package (*15*) were used to determine sequence homologies.
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22. The 5′ region of the X*mrk* proto-oncogene was amplified in a PCR reaction containing primers ND1 (5′-GCAGCTCGAGCAGCGCCATTATTAC-TGTC-3′, position 215 to 188 of the proto-oncogene cDNA sequence) and ND2 (5′-GACTTGCGCCGAGCCAGCAGACAGGCAG-GACCC-3′, position 6 to 37 of the proto-oncogene cDNA sequence). The 5′ region of the X*mrk* oncogene was amplified in a PCR reaction containing primers DA10 and DA11 (*16*). The PCR products were separated on 2% agarose gels, purified, and labeled by random priming. Southern hybridizations were performed as described (*3*), except that the filters were washed at 72°C in 0.1× SSC and 1% SDS.
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24. The primers used in the PCR reactions were OP1 (5′-ATGGAGCAGCAGTCTGACCTG-3′, position 290 to 310 of the oncogene sequence) and OP2 (5′-CCGCTCCTCCGCGCAGAAAC-3′, position 347 to 365 of the oncogene sequence), which border the region framed by the large box in Fig. 1.
25. The X*mrk* oncogene promoter was cloned into pBLCAT3 (*17*). Orientation and cloning sites were confirmed by sequencing. The sequence fused to the CAT gene spans the region from position 1 to 288 of the oncogene sequence (see Fig. 1). A detailed description of cell lines A2 and PSM is given elsewhere (*18*). DNA transfections were performed in triplicate according to (*13*) with an automated precipitator (*19*). CAT activity was measured as described (*20*), with the following modifications. Cells were lysed by three freeze-thaw cycles, and after centrifugation the protein concentration in the supernatant was determined (Protein Dye Reagent, Biorad). Identical amounts of protein were incubated overnight with $[^{14}C]$chloramphenicol and acetyl coenzyme A. The acetylated derivatives were separated from the unacetylated form by thin-layer chromatography (TLC), and the TLC plates were subsequently autoradiographed. CAT conversion rates were determined with a scintillation counter. For experiment 1, pBLCAT3 was used as an internal standard; for experiment 2, the internal standard was pBLCAT3 containing a DNA fragment from the intron of λX21-1 instead of the oncogene promoter. In both experiments, the low basal CAT conversion values obtained with the internal standards were set to "1" relative to the values obtained with the oncogene promoter.

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Multiple Output Channels in the Basal Ganglia

John E. Hoover and Peter L. Strick*

The neural circuits that link the basal ganglia with the cerebral cortex are critically involved in the generation and control of voluntary movement. Retrograde transneuronal transport of herpes simplex virus type 1 was used to examine the organization of connections in the cebus monkey between an output nucleus of the basal ganglia, the internal segment of the globus pallidus (GPi), and three cortical areas: the primary motor cortex, the supplementary motor area, and the ventral premotor area. Spatially separate regions of the GPi were labeled after virus injections into each cortical area. The GPi projects to multiple cortical motor areas, and this pallidal output is organized into discrete channels. This information provides a new anatomical framework for examining the function of the basal ganglia in skeletomotor control.

The basal ganglia are subcortical brain nuclei that are critical for the central generation and control of voluntary movement. It has been suggested that these structures are involved in the internal generation of movement, the automatic execution of motor plans, and the acquisition and retention of motor skills (*1*). Dysfunction of the basal ganglia, as occurs in Parkinson's disease, is associated with striking disorders of movement (*2*).

The input nuclei of the basal ganglia (that is, the caudate and putamen) receive substantial projections from diverse regions of the cerebral cortex, including motor, sensory, prefrontal, and limbic cortical areas. The output nuclei of the basal ganglia (that is, the GPi and the substantia nigra pars reticulata) send their axons to the thalamus and, by this route, project back upon the cortex. Thus, a major aspect of basal ganglia circuitry is its participation in multiple open and closed loops with the cerebral cortex (*3*).

Our understanding of the organization of basal ganglia loops with the cerebral cortex has evolved considerably over the last 20 years. In the past, the output of the basal ganglia was thought to terminate in a single region of the thalamus and to influence a single cortical area, the primary motor cortex (*4*). According to this view, the basal ganglia funneled information from widespread regions of the cerebral cortex into the motor system. It is now clear that the output of the basal ganglia terminates in thalamic regions that gain access to a wider region of the frontal lobe than previously suspected (*5*). Indeed, five parallel basal ganglia–thalamocortical loops have been identified, each of which is focused on a particular region of the frontal cortex (*6*). These loops were designated the skeletomotor, oculomotor, dorsolateral prefrontal, lateral orbitofrontal, and anterior cingulate circuits. We have now examined the organization of the skeletomotor circuit with a neuroanatomical technique that makes use of retrograde transneuronal transport of herpes simplex virus type 1 (HSV-1) (*7*). This technique provides a unique method for labeling a chain of synaptically linked neurons. Our results provide evidence for at least three skeletomotor circuits, each of which involves a spatially separate region of GPi and influences a different cortical motor area.

We injected the McIntyre-B strain of HSV-1 into the arm representations of either the primary motor cortex, the supple-

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mentary motor area (SMA), or the ventral premotor area (PMv) of cebus monkeys (*Cebus apella*) ($n = 6$). The surgical procedures we used have been described in detail elsewhere (8). After anesthetizing the animals with Telazol (initial dose, 20 mg per kilogram of body weight; supplemental dose, 5 to 7 mg per kilogram of body weight per hour), we performed a craniotomy to expose the left frontal lobe; then the arm representation of one of the cortical motor areas was injected (six to ten sites; 0.05 μl per site) with McIntyre-B HSV-1 (titer, 8.2×10^8 plaque-forming units per milliliter). The injections were made with a microsyringe; we determined their placement by using sulcal landmarks and intracortical stimulation (9, 10). Animals were killed approximately 5 days after inoculation (11). The brain of each animal was sectioned and processed to demonstrate the location of virus-specific antigen (12).

Injections of virus into the arm representations of either the primary motor cortex, SMA, or PMv (Fig. 1) labeled many neurons in the GPi. An average of 1300 pallidal neurons were labeled by transneuronal transport in each experiment. These labeled neurons were found in both the inner and outer portions of the GPi. Virus antigen densely filled the labeled cells, clearly marking their somata and primary dendrites (Fig. 2). The labeled primary dendrites coursed obliquely through the GPi; however, they were concentrated in the regions of the nucleus that contained labeled cell bodies.

The dorsoventral location of the labeled pallidal neurons varied depending on the location of the cortical injection site. Virus injections into the arm representation of the SMA labeled neurons in a dorsal region of the GPi (Fig. 3, animal Jo10). In contrast, injections into the arm representation of the PMv labeled neurons mainly in ventrolateral portions of the GPi (Fig. 3, animal Jo15). Neurons labeled after injections into the arm area of the primary motor cortex were located between the two groups of neurons labeled by the SMA and PMv injections (Fig. 3, animal Z10). The region of the GPi with a high density of labeled neurons was consistently limited to approximately a 1- to 2-mm segment of the nucleus in the middle of its anterior-posterior extent (13). These observations suggest that the arm representations of the primary motor cortex, SMA, and PMv each receive pallido-thalamocortical input from separate regions of the GPi (Fig. 4).

These results help to clarify currently debated issues about the anatomical organization of connections of the basal ganglia with motor areas of the cerebral cortex. It is generally agreed that a major site of termination for pallidal efferents involved in skeletomotor control is the thalamic subnucleus ventralis lateralis pars oralis (VLo) (14). The VLo projects to sites in several motor

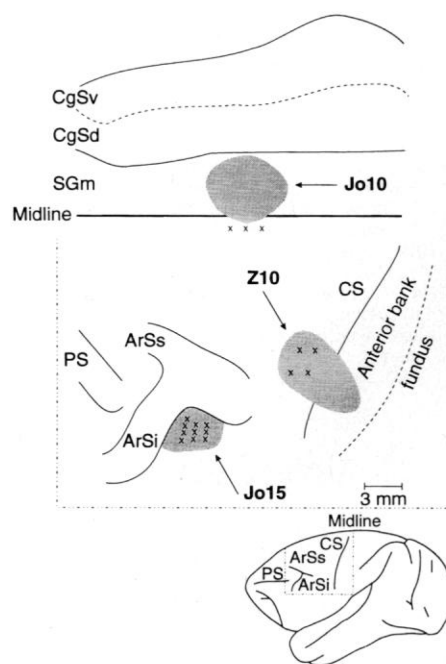

Fig. 1. Location of virus injection sites in the primary motor cortex (animal Z10), SMA (animal Jo10), and PMv (animal Jo15). The dotted lines on the inset at the lower right indicate the region of the frontal lobe that is enlarged in the diagram at the top. The medial wall of the hemisphere is illustrated as if it has been reflected upward, with the cingulate sulcus unfolded to display the cortex in its banks. The anterior bank of the central sulcus has also been opened. Small x's indicate the sites where the microsyringe needle entered the cortex for virus injection. The shaded areas indicate the spread of virus. ArSi, inferior limb of the arcuate sulcus; ArSs, superior limb of the arcuate sulcus; CgSd, dorsal bank of the cingulate sulcus; CgSv, ventral bank of the cingulate sulcus; CS, central sulcus; PS, principal sulcus; and SGm, medial portion of the superior frontal gyrus.

areas, including the SMA (15), PMv (16), and primary motor cortex (8, 17). These findings raised the possibility that a number of motor areas, rather than one in particular, are influenced by pallidal output. Our experiments provide direct support for this concept by demonstrating that at least three motor areas are targets of pallido-thalamocortical pathways (18). In addition, we found that the SMA, PMv, and primary motor cortex are each influenced by a different portion of the GPi. These results suggest that localized regions of the globus pallidus are organized into discrete output channels that are focused on selected cortical areas.

The concept that pallidal output is organized into discrete channels may provide some insight into the pathophysiology of disorders of the basal ganglia such as Parkinson's disease. This movement disorder is associated with three cardinal symptoms: tremor, rigidity, and akinesia (2). However, patients with Parkinson's disease present with vary-

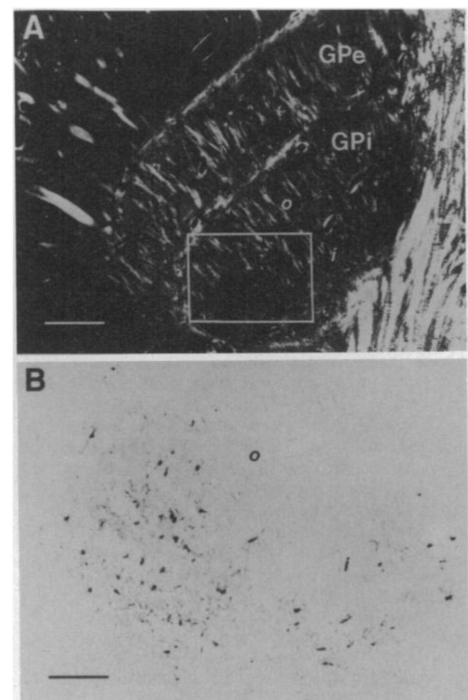

Fig. 2. Neurons in the GPi labeled by retrograde transneuronal transport of HSV-1. (**A**) A low-power, dark-field photomicrograph of the globus pallidus of animal Z10. The boxed area indicates the region of the pallidum shown at higher magnification in (B). (**B**) Neurons labeled with virus in the GPi. Scale bar: (A) 1 mm; (B) 300 μm. GPe, external segment of GP; i, inner portion of GPi; and o, outer portion of GPi. Top, dorsal; right, medial.

ing amounts of each symptom. For example, some display a syndrome that is almost purely akinetic, whereas others display a syndrome in which tremor is the dominant feature (2). Perhaps each of the cardinal symptoms results from dysfunction in separate output channels. The variability in symptoms may reflect variation in the degree to which individual output channels are affected by the disease.

The results of stereotactic lesions of the globus pallidus, made to ameliorate the symptoms of patients with Parkinson's disease, support this view. Such lesions are thought to prevent abnormal pallidal signals from reaching cortical motor areas where they would generate inappropriate descending commands for movement. When pallidal lesions were made at an anterodorsal target in the nucleus, they produced a long-term improvement in rigidity but did little to change tremor and akinesia (19). In contrast, lesions made at a more posteroventral target alleviated all three of these motor symptoms (20). One interpretation of these findings is that anterodorsal pallidotomy produces only limited improvement because the lesion affects only a restricted number of output channels, leaving some abnormal pallidal signals uninterrupted, whereas great-

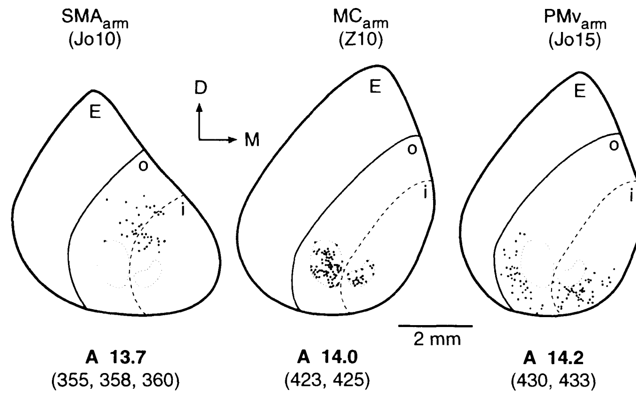

Fig. 3. The positions of neurons in the GPi labeled by retrograde transneuronal transport of HSV-1 from the SMA, primary motor cortex, and PMv. This figure illustrates representative coronal sections through the globus pallidus of animals that received injections of HSV-1 into the arm representations of the SMA (animal Jo10), primary motor cortex (MC) (animal Z10), or PMv (animal Jo15). The dots indicate the positions of labeled cells observed in two or three adjacent sections (section numbers at bottom in parentheses). For comparison, the dotted line indicates the region of the GPi containing neurons labeled from the primary motor cortex in animal Z10. The thick solid line indicates the outline of the globus pallidus. The thin solid line indicates the border between GPe and GPi. The dashed line indicates the border between the inner and outer portions of the GPi. E, external segment of the GP; i, inner portion of the GPi; o, outer portion of GPi; D, dorsal; and M, medial.

er success is achieved with posteroventral pallidotomy because the lesion interrupts the output of multiple channels.

Our results also provide some insight into the involvement of pallido-thalamocortical pathways in skeletomotor control. The finding that pallidal output innervates multiple cortical motor areas indicates that the basal ganglia have a broad influence over the generation and control of movement. Indeed, all three of the cortical areas we have examined—the SMA, PMv, and primary motor cortex—project directly to the spinal cord (*21*). Thus, the pallidal projection to each of these motor areas may provide the basal ganglia with a direct route for influencing the motor output of the spinal cord. The separation of pallidal output into discrete channels directed to specific cortical areas raises the possibility that each channel deals with some specific aspect of skeletomotor control. Perhaps this organization gives the basal ganglia the capacity to concurrently process multiple variables. The pallidal channel directed to the primary motor cortex may be involved with the control of movement parameters, such as direction and force (*22*). On the other hand, pallidal channels directed to premotor areas, such as the SMA and PMv, may be separately involved with higher order aspects of motor programming, such as the internal guidance of movement, movement sequencing, and skill acquisition (*23*).

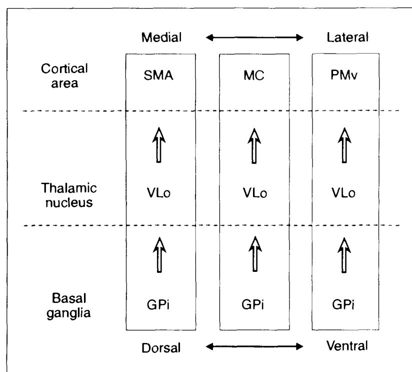

Fig. 4. Summary of the data. The three cortical motor areas we examined—the SMA, primary motor cortex (MC), and PMv—are each the target, by way of the thalamus, of separate pallidal output channels.

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9. The locations of frontal lobe motor areas, relative to sulcal landmarks in macaque monkeys, have been described in detail by R. P. Dum and P. L. Strick [*J. Neurosci.* **11**, 667 (1991)]. These motor areas are organized similarly in cebus monkeys. This has been demonstrated in two ways: (i) by injecting wheat germ agglutinin conjugated to horseradish peroxidase into the primary motor cortex of cebus monkeys and mapping the cortico-cortical connections and (ii) in other animals, by injecting the same tracer into the spinal cord and mapping the retrogradely labeled corticospinal projections (G. A. Bortoff and P. L. Strick, unpublished data). This information was used to help guide the placement of injections in the present experiments.
10. See P. L. Strick and J. B. Preston [*J. Neurophysiol.* **48**, 139 (1982)] for a detailed description of the stimulation methods we used.
11. Each animal was deeply anesthetized (ketamine hydrochloride, 25 mg/kg; pentobarbital sodium, 40 mg/kg) and transcardially perfused with 0.1 M phosphate buffer (pH 7.4) and then with 4% (w/v) paraformaldehyde in phosphate buffer and 4% paraformaldehyde in phosphate buffer with 10% (v/v) glycerol.
12. Serial coronal sections were cut through the brain at 50-μm intervals with a freezing microtome. Free-floating tissue sections were processed according to the avidin-biotin-peroxidase method (ABC; Vectastain, Vector Laboratories, Inc.) with a commercially available antibody to HSV-1 (Dako Corporation; 1:2000 dilution). Immunoreactivity was developed by incubation in a solution prepared by the mixing of equal volumes of 0.1% 3,3′-diaminobenzidine tetrahydrochloride and 0.02% hydrogen peroxide. At least every third section through the brain was treated in this manner. For more complete details, see P. L. Strick and J. P. Card [in *Experimental Neuroanatomy: A Practical Approach*, J. P. Bolam, Ed. (Oxford Univ. Press, Oxford, in press)].
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24. We thank D. I. Bernstein of the Gamble Institute of Medical Research (Cincinnati, OH) for HSV-1 (McIntyre-B); we thank S. Fitzpatrick, M. Corneille, and K. Hughes for histological assistance. Supported by the Veterans Administration Medical Research Service (P.L.S.) and NIH grants NS2957 and NS24328 (P.L.S.).

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