



The effect of cingulate lesions on social behaviour and emotion

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Abstract

Functional and structural neuroimaging of the human cingulate cortex has identified this region with emotion and social cognition and suggested that cingulate pathology may be associated with emotional and social behavioural disturbances. The importance of the cingulate cortex for emotion and social behaviour, however, has not been clear from lesion studies. Bilateral lesions in the cingulate cortex were made in three macaques and their social interactions were compared with those of controls. Subsequently, cingulate lesions were made in the three controls and their behaviour was compared before and after surgery. Cingulate lesions were associated with decreases in social interactions, time spent in proximity with other individuals, and vocalisations but an increase in manipulation of an inanimate object. The results are consistent with a cingulate role in social behaviour and emotion.

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1. Introduction

It is widely held that the cingulate cortex processes emotional and social information. For this reason when changes in cingulate blood flow or neuroanatomy have been identified in patients with depression [49] or autism [1] the findings have been thought important for understanding the nature and origins of these illnesses. There is, however, some uncertainty about the role of the cingulate cortex in normal social behaviour and emotion [18,19,28,40,45,56,61,68]. The ready identification of cingulate cortex with emotion is partly a consequence of its historical association with Papez's circuit [50] and its anatomical connexions with the amygdala (85) which has a clearly established role in emotion [2,3,7–9,33,34,43]. Direct evidence that the cingulate cortex itself is concerned with emotion and social behaviour is more difficult to evaluate.

Several studies have recorded changes in blood flow and blood oxygenation level (BOLD) signal (“activations”) in the cingulate cortex when subjects view emotionally arousing images and pictures of angry faces [13,36] or when subjects participate in some other emotionally arousing condition, such as anxiety induction [54]. Activations are also recorded in cingulate and paracingulate areas when subjects

are required to make attributions about the thoughts and beliefs of others in “theory of mind” tasks [25,27]. These studies, however, can sometimes be difficult to interpret. First, the cingulate cortex is often just part of a wider network of activated areas and the activation changes in the cingulate may be much smaller and less reliable than those recorded in other non-limbic areas of the brain, such as the visual cortex [11]. Second, even when it has been concluded that the cingulate cortex is playing a role in some aspect of emotion it has been pointed out that the activation changes in this region are difficult to interpret because of its high level of resting baseline activity [61]. Third, because cingulate and other medial frontal activations are common in many different tasks their interpretation is not transparent [35,52,53].

It is difficult to make definitive conclusions about the role of the cingulate in emotion and social behaviour by studying human patients. It is clear that many patients with emotional and social behavioural disturbances have lesions that include the cingulate cortex [12,58,62]. When cerebrovascular accidents in the territory of the anterior cerebral artery damage the cingulate cortex, however, they also tend to damage the orbitofrontal cortex [19]. Any changes in emotion or social behaviour are often attributed to orbitofrontal damage because it is clear from studies of lesions in monkeys that this region is important in emotion and social behaviour [34] and in reinforcement processing [10,26]. Cingulate lesions have been made in patients with severe psychiatric illnesses and it is reported that in some cases these have led to amelioration

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of emotional pathology [19]. The unusual pre-surgical behaviour in such cases, however, can make interpretation of the lesion effect difficult.

In the case of other brain structures linked with emotion, such as the amygdala, similar issues have been addressed by the making of lesions in non-human primates [3,8,22]. In the case of the cingulate cortex, however, the literature concerning the effects of lesions in the monkey brain is older and more confusing. Reviews of early studies have found evidence that cingulate lesions led to social and emotional impairments but have also highlighted some reports where no deficits were found [18,66]. It is clear, however, that the naming conventions used to describe cingulate areas, particular anterior and ventral areas, has varied over time and between researchers. The extent to which the more anterior and ventral cingulate divisions are included within lesions has varied considerably. Neuroimaging results, however, consistently emphasise the importance of just such cingulate regions for emotion [14]. In addition, the testing procedures that have been used in earlier studies have been very varied and are sometimes merely anecdotal.

In the present investigation, we have addressed the role of the cingulate cortex in social behaviour and emotion by attempting to make more complete lesions of the anterior

cingulate gyrus in cynomolgus macaques. We took care to include the cingulate regions rostral and ventral to the corpus callosum that can be difficult to access. We have assessed any impairments using procedures based on those used by other investigators to identify the social and emotional role of the amygdala in lesion studies [7,9,23]. In the first experiment, we looked at the behaviour of pairs of monkeys with cingulate lesions and compared it to the behaviour of pairs of control monkeys. We recorded vocalisations, gestures, interactions, time spent with a novel object and time spent in proximity to one another. In the first experiment, both animals were able to move freely throughout the testing area (Fig. 1a). In experiment 2, we attempted to confirm the measurements of time spent in proximity when one animal was constrained to a sub-section of the apparatus and only the second monkey was free to move throughout the apparatus (Fig. 1b). Experiment 3 used a similar procedure to experiment 1 but in this case a comparison was made between the pre- and post-operative performances of the same animals.

2. Experiment 1

2.1. Methods 1

2.1.1. Subjects

Six male cynomolgus macaques were used, aged between 3 and 5 years and weighing between 4 and 6 kg. The studies were carried out under project and personal licenses from the British Home Office. The animals were born and bred in captivity and housed in social groups. The animals were housed together in a group of six over an 18 months period prior to surgery in rooms with automatically regulated lighting (12 h light and 12 h dark). Lesions were made in three of the animals (CING1, CING2, CING3) and the other three animals (CING4, CING5, CING6) served as controls. After surgery the group of three experimental animals were housed together and the group of three control animals were housed together. The two groups, however, were kept separated from one another but in the same room. The animals shared a set of three section cages (Fig. 1a) in which the communicating doors were open. The studies were carried out approximately 4–7 months after the lesions were made. By this time the hair of the operated animals had re-grown over the positions of the surgical incisions. No sham lesions were made in the control animals because these animals were to be operated on in the second part of the study and it would not have been possible to justify two surgeries in these animals.

2.1.2. Surgery

The intended lesion is shown on a schematic in Fig. 2. All surgery was carried out under sterile conditions with the aid of a binocular microscope. Barbiturate anaesthesia was used during surgery. The skin, galea, and muscle were first cut and retracted. A D-shaped bone flap was then made so that one hemisphere could be exposed up to the level of the

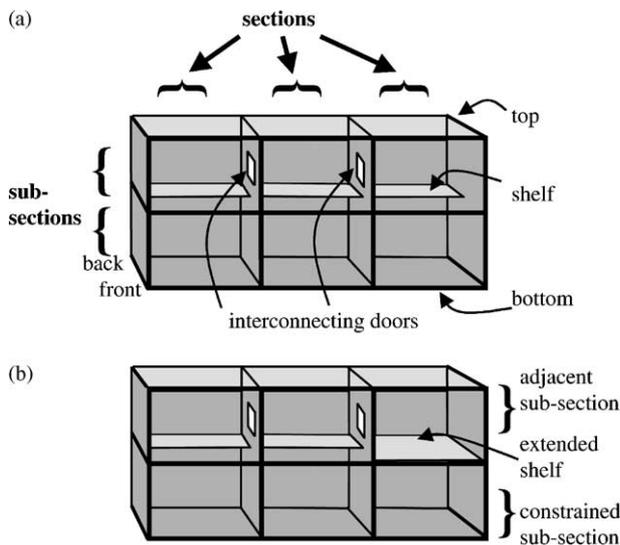


Fig. 1. (a) The cage used for testing social interaction in experiment 1: the cage was 286 cm long, 162 cm high, and 130 cm deep and subdivided into three equally sized sections with communicating doors situated in the top half of each side wall. Each section was further subdivided into equal sized top and bottom sub-sections by a wire mesh shelf that extended forwards to cover half of the cage's depth. The cage was, therefore, divisible into six different sub-sections. (b) The cage used for testing in experiments 2 and 3: the cage was identical except that now the transparent wire mesh shelf in the right section was extended so that the bottom right hand sub-section was separated from all other sections. One monkey (the constrained monkey) was put in the constrained section in the bottom right hand sub-section of the cage where it could be seen by the other monkey (unconstrained monkey) through the wire mesh from the sub-section immediately adjacent and above. The behaviour of the unconstrained monkey was then measured.

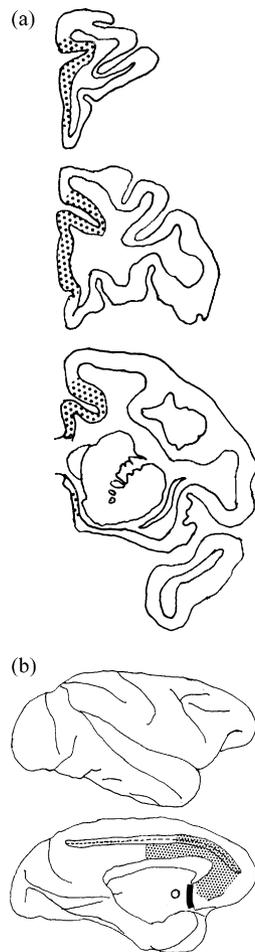


Fig. 2. (a) Intended lesion in three coronal cross-sections of one hemisphere. The most posterior of the three coronal cross-sections is shown at the bottom and is taken at the level of the bow of the arcuate sulcus. The other two sections are 5 and 10 mm anterior, respectively. (b) Lateral (top) and medial (bottom) views of a cynomolgus macaque brain adapted from Martin and Bowden [42]. The intended area of the lesion in the anterior cingulate gyrus and sulcus (opened) is indicated with stippling.

midline. The dura was then cut and retracted. It was important to be able to see the arcuate sulcus and the precentral dimple as these were used to guide the placement of the lesion's posterior limit. The lesion was made by aspiration with a fine gauge sucker. The lesion was first made in the exposed hemisphere. Veins on the medial surface that drained into the sagittal sinus were cauterized and cut. The cortex of the cingulate gyrus and sulcus was removed. The posterior limit of the lesion in the cingulate sulcus was an imaginary line drawn from the bow of the arcuate sulcus through the midpoint of the precentral dimple. The posterior limit of the lesion in the cingulate gyrus was more posterior and stopped at the level of an imaginary line drawn through the midpoint of the precentral dimple, approximately 10 mm anterior to the level of the central sulcus at the inter-hemispheric fissure. The lesion continued anteriorly along the length of the cingulate sulcus. The anterior limit of the lesion was an imaginary line between the tips of the rostral and cingulate

sulci. The posterior and supracallosal part of the lesion extended ventrally to the corpus callosum while the more anterior part of the lesion extended ventrally to the rostral sulcus. Strips of supporting tissue were spared underneath the ascending branches of the anterior cerebral artery using the method of Parker and Gaffan [51]. This ensured the blood supply to the tissue dorsal and lateral to the lesion. When the lesion was complete in the first hemisphere the falx was cut and retracted dorsally. It was then possible to make a similar lesion in the second hemisphere.

2.1.3. Histology

Once the behavioural experiments were completed the animals were deeply anaesthetized and perfused transcardially with saline followed by formal saline. The brains were blocked in the coronal stereotaxic plane posterior to the posterior end of the central sulcus and allowed to sink in sucrose–formalin solution. The brains were then cut in 50 μm sections on a freezing microtome and every 10th section was retained and stained with Cresyl Violet.

The bow of the arcuate sulcus is often taken as a landmark and reference point for the cingulate sulcus [20,46]. In Fig. 3, for each animal, we have presented coronal sections at the level of the bow of the arcuate sulcus. We have then presented two more sections anterior to this level, at distances of 5 and 10 mm. Because there is often some skewing of sections away from the true coronal plane it was necessary to define the section containing the bow of the arcuate sulcus separately for each hemisphere. Thus, each section in Fig. 3 is composed of two half sections taken from two different slides. In this way, it is possible to present histological data for the same coronal position for each hemisphere in each row of Fig. 3.

2.1.4. Apparatus

Testing was conducted in a cage (286 cm long, 162 cm high, 130 cm deep) in an otherwise empty room. The cage was a newly cleaned replica of the animals' home cage. The testing cage was in a different room to the home cage room and no other animals were present. The cage was subdivided into three equally sized sections (Fig. 1a) with communicating doors between each section and the next. Each section was further subdivided into equal sized top and bottom sub-sections by a shelf. In total the cage could be subdivided into six different sub-sections throughout which animals could move.

2.1.5. Testing procedure

Pairs of animals were tested in the apparatus at a time. The communicating doors between each of the three main cage sections were closed prior to the start of testing. One animal was then placed in the end cage on the left of the apparatus and one animal was placed in the end cage on the right of the apparatus. The communicating doors were then opened and the animals' behaviour was recorded by two investigators (KAH and MFSR) and filmed for 10 min. The observers

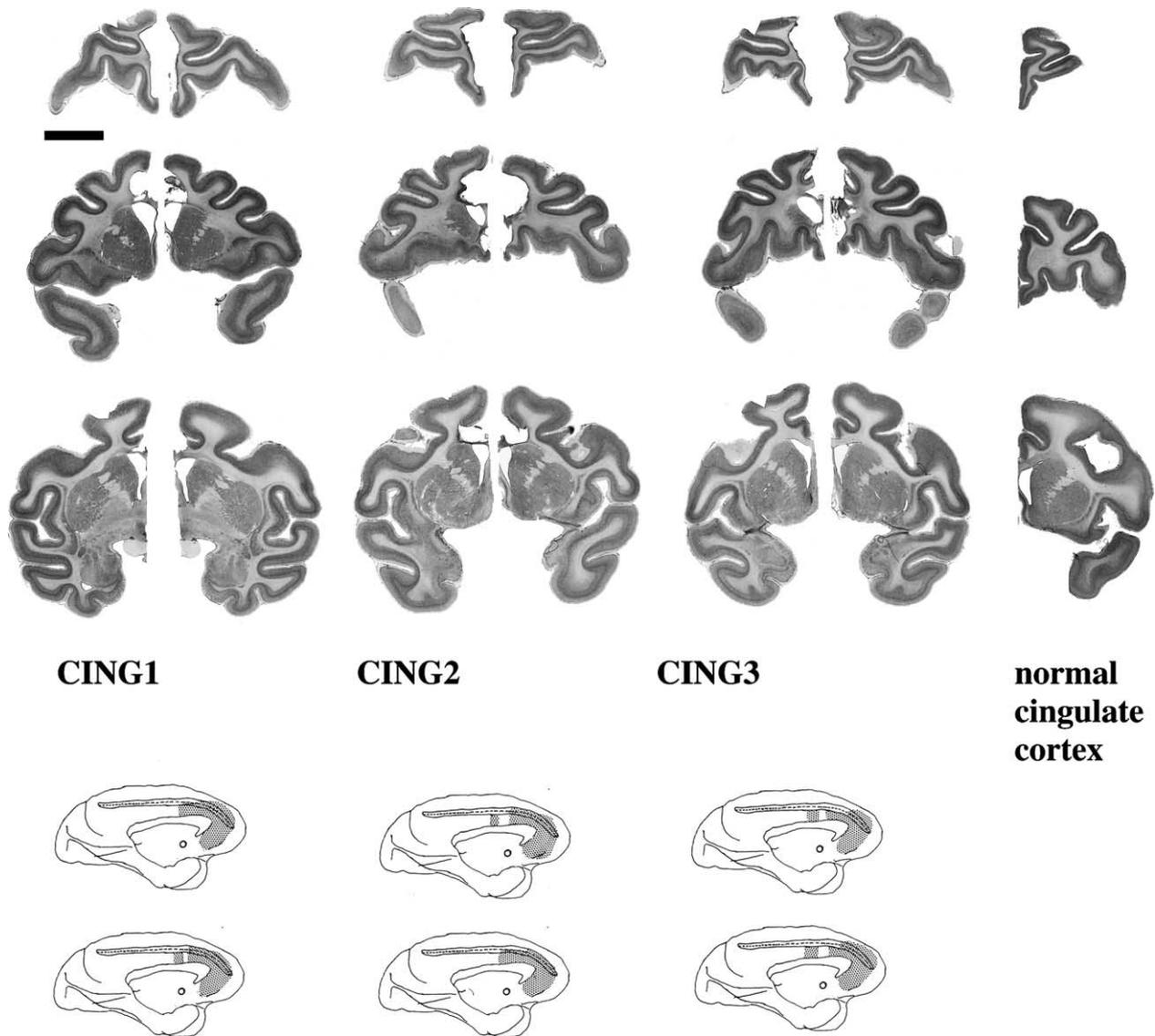


Fig. 3. Coronal sections taken through each brain (animals CING1, CING2, CING3—experiment 1). Three sections are shown for each individual. The most posterior section is shown at the bottom and is taken at the level of the genu of the arcuate sulcus which is commonly used as a landmark for describing cingulate anatomical positions [20]. The more anterior sections are 5 and 10 mm more anterior. On the far right similar sections from an intact hemisphere of a cynomolgus macaque are shown for comparison. The scale bar at the top left indicates 10 mm. At the bottom are shown approximate reconstructions of the lesions superimposed on a medial view of a standard brain from a cynomolgus macaque adapted from Martin and Bowden [42]. In each case the upper and lower sections correspond to the right and left hemispheres, respectively.

were not “blind” to the condition of the animals and they were aware of whether an animal had had a lesion. Behaviour was also recorded for a minute prior to the opening of the communicating doors. After 2 min one of eight possible toy objects was placed in one of the side cages and the toy remained at this position for the remaining 8 min of the 10 min testing session. The toy objects were small brightly coloured rubber or plastic objects and included items such as a yellow frisbee, a blue ball, a small red dumbbell, a red handle, a blue squeeze toy for pet dogs and a green hoop. The animals in the colony were used to being given such objects to play with but the particular exemplars used in the experiments were novel. The experimenters were located in front of the

test cage and recorded data using pencil and paper. The session was also recorded on videotape and proximity (time in same section or sub-section) and activity level (crossing rate) measurements were made from the videotapes.

Tested pairs always consisted of either two lesion animals or two control animals. Each control animal was tested together with each of the other control animals and each lesion animal was tested with each of the other lesion animals. Each pair was tested each day for 5 days.

We attempted to record behaviour according to criteria adapted from Bachevalier [7]. The three main recording categories used were (i) interactions between animals, (ii) facial expressions and vocalisations, (iii) toy manipulation.

(i) *Interactions between animals*: At the outset of the experiment, we intended to follow protocols similar to those used by Bachevalier [7] in an investigation of the effects of medial temporal lesions on social interactions. Bachevalier [7] defined several different categories of interaction depending on which animal approached the other.

We defined interactions as the occasions when animals were either in physical contact with one another or occasions when the animals were close enough to be within physical contact (i.e. within arm's reach of one another). Interactions were initially recorded under several different categories of approach (aggressive, submissive, neutral) and response to approach by the second animal (acceptance of approach or withdrawal). The animals were well known to one another and most approaches were judged to be in the neutral category (interobserver reliability, Pearson's $r = 0.84$, $P < 0.001$). Many approaches were judged to have been accepted (interobserver reliability, Pearson's $r = 0.82$, $P < 0.001$). Judging whether animals were actively withdrawing from interactions or simply following different individual interests was difficult and interobserver agreement was less good for this category (interobserver reliability, Pearson's $r = 0.54$, $P < 0.001$) as in earlier studies ($r = 0.63$ in the study by Bachevalier et al. [9]). Aggressive approaches were recorded rarely and even then was a lack of consistency between raters as to whether the approach was sufficiently aggressive as to merit inclusion in the aggressive category or whether it should have been assigned to the neutral category. The inclusion of a submissive approach category, in addition to the categories devised by Bachevalier et al. [9] did not prove useful. In a similar manner to earlier studies [9] a total interaction score for each monkey was calculated by summing across each dyad.

The measure of the number of interactions was supplemented by also recording the amount of time that animals spent in the same sub-section of the cage as each other and the amount of time that animals spent in the same section of the cage as each other. Because of the unambiguous nature of these measures they were made by one investigator (KAH).

(ii) *Activity levels*: Despite the apparent usefulness of proximity measures it is possible that their interpretation might be confounded by general changes in activity levels. In order to obtain a quantitative measure of activity we measured the rate at which animals crossed between the top and bottom sub-sections within each of the three cage sections (Fig. 1a). Once again, because of the clearly objective nature of these measurements they were made by one investigator (KAH).

(iii) *Facial expressions and vocalisations*: We recorded several different types of facial expressions including grimace (open mouthed submissive grimace where the lips are pulled back over closed teeth while looking at the other animal, interobserver reliability Pearson's $r = 0.91$, $P < 0.001$) and lip-smacking (interobserver reliability Pearson's $r = 0.95$, $P < 0.001$). We also intended to record open mouth submissive (open mouth while looking away from

the other animal and staying stationary or retreating) and open mouth threat (open mouth and teeth bared while approaching another individual). As mentioned above, very little aggressive behaviour occurred and either none of these behaviours (threat) or so few (open mouth submissive) were emitted that it was not possible to calculate interobserver reliability.

We recorded several different types of vocalisations including barking (aggressive loud threat), aggressive open mouthed pants, high-pitched submissive calls and contact calls. In general very few vocalisations were recorded. As mentioned above there were very few aggressive interactions and there were too few aggressive calls to calculate useful measures of inter-observer reliability. Contact calls were frequent and these were particularly prominent in the 1 min period prior to the opening of the communicating doors and immediately afterwards. In other words, these calls were most often made when the monkeys did not have visual contact with one another. At this stage in the experiment one investigator would be ready to open the communicating doors between the two animals so calls had to be recorded by just the other experimenter. Prior piloting indicated clear inter-observer agreement about the nature of such contact calls.

(iv) *Toy manipulations*: We recorded the number of times that each animal picked up and played with the toy (inter-observer reliability $r = 0.93$, $P < 0.001$).

2.1.6. Analysis

Planned between subject one-tail t -tests were used to compare the performance of the lesion and controls groups. The variance estimate and degrees of freedom were adjusted according to a Welch–Satterthwaite type correction procedure (SPSS 10) whenever Levene's test revealed that the variances of the two groups were not equal.

2.2. Results 1

2.2.1. Interactions between animals

Total interactions: Pairs of cingulate animals entered into significantly fewer interactions with one another than did the control animals ($t = 4.024$, d.f. = 4, $P = 0.008$). The results are summarised in Fig. 4a.

Time spent together: Pairs of animals with cingulate lesions spent significantly less time in the same sub-section (Fig. 4b) of the cage than did control animals ($t = 6.591$, d.f. = 4, $P = 0.002$). The difference was similarly significant when the amount of time that animals spent in the same section (Fig. 4c) of the cage was considered ($t = 6.880$, d.f. = 4, $P = 0.001$).

2.2.2. Activity levels

The cingulate animals made significantly more crossings than controls when the animals were in the same section ($t = -4.037$, d.f. = 4, $P = 0.016$) but there was no group difference in crossings when the animals were in separate

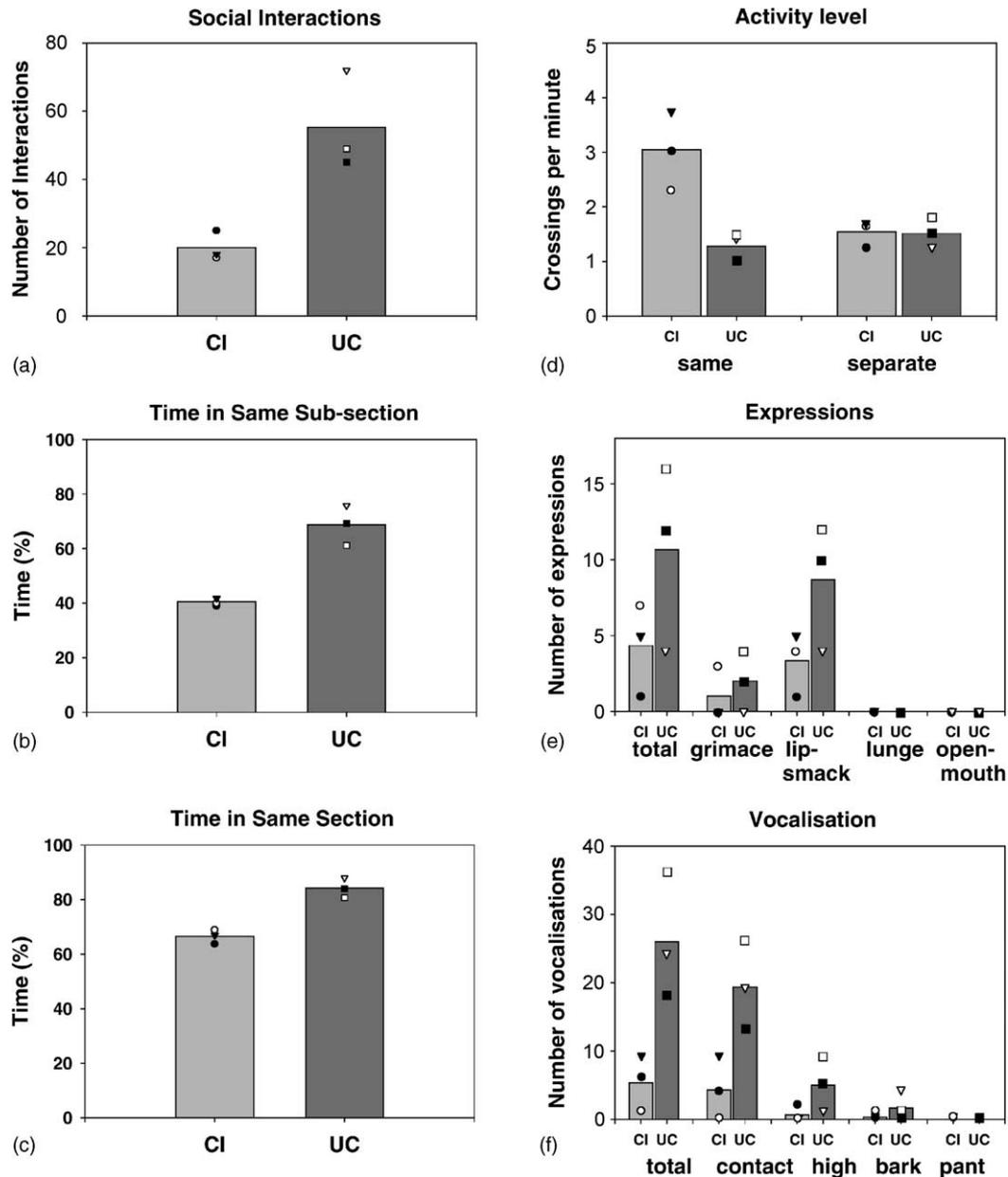


Fig. 4. Social interactions in experiment 1: (a) Number of interactions between animals. (b) Proportion of time spent by both animals in the same sub-section (the cage was subdivided into six sub-sections). (c) Proportion of time spent by both animals in the same section (the cage was subdivided into three sections). (d) Activity levels as indexed by the number of cage crossings (moving from one sub-section to the other) per minute when the animals were in the same and separate cage sections. (e) Number of facial expressions made by each animal. The total number of expressions is shown on the left of the graph and sub-totals for grimaces, lip-smacks, lunges, and open mouth threat expressions are shown to the right. (f) Number of vocalisations made by each animal. The total number is shown on the left hand side of the graph and sub-totals for contact, high-pitched calls, barks, and pants are shown to the right.

cage sections ($t = 0.136$, d.f. = 4, $P > 0.05$). The results are summarised in Fig. 4d.

2.2.3. Facial expressions and vocalisations

Facial expressions: There was a trend for animals with cingulate lesions to produce less facial expressions than control animals, however the difference did not reach significance ($t = 1.606$, d.f. = 4, $P = 0.092$). The results are summarised in Fig. 4e. Sub-dividing the facial expressions

into different types did not produce any further evidence for an impairment after cingulate lesion: there was no significant difference between the number of grimaces ($t = 0.655$, d.f. = 4, $P > 0.1$), the number of lip smacks ($t = 1.985$, d.f. = 4, $P > 0.1$), or the number of open mouth threats (none were produced by either group).

Vocalisations: The cingulate animals produced significantly fewer vocalisations than did the control animals ($t = 2.534$, d.f. = 4, $P = 0.032$). The results are summarised

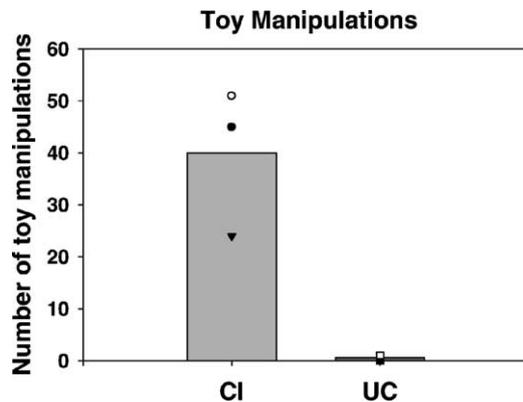


Fig. 5. Number of occasions on which each individual picked and manipulated the toy object left in the cage in experiment 1.

in Fig. 4f. The difference in vocalisation between the two groups was almost entirely accounted for by differences in contact calls that were made by control animals at the beginning of the testing session before the animals were able to see one another. When just contact calls were considered alone the difference between the groups was just at the boundary for significance ($t = 2.099$, d.f. = 4, $P = 0.052$). There was no significant difference in the number of barks ($t = 1.069$, d.f. = 4, $P > 0.1$), high-pitched calls ($t = 1.803$, d.f. = 4, $P = 0.146$), or open mouth aggressive pants (none were produced by either group).

2.2.4. Toy manipulation

The cingulate animals manipulated the toy significantly more often than did the controls ($t = 4.801$, d.f. = 2.007, $P = 0.02$). The results are summarised in Fig. 5.

3. Experiments 2 and 3—social behaviour

3.1. Methods 2

3.1.1. Subjects

The same six cynomolgus macaques were used as in experiment 1. Lesions had been made in three of the animals (CING1, CING2, CING3) and the other three animals (CING4, CING5, CING6) served as controls. The studies were carried out under project and personal licenses from the British Home Office.

3.1.2. Apparatus

Testing was conducted in the same cage as experiment 1 in an otherwise empty room. On this occasion, however, one sub-division of the cage was separated from the rest of the cage by a wire mesh (Fig. 1b). The mesh was of the same sort as the adjacent shelf and of a type commonly used in the colony for temporarily separating animals during testing and cleaning.

3.1.3. Testing procedure

Pairs of animals were tested in the apparatus at a time. One animal, the *constrained* animal was placed in the separated sub-section (Fig. 1b). The animal was unable to move from this limited area of the cage for the duration of the test. The second animal was placed at the other end of the main apparatus. As in experiment 1, the testing period began when the communicating doors within the main section of the cage were opened and the *moving* monkey was free to move throughout the apparatus. As in experiment 1, experiments lasted for 10 min. Pairs were always drawn from just the control group of animals or from just the cingulate group of animals. During each day's testing each animal would serve twice as the constrained animal and twice as the moving monkey. Just the behaviour of the moving monkey was measured. Testing was carried out over 3 days. We measured just the amount of time that the moving monkey spent in the adjacent section just above the constrained monkey as we felt that this was the clearer and less ambiguous of the two measures of interaction that we had used in experiment 1. From this section of the cage the un-constrained animal had a clear view of the constrained animal through the wire mesh of the shelf that separated them.

3.1.4. Analysis

A planned between subject one-tail t -test was used to compare the performance of the lesion and controls groups.

3.2. Methods 3

3.2.1. Subjects

The three cynomolgus macaques that had served as controls in experiments 1 and 2 (CING4, CING5, CING6) were used in experiment 3. Cingulate lesions were made in these three animals and their performances on the same testing protocol as that used in experiment 2 was compared before and after surgery. The studies were carried out under project and personal licenses from the British Home Office.

3.2.2. Surgery

Removal of the cingulate cortex was carried out in a similar manner to earlier described (see Section 2.1 for experiment 1). On analyzing the histology from the first set of lesions we had noticed that the lesions in the cingulate sulcus were not complete. In the second set of experiments, we therefore attempted to make more complete removals of even the cingulate sulcal tissue. Ensuring completeness of the sulcal lesion, however, sometimes meant that it was not possible to spare arteries on the medial surface supplying the medial aspect of the superior frontal gyrus.

3.2.3. Histology

The histology was prepared as earlier described. Once again the lesion in the cingulate gyrus was complete and as intended (Fig. 6). In addition the cingulate sulcal region

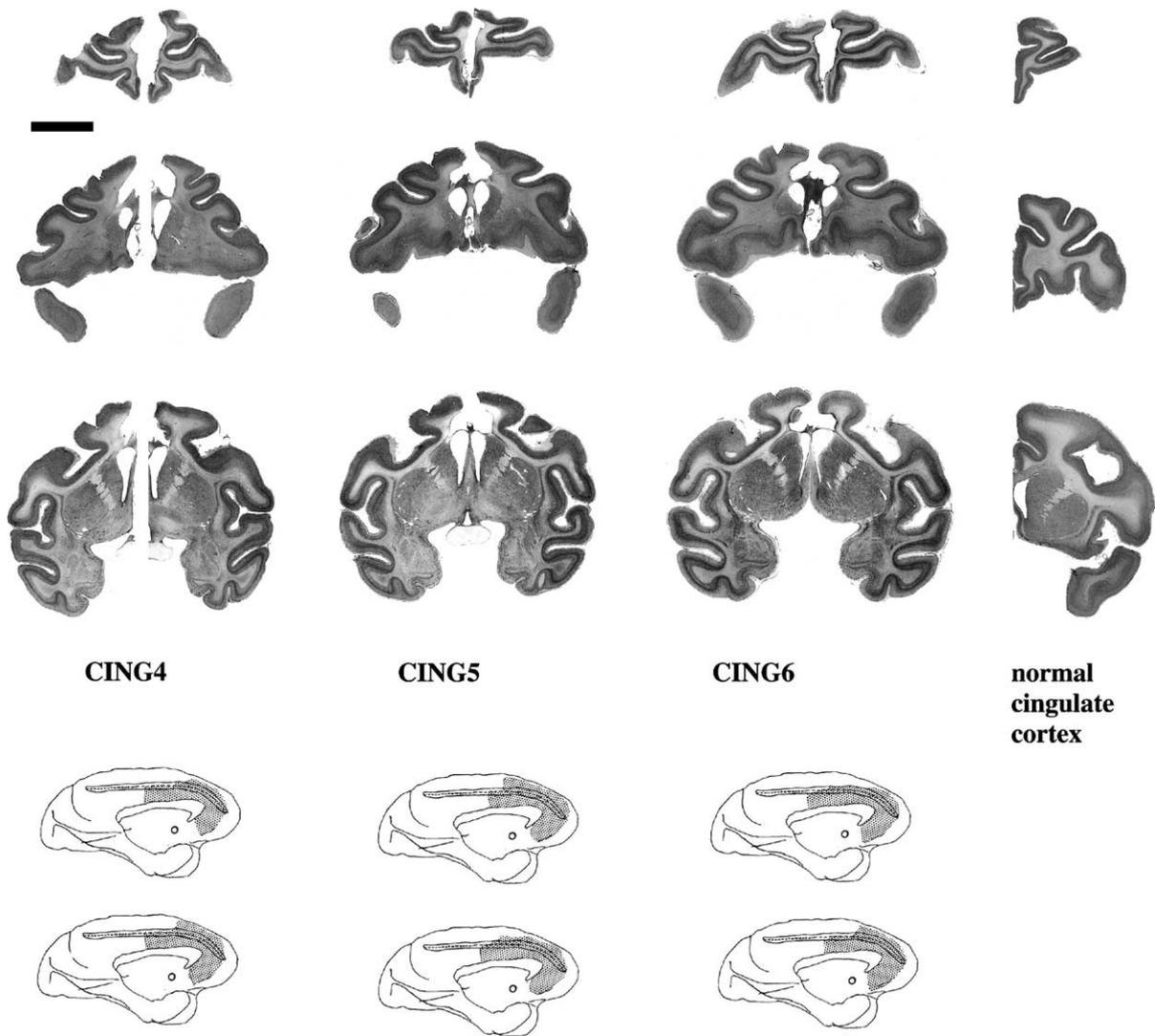


Fig. 6. Coronal sections taken through each brain (animals CING4, CING5, CING6—experiment 3). The scale bar at the top left indicates 10 mm and the same conventions are used as for Fig. 3. At the bottom are shown approximate reconstructions of the lesions using the same conventions as in Fig. 3. In each case the upper and lower sections correspond to the right and left hemispheres, respectively.

was removed completely as far posteriorly as the level of the spur of the arcuate sulcus in all three animals. There was, however, some additional damage to the medial aspect of the superior frontal gyrus.

3.2.4. Apparatus

Testing was conducted in the same apparatus as experiment 2 (Fig. 1b).

3.2.5. Testing procedure

The testing procedure was the same as that used in experiment 2. Animals were tested on two different occasions (each comprising 3 days of testing) before and after surgery.

3.2.6. Analysis

A planned within subject one-tail *t*-test was used to compare the pre- and post-operative performances within the group.

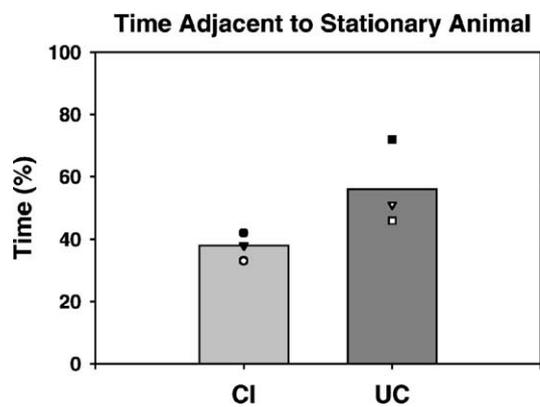


Fig. 7. Amount of time spent in the sub-section adjacent to the constrained monkey experiment 2).

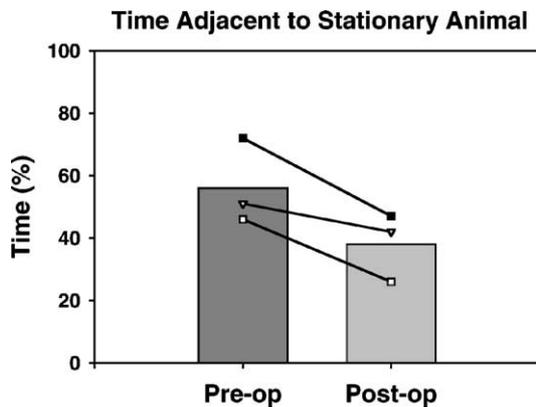


Fig. 8. Amount of time spent in sub-section adjacent to the constrained monkey in experiment 3 (pre- vs. post-operative comparison).

3.3. Results 2

Although the differences were not quite so pronounced on this occasion, we found that animals with lesions spent significantly less time in proximity to the constrained monkey than did control animals ($t = 2.228$, *d.f.* = 4, $P = 0.045$). The results are shown in Fig. 7.

3.4. Results 3

After cingulate removal the animals spent significantly less time in proximity to the constrained monkey than they had done earlier ($t = 3.809$, *d.f.* = 2, $P = 0.032$). The results are summarized in Fig. 8.

4. Discussion

Animals with cingulate lesions interacted less with one another after the lesions were made. This was measured as a significant decrease in the number of interactions and the amount of time that animals with cingulate lesions spent in the same section and sub-section of the cage (Figs. 4, 7 and 8). The changes in social behaviour interacted with changes in arousal and activity levels. Animals with cingulate lesions were more active when they were within closer proximity although we did not record a general change in activity levels (Fig. 4). Cingulate animals also made fewer vocalisations (Fig. 4). Instead of showing the usual amount of interest in the other animal the animals with cingulate lesions appeared more interested in the novel, inanimate toy objects placed in the cage; the cingulate animals manipulated these objects on more occasions (Fig. 5).

4.1. Using lesions in investigations of social behaviour

Changes in social behaviour have also been reported after medial temporal and amygdala lesions [2,7–9,23,33]. Recent investigations of the amygdala have taken care to establish

whether such changes are the result of damage to the amygdala itself, adjacent cortex, or to the fibres that pass through it [22,23,43]. These different possibilities can be partially disentangled by making amygdala lesions with excitotoxins which leave adjacent tissue and fibres of passage relatively spared. It is now clear that social behavioural changes do follow even excitotoxic lesions of the amygdala [22,23,43,55]. Some of the other deficits recorded after amygdala aspiration lesions are not found after amygdala excitotoxin lesions [41] and may instead be the consequences of damage to the fibres of passage [21].

It is not possible, however, to use excitotoxic lesion approaches when studying the cingulate cortex in a species such as the macaque. Excitotoxic lesions here would be expected to lead to cavitation and collapse of adjacent tissue. This side effect of excitotoxin injection has earlier been documented in other areas of the macaque brain [26]. Cavitation and collapse is likely to be followed by damage to branches of the anterior cerebral artery which in turn could lead to great difficulty in using the hind limbs. The technically more difficult aspiration lesion approach used in the present study may, however, damage both the cingulum bundle and the cingulate cortex. In the rat it has been shown that lesions of each of these structures lead to dissociable patterns of impairment [4,24,44,67]. Further experiments will, therefore, be needed in order to decide if damage to the cingulate cortex or the cingulum bundle is the critical determinant of the social deficits recorded in the present experiment.

4.2. Social interaction

Although the differences in interactions and time spent in proximity (Figs. 4, 7 and 8) between the groups seemed clear, it might be argued that they are statistically problematic. Any given single interaction between animal CING1 and CING2 will contribute to the score for both CING1 and the score for CING2. If CING1 and CING2 spent a minute in proximity then that minute would contribute to the time in proximity score for both animals. The observations are therefore not independent of one another.

We therefore used two further procedures, experiments 2 and 3, to confirm the lack of interaction between animals with cingulate lesions. In each case we attempted to obtain measures of each animal's tendency to approach others that were statistically independent and separate from measures made for the other animals. In experiment 2, we closed off one sub-section of the cage and placed one animal into it (Fig. 7). A second animal was then allowed to wander between all the remaining sub-sections of the cage and the amount of time it spent in the sub-section adjacent to and above the constrained animal was measured. In this way separate measurements were made for each animal that were statistically independent of one another. In experiment 3, we attempted to gain a within subject measure of social interaction changes in response to cingulate lesion. Cingulate lesions were made in each of the three animals that had

earlier served as control animals and the animals were tested, using the experiment 2 apparatus for one moving and one constrained animal, before and after surgery. We found that the cingulate lesion tended to make animals less likely to move into proximity with one another in all cases (Fig. 8).

Another way to address the same issue would have been to examine interactions between more animals. This would have required a larger number of animals in the lesion group and this may be appropriate in future studies. In the present experiment, we attempted to use the minimum number of animals needed for a statistical analysis of the results. The statistical sensitivity of the experiments, might, however, have been improved by larger group sizes. Another possibility would have been to investigate interactions between control and cingulate animals. We initially intended to make such comparisons. Under similar conditions Emery et al. [23] reported an increase in affiliative behaviour between control animals and animals with amygdala lesions. In the present study, however, we found that paired cingulate and control animals were more likely to fight. This line of inquiry was therefore discontinued to avoid unnecessary stress to the animals. It might be possible, however, to consider interactions between control and cingulate lesion animals in future experiments by using situations such as those employed by Emery et al. [23]. Emery et al. observed social interactions between physically separated monkeys so that the possibility of undue stressful and direct physical aggression was removed.

The use of proximity measures might be criticised because it is possible that its interpretation might be confounded by general changes in activity levels. In order to obtain a quantitative measure of activity, we measured the rate at which animals crossed between the top and bottom sub-sections within each of the three cage sections (Fig. 4d). The cingulate animals made significantly more crossings than controls when the animals were in the same section as one another. There was, however, no difference in crossing rate when the animals were in separate sections. The analyses, therefore, confirmed that there were differences in activity after cingulate removal but they also showed that these activity differences were not constant features of the animals' behaviour. It is therefore unlikely that a change in activity levels caused the changes in social behaviour observed after cingulate removal. Instead the results suggested that the activity levels were being modulated by social factors, such as the proximity of other individuals. When both animals were in the same section together the cingulate animals were more active but this was no longer the case once they had moved to different sub-sections.

It might be argued that it is difficult to define interactions and other social behaviours in an objective way. Inter-observer reliability in recording frequencies of neutral approaches and acceptances of such approaches was reasonably high (r between 0.82 and 0.84) but, as in earlier studies [9], there was less agreement about when animals were actively withdrawing from an approach. The recording sessions were relatively uneventful and too few aggressive

interactions were recorded to provide a useful measure of inter-observer reliability. There was a similar lack of aggressive expressions and vocalisations. It should be noted, however, that total interaction measures of the sort that we used and simple proximity measures have proven to be sensitive indices of social dysfunction after medial temporal and amygdala lesions [9,23]. The lack of aggressive behaviour during the recording sessions probably reflects the stability of the relationships between the animals investigated; they had been housed together for more than a year prior to the recording sessions.

It is likely that a larger variety of behaviour occurs during social interactions between younger animals that only spend some part of the day together [7,9] or between animals that are encountering one another for the first time [23]. The different testing environments that might be used and the age at which lesions are made may also be important variables [8,22,55].

One final caveat that must be considered is whether placement of the toy objects in the cage during the recording of social behaviour disrupted the normal pattern of social behaviour. Although this might be the case it is not clear that behavioural disruption was likely to have been specific to the operated group in experiments 1 and 2 or to the post-operative as opposed to the pre-operative testing period in experiment 3. So little is known about the effect of cingulate lesions, however, that this possibility cannot be completely eliminated. The confounding influence of such factors could, however, be removed in future experiments simply by placing toys in the cage prior to the inception of the behavioural data recording. Such sessions could be compared with others in which no toy objects are presented to the animals at any stage.

The identification of the amygdala with social behaviour is well established because it is based on repeated findings of social behavioural change after amygdectomy in a variety of animals of different ages, of differing degrees of familiarity with one another, and in different environments and contexts [2,7–9,23,33]. Whether or not the present indication of a cingulate role in social behaviour can be generalized beyond the particular conditions of the present study remains to be seen. The association between the cingulate cortex and social behaviour will be secured if corroborating data emerge in experiments using a wider variety of paradigms.

4.3. The anterior cingulate cortex in social behaviour and emotion

Because of the cingulate cortex's connexions with the amygdala and orbitofrontal cortex [65] and because it was placed in Papez' circuit [50] it is often assumed that it has been clearly established that the primate cingulate cortex plays a well defined role in emotion and/or social behaviour. Recent reviews [18,66] have found evidence in an older literature that cingulate lesions lead to social and emotional changes but there have been apparent failures

to replicate these findings. For example, Ward [68] and Glees et al. [28] reported that cingulate area 24 lesions lead monkeys to ignore other individuals and behave as if they were inanimate. In those studies, however, social/emotional deficits were not quantitatively measured. In subsequent experiments it was claimed that cingulate lesions did not lead to quantitative changes in fear and aggression or social dominance [45,56]. Lesion location and the nature of the behavioural tests, however, make these early studies difficult to interpret.

It is not clear how much of the cingulate gyrus anterior to the callosum, Ward [68] considered to be area 24. No histology was provided in that study. In other studies the amount of damage to the anterior and ventral parts of the cingulate gyrus, which are only accessed with difficulty during surgery, is very variable [28,44,55]. The most anterior and ventral cingulate regions have been particularly associated with emotional and social behaviour and pathology in human imaging studies [36,49,54]. The lesions in the present study consistently extended into the most anterior and ventral parts (areas 32 and 25) of the cingulate cortex and social behavioural deficits were consistently observed. Bechara et al. [12] made maps of the degree of lesion overlap in their patients with emotional/social behavioural impairments. Their diagrams suggest anterior and ventral cingulate cortex is consistently damaged in affected patients.

There are a number of reports of patients with lesions that include anterior cingulate cortex [12,58,64] in whom the normal patterns of social behaviour are disrupted. Because the lesions encroached on frontal areas, particularly orbitofrontal cortex, it has not been clear to what extent cingulate damage caused symptoms. The present results provide direct evidence that the cingulate cortex is essential for normal social interaction.

Changes in cingulate cortical anatomy and metabolism have been reported in patients with psychiatric illnesses that disrupt social interaction, such as autism [1] and depression [49]. The present results suggest that the changes identified in these brain areas may have causally contributed to the dysfunction. It is known that when damage to the amygdala or orbitofrontal cortex is sustained earlier in life it can lead to more widespread neural and behavioural changes [6,7,9,60] and the same may also be true in the case of the cingulate cortex.

It is possible that the cingulate cortex may exert a controlling influence on emotion and social behavioural by affecting arousal and visceral and somatic states [15–17,47]. The hypothesis that bodily sensations are central aspects of emotions can be traced to James [30]. Bechara et al. have suggested that visceromotor deficits may underlie the social dysfunction seen in some patients with ventromedial frontal lesions which extend into the cingulate cortex [12]. Although discussions of such patients have often emphasized the importance of the ventral prefrontal cortex the present results demonstrate that cingulate damage may be an important determinant of social behavioural changes.

Such a role for the cingulate cortex would be consistent with its connexions. Areas 32 and 25, along with some limited parts of the orbital frontal cortex, project particularly strongly to the hypothalamus and periaqueductal grey [5,48,57]. These regions are known to mediate the control of autonomic and stereotyped behavioural reactions including vocalisation [32]. In the present study, we found that activity levels changed as a function of proximity to other animals after the lesions were made (Fig. 4d). It is possible that the activity changes were the consequence of a lesion induced alteration in the way that social context affected arousal.

In the present investigation, we were able to confirm that cingulate lesions were followed by a decrease in vocalisation in some situations [4,39,40,63]. Electrical stimulation of the monkey cingulate cortex is known to elicit vocalisation [31]. There was a particular decrease in the number of “contact” calls that the monkeys made to each other at the beginning of the experiment in the minute prior to the opening of the doors that allowed the monkeys to approach and directly see one another. MacLean and Newman also noted decreased production of this call when squirrel monkeys were isolated from one another [40]. Cingulate lesions only seem to affect vocalisation in certain situations; Sutton et al. [63] recorded a normal response to fearful stimuli when cingulate lesions were made. It is possible that the vocalisation reduction observed in the present study might be best interpreted as just one part of a more general reduction in social interaction. A similar interpretation was also favoured by MacLean [39].

4.4. *Functional segregation within the cingulate cortex*

We also tested the animals on tests of task switching and delayed alternation performance [59]. We found that the animals were only impaired when the cingulate sulcal region at the level of the bow of the arcuate sulcus was included in the lesion. This part of the cingulate sulcus contains a region, the rostral cingulate motor area, distinguished from the surrounding cingulate gyrus by its connexions with the primary motor cortex and the spinal cord [20,29,37,38,46]. There was no evidence that rostral cingulate motor region removal was critical for the social behavioural deficits seen in the present experiments; the removal of this part of the sulcus was not complete in experiment 1 (Fig. 3) but there was still an impairment of social/emotional behaviour. Taken together with the results of the task switching and delayed alternation experiments [59] the present results indicate a separation between a more social behavioural role for the more anterior or gyral cingulate cortex and a more cognitive role for sulcal regions in and adjacent to the cingulate motor areas. The finding supports the conclusions drawn by Devinsky et al. [19], Paus et al. [53], and Bush et al. [14] who have proposed similar distinctions on the basis of anatomical connexions and reviews of disparate experiments.

5. Conclusions

In summary, the results suggest that the primate anterior cingulate cortex may play a role in social and emotional behaviour, at least under the conditions used in the present study. Future experiments are needed to investigate the generality of the present findings. In order to reveal impairments after making cingulate lesions it may be necessary to take into account changes in arousal or to use procedures that emphasise the testing of social interaction or affiliative behaviours.

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