Shape Discrimination Deficits During Reversible Deactivation of Area V4 in the Macaque Monkey

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The role of area V4 in the primate extrastriate cortex has received much attention in recent years. However, the deficit specificity following area V4 ablations has been difficult to determine due to the ablations including area V4 and additional adjacent areas, deficit attenuation and the numerous variations in the results of different research teams. In order to address these issues, we examined the role of area V4 during reversible deactivation of the lower visual field representation within this area while macaque monkeys performed simple pattern discriminations and their eye position was monitored. Specifically, the monkeys were trained to perform a match-to-sample task with the sample stimulus placed within or outside the visual field quadrant represented within the deactivated region of area V4. The sample and match stimuli had the same salience (same size or luminance). Using this approach, we identified significant simple shape discrimination deficits during deactivation of area V4 that did not attenuate with time. Deficits were also identified when the discriminanda were the same figure viewed at different orientations (rotated shapes). In contrast, no deficits were identified during simple hue discriminations. Furthermore, no saccadic eye movement deficits were identified during deactivation of area V4. Therefore, we conclude that deactivation of area V4 yields specific deficits on simple and rotated shape discriminations. These results show that area V4 is an important step in shape and form processing along the ventral visual stream leading to the inferotemporal cortex.

Introduction

Two visual processing streams through the extrastriate cortex have been identified as emanating from the primary visual cortex of the monkey (Mishkin *et al.*, 1983). The first is the dorsal stream, which projects to the parietal cortex and has been implicated in the analysis of visual space. The second is the ventral stream, which projects to the inferotemporal cortex and has been implicated in pattern and object recognition. Area V4 is generally considered to be the first visual processing area within the ventral stream following the separation of the two streams in area V2 (Mishkin *et al.*, 1983). Several studies of area V4 have examined its contribution to colour processing and form recognition. These studies revealed a role of area V4 in colour constancy (Walsh *et al.*, 1993), but a rather limited role of area V4 in colour categorization or hue discrimination (Dean, 1979; Heywood and Cowey, 1987; Walsh *et al.*, 1992b). In earlier studies, much of the contribution that area V4 was thought to make to form vision could be attributed to the fact that regions of area TEO were included in the ablations of area V4 (Heywood and Cowey, 1987). More recently, permanent deficits in the discrimination of less salient visual stimuli, through the use of occlusion or backgrounds, have been identified following area V4 destruction (Schiller, 1993; De Weerd *et al.*, 1996; Merigan, 1996, 2000). It was found that, when stimuli had the same salience, the discrimination of simple shapes was only mildly impaired following permanent deactivation of area V4 (Walsh *et al.*, 1992a; Merigan, 1996; Merigan and Pham, 1998). However, an earlier study (Schiller, 1995) carefully traced the performances of several monkeys on similar simple pattern discriminations and identified a profound deficit during the days immediately following an area V4 ablation that attenuated over time. The final outcome of this attenuation was a nearly complete recovery (Schiller, 1995). This recovery was not surprising, as deficit attenuation has been well documented in other visual areas (Newsome and Paré, 1988; Yamasaki and Wurtz, 1991; Pasternak and Merigan, 1993), although examples have also been provided of permanent deficits following restricted lesions (Lauwers *et al.*, 2000). The results from study mentioned above (Schiller, 1995) suggested that the early deficits corresponded to a pathological state of the cortex following the ablation and that the subsequent recovery was the sign of a lessening of those deficits. For instance, the secondary effects of lesions, such as paroxysmic neuronal discharges that can be recorded close to a lesion focus (Eysel and Schmidt-Kastner, 1991) or retrograde (thalamic) degeneration, could lead to peaking of the effects of the lesion.

An alternative explanation is that the initial deficit in simple pattern discriminations is due to the loss of area V4 neurons and the functional post-lesion recovery is due to a reorganization of other cortical areas that take over the role played by these neurons. Another alternative explanation would be that post-lesion recovery could be due to an animal adopting a new behavioural strategy for solving a new problem (for instance, if a target item looks different after a lesion, the animal may learn to associate this 'new' item with a reward). These explanations led us to hypothesize that reversible deactivation of area V4, in the absence of deficit attenuation, would produce consistent and reproducible deficits in simple pattern discriminations. In order to test this hypothesis, we examined the pattern discrimination abilities of macaque monkeys during cooling deactivation of area V4. Cooling deactivation permitted the specific deactivation of area V4 without the confounding variable of deficit attenuation (Lomber, 1999). In addition, we examined the impact of area V4 deactivation on hue and orientation discrimination.

Materials and Methods

Subjects and the General Plan

Five (one female and four male) naïve young adult (~4 kg) monkeys (*Macaca fascicularis*) participated. The first step in the experiment was to train the animals to sit in a primate chair and acclimate to the experimental apparatus. The second step was the surgical implantation of a scleral search coil into one eye and a stainless steel head post to the anterior one-third of the skull. The animals were then trained to perform the visual tasks. Next, a cryoloop was implanted unilaterally over dorsal area V4 of the left hemisphere in order to permit reversible deactivation with cooling (Lomber *et al.*, 1999). The animals were then tested on the visual tasks before, during and after deactivation of area V4. Finally, an overdose of barbiturates was used for euthanasia of the animals. They were then perfused with aldehydes for histology.

Normal quantities of monkey chow were available at fixed intervals during the day during training and testing and their diet was supplemented with fresh fruit at the end of each behavioural session. The animals were tested 5 days a week and water was restricted for 22 h prior to each testing session. The animals were rewarded with diluted apple juice during the testing sessions. Normal quantities of water were provided at the weekends. Care was taken to ensure that there was no significant weight loss.

Surgical Procedures

All surgical procedures were performed under sterile conditions and general anaesthesia. The animals were initially sedated with ketamine (16 mg/kg) and maintained with alphaxolone/alphadolone (Saffan®) (~15 mg/kg/h administered intravenously). The animal's head was then placed in a stereotaxic apparatus. Prior to any dermal incisions, xylocaine (1% administered subcutaneously) was administered to the site. The animals' temperature and heart rate were continuously monitored throughout each procedure and maintained at appropriate levels. Analgesic drugs (20 mg/kg/h paracetamol administered orally) and systemic antibiotics were administered after each procedure and the general state of the animal was closely monitored for any signs of pain or cerebral oedema.

In the first surgical procedure a scleral search coil, which permitted monitoring of the animals' eye position, was placed in one eye and a head post was implanted over the anterior skull, which permitted stabilization of the head during testing. The implantation of the scleral search coil was based on the Judge technique (Judge *et al*., 1980).

In the second surgical procedure, a unilateral cooling loop (Lomber *et al*., 1999) was chronically implanted over area V4 on the surface of the prelunate gyrus (Fig. 1). We defined the location of area V4 according to previously published descriptions (Gattass *et al*., 1988; Barone *et al*., 2000). A craniotomy was centred 17 mm anterior to the occipital crest and 27 mm lateral to the midline. The aperture was ~2 cm^2 and enlarged as necessary. The dorsal tip of the inferior occipital sulcus and the posterior margin of the superior temporal sulcus were used as landmarks for the placement of the loop. The dura mater was reflected in order to allow access to the prelunate gyrus. We chose one cryoloop from among several which had previously been prepared and sterilized with ethylene oxide. The loop (10 mm in length and 4 mm in width) was centred on the middle of the prelunate gyrus between the superior temporal sulcus and the lunate sulcus (Fig. 1). The anterior end of the loop abutted the tip of the inferior occipital sulcus. We estimated from an earlier study (Gattass *et al*., 1988) that the region of visual field represented by the cortex under the loop corresponded to the parafoveal contralateral inferior quadrant at an eccentricity of ~3–4° degrees (Fig. 2*A*). Once good contact had been made with the cortical surface, the loop was secured to the skull with surgical bone screws and acrylic cement. The dura was then sewn back into place above the loop or the loop was covered with artificial dura (Neuropatch®). The skull that was removed during the craniotomy was then replaced and secured with bone screws and acrylic cement that covered the entire implant, leaving the tube endings and thermocouple plug exposed. The monkey was prevented from gaining access by covering the tube endings with a cap.

Visual Stimuli

Visual stimuli and behaviour assessment were performed with the Cortex programme in the early phases (courtesy of Dr R. Desimone) and then with Tempo® software (Reflective Computing, St Louis, MO). The stimuli were displayed on a computer monitor with a 60 Hz refresh rate. The luminance background was 0.03 cd/m^2. The screen was placed 57 cm in front of the monkey's eyes. Many pairs of simple visual stimuli were used, but only one stimulus pair was tested within a session. The size of an individual stimulus was ~1° of visual angle. The stimuli had the same salience (same size or luminance, except for the white versus colour discrimination) so that no stimulus could draw more attention than the other. However, no behavioural test of salience was attempted.

Training

The animals were initially trained to sit in a primate chair (modified from Primate Products®, Miami, FL) with their head fixed. The position of their gaze was monitored with an eye coil system (Remmel Laboratories®, Ashland, MA or DNI®, Newark, DE). They first learned to fixate a small red spot (radius 0.15°) and were rewarded with diluted apple juice for maintaining their gaze (i.e. within a square window of 1.5°). A brief tone signalled the release of the reward.

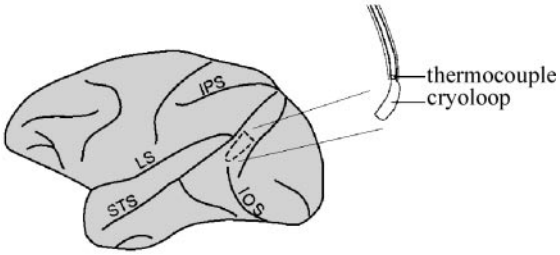

Figure 1. Schematic lateral view of the left hemisphere of the macaque cerebrum. The cryoloop was centred on the middle of the prelunate gyrus, between the superior temporal sulcus and the lunate sulcus and just dorsal to the end of the inferior occipital sulcus. STS, superior temporal sulcus; IOS, inferior occipital sulcus; LS, lateral sulcus; IPS, intraparietal sulcus. Left is anterior and top is dorsal.

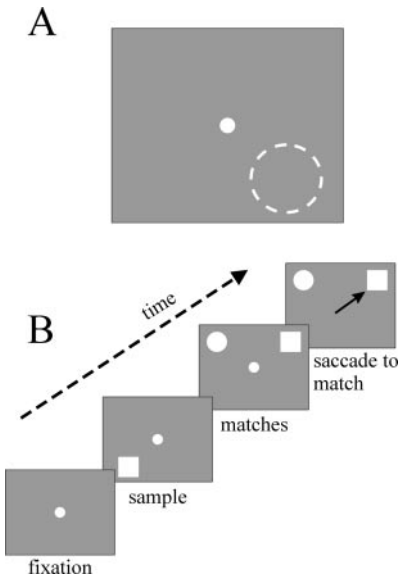

Figure 2. (*A*) The approximate portion of the visual field (parafoveal right lower quadrant) deactivated by the cooling loop is indicated by the dotted line. The central fixation spot is shown as white (red in reality, except for red–green discriminations). (*B*) Behavioural task. The monkeys first fixated a small red spot (0.4° in diameter) for 600 ms. While maintaining fixation, a sample randomly appeared in either the left or right lower quadrant for 1 s and was then extinguished. After a delay of one frame (16 ms), two stimuli (one match and one non-match) appeared in the upper quadrants. The fixation spot was extinguished after 1 s and the monkey was allowed to make a saccade to the correct match.

After mastering the simple fixation task, the monkeys were trained on a match-to-sample task. Figure 2*B* shows the temporal course of the task. While the animal fixated the central spot, a sample stimulus was presented in one of the two inferior quadrants. The sample was extinguished after a few hundred milliseconds, but the animal was required to maintain its gaze at the fixation point. Sixteen milliseconds after the extinction of the sample, two stimuli were then displayed in the upper field, one in each quadrant and one of which was the correct match. As soon as the fixation spot was extinguished, the monkey made a saccade to the correct match, fixated on it and was rewarded as above. The durations varied in the following ranges for different experimental animals: fixation spot alone 300–600 ms, sample 500–700 ms, matches 500–1000 ms and keep eyes on a response item 300–600 ms. The presentation of all stimulus configurations were interleaved in a pseudo-random manner in order to avoid more than two consecutive repetitions of the same configuration.

The sample and matches were displayed at a fixed value of eccentricity (between 2 and 4° of the visual field) during each session.

In every case, the monkeys made direct and accurate saccades to either the match or non-match stimulus. Saccades to the incorrect item were punished by an absence of reward and an increase in the inter-trial interval (up to 2 s). The standard inter-trial interval was 750 ms. If the monkey broke fixation at any time during the trial, all stimuli were extinguished and the next trial began following a penalty delay of 2 s. Any variations from this protocol are described in the Results.

Testing During Cooling Deactivation

Behavioural testing during cooling deactivation was performed as follows.

1. The monkey was placed in the apparatus and its head was secured.
2. The tube ends of the cryoloop were exposed by removing the protective cap. The tubes were then connected to the system and warm methanol was passed briefly to check for any obstructions or leaks in the methanol circulation.
3. The thermocouple was then plugged into the thermometer for monitoring the cryoloop temperature.
4. The animal then began to perform a discrimination test using a particular pair of stimuli and control data were collected.
5. While the animal was still working, the pump was turned on and cold methanol was passed through the cryoloop. In ~2 min, the temper- ature of the cryoloop was stabilized at 2 ± 2°C or 7 ± 2°C and a block of 20–30 trials per stimulus condition were collected. Trials recorded during the temperature stabilization period were not included in the analysis. A cooling deactivation period typically lasted for 15–20 min.
6. The animal then continued to work as the pump was turned off and the cortex rewarmed. A block of trials was collected during rewarming. On some days, multiple deactivations cycles were performed. In order to control for any non-specific deactivation effects, several testing sessions were performed as described above except that warm rather than cold methanol was passed through the system. This condition was termed sham cooling.

Euthanasia

At the conclusion of behavioural testing, the animals were anaesthetized with sodium pentobarbital (50 mg/kg administered intravenously) and perfused through the aorta with aldehyde fixatives in accord with the recommendations of the American Veterinary Medical Association Panel on Euthanasia (Beaver *et al.*, 2001). Note that at the time of the submission of this manuscript, two of the monkeys had not been perfused and are now being used in other projects.

Results

Deactivation Locus

The post-mortem macroscopic evaluation of the cryoloops' position confirmed that the cryoloops were placed over area V4 in the left hemisphere. Each loop was centred on the middle of the prelunate gyrus, between the superior temporal sulcus and the lunate sulcus and the anterior end of the loop was just dorsal to the tip of the inferior occipital sulcus. An earlier study measured that cooling spread to cortical tissue up to 2 mm distant from each side of the loop (Lomber *et al.*, 1999). Since the loop could be approximated to an ellipse of 10 by 4 mm, its surface was given by $\pi \times (10 + 4) \times (4 + 4)/4 = 88$ mm^2. We added an extra 3 mm^2 to our estimation since the shape was not a perfect ellipse. Then, we estimated that a total cortical area of 91 mm^2 was deactivated. Therefore, based upon the total area of V4, as described elsewhere (Felleman and Van Essen, 1991), we estimated that we deactivated 17% of area V4.

From our gross inspection of the brain, we were unable to identify any damage or alterations in the cortical surface. Therefore, we concluded that neither the presence of the cryoloops, nor their repeated deactivation over several months altered the structure or overall function of area V4.

Stimulus Detection and Eye Movements

It was necessary for us to confirm that none of the perceptual results we identified were linked to deficits in eye movements as a consequence of area V4 deactivation. In order to do this, we conducted several visual perimetry sessions in which the monkeys were required to make a saccade to a small white bar (0.4×0.13°) as soon as the fixation spot was extinguished. The bar appeared at random positions between 2 and 5° of eccentricity in any quadrant of the visual field. During these sessions, we did not attempt to determine changes in the perceptual threshold. The monkeys did not make any mistakes during cooling deactivation of area V4and all saccades were made directly to the small bar immediately following the extinction of the fixation point. This result demonstrated that the simple detection of a stimulus was preserved, as was the ability to generate accurate saccades.

Deactivation of Area V4 During Simple Pattern Discriminations

All five monkeys learned the match-to-sample task and their typical performance levels prior to any area V4 deactivations ranged from 80 to 100% correct. The percentage of correct response corresponded to the formula: number of correct responses × 100/(number of correct responses + number of errors). Errors were defined by responses to the non-match item, overshoots, undershoots or an absence of response. In practice, we observed only responses to the non-match item. Only complete trials were considered in the statistical analyses. Incomplete trials, where the animal broke fixation prematurely, were not used for computing the performance of the monkey.

The performance of the animals during deactivation of area V4 depended critically upon whether or not the sample stimulus was presented inside or outside the visual field quadrant that was affected by the cooling. Figure 3 illustrates this point and presents data collected during one complete cooling session from one monkey (M1). The monkey had to discriminate a set of bars from a square. The luminance of the bars and the square was made identical (12 cd/m^2). The lower right quadrant (dashed circle in Fig. 3) was affected by the cooling of area V4 in the left hemisphere. When the sample was presented outside the deactivated quadrant, there was no significant change in performance (Fig. 3*E–H*). In contrast, when the sample was presented within the deactivated quadrant and the correct match appeared in the same hemifield, the animals' performance dropped to chance levels (Fig. 3*C*,*D*). However, this deficit was specific to the match appearing in the same hemifield as the sample because no deficits were identified when the match appeared in the hemifield contralateral to the sample (Fig. 3*A*,*B*).

In addition to the specific deficit described above, five other important observations could be made with regard to cooling area V4 during simple pattern discriminations.

1. All errors consisted of saccades to the non-match item. The errors always consisted of saccades that were vectored directly to the non-match stimulus regardless of whether area V4 was being deactivated or not. The errors were neither due to overshooting nor undershooting of the correct match item, which would have caused the animal to fixate outside of the reward window. A non-response would have constituted an error, but in no case did any monkey refuse to respond during cooling deactivation.

2. For any given pair of stimuli, performance dropped regardless of which one of the two stimuli was selected to be the sample within the deactivated quadrant. For example, in Fig. 3 the deficit is profound regardless of whether the white square (Fig. 3*C*) or the set of bars (Fig. 3*D*) was the sample stimulus. This observation rules out the possibility that a difference in salience in these stimuli could explain the impairment.
3. The deficit identified in the ipsilateral match (Fig. 3*C*,*D*) was not due to a stereotyped behaviour or neglect of the right hemifield (saccades only made to the left side) since the animal was still able to make choices in the upper right quadrant when the sample was presented in the lower left quadrant (Fig. 3*E*,*F*). For the same reason, we could ascertain that this asymmetrical deficit was not due to a direct spread of cooling to a cortical representation of the upper right quadrant.
4. The presence of a deactivation effect was not correlated with the percentage of correct responses made during the control trials. For example, in Figure 3*C*,*E*, the initial performance levels (control) were identical (77%) and the only difference between the two configurations was the location of the sample stimulus. However, only the configuration with the sample inside the deactivated quadrant yielded a deficit (Fig. 3*C*).
5. Any deficits that were identified during area V4 deactivation were completely reversed following rewarming of the cortex for every stimulus configuration (Fig. 3*C*,*D*).

Each of these observations was also valid for other pairs of simple patterns that were discriminated during deactivation of area V4. Figure 4 illustrates another example of a deficit obtained from the same monkey (M1) as in Figure 3 using a stimulus pair consisting of a white disc and a white square that had the same salience. In Figure 4, the same stimulus pair was used in eight different cooling sessions on different days and the results were pooled. Once again, the result described in Figure 3 was observed and deficits were only found when the sample appeared in the same quadrant as the area V4 deactivation and the match stimulus appeared in the hemifield ipsilateral to the sample (Fig. 4*C*,*D*).

Deactivation of Area V4 During Discrimination of Rotated Shapes

Figure 5 presents data from a third stimulus pair that consisted of horizontal and vertical white bars. The same rectangular bar was

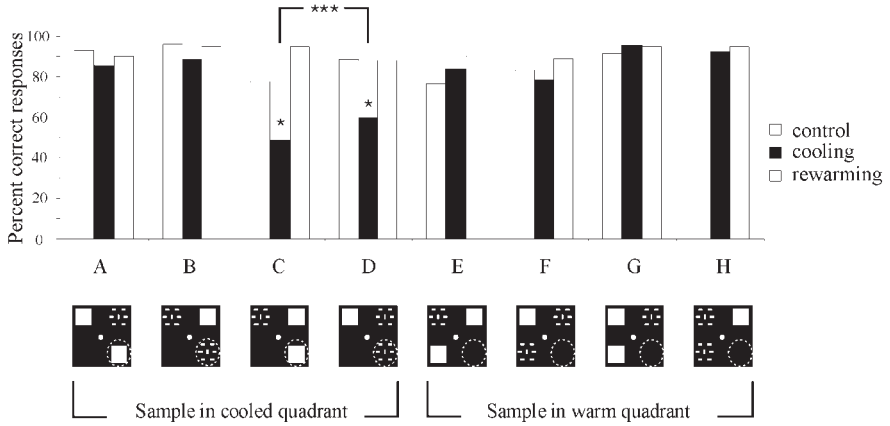

Figure 3. Histograms showing the percent correct responses for each configuration of the sample and the matches. Stimuli are shown at the bottom. For each triplet the open left bar shows data collected prior to cooling deactivation, the centre filled bar shows data collected during area V4 cooling deactivation and the right open bar shows data collected after rewarming of the cortex. The four leftmost triplets of bars (*A*–*D*) correspond to a sample appearing in the 'cooled quadrant', contralateral to the deactivated cortex (schematically delimited by a dotted circle), whereas the four rightmost triplets (*E* and *F*) represent the 'warm quadrant', ipsilateral to the deactivated cortex. The indices of significance are the level of probability obtained by the χ^2-test. $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$. The three asterisks above stimulus configurations C and D indicate statistical significance for both configurations grouped together. Number of control trials = 222, number of cooling trials = 213 and number of rewarming trials = 153.

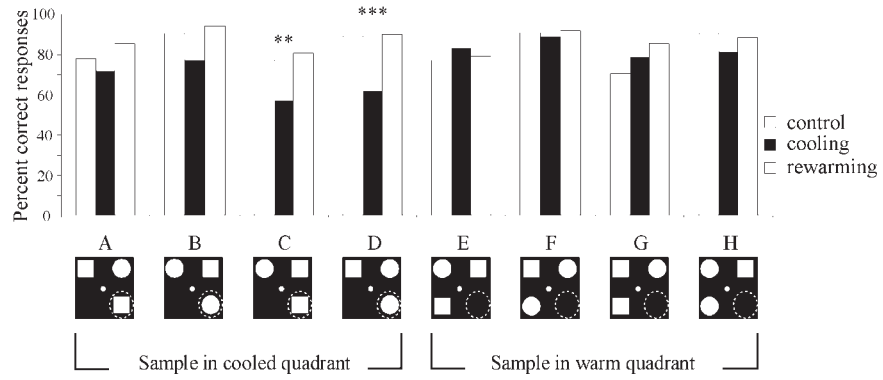

Figure 4. Histograms showing the percent correct responses for each configuration of the sample and the matches shown at bottom. For conventions, see Figure 3. Mean number of control trials = 118.375, mean number of cooling trials = 94.125 and mean number of rewarming trials = 75.7.

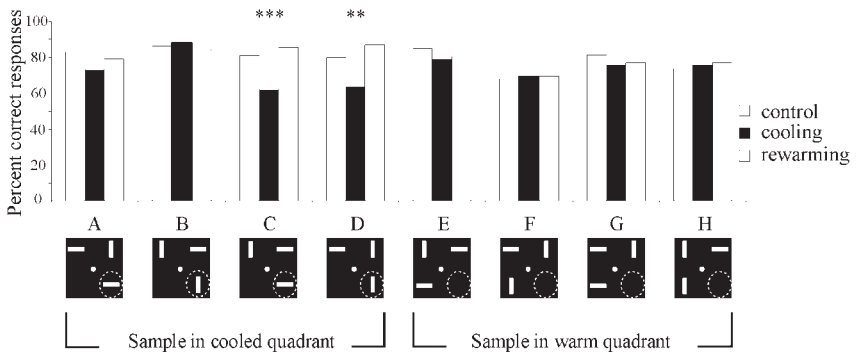

Figure 5. Histograms showing the percent correct responses for each configuration of the sample and the matches shown at bottom. For conventions, see Figure 3. Mean number of control trials = 120.8, mean number of cooling trials = 105 and mean number of rewarming trials = 90.875.

used for each stimulus in the pair and the only difference was a 90° shift in orientation (rotation). In Figure 5, the rotated stimulus pair was used in 10 different cooling sessions on different days and the results were pooled. Deficits were only identified when the sample appeared in the same quadrant as the area V4 deactivation and the match stimulus appeared in the hemifield ipsilateral to the sample (Fig. 5*C*,*D*), similar to that seen in Figure 3. Therefore, deactivation of area V4 during discrimination of rotated shapes yielded results that were nearly identical to those of simple patterns. This deficit could be interpreted either as a coarse orientation or shape discrimination deficit since we could not exclude the possibility that the monkey could judge the two objects as two different shapes.

Behavioural Controls

Training Proficiency

In order to judge the significance of the behavioural deficits during deactivation of area V4 accurately, it was necessary to establish that the animals could perform the task prior to any deactivations adequately and that the performance was stable and consistent. In doing so, we could establish that the deficits we observed were not due to fluctuations in the performance of the monkey during training. Figure 6 illustrates the stability of performance over several days for monkey M2. The stimuli are the same as those described in Figure 3 for monkey M1. Monkey M2 established stable performance for the 21 testing sessions performed prior to surgical implantation of the cooling loop. Furthermore, monkey M2's post-surgical performance was as strong as the pre-surgical performance. However, the first area V4 cooling session produced a marked drop in performance (Fig. 6*A*) on the match-to-sample task when both the sample and match were in the deactivated hemifield. This result was virtually identical to that shown for monkey M1 (Fig. 3). A similarly stable performance was identified on the stimulus configuration when the match was not in the affected quadrant and no behavioural deficits were identified (Fig. 6*B*).

Sham Cooling

Sham cooling sessions were run at various times during the testing period in order to control for any non-specific effects of the area V4 cooling deactivations. Data from one of these sessions are provided in Figure 6. The monkeys' performance during the sham cooling session was comparable to their performance on non-cooling days and pre-implantation training sessions. In addition, the animals displayed no signs of discomfort during either the sham or true cooling sessions. Therefore, we were unable to identify any non-specific effects of the cooling deactivation of area V4.

Testing for Deficit Attenuation

The animals examined in this studied were tested over many months on a variety of stimulus pairs. In order to determine whether there was any deficit attenuation over the many months of cooling, we compared performance during the first week of cooling to performance 7 months later in one animal. Figure 7 presents the data from a pair of stimuli consisting of a white disc and a white square. Even after 7 months the same characteristics of the deficits were still apparent on the discrimination task provided that the sample was presented in the deactivated quadrant and the match appeared in the hemifield ipsilateral to the sample, as described previously (Figs 3–5). A χ^2-test gave no statistical difference between the deficits of each period ($P = 0.12$). Two other sessions obtained 6 months later than the first cooling session gave similar results with the same pair of stimuli. Therefore, we did not have any evidence for deficit attenuation over the course of many months of testing. However, it should be noted that all other stimuli or animals were not tested for attenuation.

Colour Discriminations

In order to test the generality of our results, we examined the animals' ability to discriminate simple patterns that were filled with different colours. The stimuli consisted of two equiluminant red and green squares. The squares were made equiluminant with flicker photometry (using one author with normal trichromatic vision) of 6 cd/m^2. No attempt was made to determine whether the squares were equiluminant for the monkeys. There was no decrease in discrimination performance during deactivation of area V4 for any of the stimulus configurations (Fig. 8). Moreover, it did not matter whether the sample appeared inside or outside the deactivated quadrant (Fig. 8). This result was replicated in two monkeys.

Next, a more complicated stimulus was assembled using overlaid equiluminant red and green bars. This pair of stimuli produced a strong deficit during cooling (Fig. 9*M*). In fact, these two stimuli were very difficult to distinguish (authors' and authors' colleagues own experience) and the only way to identify them required the subject to pay attention to one colour or the other. This causes this task to be equivalent to an orientation discrimination task, since the animal had to identify the orientation of bars of either colour. In order to test this theory, we used a stimulus consisting of a red annulus encircling a green square and vice versa (Fig. 9*K*). Using this configuration,

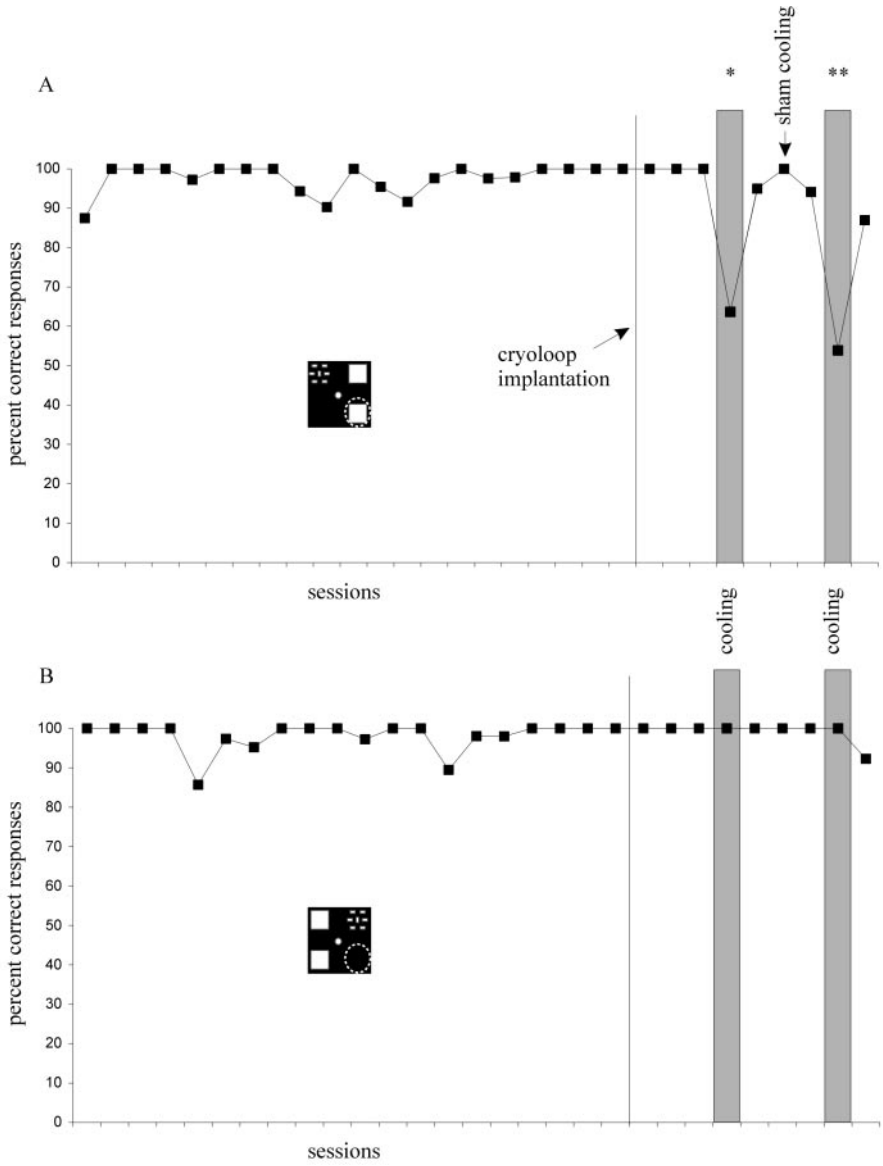

Figure 6. (*A*) Time course of training on one stimulus discrimination while the sample was placed in the cooled quadrant. The percentage of correct responses for this stimulus configuration is shown. The abscissa shows the result for all the different sessions (one per day). The performance is very good and stable, even on the first session after the cryoloop implantation (vertical line, 13 days after surgery). The cooling sessions produce a strong deficit. Note the absence of a deficit for the sham cooling session. (*B*) Time course of the training for a symmetrical configuration when the sample was placed in the warm (non-deactivated) quadrant. Cooling produced no deficit. The stimulus configurations, (*A*) and (*B*), were interleaved as indicated in the Materials and Methods.

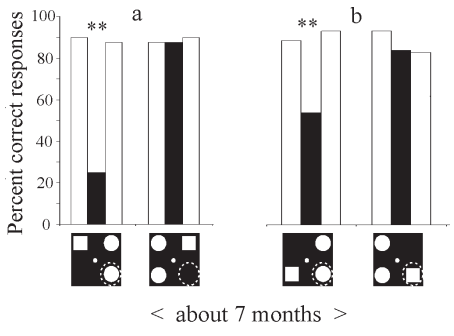

Figure 7. Deficit stability over 7 months for a given match and sample configuration. Note that in (*B*) the configuration was changed such that the sample appeared in the upper hemifield and the match in the lower hemifield. (*A*) Number of control trials = 18, number of cooling trials = 16 and number of rewarming trials = 18. (*B*) Number of control trials = 39, number of cooling trials = 39 and number of rewarming trials = 38.

the task only required colour matching since no orientation cue was present. Using this stimulus pair, deactivation of area V4 did not produce any deficit. The same conclusion could be derived from the negative results obtained with a red vertical bar and a green horizontal bar (Fig. 9*L*). The animal was presumably matching the colour cue, which is easier for monkeys than the orientation cue.

We obtained deficits using colour stimuli during area V4 cooling only when one of the discriminanda was white and the other was either a red square, green square or a green disc (Fig. 9*G*,*I*,*J*). The luminance of the stimuli was 12 cd/m^2 for white items and 6 cd/m^2 for coloured items. The pattern of the deficits could not be explained in terms of differential salience of both stimuli since the errors were evenly distributed for both discriminanda. Furthermore, the result was observed in two monkeys (M1 and M3) and negative results were obtained for monkey M2 with white square/green disc stimuli.

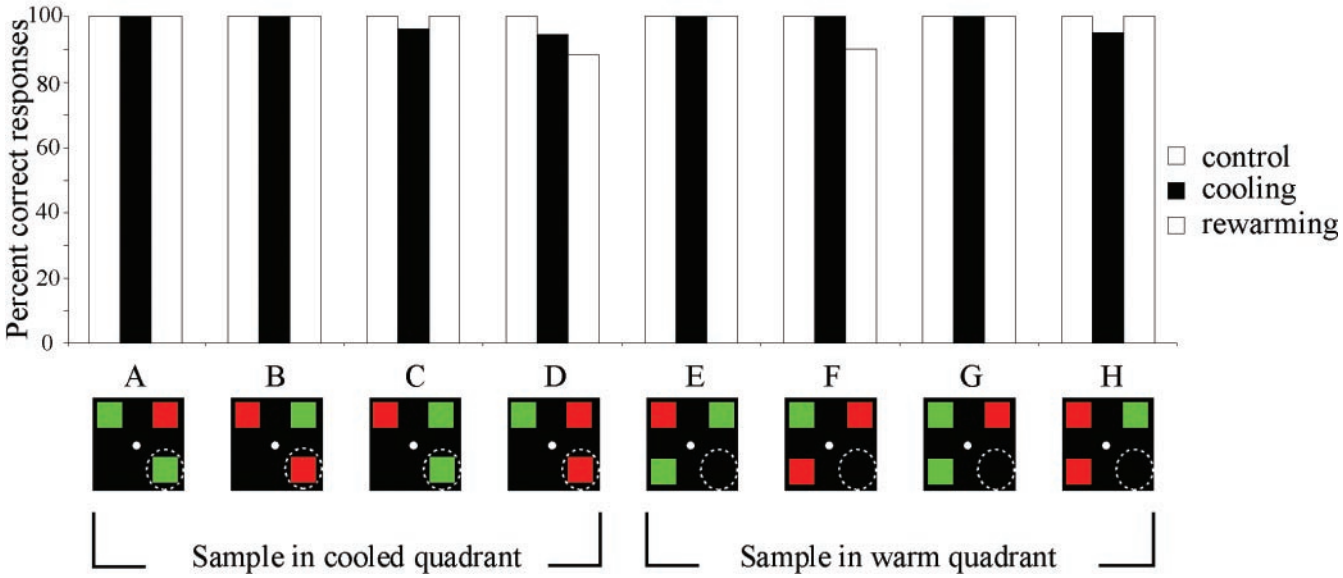

Figure 8. Histograms showing the percentage of correct responses for each configuration of the sample and the matches shown at bottom. This result is taken from one cooling session in monkey M2. For conventions, see Figure 3. Note the absence of a deficit for the equiluminant pair of stimuli. In addition, the fixation spot was actually white instead of red. Number of control trials = 40, number of cooling trials = 154 and number of rewarming trials = 117.

Other Spatial Configurations of the Sample and Matches

Other spatial configurations of the sample and matches were used (see Fig. 9). An important configuration consisted of flipping the stimuli across the horizontal meridian and suppressing the short delay between the sample and matches by making them appear at the same time (Fig. 9*D*). A deficit was present with this configuration during deactivation of area V4, which rules out any very short-term memory component between the presentation of the sample and matches. The absence of memory deficit in our task was reinforced by the observation that lengthening of the delay between the sample and matches to 1 s did not trigger a stronger deficit during cooling.

Finally we tested some configurations with the sample positioned in the fovea (Fig. 9*O–R*). In those cases, the sample appeared around (with no spatial gap) the fixation point. Under these conditions cooling produced an effect, which indicated a deficit in matching a peripheral stimulus in the cooled quadrant with a sample in the fovea. On the other hand, when both matches were in the upper quadrants (hence, the lower cooled quadrant was not visually stimulated), no deficit arose during area V4 deactivation.

Discussion

We trained macaque monkeys to perform simple pattern discriminations and identified significant shape discrimination deficits during deactivation of area V4 that did not attenuate over time. Significant deficits were also identified when the discriminanda were the same figure viewed at different orientations (rotated shapes). However, during deactivation of area V4 we identified no deficit during simple hue discriminations and no saccadic eye movement deficits.

Deficit Specificity

It was critical for us to make sure that the result was genuine and not derived from non-specific effects of the cooling application. The monkeys did not display any behavioural sign of discomfort due to the circulation of the cold fluid (as was also the case in all studies published by other authors with the same technique). Furthermore, in a given session only some of the randomly presented conditions were affected by the inactivation, leaving those stimulus configurations for which the sample was presented in the hemifield ipsilateral to the cryoloop unaffected. This means that deficits were specific to the quadrant of the visual field that was inactivated. Finally, blank cooling sessions produced negative results. These control conditions precluded any possibility of non-specific effects of the cooling loop on behaviour.

No deficit in eye movement generation or precision could explain any of our results since misreaching or the absence of saccade generation caused virtually no error. This observation was in keeping with the results of other authors (Schiller and Lee, 1994) who did not find deficits for saccades to static (or moving) targets following area V4 lesions.

Our reversible inactivations revealed stronger deficits than those usually observed following lesions. This statement holds even for recent lesion studies for which the extent of the lesion and the eye movements were well controlled. An earlier study (Schiller, 1995) also obtained deficits for simple pattern discrimination, but they were transient and only the identification of less salient stimuli remained impaired after recovery. Other authors have also revealed the absence of deficits following area V4 lesions for equi-salient simple patterns. For instance, three other studies (Walsh *et al.*, 1992a; Merigan, 1996; Merigan and Pham, 1998) reported no or moderate deficits of simple shape discrimination following an area V4 lesion. In our experiments, the monkeys were clearly impaired in the recognition of stimuli with similar salience (e.g. Figs 3 and 4). The deficit appeared to be stable throughout the cooling sessions.

An apparent discrepancy appeared in a previous study (Martin-Elkins *et al.*, 1989) where reversible cooling and bilateral inactivation of the prelunate gyrus were used. Those authors did not observe deficits in a delayed match-to-sample task using simple patterns or objects for delays of <6 min. The occurrence of eye movements (which were not controlled) to a

stimuli		minimum P level	number of sessions	number of monkeys	mean number of trials during cooling
A		P<0.001	12	3	135.7
B		P<0.001	21	2	113.6
C	delay	P<0.01	4	1	97.75
D	sample and match together	P<0.01	3	1	114.7
E		P<0.001	11	2	102.3
F		P<0.01	1	1	122
G		P<0.001	4	2	125.75
H		ns	4	2	151
I		P<0.01	1	1	103
J		P<0.001	5	2	94.2
K		ns	1	1	104
L		ns	1	1	119
M		P<0.001	3	1	125.6
N		P<0.01	1	1	106
Below, sample is in fovea					
O	both matches in lower field	P<0.001	5	2	66.6
P	both matches in upper field	ns	1	1	47
Q	one match upper field one match lower field	P<0.001	3	1	103.33
R	both matches in lower field	P<0.05	2	1	38.5

Figure 9. Deficit summary for all monkeys during area V4 reversible deactivation for each stimulus configuration and pair. For the purposes of presentation, every stimulus is delimited by a black contour that was absent in reality. Each stimuli of a pair were made the same size. ***$P < 0.001$ for at least one stimulus configuration and in at least one cooling session. **$P < 0.01$ for at least one stimulus configuration and in at least one cooling session. *$P < 0.05$ for at least one stimulus configuration and in at least one cooling session. ns, not significant. Monkey M1 was tested for all configurations except the last one (stimulus R). Note that, for stimuli O–R, the mean number of trials was lower since the sample was foveal.

spared part of the visual field may have helped the monkeys cope with the task.

We observed a marked deficit caused by area V4 inactivation in the discrimination of perpendicular bars. This is particularly interesting considering that some authors have not seen deficits in the discrimination of oriented bars unless they reduced the discriminability of the bars by adding texture noise or distractors (De Weerd *et al.*, 1996, 1999; Merigan 1996, 2000), however see one study of interest (Heywood and Cowey, 1987). Two possibilities may explain the discrepancy between our results and earlier studies. First, it has been suggested (Merigan, 2000, p. 957) that a very short post-lesional recovery, which could not be tested in that study, could prevent the observation of some deficits for stimuli that produce no deficit after recovery. Second, the tasks may not be directly comparable, as our task required a comparison of two shapes in different loci (the sample and the good match) whereas in a two-alternative forced-choice task, the monkey had to identify a shape according to their internal reference (vertical or horizontal). This was also the case in other studies (De Weerd *et al.*, 1996, 1999) in which the monkey had to refer to the internal representation of a learned vertical bar to which it was required to respond. It is interesting that strong deficits in orientation discrimination when the monkeys had to judge between two perpendicular stimuli that are presented at the same time (WGTA plates) have been reported (Heywood and Cowey, 1987; Walsh *et al.*, 1992a), whereas in the experiments reported above (De Weerd *et al.*, 1996, 1999; Merigan, 2000) the monkey was confronted with only one stimulus of which it judged the orientation with respect to an internal reference it had learned

before. Further experiments are needed in order to explore the extent to which area V4 plays a more important role in the comparison of several objects in different spatial locations or in the visual comparison of an object to an inner representation.

Colour Discrimination

The fact that inactivation of area V4 does not impair matching of equiluminant stimuli is somewhat surprising considering the view that area V4 is proposed to be the colour centre (Bartels and Zeki, 2000). It should be emphasized that data similar to that in Figure 8 were obtained during a session placed between two other cooling sessions which gave significant effects with other pairs of discriminanda and with the same experimental conditions (e.g. cooling temperature) as during these two sessions. The lack of effects on colour discrimination is in agreement with the results of other studies that did not find deficits for simple hue discriminations following area V4 lesions (Heywood and Cowey, 1987; Heywood *et al.*, 1988; Walsh *et al.*, 1992b; Schiller, 1993). Thus, the lack of effects reported by those authors cannot be considered as resulting from post-lesion recovery. Our results therefore suggest that areas other than area V4 are crucial for colour vision, which is in keeping with other authors who have found clear and profound colour deficits following lesions of cortical regions anterior and ventral to area V4 (Dean, 1979; Cowey and Heywood, 1995; Heywood *et al.*, 1995, 1998; Cowey *et al.*, 1998, 2001; Huxlin *et al.*, 2000).

Colour selectivity is elaborate enough in areas V1 and V2 (Yoshioka *et al.*, 1996) to allow for hue discrimination, at least on the basis of wavelength discrimination. Evoked potential studies have shown that processing of colour stimuli is presumably normal after area V4 lesions (Kulikowski *et al.*, 1994). These studies and others showing the impairments of colour vision following IT lesions point to a cortical system that permits colour processing without area V4, presumably via bypass connectivity from area V2 to IT (Distler *et al.*, 1993; Nakamura *et al.*, 1993).

One common pitfall in the use of colour stimuli is that, although equiluminant for human trichromats, the stimuli might not be equiluminant for the monkey. It has indeed been shown that the red–green equiluminance point of rhesus macaque monkeys differs from that of humans (Dobkins *et al.*, 2000); see however another study that found closer estimates for *M. fascicularis* (Morrone *et al.*, 1994). In our experiments there was indeed a deficit (for monkey M1) when the stimuli were not equiluminant (red square or green circle versus white square discrimination) (see Fig. 9*G*,*I*,*J*) and the errors were evenly distributed for both stimuli. The fact that there is no deficit for red–green discrimination and a deficit for white versus green or red is an odd result that cannot be easily explained. More data would be required in order to conclude that the deficit comes from the fact that the animal had to discriminate a coloured item from a non-chromatic colour item (white). Another possibility is simply that red and green are more separated in CIE colour space than are white and green or red. If this is the case, our results would be in agreement with an earlier study that found disruption of hue discrimination with stimuli that were close in colour space (Schiller, 1993).

In the case of one of the studies mentioned above (Merigan, 1996), hue matching deficits were absent when the monkey had been pre-trained in the region of the visual field that was to be lesioned. However, our results (Fig. 6) showed that pre-training was not a prerequisite for an absence of deficit when area V4 was lesioned. Furthermore, the colour discrimination task was rather easy for the monkeys and required less training than shape discrimination. If the amount of training was important, we should have seen a stronger effect with red–green discrimination than for shape discrimination.

Our observation of spared colour processing along with clear alteration in shape processing is interesting to compare to clinical studies of apperceptive agnosias in human patients. Although visual form agnosia is a complex disorder that generally arises from widespread lesions following carbon monoxide poisoning, a clear dissociation of shape perception deficits with preserved colour vision (Vecera and Gilds, 1997; Heider, 2000) can be observed that is in keeping with our results.

Asymmetrical Pattern of the Deficit

The deficits we observed tended to be asymmetrical in the sense that the monkeys were particularly impaired when they had to match stimuli in two different quadrants of the same hemifield (while only the inferior quadrant was inactivated) (see Figs 3–5). This asymmetrical deficit pattern appeared very clearly in more than half of the sessions and in each monkey, but was not systematically reproduced across sessions, with the same pair of discriminanda and the same range of temperature. The other pattern of result that could be observed consisted of an impairment for all four configurations with the sample in the cooled quadrant (see the non-significant diminution of performance in Fig. 4*A*,*B*).

The observation of an asymmetrical deficit pattern, while not easy to understand, suggests that a role of area V4 could be that of comparing two objects in different parts of the visual field and judging them as having the same shape. Earlier results (Schiller, 1993) are consistent with this idea since monkeys with area V4 lesions that are retrained in a different portion of the visual field do not seem to be able to transfer their retrained discriminations to another part of the visual field affected by the lesion.

The hypothesis of area V4 as a shape comparator was reinforced by another study (Haenny and Schiller, 1988). Those authors reported that, when monkeys are required to detect the second presentation of a stimulus in a series of items, area V4 neurons have an enhanced response during the second presentation in the receptive field. Our behavioural deficits on a matching task during area V4 deactivation fitted with this property of area V4 neurons, with the caveat that the similarity needs to be detected across different loci of the same visual hemifield.

The mechanisms of shape comparison between different positions in visual space may be even more complicated when one takes into consideration the large suppressive receptive field surrounds of area V4 cells that can extend up to 16° into the ipsilateral visual field (Desimone *et al.*, 1993). We cannot directly know whether they play a role in the asymmetrical result clearly obtained in many cooling sessions. It may be possible that unilateral cooling leads to an imbalance in favour of the warm contralateral cortex by inactivation of the suppressive surrounds. So far we cannot advance a convincing explanation for why the impairment is mostly seen when the match is presented in the upper quadrant of the same hemifield as the sample, particularly because this asymmetry is not present every time a deficit occurs. We can discard the possibility of either a simple stereotyped behaviour appearing during cooling (i.e. ignoring one side) or a direct cooling spread to the upper field since responses to this quadrant occur when the sample is in the warm controlateral cortex.

In conclusion, our results point to clear deficits in visual shape processing. It should be stressed that the discriminations used in this study were coarse ones (simple shapes or colour dis-

criminations). It would be interesting to explore whether more subtle discriminations could reveal further deficits even in hue discriminations. Future studies need to be undertaken in order to understand better to which point the deficit is linked to apperceptive agnosia. When the monkey is making matching errors, we ignore what they perceive exactly in the impaired quadrant. In other terms, it would be interesting to use altered forms of the matches in order to try to restore a correct performance (for example if the sample is a bright white square, then degrade the correct match in luminance or shape until the monkey starts to make correct responses again).

Notes

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