GTTCCTGACCTTGATGTG-3'. The final concentration of each primer in the reaction mixture was 1.0 μM. The 200 ng of DNA served as template under reaction conditions recommended by the manufacturer (United States Biochemical) except that two sets of primers were used and 0.4 μl of (α-^{32}P)dCTP 800 Ci/mmol were added to each reaction. Amplification was for 20 cycles with an annealing temperature of 58°C for the *MDR*1 primers and 55°C for the Neo primers. We separated amplified products on a 5% nondenaturing polyacrylamide gel and visualized them by autoradiography of the dried gel.

15. Mice were killed by cervical dislocation, and bone marrow cells were flushed from long bones. Total cellular RNA (cRNA) was prepared by extraction in guanidine isothiocyanate and treated with DNase I. The 2 μg of RNA were reverse transcribed and PCR amplified as previously described [B. P. Sorrentino *et al.*, *Nucleic Acids Res.* **18**, 2721 (1990)]. Using the above described β-globin or MDR primers in separate reactions, we amplified for 30 cycles with an annealing temperature of 58°C.
16. Taxol was obtained from the Developmental Therapeutics Branch of the National Cancer Institute and was dissolved in 30% dimethyl sulfoxide (DMSO), 30% ethanol, and 40% sterile water immediately before use. This carrier had no effect on peripheral blood counts. White blood counts were measured both by manual counting and automated Coulter analysis of each sample. White blood cell differentials on Wright stained blood films were performed on 100 cells per sample and used to calculate the absolute neutrophil counts.
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Infection of *Macaca nemestrina* by Human Immunodeficiency Virus Type–1

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After observations that *Macaca nemestrina* were exceptionally susceptible to simian immunodeficiency virus and human immunodeficiency virus type–2 (HIV-2), studies of HIV-1 replication were initiated. Several strains of HIV-1, including a recent patient isolate, replicated in vitro in peripheral blood mononuclear cells (PBMCs) and in CD4-positive *M. nemestrina* lymphocytes in a CD4-dependent fashion. Eight animals were subsequently inoculated with either cell-associated or cell-free suspensions of HIV-1. All animals had HIV-1 isolated by cocultivation, had HIV-1 DNA in their PBMCs as shown by polymerase chain reaction, and experienced sustained seroconversion to a broad spectrum of HIV-1 proteins. *Macaca nemestrina* is an animal model of HIV-1 infections that provides opportunities for evaluating the pathogenesis of acute HIV-1 replication and candidate vaccines and therapies.

A relevant animal model (*1*) for human lentivirus infection (*2*, *3*) is needed for the evaluation of the effectiveness of vaccines and drugs for HIV-1 infection. Asian macaques infected by the related simian immunodeficiency virus (SIV) (*4*, *5*) or HIV-2 (*6*) have been used to study the acquired immunodeficiency syndrome (AIDS). Although this animal model has contributed to our understanding of viral pathogenesis, SIV and HIV-2 differ from HIV-1 in their sequence homology and antigenic structure (*7*). These viral sequence differences are especially pertinent to HIV-1 vaccine development, in which an animal model that would allow testing of candidate HIV-1 vaccines would be particularly useful. Among nonhuman primates, only the endangered chimpanzee and gibbon ape are susceptible to HIV-1 infection (*8*–*10*). We evaluated *Macaca nemestrina* (pigtail macaques) as a model for HIV-1 infection on the basis of clinical and virological observations that suggested this species was more severely affected by SIV and HIV-2 infection than *Macaca mulatta* or *Macaca fascicularis* (*11*).

Four HIV-1 strains were tested for their ability to replicate in macaque PBMCs in vitro: HIV-1_{LAI}, obtained from the Pasteur Institute (*2*, *12*), HIV-1_{NL4-3} [a molecular clone (*13*)], HIV-1_{JR-CSF} [the molecular clone of an isolate originally cultured from the cerebrospinal fluid of a patient with AIDS dementia (*14*)], and HIV-1_{RO} (a recent patient isolate). Stocks of each isolate were prepared and titrated on human PBMCs (*15*). We initially assessed the ability of HIV-1_{LAI} to infect the PBMCs from three species of macaques and humans. In addition, we infected the macaque PBMCs with SIV_{mne} (*16*) and heat-inactivated HIV-1 and SIV. The HIV-1_{LAI} replicated efficiently in both *M. nemestrina* and human PBMCs, whereas no detectable replication occurred in *Macaca mulatta* or *Macaca fascicularis* PBMCs (Table 1). The SIV_{mne} replicated in PBMCs from all three macaque species, but viral antigens were not detected in the cultures infected with the heat-inactivated viruses. Furthermore, HIV-1_{NL4-3}, HIV-1_{JR-CSF}, and HIV-1_{RO} replicated in the *M. nemestrina* cells as assayed by HIV-1 antigen production (Table 1).

We further characterized the in vitro infection by determining whether *M. nemestrina* CD4-positive cells were the target of HIV-1 infection. Unfractionated PBMCs and purified CD4 cells, isolated from *M. nemestrina* and humans, were infected with HIV-1_{LAI}. The HIV-1 replicated with similar efficiency and kinetics in the macaque and human CD4-positive cells. The HIV-1 antigen in culture supernatants was detected sooner and at higher titers in the CD4-enriched cell populations (Table 1) as compared to that in the unfractionated cells, which is consistent with the known affinity of HIV-1 for CD4-positive cells in humans (*17*). We abrogated the infection by first treating the virus with human recombinant soluble CD4. Syncytial formation was observed microscopically in these cultures, although infrequently when compared to that in human CD4 cells infected by HIV-1 (*18*).

To provide further evidence of HIV-1 replication in *M. nemestrina* PBMCs, we examined de novo viral protein synthesis by radioimmunoprecipitation analysis (*19*). HIV-1–specific protein synthesis could be detected by day 5 in the macaque PBMCs (Fig. 1). The migration of the proteins from HIV-1–infected cells was distinct from the migration of proteins from SIV- and HIV-2–infected cells; this was particularly true for the envelope glycoprotein and the major gag proteins. Further, monoclonal antibodies specific for HIV-1 *env* and *gag* gene products (*20*) recognized viral proteins synthesized in cells infected by HIV-1, but not in cells infected by SIV_{mne} or HIV-2 (*18*).

We evaluated whether the in vitro HIV-1 infection in macaque PBMCs resulted in the formation of complete virus particles. Seven days after infection, electron microscopy revealed that large amounts of mature virions with the characteristic lentivirus morphology were associated with cells or cellular processes. Occasional budding forms associated with the plasma membrane of cultured

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macaque lymphocytes were also seen (*21*).

To verify that infectious virions were produced in PBMCs from M. *nemestrina*, we passaged culture supernatant from day 7 HIV-1_{LAI}–infected PBMCs to two susceptible, CD4-expressing human cell lines, AA2 (*22*) and C8166 (*23*), and to human PBMCs (Table 2). Production of HIV-1 antigen was demonstrated for all cells, which reflects the presence of infectious virus in the cell-free culture supernatant from the M. *nemestrina* PBMC culture on day 7. Culture supernatant from day 0 did not cause infection, which confirms that the passaged virus originated from the replication of HIV-1 in M. *nemestrina* PBMCs and not as part of the original inoculum.

To test whether M. *nemestrina* was susceptible to HIV-1_{LAI} infection, we inoculated two macaques (F89111 and F89115) with previously infected autologous PBMCs suspended in cell-free stock virus supernatants (*24*). To recover virus from the inoculated macaques, we cocultivated PBMCs with C8166 cells. The HIV-1 antigen was detected in coculture supernatants from animal F89111 at weeks 1, 2, 4, 8, and 24 and in coculture supernatants from animal F89115 at 8 weeks after inoculation. The virus recovered at week 24 from F89111 was titrated and reinoculated onto M. *nemestrina* PBMCs and C8166 cells. Immunoprecipitation analysis from radiolabeled extracts with the use of HIV-1–specific antisera (Fig. 1) revealed a pattern similar to that reported earlier (*19*), which confirms that the recovered virus was indeed HIV-1.

DNA from PBMCs was analyzed directly by polymerase chain reaction (PCR) for proviral sequences in HIV-1–infected animals. Primer pairs that flanked HIV-1–

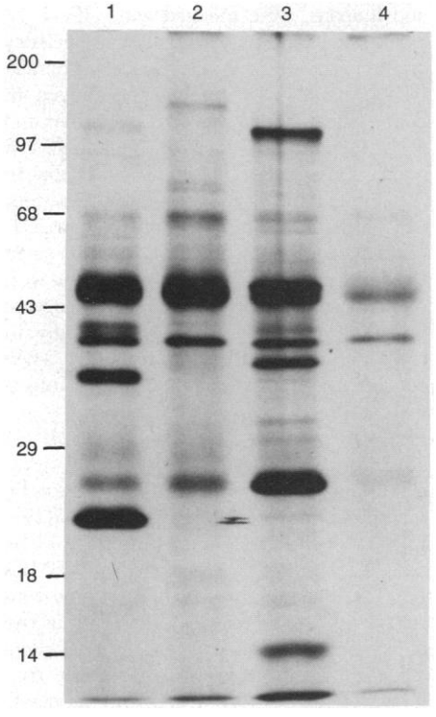

Fig. 1. Examination of de novo viral protein synthesis in *M. nemestrina* PBMCs infected by HIV-1_{LAI} (lane 1), HIV-2_{EHO} (lane 2), or SIV_{mne} (lane 3) at a multiplicity of 0.1 TCID per cell, or mock-infected (lane 4). At 7 days after inoculation, 2 × 10^6 viable cells were labeled with [^{35}S]methionine-cystiene for 4 hours. Cell extracts were prepared and analyzed by immunoprecipitation (*16*) with pooled human sera from HIV-1–infected patients (lane 1), pooled sera from HIV-2–infected patients (lane 2), pooled macaque sera from SIV-infected animals (lane 3), or a combination of pools for a mock-infected extract (lane 4). Molecular size markers are in kilodaltons.

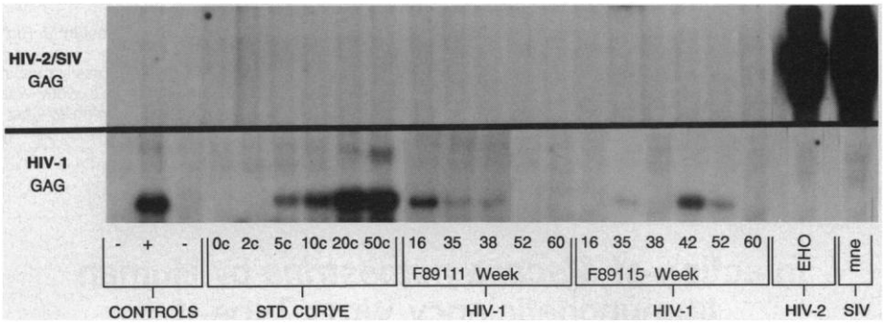

Fig. 2. Oligonucleotide solution hybridization (OSH) for the HIV-1 *gag* gene region. The cell DNA-coded controls and the 0, 2, 5, 10, 20, and 50 copy number (c) standards were prepared as described (*35*). All HIV-1–inoculated animals were negative for SIV and HIV-2 viral sequences (top panel) and positive for HLA-DQ α DNA sequences (*36*). We detected HIV-2 and SIV gag sequences in PBMC DNA from infected control animals (top right) by reacting the DNA with the respective primer and probe sets (*25*). The results for the two HIV-1–infected *M. nemestrina*, F89111 and F89115, are shown in the bottom panel for each time point. DNA from HIV-2_{EHO}– and SIV_{mne}–infected animals was also reacted with HIV-1 gag gene primers and probe, with the results shown on the lower right. The OSH results were concordant with the nonradioisotopic results with both the Genprobe and Roche assays (*18*). Proviral DNA content quantified relative to external copy number standards consistently ranged between two and ten copies in 1 × 10^6 PBMCs. These data were similar to the radioisotopic results shown.

Table 1. In vitro analyses of viral antigen synthesis [picograms per milliliter (pg/ml)] in human and macaque PBMCs or CD4-purified cells infected by HIV-1_{LAI}, HIV-1_{NL4-3}, HIV-1_{JR-CSF}, HIV-1_{RO}, SIV_{mne}, or heat-inactivated (60°C, 30 min) HIV-1_{LAI} (ΔHIV-1) and SIV (ΔSIV). PBMCs from human and three *Macaca* species were purified from Ficoll-hypaque density gradients, stimulated with PHA for 72 hours, and infected at a multiplicity of 0.5 TCID per cell. After viral attachment, the cells were washed four times and incubated in RPMI 1640 containing 20% fetal bovine serum and interleukin-2. Amounts of viral antigens were determined by whole-virus antigen capture (HIVAG-1™, Abbott Laboratories, Chicago, Illinois) at 0, 3, 7, 10, and 14 days (for most of the experiments) after infection (*23*). The average of values from supernatants of triplicate cultures is shown. Background values for viral antigens at day 0 (range, 5 to 100 pg/ml) were subtracted from the measured values for each time point. ND, not determined.

Virus	Cell origin	HIV-1 viral antigens (pg/ml) at days after infection			
		3	7	10	14
HIV-1_{LAI}	*M. nemestrina*	429	2494	2610	2034
	CD4 cells–*M. nemestrina*	>1000	>1000	>1000	>1000
	M. mulatta	<10	<10	<10	<10
	M. fascicularis	<10	<10	<10	<10
	Human	2617	3070	3842	3610
	CD4 cells–human	>1000	>1000	>1000	>1000
HIV-1_{NL4-3}	*M. nemestrina*	368	>1000	ND	ND
	Human	1100	1428	ND	ND
HIV-1_{JR-CSF}	*M. nemestrina*	233	>1000	ND	ND
	Human	728	1800	1920	1871
HIV-1_{RO}	*M. nemestrina*	<10	<10	162	>500
	Human	290	>500	>500	>500
SIV_{mne}	*M. nemestrina*	302	287	275	268
	M. mulatta	222	238	247	316
	M. fascicularis	257	180	150	167
ΔHIV-1	*M. nemestrina*	<10	<10	<10	<10
ΔSIV_{mne}	*M. nemestrina*	<10	<10	<10	<10

specific gene regions (25) were used for amplification. The amplified products were then hybridized to specific oligonucleotide probes (25), and the probe-product DNA heteroduplexes were detected by both radioisotopic (Fig. 2) and nonradioisotopic (26) detection assays. Primers flanking the human leukocyte antigen–DQ (HLA-DQ)α locus were used for internal calibration, and samples from HIV-1–infected M. *nemestrina* obtained at 16, 35, 38, 42 (F89115 only), 52, and 60 weeks after inoculation were analyzed. HIV-1 *gag* sequences were detected 16, 35, and 38 weeks after inoculation for animal F89111 and 35, 42, and 52 weeks after inoculation for animal F89115 (Fig. 2). These sequences were not detected in PBMCs from either HIV-2– or SIV_{mne}-infected animals, which confirms the HIV-1 specificity of the primer pairs. HIV-2 and SIV proviral sequences, however, were detected in samples from HIV-2– or SIV-infected positive control animals (Fig. 2). Furthermore, HIV-1 env and long terminal repeat (LTR) sequences were detected at 16 and 38 weeks in DNA from animal F89111 (18).

Table 2. Passage of infectious $HIV\text{-}1_{LAI}$ from *M. nemestrina* PBMC culture supernatant to human CD4-bearing cell lines and PBMCs. HIV-1–infected *M. nemestrina* PBMC culture supernatants from 7 days after inoculation were concentrated by centrifugation (50,000*g* for 1 hour) (37) and used to infect cell lines and human PBMCs. After a 2-hour viral attachment, the cells were washed and placed in culture as described (Table 1). Supernatant samples were assayed for HIV-1 antigens by whole-virus antigen capture (HIVAG-1™); average values from triplicate cultures are shown. Cells from each culture were washed free of unattached virus on day 0. When supernatants from day 0 were concentrated and used to infect AA2, C8166, or human PBMCs, amounts of HIV-1 antigen remained less than 10 pg/ml during days 1 through 4.

Cells infected	HIV-1 antigens (pg/ml) at days after infection			
	1	2	3	4
AA2	<10	260	>1000	>1000
C8166	<10	<10	90	100
Human PBMCs	<10	<10	20	90

Both animals developed and maintained a full complement of antibodies to HIV-1 envelope and gag proteins (Fig. 3) (27). Seroconversion in both animals was also detected by a whole-virus enzyme-linked immunosorbent assay (ELISA) (Genetic Systems, Redmond, Washington), where R values (28) peaked at 5.5 during week 16 for F89115 and 1.5 during week 24 for F89111. Sera from animals F89111 and F89115 collected at week 46 had a neutralizing antibody titer of 1:20 and 1:40 (29), respectively. Both animals developed moderate lymphadenopathy early after HIV-1 inoculation, and intermittent febrile episodes were noted. Absolute CD4 cell counts remained normal in the two macaques through 80 weeks after inoculation.

In a second and third experiment, we infected six additional M. *nemestrina*. Four animals were inoculated as before with a mixture of infected autologous PBMCs and cell-free virus (24), two with $HIV\text{-}1_{LAI}$ and two with the molecularly cloned $HIV\text{-}1_{NL4\text{-}3}$. In the third experiment, two animals were inoculated with cell-free $HIV\text{-}1_{LAI}$ alone. In these experiments, we cocultured each infected animal's PBMCs at weekly intervals with phytohemagglutinin (PHA)-stimulated human PBMCs rather than the T cell line used in the first study, as human PBMCs are more sensitive for the isolation of HIV-1 from patients with HIV-1 infection (30). During the 10 weeks after inoculation, HIV-1 was isolated from 85 to 100% of the PBMC specimens obtained at weekly intervals, including those from animals inoculated with cell-free virus. HIV-1 was recovered from one animal's plasma at weeks 2, 3, and 4 after inoculation. In addition, HIV-1–specific proviral gag DNA was detected in the PBMCs from all the samples from the six animals in experiments two and three at 2 through 10 weeks after inoculation. No HIV-1 DNA was detected in preinoculation samples from any of the animals tested. Clinically, the six animals had moderate lymphadenopathy at 1 to 2 weeks after inoculation, and two of the animals developed a rash, predominantly over the inguinal and abdominal regions, similar to that described for SIV-infected macaques (4). All animals seroconverted as measured by protein immunoblot assay to a full spectrum of HIV-1 proteins by 3 weeks after inoculation.

We have shown that M. *nemestrina* are susceptible to infection by HIV-1: all eight infected M. *nemestrina* in these experiments demonstrated acute and persistant in vivo infection by the two HIV-1 strains used to date. Our ability to isolate virus 1 to 24 weeks after inoculation, the presence for up to 52 weeks of HIV-1–specific DNA in PBMCs, and the persistent antibody response to HIV-1 envelope and gag proteins are results similar to those reported for HIV-1–infected chimpanzees (31). In contrast to the chimpanzee model, cell-free virus was found in the plasma from one of our eight macaques (31). We and others have been unable to infect either M. *mulatta* (10, 32) or M. *fascicularis* (33) PBMCs, despite reports of limited in vitro HIV-1 infection of PBMCs from M. *mulatta* (33, 34). Gajdusek and co-workers have reported that, although M. *mulatta* and M. *fascicularis* failed to seroconvert after an in vivo HIV-1 challenge, HIV-1 was recovered from M. *mulatta* leukocytes in vitro (39). Whether these discrepancies are a result of differences in HIV-1 strains, individual animals, or PBMC culturing techniques remains to be determined. Our ability to infect M. *nemestrina*, however, indicates that this animal model should be useful for defining the initial events of HIV-1 infection. The immune system of M. *nemestrina* is very similar to that in chimpanzees and humans. As such, cellular and humoral immune responses to candidate vaccines can be evaluated. It will be necessary to define the optimal dose, route of infection, and pathogenicity of a variety of HIV-1 strains.

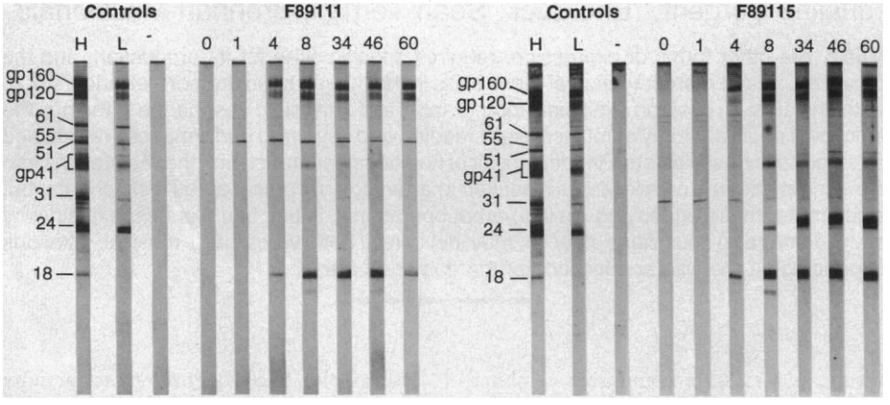

Fig. 3. Protein immunoblot analysis of sera from two HIV-1–inoculated *M. nemestrina*. Sera from animals F89111 and F89115, obtained at the weeks indicated after inoculation, were reacted with nitrocellulose strips that contained HIV-1 antigens (23) (Epitope, Beaverton, Oregon) and processed according to the manufacturer's directions. Control strips, shown on the left, were reacted with sera that contained high (H) and low (L) levels of antibodies to HIV-1 (anti–HIV-1) or with negative control serum. The relative positions and molecular weights (in kilodaltons) of specific HIV-1 proteins or glycoproteins are shown on the left. Uninfected (week 0) *M. nemestrina* sera were unreactive with HIV-1–specific antigens.

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25. The positions of the oligonucleotide primers are numbered relative to the HIV-1 HXB2, HIV-2_{ROD}, and SIV_{mne} isolates in the Human Retroviruses and AIDS Database. Primer SK38-SK39 and probe SK-19, primer SK145-SK431 and probe SK102, primer SK68-SK69 and probe SK-70, and primer AA55-M667 and probe KG1 [P. Gupta, L. Kingsly, R. Anderson *et al.*, *AIDS* **6**, 143 (1992)] were the two gag, env, and LTR HIV-1 specific primers, respectively [C.-Y. Ou *et al.*, *Science* **239**, 295 (1988)]. VB312 [gag nucleotides (nt) 539 to 566 = 5′-GTGGGAGATGGGCGCGAGAAACTC-CGCT-3′ and gag nt 837 to 810 = 5′-CACGCA-GAAGAGAAAGTGAAAGATACT-3′] and VB306-VB310 (gag nt 603 to 630 = 5′-AGGTTACGGC-CCGGCGGAAAGAAAAAGT-3′ and gag nt 780 to 753 = 5′-ACAGGTTCAGAAAATTTAAAAAGCC-TTT-3′) were the outer and inner sets of HIV-2 gag gene primers, respectively. VB308 (gag nt 684 to 714 = 5′-AACCTTCTTTTGACTCCAACAGGCTC-TCTG-3′) was the HIV-2 gag gene probe [C.-Y. Ou, personal communication]. The HIV-2 nested primer pairs are homologous to and cross-hybridize with SIV gag sequences but not with HIV-1 (*26*). SIVgag1-SIVgag2 (gag nt 587 to 619 = 5′-ATTAGGCTACGACCCGGCGGGAAGAAAAAG-TAC-3′ and gag nt 1537 to 1505 = 5′-CAGCAC-TAGCTTGCAATGTGGGTTAGCATTTTG-3′) and SIVgagGP (5′-CTGTCAGAAGGCTGCACCCCCTA-TGACATTAATCAGATGT-3′) were the SIV gag gene primers and probe. Primers flanking the HLA-DQ α locus were used to assess the ability of the sample to be amplified and served as an internal calibration. PCR was performed as described (*35*) with an automated thermal cycler programmed for 30 cycles at 94°C for 50 s, 55°C for 50 s, and 72°C for 90 s. Nested PCR was performed as described [M. R. Furtado, B. Balachandran, P. Gupta, S. M. Wolinsky, *Virology* **185**, 258 (1991)] with an automated thermal cycler programmed for 35 cycles at 95°C for 60 s and 60°C for 3 min. Negative cell DNA and reagent controls were run in parallel. Specific product DNAs were detected by a radioisotopic and two nonisotopic (Roche, Fair Lawn, NJ, and Genprobe, San Diego, CA) assays (*35*). All experiments were performed at least in duplicate.
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Distributed Neural Network Underlying Musical Sight-Reading and Keyboard Performance

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Music, like other forms of expression, requires specific skills for its production, and the organization and representation of these skills in the human brain are not well understood. With the use of positron emission tomography and magnetic resonance imaging, the functional neuroanatomy of musical sight-reading and keyboard performance was studied in ten professional pianists. Reading musical notations and translating these notations into movement patterns on a keyboard resulted in activation of cortical areas distinct from, but adjacent to, those underlying similar verbal operations. These findings help explain why brain damage in musicians may or may not affect both verbal and musical functions depending on the size and location of the damaged area.

Music is a message comprising combinatory rhythmic patterns of discrete pitches communicated by a composer to a listener, often through an interpreter. Music and speech have certain aspects in common: Both are used expressively and receptively; both involve fine sequential motor activity for their production; both are constructed of perceptually discrete sounds that can be represented in a writing system. Like speech, music is governed by culture-dependent combinatorial rules; that is, one can speak of a musical grammar in the mind of the composer, performer, and listener that in many respects parallels the grammar

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