was present. This confirmed the postulated introns 1 and 2 (Fig. 1), and further argues that the repeated region in the putative third cytoplasmic loop is not part of an intron (formerly indicated as intron 3; ref. 1).

In addition, we reconstructed full-length cDNA clones for the variant forms into the expression vector pCD-PS (Fig. 3). Transient expression of the three forms in COS-7 cells and subsequent characterization of the D4 receptor RNA expressed in these cells demonstrated the expected size and size differences between the three forms, indicating that none of the expressed D4 receptor RNAs are further processed (Fig. 3). Furthermore, two bands were observed that correlate with the use of either the endogenous D4 receptor or the SV40 (vector derived) polyadenylation site (Fig. 3). These observations provide additional evidence that the variable sequence is not part of an intron. Furthermore, it indicates that in the transient expression system the expression of the three different clones would result in the formation of three structurally different receptors.

The amino acid sequences deduced from the different genomic and cDNA clones imply the existence of three different forms of the D4 receptor with a variably sized putative third cytoplasmic loop. Interestingly, this region of the human D4 receptor is not found in the rat homologue of the D4 receptor, making this variation specific to humans (data not shown).

Pharmacological analysis showed that all three variants displayed saturable [3H]siporperin binding (300–1,000 fmol mg−1) with similar dissociation constants (KD) in the absence of sodium chloride (KD = 55 pM; Fig. 4a). But in the presence of 120 mM sodium chloride, the dissociation constant was roughly doubled for D4.2 and D4.4 but not for D4.7 (Fig. 4a).

Agonists and antagonists inhibited [3H]siporperin binding (in the presence of sodium chloride) to these different D4 receptor variants in a concentration-dependent manner (dopamine, dissociation constant (Kd) for high-affinity state ~25 mM, Kd in presence of guanilylimidodiphosphate ~460 nM; bromocriptine, Kd ~272 nM; haloperidol, Kd ~2.5 mM; raclopride, Kd ~1.6 μM; clozapine, Kd = 25 mM). Furthermore, all three variants exhibited a guanine nucleotide-sensitive high-affinity form of the receptor on competition with dopamine, suggesting that all these variants can functionally couple to G proteins.

Clozapine competition of [3H]siporperin binding showed that D4.2 and D4.4 had significantly (6- to 8-fold) lower dissociation constants compared with the absence of sodium chloride in the binding assay than they had in its presence. But D4.7 did not exhibit the same sodium chloride sensitivity. The absence of sodium chloride clearly revealed the different pharmacological characteristics between the two shorter variants and D4.7 (Fig. 4). The sodium chloride-mediated effects for clozapine on the D4 variants were not modulated by guanine nucleotides.

The identification of three dopamine D4 receptor variants in the human population is the first example of a polymorphic variation for catecholamine receptors. The fact that the polymorphism is in the domain of the third cytoplasmic loop suggests the possibility of differences in G-protein interaction among the different forms. Polymorphisms due to variable length of repeated sequences in genes have been implicated in diseases such as X-linked spinal and bulbar atrophy11, fragile X-syndrome12 and myotonic dystrophy13. The D4 receptor gene has been localized to chromosome 11p15.5 distal from the Harvey ras-1 locus14, a region that has been implicated in bipolar affective disorder15 and long QT syndrome16. The observed dopamine D4 receptor polymorphism may have important implications in susceptibility to neuropsychiatric disorders such as schizophrenia and manic depression, as well as response to drug treatment.

Note added in proof: Genetic typing of an additional 200 individuals revealed examples of two more allelic forms of the D4 gene corresponding in size with a three- and five-fold repeat sequence.

Objective analysis of the topological organization of the primate cortical visual system

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The primate cortical visual system is comprised of many structurally and functionally distinct areas1–3, each receiving and sending about 10 projections from and to other cortical areas4. The visual cortex is thus served by many cortical–cortical connections to form a network of complex topological complexity. Thus the gross organization of this cortical processing system presents a formidable topological problem: although the spatial position of the areas in the brain is reasonably well established, the gross 'processing architecture' defined by the connections, is less well understood. Here I report an optimization approach that gives both qualitative and quantitative insight into the connectional topology of the primate cortical visual system. This approach supports suggestions that the system is divided into a dorsal 'stream' and a ventral 'stream' with limited cross-talk, that these two streams recombine in the region of the principal sulcus (area 46) and in the superior temporal polysensory areas, that the system is hierarchically organized, and that the majority of the connections are from 'nearest-neighbour' and 'next-door-but-one' areas.

The cortical sheet can be divided into different areas according to several different parcellation schemes (see, for example, refs 1, 4, 5). I used the most recent parcellation1, and examined a matrix of connections between these areas of the macaque cortical visual system (Table 1). The connection matrix was analysed by an optimization method7,9 that yields a configuration of points that correspond to the cortical areas. The derived structure optimally fits the matrix so that the distances between the points of the structure are as close as possible to the reverse rank order of the 'proximities' between areas in the matrix in Table 1. Thus in the derived structure the length of known connections is at a minimum given the co-constraint that the length of nonexistent (and presently unreported) connections is at a maximum. Hence points representing areas that have very similar afferent and efferent cortico–cortical connections
are close together, whereas points representing areas that have very different patterns of connections are far apart. The details of the analysis are indicated in the legend to Fig. 1, which shows the best-fit structure for the connectivity matrix in Table 1. Points representing the areas of the cortical visual system are shown connected by the projections to and from the corresponding areas.

Several organizational features of the connectional topology of the system are immediately apparent. The two dimensions of the structure correspond roughly to the posterior-anterior (left to right in Fig. 1) and to the dorsal-ventral (top to bottom) spatial distribution of the areas in the brain. For example, areas of the caudal superior temporal sulcus and of the posterior parietal cortex appear concentrated in the top part of the diagram, whereas areas of the inferotemporal cortex are located in the lower part. Because no information regarding the spatial position of the areas entered the analysis (only information concerning the areas to which each area is connected) this suggests that the spatial position of an area in the brain is a good predictor of the areas to which it is likely to be connected, and that nearby areas tend to innervate another (see below).

Beginning at the far left of Fig. 1, where primary visual cortex (V1) is located, visual signals pass to a cluster of prefrontal areas. This 'prefrontal group' consists of areas V2, V3, VP, V4t, V3A, MT and, perhaps surprisingly, area PIP. Areas V3A and MT are topologically less peripheral than other members of this group, and MT is only distinguished topologically by its one-way projections to frontal cortex area 46 and to the frontal eye fields (FEF). Every area of the 'prefrontal group' sends output connections to a further cluster of areas comprising FST, MSTd, MSti, VIP, PO, LIP and DP. The projections from the 'prefrontal group' to this group appear highly redundant, which might account for the fact that partial damage to these prefrontal areas does not seriously disrupt spatial vision. Signals from the 'MST/posterior parietal complex' then pass to the FEF, area 7a, the posterior part of the superior temporal polysensory area (STP), and eventually to area 46 and the anterior STP (STPa).

Moving ventrally from V1, signals are relayed by V4 and VOT into the 'inferotemporal (IT) complex'. As V4 is the principal gateway for signals entering the IT, it would seem unlikely on topological grounds that V4 is involved only in colour vision. The 'IT complex' seems to be hierarchically organized, in the sense that more anterior stations are topologically further from the periphery, and is associated with parahippocampal areas TF and TH. The topologically 'higher' areas of IT, where some cells respond with high specificity for particular visual patterns, project to area 46 and to STPa.

Connections between the dorsal and ventral streams are much less dense than those within each stream, and opportunities for cross-talk do not exist at every station. Both streams, however, project selectively to area 46 and to STPa. Area 46, for example, receives signals which presumably concern what an object is (from IT), where it is (area 7a, LIP), its movement in visual space (MT, MSTd, MSti), its colour (V4) and its relation to movements of the eyes (FEF).

I used the mathematical tractability of the structure in Fig. 1 to investigate quantitatively whether the topological organization of the visual cortex reflects the dichotomy, reorganization, hierarchy and border relations suggested by qualitative inspection. This was accomplished by a regression-like procedure, PROCRUSTES rotation, which compared artificial model configurations, numerically embodying each of the proposed
organizational features, against the structure in Fig. 1. The statistical rarity of each comparison was assessed by approximate randomization20,21 (see Table 2 legend).

Possible border relations embedded in the organization of the visual system were investigated by constructing matrices analogous to Table 1. The first matrix was derived by scoring hypothetical connections between areas that share a common border as ‘1’ and all other possible connections as ‘0’: the ‘nearest-neighbour’ model (all connections were assumed to be reciprocal). This nearest-neighbour wiring matrix accounted for 61 out of 301 connections in Table 1 (27%). A second matrix was derived by scoring possible connections as a ‘1’ if the areas share a common border or if they are separated by only one intervening area which abuts both areas: the ‘nearest-neighbour or next-door-but-one’ model. Of the 301 connections in Table 1, 169 (55%) were connections between nearest-neighbour or next-door-but-one areas. Both these matrices were submitted to the same procedure used to derive the structure in Fig. 1, and the resulting configurations were compared with Fig. 1 by PROCRUSTES rotation.

The hierarchic ladder derived from the laminar origin and termination patterns of projections1,6 was used to construct a unidimensional hierarchic model, by associating an integer value with each area according to its height above V1 in the Fellman and Van Essen scheme. This was the ‘hierarchic’ model. To model the dichotomization of the system into a dorsal and ventral stream, and the subsequent reconvergence of these two streams, I assigned each area to one of three categories. Posterior parietal areas, caudal superior temporal areas and areas associated with eye movement (such as FEF) were assigned a ‘3’. ‘Shared’ areas, such as V1 and V2 (representing the ‘shared’ origins of the streams in occipital cortex), and STPs and area 46 (representing the reconvergence of the streams), were assigned a ‘2’. Areas of the ventral stream were assigned a ‘1’. This constituted the ‘two streams and reconvergence’ model. Finally, I derived a ‘combined hierarchical, two streams and reconvergence’ model by making a two-dimensional configuration in which the hierarchical model was dimension 1 and the two streams model was dimension 2.

Table 2 shows the results of the quantitative comparison of these models with the structure in Fig. 1. All five models were related to the structure derived for the real cortical visual system at a level that would not be expected by chance (less than 1 in 600 probability). The two models that represented border relations between areas explained about the same amount of variability (30%) as the hierarchical model. The model that represented dichotomization and reconvergence, however, explained almost half the variability, whereas the combined model accounted for almost three quarters of the variability in Fig. 1.

These results suggest that, despite the enormous complexity of the cortical visual system, at this gross level it may be organized according to four principles: (1) It is dichotomized into two streams, (2) both streams are hierarchies, (3) the streams reconverge in area 46 and STPs, and (4) neighbouring areas tend to innervate one-another.

Two of these organizational features derived from topological analysis of the patterns of connections, the two streams and hierarchical features, corroborate organizational principles derived from different information sources, such as lesion studies19 and from the laminar termination patterns of cortico-cortical projections1, respectively. The finding that border relations may be present in the wiring pattern of the system might reflect the evolutionary advantage of keeping wiring to a minimum2,23, although the redundancy of connections from the prestitiate areas to the areas of the caudal superior temporal sulcus and the posterior parietal cortex suggests that wiring economy is not a strong constraint. Alternatively, the tendency of areas to innervate their neighbours may reflect a parsimonious developmental process.

Some recent models of vision in which the coherence of visual perception is brought about by the synchronization of oscillatory activity in neurons distributed widely in the brain24 have been motivated in part by the apparent lack of a brain structure at which the reconvergence of processed visual information could occur. These results emphasize that there are cortical sites, namely STP25,26 and area 46, at which reconvergence may take place. This feature of the primate cortical visual system may allow primates to form coherent representations of their visual environment without the need for stimulus-related oscillatory neuronal activity27,28.
FIG. 1 The topological organization of the macaque cortical visual system. Reciprocal connections are coloured red, one-way projections going from left to right are coloured blue and one-way projections going from right to left are green. A total of 301 connections is represented, of which 62 are one-way. This non-arbitrary structure is a best-fit representation in 2 dimensions of the connectional topology of this system, in which the positions of areas are specified by their positions being ones that minimize the distance between connected areas and maximize the distance between areas that are not connected. The analysis represents in a spatial framework the organization of the network of cortico–cortical connections between elements of the visual cortex. In detail, the structure was derived by submitting the proximity matrix in Table 1 to non-metric multidimensional scaling\textsuperscript{7–9}, using ALSCAL\textsuperscript{10}. Solutions with the level of measurement specified as nominal and ordinal were derived, to assess whether a least-squares categorical transformation was required\textsuperscript{9}, but there was no perceptible difference between them. Analogous solutions were derived with different assumptions about the connections which have not been observed (assumed not to exist in Fig. 1). The first alternative solution used a missing data estimation procedure\textsuperscript{30} to estimate the ‘unknown’ connections, the second coded ‘unknowns’ at intermediate values between those for ‘known’ and known not to exist’ connections, and the third assumed that all ‘unknowns’ will be found to exist. These solutions in all cases showed a similar structure to Fig. 1, all explaining more than 76% of its variability, but with areas whose connections are poorly understood (such as VOT and V4t) slightly displaced. Ordinal solutions in one to five dimensions were derived so that solutions with different dimensions could be compared in a ‘score’ test. This test showed diminishing returns in numbers of dimensions greater than two.


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Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen

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IMMUNIZATION with myelin basic protein (MBP) induces experimental allergic encephalomyelitis (EAE), a prototype of CD4\textsuperscript{+} T-cell mediated autoimmune disease. In rodents, MBP-reactive T-cell clones are specific for a single, dominant determinant on MBP and use a highly restricted number of T-cell receptor genes\textsuperscript{1–3}. Accordingly, EAE has been prevented by various receptor-specifitic treatments\textsuperscript{4–6}, suggesting similar strategies may be useful for therapy of human autoimmune disease. Here we report that in SJLxB10.PL\textsuperscript{F} mice, immune dominance of a single determinant, MBP:Ac1–11, is confined to the inductive phase of EAE. In mice with chronic EAE, several additional determinants of MBP in peptides 35–47, 81–100 and 121–140 recall proliferative responses. Most importantly, reactivity to the latter determinants was also detected after induction of EAE with MBP peptide Ac1–11 alone; this demonstrates priming by endogenous MBP determinants. Thus, determinants of MBP that are cryptic\textsuperscript{7–11} after primary immunization can become immunogenic in the course of EAE. Diversification of the autoactive T-cell repertoire due to

‘determinant spreading’ has major implications for the pathogenesis of, and the therapeutic approach to, T-cell driven autoimmune disease.

We tested the fine specificity of the T-cell response to MBP in SJLxB10.PL\textsuperscript{F} mice which are highly susceptible to EAE. Because immunization with autologous MBP is inefficient in priming an autoreactive T cell response we, like others\textsuperscript{12–14}, used a xenogeneic peptide for priming; guinea-pig MBP shares 91% sequence homology with the murine protein. First, we followed the inductive phase of the immune response in draining lymph nodes after immunization with MBP and pertussis toxin (PTX). Injection of PTX is a prerequisite for induction of EAE in mice, owing to its disruption of the blood–brain barrier\textsuperscript{15}. At the peak of the local response, there was immune dominance of a single determinant (peptide MBP: Ac1–11) (Fig. 1a). Neither peptide 81–100, the immunodominant MBP determinant in the SJL (H\textsuperscript{2}) strain\textsuperscript{16}, nor MBP peptides 35–47 and 121–140, which are both immunogenic and encephalitogenic in the B10.PL (H–2\textsuperscript{b}) mice\textsuperscript{17,18}, induced a response in T cells from F\textsubscript{1} mice after immunization with MBP (Fig. 1a). Therefore, peptides 35–47, 81–100 and 121–140 behaved as cryptic determinants. The fine specificicity of the T-cell response to MBP in the splenocyte population on day 9 was essentially identical (Fig. 1b). As a prototype ‘foreign’ antigen, we used hen eggwhite lysozyme (HEL) for control immunizations. In HEL/PTX-immunized F\textsubscript{1} mice, peptide HEL: 30–53 was immunodominant (Fig. 1d, e). Importantly, HEL peptides 11–25 and 116–129, which are both dominant in the SJL parental strain (and which are immunogenic when injected as peptides into F\textsubscript{1} mice, P.V.L. and T.F., unpublished observations), behaved as cryptic determinants in the F\textsubscript{1} mice (Fig. 1d, e). Thus, in both the MBP and HEL systems, immune dominance of a single determinant occurred despite the presence of several potential T-cell determinants.

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