ture of impulse-mediated and graded transmission observed within the ganglion may reflect these two functional features. Similar considerations suggest that mixtures of impulse-mediated and graded transmission might occur in other systems where projection neurons participate in local circuit interactions (*19*).

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10. Stomatogastric neurons are monopolar; their major process dive into neuropil, branch, and then exit the ganglion. Synaptic sites are diffusely distributed on collateral branches in the neuropil [D. King, *J. Neurocytol.* **5**, 207 (1976); *ibid.*, p. 239]. Overshooting impulses are rarely observed with microelectrode penetrations of ganglionic neurites but can be observed where the pyloric motor axons exit the posterior of the ganglion (J. Miller, unpublished data).
11. The stomatogastric ganglion, the esophageal ganglion, and the commissural ganglia, their connectives, and some motor neurons were dissected free from the dorsal surface of the stomach and maintained in physiological saline. The stomatogastric ganglion and the base of the posterior output (dorsal ventricular) nerve were desheathed with fine forceps and conventional intra- and extracellular recording procedures were followed. Tetrodotoxin ($1.6 \times 10^{-5}M$) (Sankyo) was perfused across the base of the output nerve from one drawn pipette into another 100 to 400 μm in diameter. A stock toxin solution was diluted with saline, marked with fluorescein, and sometimes made isotonic with sucrose. Transverse lighting of the preparation facilitated visualization of the toxin solution as it traversed the path between pipettes.
12. One alternative explanation is that the graded control of transmitter release is the predominant mode of synaptic transmission in vigorously cycling preparations.
13. During the course of localized toxin experiments (5 to 15 minutes), toxin diffused deeper into the ganglionic tissue, first interfering with impulse initiation, then with the spontaneous generation of oscillating generator potentials, and later blocking the invasion of nerve impulses evoked by stimulation of the anterior input nerve.
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15. K. Graubard, J. Raper, D. Hartline, in preparation. The criteria were (i) reversal of the postsynaptic waveform with the injection of hyperpolarizing current, (ii) abolition of the postsynaptic waveform in low-calcium–high-magnesium saline, and (iii) the selective blockade of some synaptic pathways with picrotoxin.
16. Plateau potentials could reach more depolarized levels in the absence of impulse generation than under normal conditions if the regions subserving impulse and plateau generation are electronically close to each other, allowing competition between their underlying ionic currents (*7*). Under these conditions, impulse generation could decrease net transmitter release by interfering with the full expression of the plateau characteristic.
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Transmitter-Specific Retrograde Labeling in the Striato-Nigral and Raphe-Nigral Pathways

Abstract. *Injecting radioactive transmitters into the rat substantia nigra led to retrograde neuronal labeling either in the dorsal raphe nucleus, after ^{3}H-labeled serotonin injection, or in the caudoputamen, after ^{3}H-labeled γ-aminobutyric acid injection. This differential labeling in projections whose transmitter has been established provides the basis for a histochemical tracing method indicating both connectivity and transmitter specificity of neural pathways.*

We have proposed that the connectivity and the chemospecificity of neural pathways could be established by transmitter-related retograde tracing (*1*). Such selective tracing could be based on specific terminal uptake after the administration of radioactively labeled transmitter, retrograde axonal migration, and retention of labeled material. The hypothesis of selective tracing originated from the observation of perikaryal labeling of neurons in the pigeon optic lobe after [^{3}H]glycine was applied to the terminal area (*1*). The release of exogenous and endogenous glycine when this pathway is electrically stimulated indicates its glycinergic nature (*2*). Furthermore, retrograde labeling has been found after administration of γ-[^{3}H]aminobutyric acid (GABA) in two neuronal systems, in which the transmitter has not been determined by well-established criteria (*1*, *3*). We have now tested our hypothesis in mammalian pathways characterized with respect to their transmitter.

The rat striato-nigral and raphe-nigral projections were chosen as models. Electrophysiological and biochemical investigations leave little doubt that GABA is a transmitter in the striato-nigral pathway (*4*, *5*). There is also good evidence for a serotoninergic projection from the raphe nucleus to the substantia nigra (*6*). According to the hypothesis tested, injection of [^{3}H]GABA into the substantia nigra should lead to perikaryal labeling in the caudoputamen, whereas application of [^{3}H]serotonin should label nerve cell bodies in the raphe nucleus.

We tested these predictions in female albino rats (160 to 190 g) anesthetized with Nembutal (40 mg per kilogram of body weight, injected intraperitoneally) for stereotaxic injections (coordinates were 2.6 mm anterior to the interaural line, 2.0 mm lateral to the midline, and 2.0 mm below the horizontal zero plane) (*7*). Two animals received [^{3}H]GABA (15 μCi in 0.05 μl, 5.6 mM, or 10 μCi in 0.1 μl, 1.85 mM, 4-amino-*n*-[2,3-^{3}H]butyric acid, 54 Ci/mmole; Radiochemical Centre, Amersham) and another two [^{3}H]serotonin (13 to 16 μCi in 0.05 μl, 24 to 30 mM, 5-hydroxy[G-^{3}H]tryptamine creatinine sulfate, 10.7 Ci/mmole; Radiochemical Centre). As a control, the unspecific retrograde marker substance horseradish peroxidase (HRP) [0.05 μl of 30 percent (weight to volume); Boehringer RZ3] was used to treat two rats. The survival time was 6 hours in the GABA and serotonin cases and 1 day in the HRP experiments. The animals were intracardially perfused, first with 5 percent Rheomacrodex solution (Pharmacia Uppsala) for 20 to 30 seconds and then with 400 ml of 5 percent phosphate-buffered (0.16M, pH 7.4) glutaraldehyde fixative. Transverse frozen sections (50 μm thick) were prepared for light microscopic autoradiography according to standard techniques and exposed in the dark for 6 and 12 weeks or stained for peroxidase activity (*8*).

The two injection sites after [^{3}H]-serotonin application differed in size from each other. Nevertheless, labeling

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was maximal over the lateral part of the substantia nigra pars reticulata. Especially with the larger injection site, the background activity over a large part of the brain was high. In both [^{3}H]serotonin cases, labeled perikarya were observed predominantly in the ipsilateral half of the dorsal raphe nucleus (Fig. 1, A and B). A small number of neurons were labeled in this area. Occasionally accumulations of silver grains with the shape of proximal dendrites were detected in the neuropil surrounding labeled perikarya. No labeled neurons were seen in other monoamine cell groups (*9*). The distribution of labeled cell bodies was similar to the one found in earlier (*4*, *5*) and in our HRP experiments. These observations are consistent with the hypothesis that the serotoninergic raphe-nigral pathway was selectively labeled.

Retrograde perikaryal labeling in the nucleus raphe dorsalis after the injection of [^{3}H]serotonin into the rat caudoputamen has been described by Leger *et al.* (*10*). They found modestly labeled cells in this area and heavily labeled neurons in the substantia nigra. Thus, retrograde perikaryal labeling after the application of [^{3}H]serotonin was not restricted to serotoninergic pathways, but was also obtained in a dopaminergic system (*9*). After [^{3}H]dopamine was injected into the striatum, we found preliminary evidence of heavy perikaryal labeling in the substantia nigra and weak labeling in the nucleus raphe dorsalis. This crossed specificity of the labeling patterns after [^{3}H]dopamine or [^{3}H]serotonin injections could possibly be explained by the well-established crossed specificity of the uptake systems in dopaminergic and serotoninergic terminals even at very low concentrations (*11*).

Further limitations in the chemospecificity of retrograde perikaryal labeling were indicated, however, by exceptional labeled pyramidal cells in layer 5 of the cortex in one of our [^{3}H]serotonin experiments (large injection site). Bunney and Aghajanian (*5*) described a cortico-nigral pathway in the rat by retrograde tracing after nigral HRP injection, and our experiment confirmed their finding. Thus, the labeled cortical perikarya after nigral [^{3}H]serotonin application presumably belonged to this connection in which there is no indication that serotonin is a transmitter. As no significant labeling was found in these experiments over striatal and pallidal neurons, a certain degree of chemospecificity in retrograde labeling after [^{3}H]serotonin injection could nevertheless be hypothesized.

Labeling from the [^{3}H]GABA injection sites was more restricted than that in the [^{3}H]serotonin experiments and was maximum in the zona reticulata of the substantia nigra—very lateral for the smaller and slightly more medial for the larger injection. In a series of successively more anterior sections, accumulations of silver grains with the shape of a strong fiber bundle were observed over the internal capsule. In the region of the globus pallidus, we found a few smaller bundles divided up into even smaller ones in a restricted area of the caudoputamen (Fig. 1, C and D). Clusters of labeled perikarya were detected there [between about 5.4 and 6.4 mm anterior to the in-

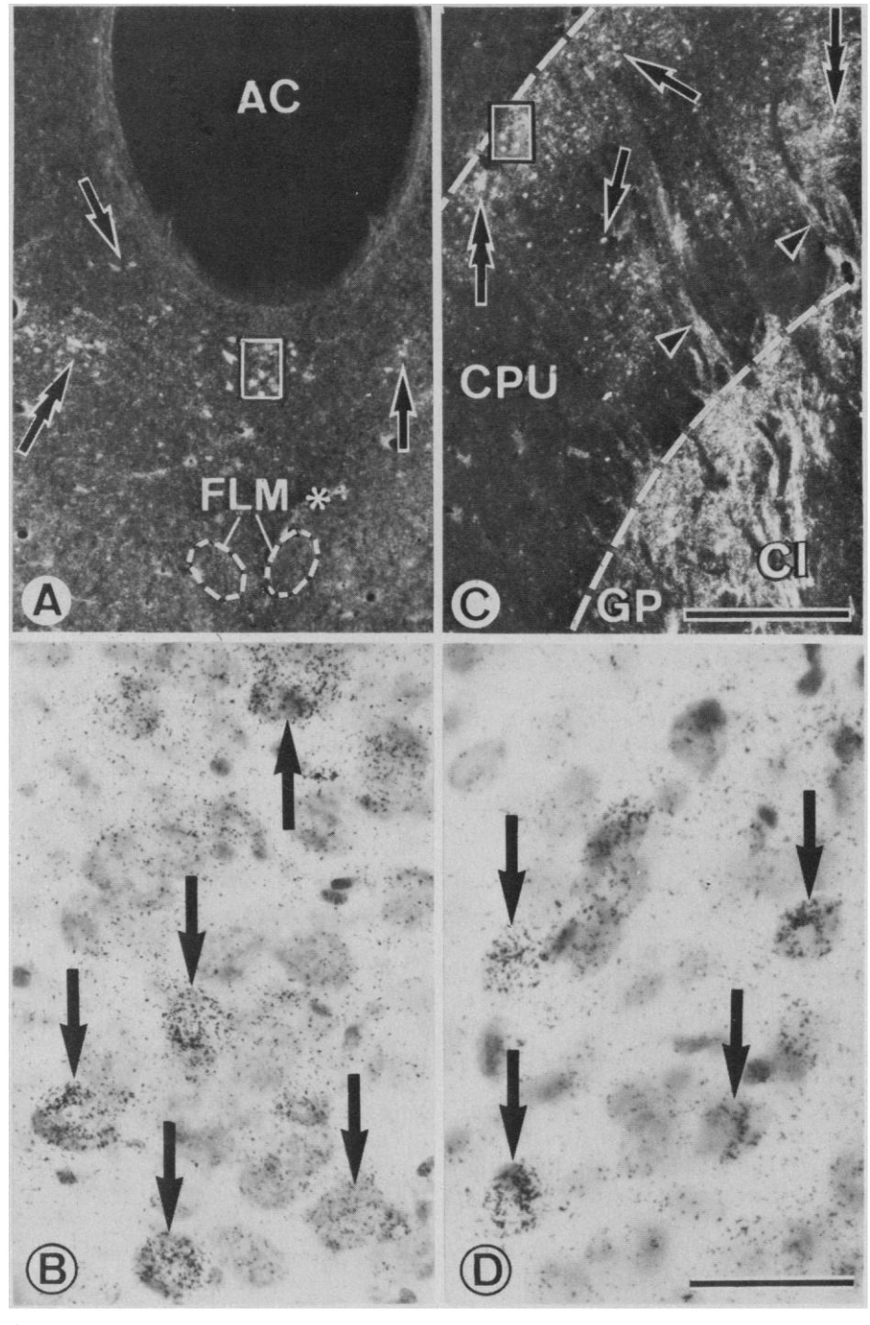

Fig. 1. Light microscopic autoradiographs showing neuronal perikarya labeled by retrograde transport (arrows) 6 hours after nigral injection of [^{3}H]serotonin (A and B) or of [^{3}H]GABA (C and D). (A) Pattern of labeling in the nucleus raphe dorsalis. Clustered perikarya are indicated by double arrows. Abbreviations: *AC*, aquaeductus cerebri; *FLM*, fasciculus longitudinalis medalis. The asterisk marks a blood vessel. The rectangle is enlarged in (B). (B) Accumulations of silver grains over neural cell bodies. (C) Labeling of perikarya and small fiber bundles (arrow heads) in the caudoputamen (*CPU*). Many strongly labeled fibers can be seen in capsula interna (*CI*). Abbreviation: *GP*, globus pallidus. (D) Enlargement of rectangle in (C). (A and C) Dark-field illumination; bar, 500 μm. (B and D) Bright-field illumination; cresylecht violet staining; bar, 50 μm.

teraural line (*7*)]. The number of silver grains overlying a neuron varied considerably from one cell to another. The silver grains overlying a perikaryon were often not single but clustered in small groups. Only medium-sized (12 to 15 μm) (none of the rarer "giant") neurons were labeled. The majority of labeled perikarya were localized in a more external part of the caudoputamen, close to the unlabeled subcortical white matter. The labeling in fiber bundles in the neuropil and around labeled cell bodies was probably at least partly due to the migration of radioactive material in the axons back to the striatal perikarya. In the globus pallidus, the high density of silver grains over fiber bundles did not permit us to determine whether certain neurons were labeled (*12*). In the [^{3}H]GABA cases we saw no perikaryal labeling in the cortex or in the nucleus raphe dorsalis. In both of these areas, labeling was easily detected after HRP was injected into the nigra. In the HRP cases, the labeling in the caudoputamen extended over a wider rostro-caudal region than it did in the [^{3}H]GABA experiments. The type of neurons labeled was the same in both kinds of experiments. The results obtained with [^{3}H]GABA suggest that striato-nigral neurons containing GABA were selectively labeled, although it was not possible to control for false-positive perikaryal labeling in striatal neurons with neurotransmission mediated by substance P (*13*). Substance P neurons had been localized, however, in more rostral portions of the caudoputamen than the ones in which the labeled perikarya were seen (*14*).

No perikaryal labeling was observed after nigral injection of GABA precursors like glutamate (0.05 μl of L-[G-^{3}H]glutamic acid, 14 μCi; specific activity, 28 Ci/mmole; Radiochemical Centre) or glutamine (0.05 μl of L-[G-^{3}H]glutamine, 15 μCi; specific activity, 21 Ci/mmole; Radiochemical Centre) (*15*). These negative findings might indicate that newly synthetized transmitter did not get into compartments for retrograde axonal migration in amounts high enough to be detected in the perikaryon. The results demonstrated, in addition, a high degree of chemospecificity for the retrograde perikaryal labeling.

The labeling of nigral afferents originating in the cortex, the caudoputamen, and the nucleus raphe dorsalis only with the unspecific tracer HRP confirms earlier studies. A differential pattern was found, however, with radioactive transmitters. After [^{3}H]serotonin injection into the substantia nigra, neurons were retrogradely labeled in the dorsal raphe nucleus but not in the caudoputamen, whereas after [^{3}H]GABA injections the labeling pattern was the opposite. These results support the hypothesis of transmitter-specific retrograde tracing. Certain limitations in the chemospecificity are suggested in the case of serotonin by slight cortical labeling. Although the new method is not yet established, it may prove a useful tool for indicating simultaneously the transmitter and the connectivity of labeled neurons. Whether transmitter-specific retrograde labeling is based on selective uptake and active axonal transport and whether it is biologically meaningful (for example, in signaling the state of the terminal to the neuronal perikaryon) remains to be investigated.

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Hybrid Ape Offspring of a Mating of Gibbon and Siamang

Abstract. *The serendipitous mating of a male gibbon,* Hylobates moloch, *and a female siamang,* Symphalangus syndactylus, *has produced two female offspring born 1 year apart. The hybrid karyotype of 47 chromosomes comprises the haploid complements of the parental species, 22 for the gibbon and 25 for the siamang. Chromosomal G and C banding comparisons revealed no clear homologies between the parental karyotypes except for the single chromosome in each species containing the nucleolus organizer region. The lack of homology suggests that the structural rearrangement of chromosomes has played a major role in the process of speciation for these lesser apes.*

On 11 August 1975 a siamang, *Symphalangus syndactylus*, gave birth to a female offspring fathered by a silver-gray gibbon, *Hylobates moloch*. Karyotypic analysis of the offspring, locally termed a siabon (*1*), reveals a complement of 47 chromosomes. This is the first reported viable hybrid of apes (*2*). The unexpected mating was made possible when in 1971 two adolescent female siamangs and one adolescent male gibbon were housed together at Atlanta's Grant Park Zoo as an exhibit of lesser apes. The siabon was abandoned by the mother at 3 months of age and was raised at the Primate Behavior Laboratory of Georgia State University apart from other apes. She has been given routine care and maintained in excellent health. A second female offspring, born 30 August 1976 to the same pair, died at 4 1/2 months of age from complications of an infection that

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