suggest that herbicide-tolerant oilseed rape poses no greater threat to the environment than the conventional crop, it would be prudent to reserve judgment on the risks that might be posed by other crop species or different transgenic constructs (such as transformations like drought tolerance or pest resistance, which might be expected to enhance plant performance in natural habitats). □

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Spatial working memory in humans as revealed by PET

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THE concept of working memory is central to theories of human cognition because working memory is essential to such human skills as language comprehension and deductive reasoning[1–4]. Working memory is thought to be composed of two parts, a set of buffers that temporarily store information in either a phonological or visuospatial form, and a central executive responsible for various computations such as mental arithmetic[5,6]. Although most data on working memory come from behavioural studies of normal and brain-injured humans[7], there is evidence about its physiological basis from invasive studies of monkeys[8–10]. Here we report positron emission tomography (PET) studies of regional cerebral blood flow in normal humans that reveal activation in right-hemisphere prefrontal, occipital, parietal and premotor cortices accompanying spatial working memory processes. These results begin to uncover the circuitry of a working memory system in humans.

The two main conditions of the experiment are illustrated in Fig. 1. The volunteers were trained on the perception and memory tasks and then prepared for PET scanning. The experiment consisted of six scans: two perception conditions, two memory conditions, and two scans of other conditions not relevant to the present experiment. Figure 2 illustrates the protocol for a scan.

Subjects were quite accurate in their behavioural performance in this task, making no errors in the perception condition, and only 15.8% errors in the memory condition. Of these errors, roughly half occurred on those trials in which the probe did not encircle a target location. Interestingly, these 'miss' errors were more frequent the closer the probe was to a previously occupied location (28.9% of them occurred for 'near' probes versus 7.5% for 'far' probes). This is to be expected if subjects used a spatial memory buffer to mediate their performance, making a probe that appeared relatively close to the location of a previous target difficult to reject on the basis of having encircled that location.

The major difference between the perception and memory conditions was that only the latter required subjects to store location information; hence, the differences between these two conditions provide information about the localization of processes associated with memory. Figure 3 presents four brain images showing the brain activation levels that resulted from subtraction of the perception from the memory condition.

The top left image in Fig. 3 shows one of the areas of reliable activation, in the right prefrontal cortex. This focus is consistent with reports of single-cell activity in monkeys in a similar area, in the vicinity of the principal sulcus[10]. But the reported activity in monkeys is bilateral, in contrast to the largely unilateral activation in our human subjects. A second area of activation shown in the bottom left image of Fig. 3 was centred in the posterior portion of the parietal cortex of the right hemisphere.

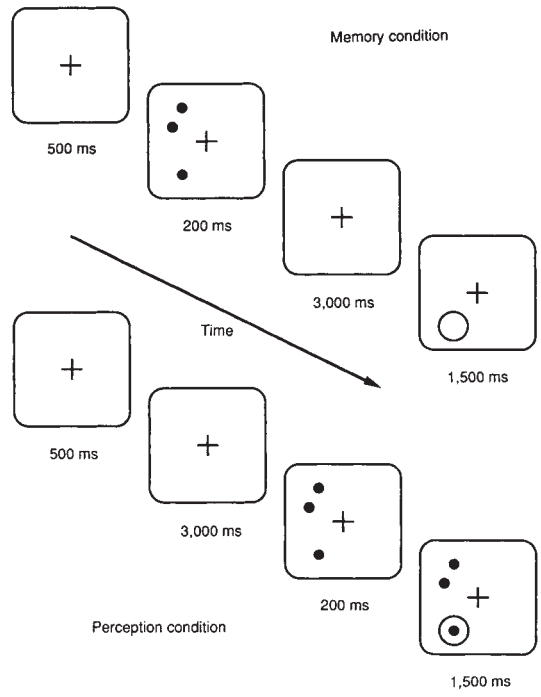

FIG. 1 The upper part of the figure illustrates the events in the 'memory' condition of the experiment. Subjects began by fixating a cross in the centre of the screen for 500 ms. The cross was then supplemented by three dots appearing on the circumference of an imaginary circle of 14 degrees diameter, centred on the cross. The dots remained in view for 200 ms (too short an interval to permit a successful saccade to the dots on average), following which the fixation cross continued to appear for a retention interval of 3,000 ms. This was followed by a probe for location-memory which consisted of a single outline circle that either encircled the location of one of the previous dots (with probability 0.50) or not. The probe circle was presented for 1,500 ms; during this interval, subjects pressed a response button once or twice to indicate whether or not the probe marked the location of a dot. The probe circle was either centred directly over the previous location of a dot, or it missed the nearest dot location by 15–40 degrees. The lower part of the figure shows the events on each trial of the 'perception' condition. Trials again began with a fixation cross, which remained in view for 3,500 ms (the duration of the fixation plus retention intervals in the memory condition). This was followed by three dots presented for 200 ms, followed immediately by an interval of 1,500 ms during which the three dots and probe circle were presented simultaneously. As in the memory condition, subjects pressed a response button once or twice to indicate whether or not the probe encircled a dot. The order of administration of conditions was counterbalanced across subjects.

Protocol for a PET scan

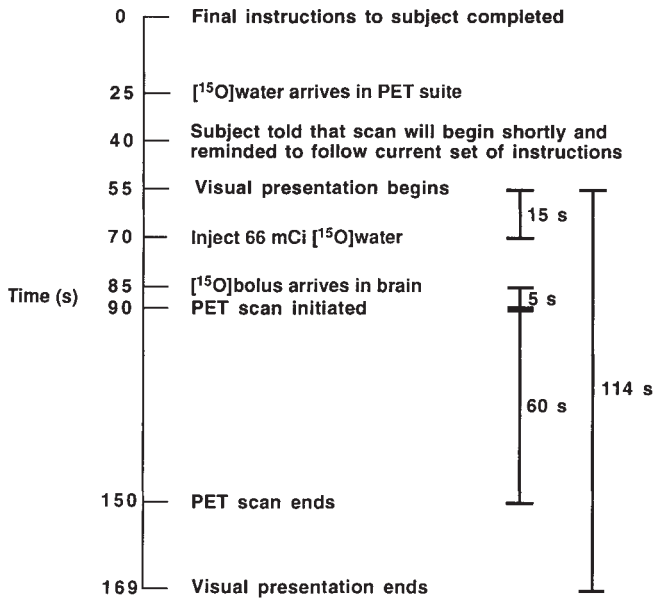

FIG. 2 Each scan consisted of 20 trials, with the first 3 presented before the injection of the radionuclide (the total duration of these 3 trials was ~15 s). Immediately after these trials, an intravenous bolus administration of 66 mCi of [^{15}O]water was given, after which ~15 s elapsed before the radionuclide reached the brain. Trials continued to be administered during this interval. Recording of activity began 5 s after the count rate was observed to increase above the background level and continued for 60 s thereafter. Injections for subsequent scans were separated by 14-minute intervals (seven half-lives), permitting the ^{15}O to decay to less than a 1% background level.

This area has previously been reported to be engaged by visual tasks that require object localization[11,12]. A third area of activation (shown in the top right image of Fig. 3) is in the right occipital cortex. This area has been implicated in the creation of images in humans[13,14]. The final focus of activation, illustrated in the bottom right image, is in right premotor cortex. We do not believe that activation in this region is due to the motor requirement of the present task (responding with button pushes) because this requirement was identical in both the perception and memory conditions and therefore should have been subtracted out. More importantly, the premotor activation revealed in our data is in the right hemisphere, yet all subjects responded with their right hands.

The statistically significant areas of increased activation reported above are all located in the right hemisphere. There was increased activation as well in the homologous areas in the left hemisphere (as suggested by examination of some of the images of Fig. 3), although none of the areas in the left hemisphere reached statistical significance by our criterion. Nevertheless, there is reason to be cautious about the localization of the relevant processes to only one hemisphere (see ref. 15 for related evidence).

The patterns of brain activation revealed in this experiment suggest possible models of spatial working memory processes that are broadly consistent with the modal conceptualization of working memory derived from behavioural experiments[5]. One possible model postulates that subjects create an internal image of the target dots at the time these targets are presented, using mechanisms in the occipital lobe. An internal image of the dots might correspond to a matrix-like representation that includes information about target location for all the dots simultaneously. This image might then be used to calculate the dot coordinates by mechanisms in parietal cortex. Storage processes of the prefrontal cortex might then be responsible for keeping the image in memory during the retention interval. Further tests of this and similar models will be possible with further refinements of the tasks given to subjects while their brain activity is recorded.

The present experiment provides data once again implicating lateral prefrontal cortex in the maintenance of spatial information for short periods of time, thereby extending previous research conducted on monkeys[10]. Although it is becoming increasingly clear that prefrontal cortex is involved in working

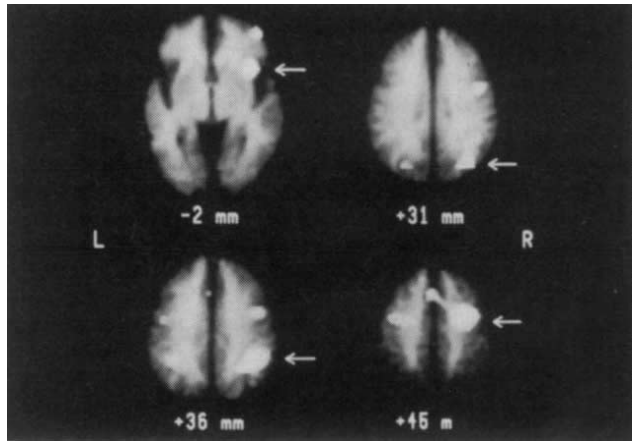

FIG. 3 PET images of the four statistically significant activation sites, each superimposed on a magnetic resonance image of a composite brain for the purpose of illustrating the anatomical localization of activation foci. The images represent the subtraction of the perception from the memory condition across 18 subjects. The areas of activation highlighted by arrows are those that exceeded a level of statistical significance of $P < 0.05$ by t-test after correction for multiple comparisons. All four significant foci of activation were in the right hemisphere. These were in prefrontal cortex (Brodmann's area 47, stereotaxic coordinates: −35, 19, −2), parietal cortex (Brodmann's area 40, stereotaxic coordinates: −42, −40, 36), occipital cortex (Brodmann's area 19, stereotaxic coordinates: −30, −76, 31), and premotor cortex (Brodmann's area 6, stereotaxic coordinates: −34, −1, 45) (coordinates are presented in the order: left to right, posterior to anterior, and ventral to dorsal). The data were derived by the following method. The PET images for each subject were transformed to a stereotaxic coordinate sytem[17,18] and standardized to an atlas brain by linear scaling[19]. After normalizing pixel values for global flow rate differences among scans[20], the data were averaged across the 18 subjects, giving mean and variance values for each condition. The average image for the perception condition was subtracted from that of the memory condition to reveal differences in activation between these conditions. The difference image was analysed for statistical significance on a pixel-by-pixel basis using t-statistics, followed by a multiple-comparison adjustment based on the Bonferroni method[21]. A one-tailed adjusted value of $P < 0.05$ was used as a criterion for reliability.

memory, it is not yet clear what its role is. One obvious possibility (assumed in the preceding model) is that the neuronal activity recorded in prefrontal cortex is itself the internal representation of spatial location that is maintained during a retention interval. According to this view, spatial location may be coded by individual neurons, or it may be coded by populations of such neurons, as is apparently characteristic of other cortical areas[16]. It is also possible that the prefrontal activity that we and others have recorded represents a pointer or index to other circuitry that is responsible for maintaining the actual engram for spatial location. This other circuitry may itself be coherent and well localized (for example, consisting of an image stored in occipital cortex), or it may be distributed among a number of interlinked areas of cortex. □

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Extent to which homology can constrain coding exon junctional diversity in *V(D)J* recombination

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AMONG site-directed DNA recombination systems, *V(D)J* recombination is noteworthy in that identical reactants yield different recombination products at the junction of joined segments. This variation is the basis for diversity at the base of antigen receptor binding pockets and corresponds to *V-(D)-J* DNA junctions. An abundance of certain junctions has been noted[1–5]. It has been proposed that these junctions are favoured because they occur where short regions of homology in participating coding ends might align preferentially[1]. Here we use a system that is entirely free from cellular selection to show that the diversity of coding joints can be severely restricted when the coding ends participating in the reaction have short regions of homology. This constraint on diversity is diminished but not eliminated by terminal deoxynucleotidyl transferase, a mechanistic feature that has implications for the establishment of the immune repertoire.

We have assessed the frequency of coding end homology

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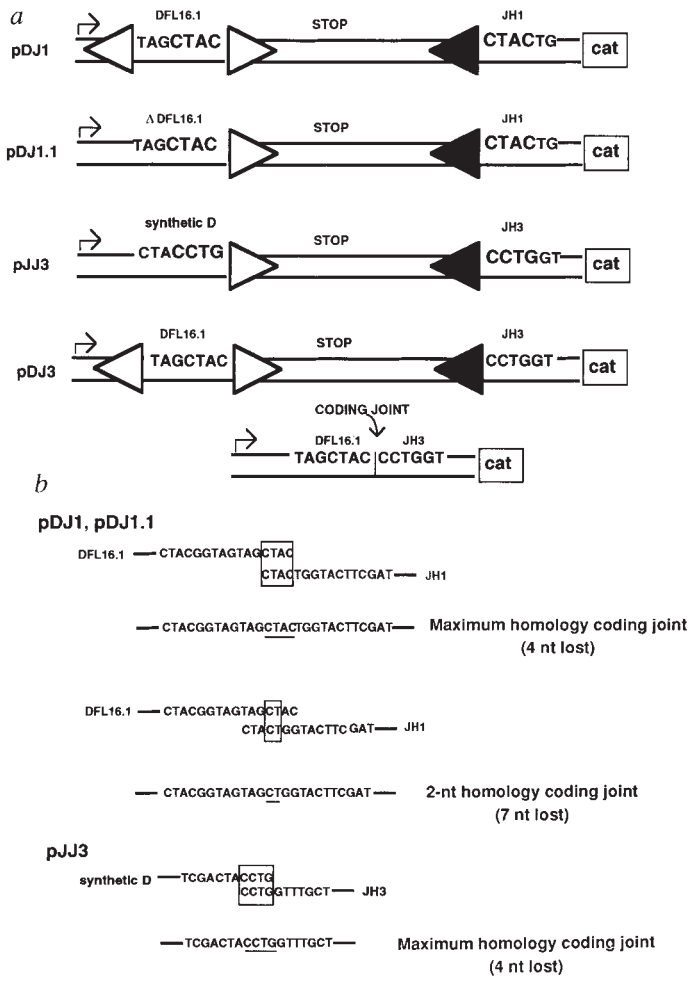

FIG. 1 *DJ* recombination substrates and their recombinant products. *a*, Substrates. Essential features of these recombination substrates have been described[13]. *V(D)J* recombination signal sequences (the signal with the 12-bp spacer is depicted as an open triangle and the 23-bp spacer signal is shown as a filled triangle) are separated by a stop sequence, the prokaryotic transcription terminator *oop*, and are placed between the chloramphenicol acetyltransferase gene (*cat*) and a prokaryotic promoter (P_{lac}), represented by an arrow. A small portion of the coding end nucleotide sequence is displayed. After excision of the signals and *oop* during *V(D)J* recombination in mammalian cells, the recombinant plasmid expresses *cat* upon transformation of *Escherichia coli*. Substrates contain the β-lactamase gene, which confers ampicillin resistance in *E. coli*. Therefore, recombinants can be selected on ampicillin/chloramphenicol plates. *b*, Illustration of coding joints. The maximum homology coding joint is formed when the 4-nucleotide (nt) region of sequence homology at the coding ends of pDJ1, pDJ1.1 or pJJ3 (boxed) is used in coding joint formation. The 2-nucleotide homology (boxed) coding joint shown results when 2, 3 or 4 nucleotides are lost from one coding end and 5, 4 or 3 are lost from the other coding end of pDJ1, pDJ1.1. Retained homology is underlined.
METHODS. pDJ1 was constructed by cloning oligonucleotides containing the DFL16.1 and and JH1 of the BALB/c IgH locus into the *Sal*I and *Bam*HI sites, respectively, of pJH298 (ref. 13). pDJ1.1 is essentially the same as pDJ1, except that the ΔDFL16.1 element does not contain a signal sequence 5′ of the coding sequence and does contain consensus *V(D)J* recombination signal sequences[12]. This modification was necessary to obtain coding joints in fibroblasts co-transfected with RAG1 and RAG2 expression vectors. pDJ3 contains the same D element as pDJ1 and has an oligonucleotide containing JH3 from the BALB/c IgH locus inserted in the *Bam*HI site. Construction of substrates containing DFL16.1, JH1, and JH3 are detailed in ref. 27. pJJ3 is a modification of pDJ3 in which the *Sal*I insert has been replaced with an oligonucleotide (5′-TCGACTACCTGCACAGTGCTATATCCATCAGCAAAAA-CCTGCAG-3′) that creates a synthetic D element.