cells do not hybridize to the HPB-MLT V region (Fig. 3B and Table 1). Because the type IV rearrangement is very common (Table 1) we suggest that this rearrangement involves V-J recombination with the V region of another non–cross-hybridizing family of V region genes. Thus, the human γ chain genes can rearrange with at least four different V region genes. The maximum number of V region genes is not defined by these experiments; however, we suggest that there will be approximately 15 based on the number of V regions in the HPB-MLT family and the number of different rearrangements observed. This appears to be significantly more recombinational diversity than is available to the murine γ genes (*2, 3*).

The role of the γ chain gene remains uncertain. However, we note that the gene is rearranged in human T cells with diverse cell surface phenotype (CD4, CD8; Table 1). Further studies to determine whether these rearrangements lead to expression of functional γ chains in these T cells may help to define the role of this polypeptide.


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# T-Cell Recognition of Ia Molecules Selectively Altered by a Single Amino Acid Substitution


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**T lymphocytes recognize foreign antigen together with allele-specific determinants on membrane-bound class I and class II (Ia) gene products of the major histocompatibility complex. To identify amino acids of class II molecules critical to this recognition process, the genes encoding the β chains of the I-$A^k$ molecule were cloned from a wild-type B-cell hybridoma and from an immunoselected variant subline showing distinct serological and T-cell stimulatory properties. Nucleotide sequencing and DNA-mediated gene transfer established that a single base transition (G → A) encoding a change from glutamic acid to lysine at position 67 in the I-$A^k_\beta$ molecule accounted for all the observed phenotypic changes of the variant cells. These results confirm the importance of residues 62 to 78 in the amino terminal domain of I-$A_\beta$ for class II–restricted T-cell recognition of antigen and demonstrate the ability of a single substitution in this region to alter this recognition event.**


CLASS II (OR IA) MAJOR HISTOCOMPATIBILITY molecules are integral membrane glycoproteins that exist as heterodimers of two noncovalently associated chains, α (33 kD) and β (29 kD). In the mouse, two isotypic forms of Ia have been demonstrated: I-A ($A_\beta A_\alpha$) and I-E ($E_\beta E_\alpha$) (*1, 2*). These molecules are predominantly expressed on B lymphocytes, some macrophages and T lymphocytes, dendritic cells, and thymic epithelial cells (*3, 4*). There is evidence that the primary role of Ia molecules is to act as corecognition elements during antigen-specific interactions of helper-inducer T lymphocytes with accessory cells (macrophages or dendritic cells) or with B lymphocytes [reviewed by Schwartz (*5*)]. A striking feature of class II molecules is their extensive intraspecies polymorphism, and it is this allelic variation that is directly involved in the Ia-restricted recognition of antigen by T cells. Nucleotide sequence analysis of cloned class II genes has revealed that the principal amino acid polymorphisms among alleles are in the $NH_2$-terminal domains of the I-$A_\beta$, I-$A_\alpha$, and I-$E_\beta$ chains (*6–12*). [The I-$E_\alpha$ chain shows relatively little variation among haplotypes (*13, 14*).] The amino acid substitutions appear clustered in three or four hypervariable regions spread across the entire domain.

Identification of the location of allelic variation in these molecules is the first step toward understanding the relation between the fine structure of Ia and its immunological function. Further analysis is needed to map more precisely the regions and residues participating in haplotype-restricted T-cell antigen recognition and to assign functions to the mapped regions with respect to such features as interaction with antigen or direct binding to the T-cell receptor. One of the two major approaches to exploring these issues is the use of DNA-mediated gene transfer technology to obtain expression of class II genes recombined in vitro ("exon shuffled") by cells capable of antigen presentation. The alterations in structure are then systematically correlated with those seen in function. This method has proved successful in confirming the predicted unique role of the $NH_2$-terminal I-$A_{\beta 1}$ or I-$E_{\beta 1}$ domains in Ia-restricted T-cell stimulation (*15, 16*).

The other approach is to identify mutations in class II genes that lead to altered immunologic function and then to determine the precise nature of the structural change in the mutated gene. However, in contrast to the many spontaneous mutations that have been described in genes encoding class I (H-2K) major histocompatibility complex (MHC) molecules, only one has been found affecting a class II gene (*17*). The B6.CH2$^{bm12}$ mouse has an altered I-$A^b_\beta$ gene, with three nucleotide changes compared to the wild type giving rise to three amino acid substitutions—at positions 67, 70, and 71 (*18*). The changes have resulted in an altered Ia serologic reactivity, a new immune response phenotype, mixed lymphocyte reactivity against cells of the parent strain, and a distinct pattern of MHC-restricted antigen presentation by cells bearing the mutant I-A molecule (*19*). It is not yet known, however, whether all the changes in the bm12 I-$A_\beta$ molecule are required for each altered trait. Thus, it would be useful to have a panel of cells bearing mutant class II


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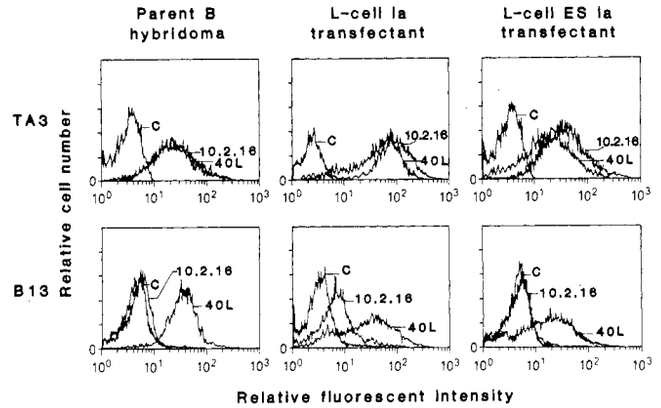


Fig. 1. Flow microfluorimetric analysis of B-cell hybridomas and $Ia^+$ L-cell transfectants with a panel of anti–I-$A^k$ monoclonal antibodies. The DAP.3 thymidine kinase–negative L-cell subline was cotransfected by the calcium phosphate precipitation method with I-$A_\beta$ (derived from either TA3 or the mutant cell B13), I-$A^k_\alpha$, and the herpes thymidine kinase gene (*25, 26*). Expressible forms of the I-$A^k_\beta$ and the exon-shuffled I-$A^k_{\beta 1}$, $\beta 2^d$, $TM^d$, $ICI^d$, $IC2^d$ genes (referred to as ES) were constructed as described (*15*). Transfectants were selected in HAT (hypoxanthine, aminopterin, and thymidine) medium (*26*). All cells were stained with anti–I-A reagents as described (*15*). The following monoclonal antibodies were used as hybridoma culture supernatants: 10.2.16 (*27*), 40L (*28*), and H116.32 (*29*). C refers to the medium control. Cells were sorted for high expression of I-$A^k$ with a flow microcytometer (Epics).

molecules with single amino acid changes and known functional differences from wild-type cells in order to correlate these amino acid changes with specific functional differences.

To generate such cell lines, Glimcher *et al.* (*20*) mutagenized an $Ia^+$ B-cell hybridoma capable of antigen presentation to T cells, then negatively selected these cells by complement-mediated lysis with a monoclonal antibody directed to one I-$A^k$ determinant. Surviving cells were positively selected by fluorescent-activated cell sorting after being stained with a second monoclonal antibody directed to a distinct I-$A^k$ specificity. This protocol led to cells that had lost one I-$A^k$ epitope but had retained others. Such variant cells were found to be incapable of presenting antigen to some, but not all, T hybridomas that normally could be stimulated by the parent cell (*20*). To determine whether the change in antigen-presenting function of the serologic variants was in fact related to one or more mutations in class II genes and to identify the precise structural basis for the serologic and functional changes, we cloned the presumed target gene (I-$A^k_\beta$) from one such Ia-variant B hybridoma. DNA-mediated gene transfer and nucleotide sequence analysis were then used to characterize the relevant mutation underlying the phenotypic change of the variant cell line.

TA3, the wild-type B-cell hybridoma, was derived from a fusion between a BALB/c B-cell lymphoma M12.4.1 ($H\text{-}2^d$) and lipopolysaccharide-stimulated B cells from $(BALB/c \times A/J)F_1$ mice ($H\text{-}2^{d/a}$). This resulted in cells that had one copy of I-$A^k_\beta$ and I-$A^k_\alpha$ genes (*20*). The mutant cell lines discussed in this report, A19 and B13, were obtained in the same experiment after ethylmethane sulfonate treatment of TA3 and immunoselection for the loss of the I-$A^k_\beta$ determinant seen by the 10.2.16 monoclonal antibody. These two clones appear to be siblings derived from a single mutational event. The virtually complete loss of the 10.2.16 epitope on these variants coincided with the loss of some I-$A^k_\beta$ determinants, but retention of other I-$A^k_\beta$ and all I-$A^k_\alpha$ specificies tested. Functional analysis of these cells showed a difference in their ability to present antigen to certain T-cell hybridomas in the context of I-$A^k$. Antigen presentation to I-$A^d$–, I-$E^d$–, and I-$E^k$–restricted T-cell hybridomas was not affected; this argues against a generalized antigen presentation defect in these cells.

To clone the I-$A^k_\beta$ genes from the wild-type and variant cell lines, which have about three I-$A^d_\beta$ genes to one I-$A^k_\beta$ gene per cell, we took advantage of the distinct sizes of the Eco RI restriction fragments containing the 5′ flanking and coding regions of I-$A^k_\beta$ and I-$A^d_\beta$ (17 and 6 kb, respectively). Southern blot analysis (*21*) of Eco RI–digested DNA from these cells showed no obvious size differences between I-$A^k_\beta$ fragments from parent or variant cells, indicating that the mutation caused no gross deletions or insertions in these genes. High molecular weight DNA from the three cell lines was digested with Eco RI and size-selected on a 5 to 20 percent NaCl gradient to enrich for 17-kb sequences. These fragments were cloned into Charon 4A phage arms, and the resultant libraries were screened with a probe specific for the I-$A_{\beta 1}$ exon. Hybridizing phage were picked, and the identity of the inserts as I-$A^k_\beta$ genes was verified by restriction map analysis.

DNA-mediated gene transfer was used to determine whether the I-$A^k_\beta$ genes were actually responsible for the differences seen

Table 1. Stimulation of I-$A^k$–restricted T hybridomas by B hybridomas and I-A expressing L cells. T hybridoma cells ($10^5$) were cocultured with the designated stimulator cells in 0.2 ml of complete medium in the presence of native hen egg lysozyme (HEL) (100 μg/ml). After 24 hours of culture, supernatants were collected and assayed for interleukin-2 content in a secondary culture of HT-2 cells. HT-2 cells ($4 \times 10^3$) were cultured for 36 hours in the presence of 40 percent primary culture supernatant, and the degree of stimulation was measured by the incorporation of [$^3$H]thymidine into DNA. Varying numbers of stimulators of each type were used. Only the maximum response obtained is shown. TA3, A19, and B13 are the parent B-cell hybridomas. TA3-$A^k_\beta$-Tx, A19-$A^k_\beta$-Tx, and B13-$A^k_\beta$-Tx are L cells transfected with I-$A^k_\alpha$ and with the I-$A^k_\beta$ gene derived from the designated donor cell. TA3-ES-Tx, A19-ES-Tx, and B13-ES-Tx are L cells transfected with I-$A^k_\alpha$ and with an exon-shuffled I-$A_\beta$ gene. Leader and $\beta_1$ domain only were derived from the designated donor cell as described in the text. Other abbreviations: APC, antigen-preventing cells; N.D., no data.

| APC | T-cell hybridoma responses (count/min) | | | | | |
|---|---|---|---|---|---|---|
| | Exp. 1 | | Exp. 2 | | Exp. 3 | |
| | HEL-C10 | HEL-A2 | HEL-C10 | HEL-A2 | HEL-C10 | HEL-A2 |
| TA3 | 52,792 | 53,102 | 214,179 | 110,119 | 21,935 | 29,973 |
| TA3-$A^k_\beta$-Tx | 48,623 | 39,690 | 94,741 | 37,760 | 42,883 | 19,123 |
| TA3-ES-Tx | N.D. | N.D. | 76,470 | 83,062 | N.D. | N.D. |
| A19 | 561 | 32,610 | 1,968 | 23,426 | 2,913 | 21,356 |
| A19-$A^k_\beta$-Tx | 404 | 40,834 | 265 | 17,703 | 2,483 | 21,587 |
| A19-ES-Tx | 980 | 25,220 | N.D. | N.D. | N.D. | N.D. |
| B13 | 315 | 30,634 | 535 | 25,852 | 3,973 | 15,704 |
| B13-$A^k_\beta$-Tx | 763 | 41,648 | N.D. | N.D. | 1,402 | 15,913 |
| B13-ES-Tx | N.D. | N.D. | 308 | 21,217 | 493 | 22,988 |

between TA3 and A19/B13. Expressible forms of the cloned genes were prepared by ligating the 17-kb I-$A^k_\beta$ gene fragments to a pSV2gpt vector containing the missing 3′ untranslated region of I-$A^k_\beta$. Constructs with the proper orientation were isolated, and DNA was prepared and used for cotransfection of L cells with I-$A^k_\alpha$ and the herpes thymidine kinase gene *(tk)*. Additional transfectants were prepared from I-$A^k_\alpha$, *tk*, and recombinant I-$A_\beta$ genes containing the leader and $\beta_1$ exons encoding the $NH_2$-terminal domain of I-$A^k_\beta$ (donated from either wild-type TA3 or variant A19/B13 cells), spliced to the exons encoding the remainder of I-$A^d_\beta$. Earlier work established that an exon-shuffled wild-type I-$A_\beta$ gene shows the functional and serologic properties of the $\beta_1$-domain donor, including the expression of the 10.2.16-reactive epitope relevant to the present studies *(15)*.

Figure 1 illustrates representative results of flow microfluorimetric (FMF) analysis of the L-cell transfectants, after selection of suitable I-A–positive sublines by staining with H116.32, a monoclonal antibody to I-$A^k_\alpha$ (anti-$A^k_\alpha$). The FMF results demonstrate that the presence of the $\beta_1$ domain from the I-$A^k_\beta$ gene of the A19/B13 variants leads to the same serologic profile as seen with the original variant B hybridomas. Thus, all cells stain with anti-$A^k_\alpha$ antibodies, as well as with the 40L anti-$A^k_\beta$ antibody. The variant B hybridomas and transfectants containing either the intact or exon-shuffled I-$A^k_\beta$ gene from these variants show little or no staining with 10.2.16. This is in contrast to the TA3 cells, or wild-type I-$A^k_\beta$ transfectants, which show 10.2.16 staining comparable to that of other anti-$A^k_\beta$ or anti-$A^k_\alpha$ antibodies.

This panel of transfectants was also used in studies of antigen presentation to assess whether the relation between I-$A^k_{\beta 1}$ and serology would hold for I-$A^k_{\beta 1}$ and T-cell antigen recognition. The ability of transfected L cells to present antigen to T hybridomas in the context of their newly expressed I-A molecules has been reported *(22, 23)*. Table 1 summarizes several experiments carried out with a pair of T hybridomas specific for hen egg lysozyme (HEL), which show distinct patterns of response to HEL and either TA3 or the A19/B13 variant B hybridomas. The data reveal that, as for the FMF studies, the presence of the I-$A^k_{\beta 1}$ domain from the variant cell lines determines the T-cell stimulatory pattern of the transfectants, with those possessing the variant domain unable to activate the HEL-C10 hybridoma for interleukin-2 release. All B hybridomas and transfectants could stimulate the I-$A^k$–restricted HEL-A2 cell, demonstrating that no I-A–independent general presentation defect accounts for the phenotype of the variant cell lines.

The serologic and functional data thus indicated that the relevant mutation in the A19/B13 lines lay in the I-$A^k_{\beta 1}$ domain. The leader and $\beta_1$ exons encoding this domain of the wild-type and variant I-$A_\beta$ molecules were therefore subjected to nucleotide sequence analysis. Figure 2A shows the sequencing strategy and the results obtained for the critical region of the $\beta_1$ exon. The wild-type and mutant genes differ by a single base change, a G → A transition in the first nucleotide of the codon for amino acid 67 [amino acid 67 in I-$A^k_\beta$ corresponds to position 69 in other I-$A_\beta$ chains (see Fig. 2B)]. The mutation changes the encoded residue from glutamic acid to lysine.

The results of gene transfer and nucleotide sequencing of the I-$A^k_\beta$ genes of wild-type and immunoselected variant B hybridomas thus reveal that a single amino acid substitution in the $NH_2$-terminal domain accounts for all serologic and T-cell antigen presentation changes seen with the original variant B hybridomas. Several lines of evidence have previously indicated that the region comprising amino acids 62 through 78 of the I-$A_\beta$ chain is an important one for antigen presentation. Sequence comparisons of the I-$A_\beta$ chains of several haplotypes have shown this to be one of three or four regions demonstrating the intraspecies hypervariability believed to be involved in creating the allele-specific regions critical to class II–restricted T-cell stimulation *(1, 2, 7–12)* (see Fig. 2B). Furthermore, the bm12 mutation affects amino acids at positions 67, 70, and 71 of the I-$A^b_\beta$ chain and leads to an altered antigen-presenting phenotype *(18, 19)*. Finally, the hypervariability of this region is also observed in haplotype comparisons of mouse I-$E_\beta$ chains and of human DR and DQ β chains *(1)*. These results affirm the importance of this region for class II MHC–restricted antigen presentation and demonstrate that even a single amino acid change in this region can affect T-cell recognition of

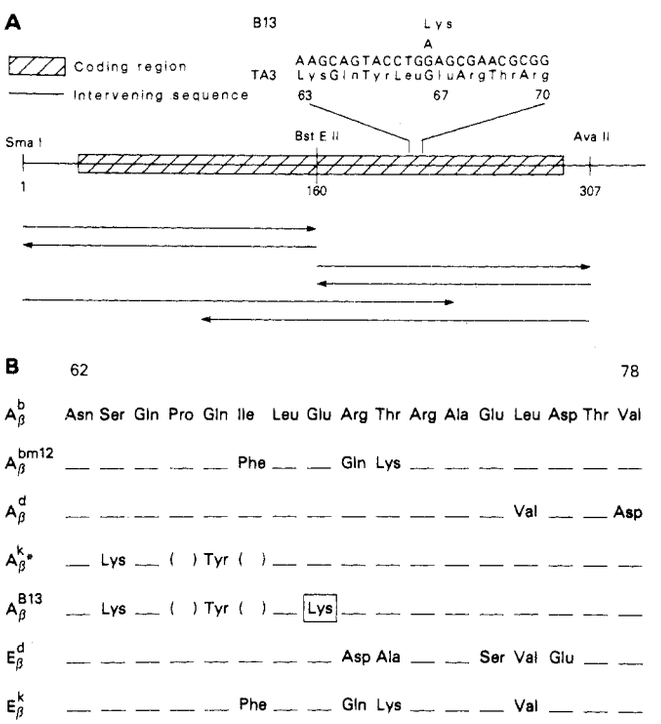


Fig. 2. (A) Sequencing strategy for the β1 exon of I-$A^k_\beta$. Sma I–Ava II, Sma I–Bst EII, and Bst EII–Ava II fragments were isolated from an 0.5-kb Sma I fragment containing the β1 exon plus some intervening sequence. Protruding 5′ ends were made flush by a fill-in reaction with the Klenow fragment of DNA polymerase. The fragments were subcloned into the Sma I site of M13 mp8, and nucleotide sequencing was carried out by the dideoxy chain termination method *(30)*. The first four amino acids of the β1 domain of the mature I-$A_\beta$ polypeptide are encoded in the leader exon. The nucleotide sequence specifying these amino acids was determined by subcloning, into M13 mp8, a 2.5-kb Pvu II fragment containing the entire leader sequence. This fragment was identified by Southern blot analysis with a $DQ_\beta$ leader probe *(31)*. Sequencing reactions were performed, as described above, with a 17-base oligonucleotide primer complementary to a region 42 bases to the 5′ side of the start of the β1 coding sequence. The sequence of the leader exon encoding the amino terminus of I-$A^k_\beta$ (GGAAACTCCGAAA → GlyAsnSerGlu) is identical for TA3 (wild-type) and B13 (mutant). (B) Comparison of amino acids 62 to 78 in mouse β chains as determined from published sequences *(7–12)*. The numbering of amino acids in $A^k_\beta$ chains is different from that in other β chains because of the deletion of two amino acids. ( ) indicates deletion of an amino acid at this position.

antigen in the context of class II MHC molecules.

It is not yet possible to know whether a functionally significant amino acid change of the type reported here mediates its effect by altering the overall conformation of the I-A molecule, by changing the local tertiary structure of I-$A_\beta$, or by substituting a new side chain directly at a site physically involved in T-cell receptor-antigen-Ia interaction. Nonetheless, it is clear that even a nonconservative change in a normally invariant residue (Glu at I-$A^k_\beta$ position 67; see Fig. 2B) does not destroy all immunologic activity of the resultant I-A, since certain anti-$A^k_\beta$ monoclonal antibodies and I-$A^k$–restricted T cells still recognize such molecules. These findings suggest that this mutation does not change the gross three-dimensional structure of the I-A molecule. If so, then one may speculate that the loss by A19/B13 of antigen-presenting function to HEL-C10 is related to the local alteration of a site directly involved in either binding by the T-cell receptor (the so-called "histotope") or interaction with HEL-derived "processed" antigen (the "desetope") (*24*). Further experiments are required to distinguish between these possibilities.

The approach taken here cannot map all potentially important portions of I-A molecules, since it is constrained by the serologic reagents used and the panel of T hybridomas tested. However, it serves as an important complementary approach to site-directed mutagenesis and in vitro recombination. Used together, these techniques should continue to yield important insights into the structural basis for MHC-restricted T-cell recognition of antigen.

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# Quantitative Analysis of D2 Dopamine Receptor Binding in the Living Human Brain by PET


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**D2 dopamine receptors in the putamen of living human subjects were characterized by using the selective, high-affinity D2 dopamine receptor antagonist carbon-11–labeled raclopride and positron emission tomography. Experiments in four healthy men demonstrated saturability of [$^{11}$C]raclopride binding to an apparently homogeneous population of sites with Hill coefficients close to unity. In the normal putamen, maximum binding ranged from 12 to 17 picomoles per cubic centimeter and dissociation constants from 3.4 to 4.7 nanomolar. Maximum binding for human putamen at autopsy was 15 picomoles per cubic centimeter. Studies of [$^{11}$C]raclopride binding indicate that clinically effective doses of chemically distinct neuroleptic drugs result in 85 to 90 percent occupancy of D2 dopamine receptors in the putamen of schizophrenic patients.**


BINDING OF LABELED LIGANDS TO neurotransmitter receptors in the living human brain has been demonstrated with positron emission tomography (PET) (*1–3*). Models have been proposed for the measurement of binding variables in PET-scan studies, but their applicability has been limited by the slow achievement of equilibrium with available ligands (*4–7*). Receptor densities and affinity characteristics have therefore not been determined for any neurotransmitter receptor system in the living human brain. In vitro methods have supplied evidence for increased densities of D2 dopamine receptors in the dopamine-rich basal ganglia of deceased schizophrenics (*8–11*). A quantitative in vivo method would help to clarify the importance of these alterations in the pathophysiology of schizophrenic disorders.

We previously described the usefulness of a new ligand, [$^{11}$C]raclopride, for PET-scan studies of D2 dopamine receptors in the human brain (*3*). Unlike other ligands used for such studies, raclopride has a high selec-

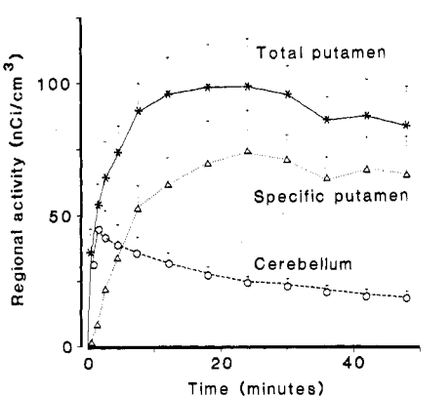


Fig. 1. Radioactivity in the putamen and cerebellum in six healthy men after intravenous injection of [$^{11}$C]raclopride (2.7 mCi). The radioactivity was observed sequentially for 1- to 6-minute periods during 50 minutes after the injection. The radioactivity in the cerebellum was assumed to reflect free and nonspecific binding. Specific binding in the putamen was calculated as the difference between radioactivity in the putamen and that in the cerebellum. Values are means ± standard deviations.


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