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17. Sediment samples of approximately 25 cm^3 were dried at 50°C, disaggregated in a hot Calgon solution, wet-sieved through a 63-μm screen and dried at 50°C. Whole foraminifera tests (mixed planktonic species; size fraction, 150 μm), were hand-picked. Between 1 and 3 mg of foraminifera were used for isotopic analysis; about 1 mg was dissolved for atomic absorption spectrophotometry. Pore water sample size was 0.5 ml.
18. For isotopic analysis, foraminifera tests were cleaned by repeated sonication in ultrapure water. The cleaned tests were dissoved in 2.5*N* HCl, and the strontium fraction was separated by cation exchange column. This solution was loaded in a drop of tantalum oxide–phosphoric acid slurry on a single rhenium filament for isotopic analysis. Pore water samples were evaporated to dryness and salts taken up in 2.5*N* HCl before undergoing the same analytical procedure. Analyses were done at URI on a VG Micromass 30B single collector, double focusing, thermal ionization mass spectrometer. Measured blanks for the procedure are negligible, giving uncertainties less than the in-run errors in Tables 2 and 3 for the 95 percent confidence level. A split of each foraminifera sample was dissolved in dilute HCl and analyzed for strontium and calcium by flameless and flame atomic absorption spectrophotometry, respectively. From these data, Sr/Ca ratios were calculated.
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46. Mass balance calculations involved mixing meteoritic and vaporized crustal strontium into the volume of the ocean, and then calculating the change in the seawater $^{87}Sr/^{86}Sr$. End member cases considered were (i) 0.1 times bolide mass of crustal material vaporized (*43*), and (ii) 10 times bolide mass of crustal material vaporized. We assumed full solubility and dispersal of vaporized meteoritic and crustal strontium in seawater; a bolide size (*39*) of 10^{18} g; strontium in ocean (*2*), 87×10^{-6} mol/kg; volume of ocean (*2*), 1.37×10^{21} liters; $^{87}Sr/^{86}Sr$ of Late Cretaceous ocean, 0.707865; $^{87}Sr/^{86}Sr$ of continental dust, 0.722 (average crust) and 0.800 (upper limit); strontium in upper continental crust (*47*), 350 μg/g. Resulting model predictions for the increase in seawater $^{87}Sr/^{86}Sr$ are (i) average crustal $^{87}Sr/^{86}Sr$, 0.00000003; upper limit $^{87}Sr/^{86}Sr$, 0.0000003; (ii) average crustal $^{87}Sr/^{86}Sr$, 0.0000026; upper limit, 0.000030. The observed change is about 0.0001.
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49. We thank P. Delaney, P. A. Baker, J. P. Kennett, J. Zachos, and the Deep Sea Drilling Project for samples; H. Elderfield and J. Gieskes for encouragement during the early stages of this work; R. Matthews and K. Turekian for discussions on the K/T boundary; R. Kingsley, B. Hannan, M. Cole, G. Waggoner for assistance with mass spectrometry; and R. B. Beach for assistance in the preparation of this manuscript. Supported by NSF grants OCE82-07787 and -8501916.

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Evidence for the Involvement of *GM-CSF* and *FMS* in the Deletion (5q) in Myeloid Disorders

MICHELLE M. LE BEAU,* CAROL A. WESTBROOK, MANUEL O. DIAZ, RICHARD A. LARSON, JANET D. ROWLEY, JUDITH C. GASSON, DAVID W. GOLDE, CHARLES J. SHERR

By in situ chromosomal hybridization, the *GM-CSF* and *FMS* genes were localized to human chromosome 5 at bands q23 to q31, and at band 5q33, respectively. These genes encode proteins involved in the regulation of hematopoiesis, and are located within a chromosome region frequently deleted in patients with neoplastic myeloid disorders. Both genes were deleted in the 5q- chromosome from bone marrow cells of two patients with refractory anemia and a del(5)(q15q33.3). The *GM-CSF* gene alone was deleted in a third patient with acute nonlymphocytic leukemia (ANLL) who has a smaller deletion, del(5)(q22q33.1). Leukemia cells from a fourth patient who has ANLL and does not have a del(5q), but who has a rearranged chromosome 5 that is missing bands q31.3 to q33.1 [ins(21;5)(q22;q31.3q33.1)] were used to sublocalize these genes; both genes were present on the rearranged chromosome 5. Thus, the deletion of one or both of these genes may be important in the pathogenesis of myelodysplastic syndromes or of ANLL.

COLONY-STIMULATING FACTORS (CSF's) are required for the growth and maturation of myeloid progenitor cells in vitro (*1, 2*). The CSF's are classified according to their cell specificity; hematopoietic progenitor cells respond to CSF's by producing different types of mature blood cells. For example, in the murine system, multi-CSF (IL-3) stimulates the progenitor cells of most of the hematopoietic cell lineages (*3*), whereas GM-CSF stimulates the proliferation of cells from the granulocyte, granulocyte/macrophage, and macrophage lineages (*4*). M-CSF (CSF-1) (*5*) and G-CSF (*6*) primarily stimulate cells committed to the macrophage and granulocyte lineages, respectively. Human GM-CSF has been purified from medium conditioned by a T-lymphoblast cell line (*7*), and complementary DNA (cDNA) clones were isolated from an expression library prepared from messenger RNA (mRNA) (*8*). The purified recombinant GM-CSF has all of the biological properties attributed to natural GM-CSF (*9*). More recently, genomic sequences encoding human GM-CSF have been cloned (*10*). CSF's exert their effects on hematopoietic cells via specific receptors. At present, the cellular receptors specific for CSF's have not been isolated; howev-

M. M. Le Beau, C. A. Westbrook, M. O. Diaz, R. A. Larson, J. D. Rowley, Joint Section of Hematology/Oncology, Department of Medicine, University of Chicago, Chicago, IL 60637.
J. C. Gasson and D. W. Golde, Division of Hematology/Oncology, School of Medicine, University of California, Los Angeles 90024.
C. J. Sherr, Department of Tumor Cell Biology, St. Jude Children's Research Hospital, Memphis, TN 38105.

*To whom correspondence should be addressed at Box 420.

er, the proto-oncogene *FMS* encodes a protein that appears to be related or identical to the receptor for CSF-1 (*11*).

Because GM-CSF plays an important role in the differentiation and proliferation of white blood cells, the gene encoding this protein may be involved in the nonrandom chromosomal abnormalities associated with neoplastic hematologic disorders. To determine the chromosomal localization of the *GM-CSF* gene, we performed in situ chromosomal hybridization to normal human metaphase cells with the probe pCH-5.2, which contains the entire human *GM-CSF* gene (*10*). This hybridization resulted in specific labeling only of chromosome 5. Of 100 metaphase cells examined, 20 were labeled on region q2 or q3, specifically at bands q23 to q32 of one or both chromosomes 5 (Fig. 1, left). This labeling was significant as determined by χ^2 analysis ($P < 0.0005$). A total of 32 grains were observed on this chromosome; of these, 21 (66 percent) were clustered at bands 5q23 to q32 and represented 15 percent (21/140) of all labeled sites. This localization is very similar to that obtained independently by Huebner *et al.* with the same probe (*10*).

Another gene, the proto-oncogene *FMS*, had previously been localized to this same region of chromosome 5, namely to band q34 (*12*). The retroviral oncogene v-*fms* (McDonough strain of feline sarcoma virus) encodes a glycoprotein with an associated tyrosine-specific protein kinase activity whose transport to the cell membrane is required for malignant transformation (*13*). The product of the feline *FMS* proto-oncogene is also a transmembrane glycoprotein with associated tyrosine kinase activity (*14*), whose expression in adult cats appears to be limited to cells of the monocyte-macrophage lineage (*11*). As expected, *FMS* mRNA is present at high levels in human peripheral blood monocytes (*15*). *FMS* encodes a protein that is related and possibly identical to the receptor for CSF-1 (*11*). Thus, the *FMS* product appears to play an integral role in proliferation and differentiation of cells of the mononuclear phagocytic lineage.

The *FMS* locus is included within the region of chromosome 5 that is deleted in bone marrow cells from three patients with the refractory anemia 5q− syndrome (*16*); the distal breakpoint in each patient was thought to be proximal to the previously mapped location at 5q34 (*12*). To clarify the sublocalization of *FMS* on human chromsomes, we performed in situ chromosomal hybridization of an *FMS*-specific probe to normal human metaphase cells. In this case, also, we noted specific labeling only of chromosome 5. Of 100 metaphase cells, 31 were labeled on region q2 or q3 (bands q22 to q35) of one or both chromosome 5 homologues (Fig. 1, right; $P < 0.0005$). Clusters of grains were noted at two regions; the larger cluster, observed at bands q31 to q33, represented 15.3 percent (23/150) of all labeled sites. A smaller cluster of grains was noted at band q35, representing 4.7 percent (7/150) of all labeled sites. Thus, *FMS* is located at bands 5q31 to q33.

Loss of a whole chromosome 5 or loss of a part of the long arm of chromosome 5 [deletion (5q)] has been observed in the malignant cells of patients with acute nonlymphocytic leukemia (ANLL) arising either de novo (ANLL–de novo) or secondary to cytotoxic therapy for a previous malignant disease (t-ANLL) (*17*). The relatively high frequency of loss of chromosome 5 or del(5q) in patients with t-ANLL, and the relative absence of these abnormalities in the leukemic cells of patients with ANLL–de novo who are under the age of 30, has led to the suggestion that these abnormalities may be a marker of mutagen-induced leukemia (*18–20*).

A distinct clinical, morphological, and cytogenetic syndrome associated with a deletion of 5q (bands q13 to q33) is also seen in older patients, especially in females (*21*). Morphologically, this 5q− syndrome is characterized by refractory anemia, the presence in the bone marrow of abnormal megakaryocytes with mono- or bilobulated nuclei, and normal or elevated platelet counts. Patients who have only a del(5q) tend to have a relatively mild clinical course that usually does not progress to acute leukemia, whereas in patients with leukemia, the del(5q) is usually accompanied by additional chromosomal abnormalities.

The chromosome 5 deletions observed in patients with the 5q− syndrome, ANLL–de novo, and t-ANLL appear to be relatively similar; there is a proximal breakpoint commonly in q13 to q15 and a distal breakpoint within region q3, at bands q31 to q35 (*19–21*). In a series of 50 patients [all of whom had a del(5q)] with t-ANLL, ANLL–de novo, or a myelodysplastic syndrome (MDS), 25 patients appeared to have a deletion of all of q33, suggesting a break in 5q33.3 (*19, 22*). Breakpoints in 5q31–32 or

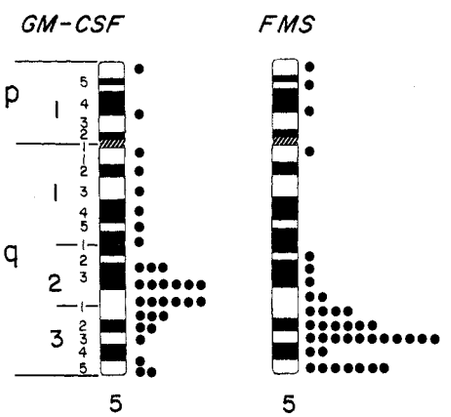

Fig. 1. Distribution of labeled sites on chromosome 5 in normal metaphase cells that were hybridized to *GM-CSF* or to *FMS* probes. Human metaphase cells prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes were hybridized with the ^{3}H-labeled *FMS*- and *GM-CSF*–specific probes. For the in situ hybridizations, we used a 3.0-kilobase (kb) Eco RI fragment containing 5′ sequences of the human *FMS* gene (*16*) cloned in pUC9; for the *GM-CSF* probe (pCH-5.2), a 5.2-kb Hind III fragment of genomic DNA containing three exons of the *GM-CSF* gene cloned in pBR322 was used (*10*). Radiolabeled probes were prepared by nick translation of the plasmid DNA's with tritiated deoxynucleoside triphosphates to specific activities of 9.0×10^7 dpm/μg (*GM-CSF*) and 2.0×10^8 dpm/μg (*FMS*). Hybridization was performed as described previously (*25*). Metaphase cells were hybridized at 20 and 4 ng of probe per milliliter (*GM-CSF*) and at 4 and 2 ng of probe per milliliter (*FMS*) of hybridization mixture.

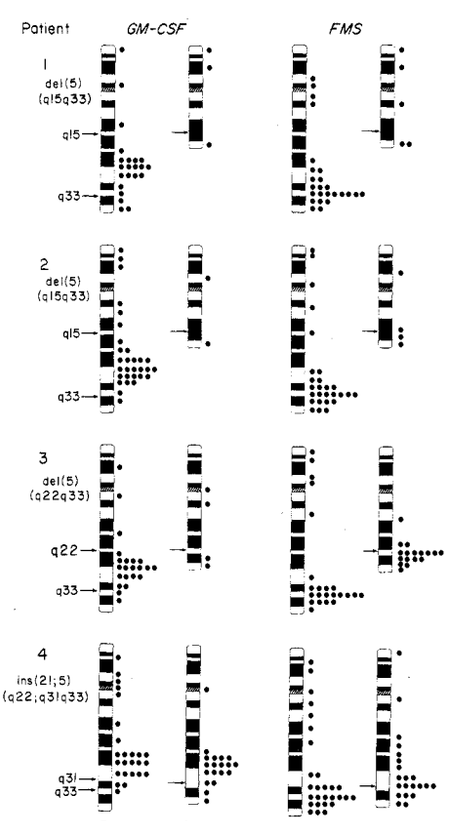

Fig. 2. Distribution of labeled sites on the normal chromosome 5 and on the rearranged homologues in metaphase cells from patients 1, 2, and 3 with a del(5q) and from patient 4 with an ins(21;5) that were hybridized with *GM-CSF* or *FMS* probes. The arrows to the right of the normal chromosome 5 (far left) illustrate the breakpoints and the segment that is deleted in each patient. The arrows on the 5q− chromosomes identify the breakpoint junctions.

Table 1. Hybridization of *GM-CSF*– and *FMS*-specific probes to metaphase cells from bone marrow of three patients with a del(5q) and from one patient with an ins(21;5). For each probe, 100 cells were analyzed from patients 2 to 4; for patient 1, 82 cells were examined with *GM-CSF* and 62 cells with *FMS*.

Patient	Total number of labeled sites	*GM-CSF*–labeled sites (%)				Total number of labeled sites	*FMS*-labeled sites (%)			
		Normal chromosome 5		5q−			Normal chromosome 5		5q−	
		Total	Bands q23 to q33	Total	Bands q23 to q33		Total	Bands q31 to q35	Total	Bands q31 to q35
1	101	22 (21.8)*	15 (18.3)	3 (2.9)	—	84	25 (29.8)*	20 (23.8)	5 (6)	2 (2.4)†
2	131	30 (23)*	20 (15.3)	2 (1.5)	—	134	27 (20)*	22 (16.4)	4 (3.0)	3 (1.5)†
3	128	22 (17.2)*	18 (14)	4 (3)	—	143	23 (16)*	18 (12.6)	16 (11.2)*	14 (9.8)‡
4	150	25 (16.7)*	18 (12)	19 (12.7)*	15 (10)†	164	28 (17)*	21 (12.8)	21 (12.8)*	14 (8.5)§

*χ^2 value corresponds to $P < 0.0005$. The χ^2 analysis tests the hypothesis that labeling is random over the entire genome. In all other cases, χ^2 values corresponded to $P > 0.05$. †Bands q15 to q33.3 are deleted. ‡Bands q22 to q33.1 are deleted. §Bands q31.3, q32, and q33.1 were inserted into the long arm of chromosome 21.

in 5q34–35 were observed in 15 and in 10 patients, respectively. The single most common deletion extended from 5q13 to 5q33. In three other reports describing del(5q), 5q33 was the most common breakpoint (*20*). We showed in 17 patients with a del(5q) and t-MDS or t-ANLL that, although the breakpoints were somewhat variable, one segment of 5q was consistently lost in all patients. This segment, called the critical region, includes bands q23 to q32 (*19*), the site of *GM-CSF*. Thus, it is likely that the loss of genes located within this critical region is important in the pathogenesis of these hematologic disorders.

In hybridizations of the *GM-CSF* probe to metaphases from bone marrow cells of patients 1 and 2 [who have the 5q− syndrome; del(5)(ql5q33.3)], and patient 3 [who has t-ANLL characterized by del(5)(q22q33)], specific labeling was observed on the normal chromosome 5, but not on the rearranged homologue (Fig. 2, left, and Table 1). Thus, the *GM-CSF* locus was deleted in these patients as a result of an interstitial deletion of 5q. These findings contrast with those observed in the hybridization of the *GM-CSF* probe to metaphase cells from patient 4 who has ANLL–de novo and a rearranged chromosome 5 resulting from an insertion rather than a deletion [ins(21;5)(q22;q31.3q33.1)]. Metaphase cells from this patient are particularly useful for the sublocalization of *GM-CSF* and *FMS*, since the ins(21;5) results in a rearranged chromosome 5 that is missing band q32 as well as a small part of the adjacent bands. Here, clusters of grains were found on the long arm on both the normal chromosome 5 and on the rearranged homologue. The rearranged chromosome 21, containing bands 5q32 along with distal 5q31 and proximal 5q33, was not labeled significantly ($P > 0.8$).

In each of the hybridizations of *GM-CSF* described above, the cluster of grains on the normal chromosome 5 extended from bands 5q23 to q33; however, the majority of labeled sites were noted on 5q23 and q31. Therefore, we would expect the *GM-CSF* locus to be within the deleted segment in patients who have an interstitial deletion of chromosome 5 for which the distal breakpoint is in 5q33. The presence of this gene on the rearranged chromosome 5 from patient 4, which is missing band q32 and the proximal portion of q33, strongly suggests that *GM-CSF* is located either in band 5q23 or in q31.

FMS was located within the deleted segment in patients 1 and 2, in whom the distal breakpoint occurred in band q33.3 (the distal portion of q33), whereas this gene was present in the del(5q) chromosome in patient 3, and in the rearranged chromosome 5 in patient 4, whose distal breakpoints were proximal to 5q33.2 or 5q33.3 (Fig. 2, right, and Table 1). This result indicates that *FMS* is located in band 5q33.2 or q33.3, rather than in 5q34-35 as described previously (*12*).

Our results and those of previous studies (*16*) have demonstrated that *GM-CSF* and *FMS* are included in the segment that is deleted from chromosome 5 in the 5q− syndrome and in ANLL. Thus, these genes, which code for proteins involved in hematopoiesis, may play some role in the pathogenesis of these disorders. The extent of the chromosomal deletions observed in the 5q− syndrome and in acute leukemia appears to be similar, with a proximal breakpoint in bands q13 to q15 and a distal breakpoint in band q33. However, in several patients with refractory anemia, ANLL–de novo, or t-ANLL (including patient 3), we have observed a distal breakpoint in band q31, q32, or q33.1, suggesting that the *FMS* locus in q33 is preserved in these leukemic cells.

The relative variability of the breakpoints noted in the deletion of chromosome 5 suggests that the loss of a critical DNA sequence may be essential for malignant transformation rather than the consistent juxtaposition of two genes as in the case of c-*abl* and *bcr* in chronic myelogenous leukemia (*23*). The identification of a critical region (a chromosomal segment that is deleted in all patients with this rearrangement) further supports this hypothesis. The gene-dosage effect of a deletion of a *GM-CSF* or *FMS* allele may be sufficient to result in a reduction in the concentration of the gene products. However, this effect is not inevitable in that these genes may be regulated in such a way as to maintain a constant product output, irrespective of their dosage. A more likely outcome of a deletion would be the loss of a wild-type gene, thereby allowing the expression of a recessive mutant allele on the homologous chromosome. A similar mechanism has been proposed for the pathogenesis of retinoblastoma and Wilms' tumor (*24*).

For hematologic disorders associated with a del(5q), *GM-CSF* and *FMS* are appropriate candidates for genes whose altered function may result in abnormal hematopoiesis. However, because some patients appear to have a distal breakpoint at q31 to q33.1, apparently leaving the *FMS* locus intact, *GM-CSF* and *FMS* may have different roles in these disorders. Moreover, we cannot exclude the possibility of involvement of an additional gene or genes coding for other CSF's (or their receptors) that may be clustered on 5q, along with other genes of unrelated functions. Detailed molecular characterization of the *GM-CSF* and *FMS* loci and their encoded products in patients with these disorders is warranted.

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Selectivity of the Parkinsonian Neurotoxin MPTP: Toxic Metabolite MPP$^+$ Binds to Neuromelanin

ROBERT J. D'AMATO, ZOE P. LIPMAN, SOLOMON H. SNYDER*

Methylphenyltetrahydropyridine (MPTP) selectively destroys neuronal cell bodies in the melanin-containing substantia nigra of humans and other primates. We show that methylphenylpyridine (MPP$^+$), an active metabolite of MPTP which is accumulated intraneuronally by the catecholamine uptake system, binds with high affinity to melanin and neuromelanin. MPP$^+$ bound intracellularly to neuromelanin may be released gradually, resulting in subsequent damage to the neurons of the substantia nigra.

METHYLPHENYLTETRAHYDROPYRIDINE (MPTP), a by-product in the synthesis of certain illicit opiates, selectively destroys dopamine neurons of the substantia nigra and produces parkinsonism in human subjects (*1, 2*) and parkinsonian symptoms in animals (*3, 4*). The selective destruction of these neurons by MPTP appears to depend on several factors. MPTP initially binds with high affinity to the enzyme monoamine oxidase (type B) (*5–9*). The conversion by monoamine oxidase of MPTP to methylphenylpyridine (MPP$^+$) is required for neurotoxicity, since prior treatment of animals with monoamine oxidase inhibitors prevents MPTP neurotoxicity (*10, 11*). The initial selectivity of MPP$^+$ for catecholamine neurons appears attributable to the high affinity of MPP$^+$, but not MPTP, for the catecholamine uptake system. Accordingly, catecholamine neurons can concentrate MPP$^+$ to levels many times greater than the surrounding extracellular fluid (*12*).

Several features of MPTP toxicity remain difficult to explain. There are marked species differences in susceptibility to MPTP neurotoxicity. In humans and monkeys, there is an extensive loss of neurons in the substantia nigra at very low doses of MPTP, while in rodents, even at higher doses, there are no permanent deficits. In mice depletion of striatal dopamine can be produced by high doses of MPTP, but neuronal cell bodies are not destroyed (*13, 14*). Monkeys and humans have a high content of neuromelanin in the substantia nigra while little or no neuromelanin exists in the substantia nigra of rodents. Furthermore, the susceptibility of monkeys to MPTP appears to increase with age (*15*) corresponding to the age-related increase of neuromelanin in their substantia nigra. Lyden *et al.* (*16*) described binding of MPTP to synthetic melanin, but only low affinity interactions were observed. We now report high affinity binding of MPP$^+$, the active metabolite of MPTP, to synthetic melanin and neuromelanin which can explain important aspects of MPTP neurotoxicity.

Melanin can arise by the autoxidation and polymerization of tyrosine, dihydroxyphenylalanine (dopa), dopamine, or norepinephrine. In skin, the synthesis of melanin is catalyzed by the enzyme tyrosinase, while in the brain it is not clear whether the formation of neuromelanin is enzymatic or nonenzymatic. Das *et al.* (*17*) showed that neuromelanin of the substantia nigra is similar to the type formed by dopamine autoxidation. Initially we examined ligand binding to synthetic melanin that was synthesized from dopamine or norepinephrine by autoxidation to model neuromelanin of the substantia nigra or locus coeruleus, respectively (*18*). [^{3}H]MPP$^+$ bound with high affinity to dopamine melanin (Fig. 1). Scatchard analysis of equilibrium-saturation data re-

Table 1. Equilibrium constants for MPP$^+$ and MPTP binding to melanin. Binding constants were calculated by computer fit (*31*). Saturation analysis was performed for [^{3}H]MPP$^+$ and [^{3}H]MPTP binding to various melanin preparations using 14 concentrations of ligand as described in (*32*). Values reported are the means ± standard error of four independent determinations. DA, dopamine; NE, norepinephrine.

	K_D (n*M*)	B_{max} (nmol/mg melanin)
[^{3}H]MPP$^+$		
DA Melanin	28.0 ± 4.0	1.00 ± 0.17
NE Melanin	32.0 ± 3.1	0.49 ± 0.08*
[^{3}H]MPTP		
DA Melanin	39.0 ± 5.3	0.27 ± 0.06
NE Melanin	37.0 ± 3.2	0.03 ± 0.001†

*Differs from DA melanin value, $P < 0.05$. †Differs from DA melanin value, $P < 0.001$. A modified Student's *t*-test which accounts for unequal variance between groups was used.

R. J. D'Amato and Z. P. Lipman, Department of Neuroscience, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205. S. H. Snyder, Departments of Neuroscience, Pharmacology and Experimental Therapeutics, and Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, 725 North Wolfe Street, Baltimore, MD 21205.

*To whom correspondence and reprint requests should be addressed.