European Journal of Neuroscience, Vol. 11, pp. 4403–4418, 1999

Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys

Martine Meunier,[1] Jocelyne Bachevalier,[2] Elisabeth A. Murray, Ludiše Málková[3] and Mortimer Mishkin
Laboratory of Neuropsychology, National Institute of Mental Health, Bethesda, MD, USA
[1]Institut Des Sciences Cognitives, CNRS, Bron, France
[2]University Of Texas Health Science Center, Houston, TX, USA
[3]Georgetown University Medical Center, Washington DC, USA

Keywords: emotions, ibotenic acid lesions, medial temporal cortex, rhesus macaques, rhinal cortex

Abstract

All previous reports describing alterations in emotional reactivity after amygdala damage in monkeys were based on aspiration or radiofrequency lesions which likely disrupted fibres of passage coursing to and from adjacent ventral and medial temporal cortical areas. To determine whether this associated indirect damage was responsible for some or all of the changes described earlier, we compared the changes induced by aspiration of the amygdala with those induced by fibre-sparing neurotoxic lesions. Four different stimuli, two with and two without a social component, were used to evaluate the expression of defence, aggression, submission and approach responses. In unoperated controls, defence and approach behaviours were elicited by all four stimuli, 'social' and inanimate alike, whereas aggression and submission responses occurred only in the presence of the two 'social' stimuli. Furthermore, all defence reactions were reduced with an attractive inanimate item, while freezing was selectively increased with an aversive one. Relative to controls, monkeys with neurotoxic amygdala lesions showed the same array of behavioural changes as those with aspiration lesions, i.e. reduced fear and aggression, increased submission, and excessive manual and oral exploration. Even partial neurotoxic lesions involving less than two-thirds of the amygdala significantly altered fear and manual exploration. These findings convincingly demonstrate that the amygdala is crucial for the normal regulation of emotions in monkeys. Nevertheless, because some of the symptoms observed after neurotoxic lesions were less marked than those seen after aspiration lesions, the emotional disorders described earlier after amygdalectomy in monkeys were likely exacerbated by the attendant fibre damage.

Introduction

Bilateral temporal lobectomy produces a complex set of symptoms in monkeys which, as reported by Klüver & Bucy (1938, 1939), includes changes in, or absence of, anger and fear, excessive examination of objects (hypermetamorphosis), often with the mouth (hyperorality), hypersexuality, changes in dietary habits, and loss of social interactions. Subsequent investigations revealed that these socio-emotional changes are associated with damage in the region of the amygdala (Weiskrantz, 1956; Horel *et al*., 1975; Aggleton & Passingham, 1981; Zola-Morgan *et al*., 1991; Kling & Brothers, 1992), leading to the idea that this structure plays a critical role in processing the affective significance of sensory information (Weiskrantz, 1956; Jones & Mishkin, 1972; Spiegler & Mishkin, 1981; Gaffan *et al*., 1988).

This idea has received additional support from studies in both humans and rodents. Studies of patients with bilateral damage to the amygdala (e.g. Adolphs *et al*., 1994; Cahill *et al*., 1995; Phelps *et al*., 1997; Scott *et al*., 1997; Broks *et al*., 1998) as well as recent neuroimaging studies in normal individuals (e.g. Breiter *et al*., 1996; Fried *et al*., 1997; Zald & Pardo, 1997; Morris *et al*., 1998; Whalen *et al*., 1998) have demonstrated that the amygdala is involved in functions, e.g. evaluation of the facial expressions of emotion. In rodents, experimental studies have begun to unveil the intra-amygdala circuitry and neurochemistry that underlie emotional responses, e.g. conditioned fear (for reviews see Davis, 1992; LeDoux, 1992; McGaugh *et al*., 1992; Gallagher & Chiba, 1996).

Despite the wealth of evidence pointing to a critical contribution to emotion by the amygdala, one fundamental issue remains to be settled. In non-human primates, all earlier studies reporting striking emotional changes following amygdalectomy were based upon aspiration lesions (e.g. Weiskrantz, 1956) or, in rare instances, radiofrequency lesions (e.g. Aggleton & Passingham, 1981; Zola-Morgan *et al*., 1991). Ablations by aspiration necessarily include direct damage to both the piriform cortex and the anterior portion of the entorhinal cortex; but they also lead to extensive indirect damage to other ventral and medial temporal areas by transecting fibres that course through or nearby the amygdala (Murray, 1996; Goulet *et al*., 1998). Specifically, aspiration removals of the amygdala interrupt efferent projections of cells located in the temporal polar, entorhinal, perirhinal and area TE cortical fields en route to the medial thalamus (Goulet *et al*., 1998), as well as projections from the three latter temporal regions to the orbital frontal cortex (Baxter *et al*., 1998). Stereotaxic radiofrequency lesions of the amygdala minimize direct damage to the temporal cortex, but likely disrupt fibres of passage in the same manner as aspiration resections. By using more selective, neurotoxic, lesions, recent studies in monkeys have revealed that many of the cognitive deficits formerly attributed to amygdala removal result instead from this associated damage to projection

Correspondence: Dr M. Meunier, as above.
E-mail: Meunier@Isc.Cnrs.Fr

Received 22 March 1999, revised 18 August 1999, accepted 23 August 1999

TABLE 1. Estimated damage (percentage of normal volume) in subjects that received bilateral lesions of the amygdala

Subject	Previous memory study involving same subject	Amygdala				Entorhinal cortex				Perirhinal cortex			
		L%	R%	X%	W%	L%	R%	X%	W%	L%	R%	X%	W%
A_{ASP}-1	Our unpublished results	83	96	89.5	79.7	3	0	1.5	0	0	0	0	0
A_{ASP}-2	Murray *et al.* (1993)	97	99	98.0	96.0	58	44	51.0	25.5	0	0.5	0.25	0
A_{ASP}-3	Murray & Gaffan (1994)	94	99	96.5	93.0	19	11	15.0	2.1	0	0	0	0
A_{ASP}-mean				94.7	89.6			22.5	9.2			0.1	0
A_{IBOc}-1	Málková *et al.* (1997)	100	96	98.0	96.0	42	8	25.0	3.4	6	1	3.5	0.1
A_{IBOc}-2*	Murray *et al.* (1996)	91	98	94.5	89.2	57	53	55.0	30.2	2	4	3.0	0.1
A_{IBOc}-3		99	100	99.5	99.0	7	43	25.0	3.0	0	4	2.0	0
A_{IBOc}-mean				97.3	94.7			35.0	12.2			2.8	0.1
A_{IBOp}-1*	Murray *et al.* (1996)	40	63	51.5	25.2	2	70	36.0	1.4	0	6	3	0
A_{IBOp}-2	Málková *et al.* (1997)	92	50	71.0	46.0	4	0	2.0	0	2	0	1	0
A_{IBOp}-3	Málková *et al.* (1997)	54	93	73.5	50.2	0	1	0.5	0	0	0	0	0
A_{IBOp}-mean				65.3	40.5			12.8	0.5			1.3	0

L%, percentage damage in the left hemisphere; R%, percentage damage in the right hemisphere; X%, average of L% and R%; W% = (L% × R%)/100 (Hodos & Bobko, 1984). Note that W% gives much lower values than X% when lesions are grossly asymmetrical (e.g. amygdala in group A_{IBOp} or entorhinal cortex in A_{IBOc}) and so may provide a better index when, as here, largely unilateral damage is presumed to be behaviourally ineffective. Like operated subjects, unoperated controls had previously participated in memory studies (N-1 and -2, Murray, 1987; N-3, Meunier *et al.*, 1993; N-4–6, Murray *et al.*, 1996). *Two subjects that, like case N-4, were imported from outside the USA (see section on Subjects).

fibres from adjacent cortex (see Murray, 1996; Goulet *et al.*, 1998). Because no such re-evaluation has been carried out for the effects of amygdalectomy on emotions, there is a possibility that here, too, the responsible damage is to the fibres of passage rather than to the amygdala itself. To test this possibility, we compared the emotional behaviour of monkeys with neurotoxic lesions of the amygdala that spared fibres of passage with that of monkeys with conventional, aspiration removals of the amygdala. The two experimental groups and their normal controls were exposed to four different stimuli (a human, a conspecific stimulus, a negative item and a positive item) to evaluate the expression of defence, aggression, submission and approach responses.

A preliminary report of this work has appeared elsewhere (Meunier *et al.*, 1996).

Materials and methods

Subjects

This study was approved by the NIMH Animal Care and Use Committee. The subjects were 15 adult male rhesus monkeys (*Macaca mulatta*), weighing from 4.1 to 5.9 kg at the time of surgery or at the corresponding period of time for the unoperated controls. They were housed individually in rooms with automatically regulated lighting (12 h light : 12 h dark cycle), and were maintained on a diet of monkey chow (no. 5038, PMI Feeds, St Louis, MO, USA) supplemented with fruit. Food was given *ad libitum* once a day after completion of the behavioural testing; water was always available. The animals included six unoperated control monkeys (N), three monkeys with aspiration lesions of the amygdala (A_{ASP}) and six with neurotoxic lesions of the amygdala (A_{IBO}). Twelve of the monkeys were born in domestic breeding colonies, and the others were imported from outside of the USA (Table 1). Evaluation of the animals' emotional reactivity took place 3–11 months after surgery (average, 7.3 months). The data were collected over the course of ~ 8 years as monkeys participating in other studies became available for this one (Table 1).

Surgery

Surgical procedures were similar to those described in earlier studies from our laboratory using aspiration (e.g. Murray *et al.*, 1993) or neurotoxic amygdala lesions (e.g. Málková *et al.*, 1997). Monkeys were immobilized with ketamine hydrochloride (10 mg/kg i.m.) and anaesthetized with either pentobarbital sodium (25–35 mg/kg i.v.), which was supplemented throughout surgery as required, or isofluorane gas (1–2%, to effect). After induction of anaesthesia, the animals were treated with atropine sulphate (0.04 mg/kg i.m.) to reduce secretions. Surgery was carried out aseptically, and heart rate, respiration rate and body temperature were monitored throughout the procedure. In some operations, blood pressure, expired CO_2 and blood oxygen levels were also monitored. After the aspiration or neurotoxic lesions were completed, the wound was sutured in anatomical layers. All monkeys received dexamethasone phosphate (0.4 mg/kg) and Di-Trim (0.1 mL/kg, 24% solution, i.m.; Syntex Animal Health, West Des Moines, IA, USA) for 1 day before surgery, and daily for 1 week after surgery, to reduce swelling and prevent infection, respectively. Monkeys also received acetaminophen (40 mg) for 3 days after surgery as an analgesic.

For the bilateral aspiration lesions of the amygdala, which were carried out in a single stage, a bone opening was made in the appropriate portion of the cranium, and the dura mater was incised and reflected. The frontal lobe was gently retracted from the orbit with a brain spoon to expose the anterior medial temporal lobe, and then the amygdala was removed by direct aspiration of tissue with the aid of an operating microscope. The boundaries of the aspiration lesion of the amygdala were the rostral face of the hippocampus, caudally, the tissue level with the top of the rostral face of the hippocampus, dorsally, the fundus of the rhinal sulcus, ventrolaterally, and the white matter of the temporal stem, laterally. The ablations therefore included not only the amygdala, but also the piriform and periamygdaloid cortex, medially, and, in some hemispheres, the anterior portion of the entorhinal cortex, ventrally.

For the neurotoxic lesions of the amygdala, each monkey was first given a brain scan using magnetic resonance imaging (MRI). The MRI scans were used to calculate the stereotaxic coordinates for a series of injection sites in the amygdala. The number of sites and their locations were tailored to each individual but, in all subjects, the sites were separated by ~ 2 mm in each plane. During surgery, a large bone flap was turned over the dorsal aspect of the cranium. Small slits were cut in the dura to allow the needle of a 10-μL Hamilton syringe, held

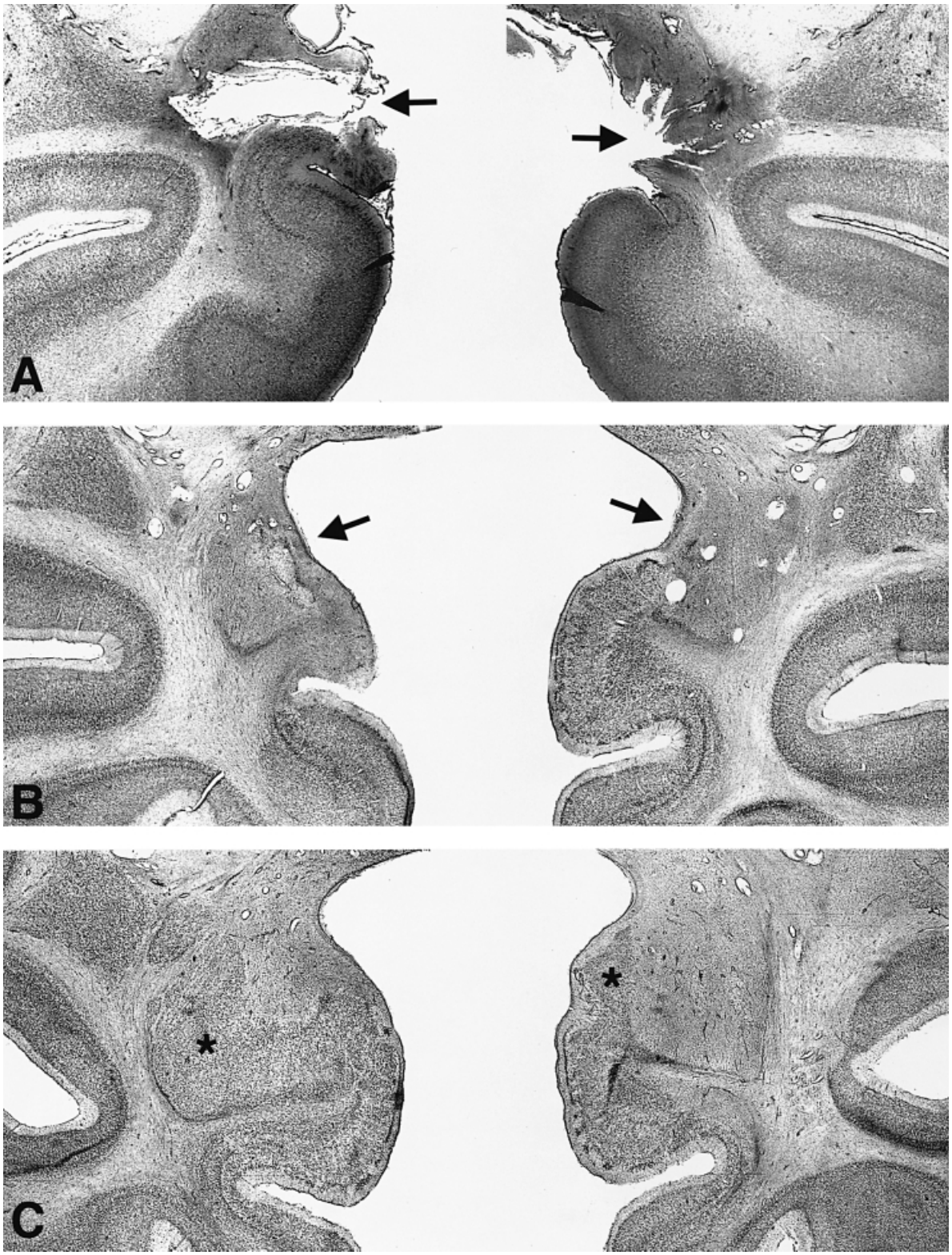

FIG. 1. Photomicrographs of Nissl-stained coronal sections through the medial temporal lobe (at +17 mm from the interaural plane) from one subject of each operated group. (A) Sections from case A_{ASP}-2 following an aspiration removal of the amygdala. Arrows point to the space that would normally be occupied by the amygdala and entorhinal cortex, and that is now partially filled by the surrounding tissue. (B) Sections from case A_{IBOc}-1 with a complete neurotoxic lesion of the amygdala. Arrows points to the amygdala, which sustained massive cell loss and is now shrunken and gliotic. In this case, there is inadvertent damage to the entorhinal cortex on the left side. (C) Sections from case A_{IBOp}-3 with a partial neurotoxic lesion of the amygdala. Asterisks are located in spared regions of the amygdala.

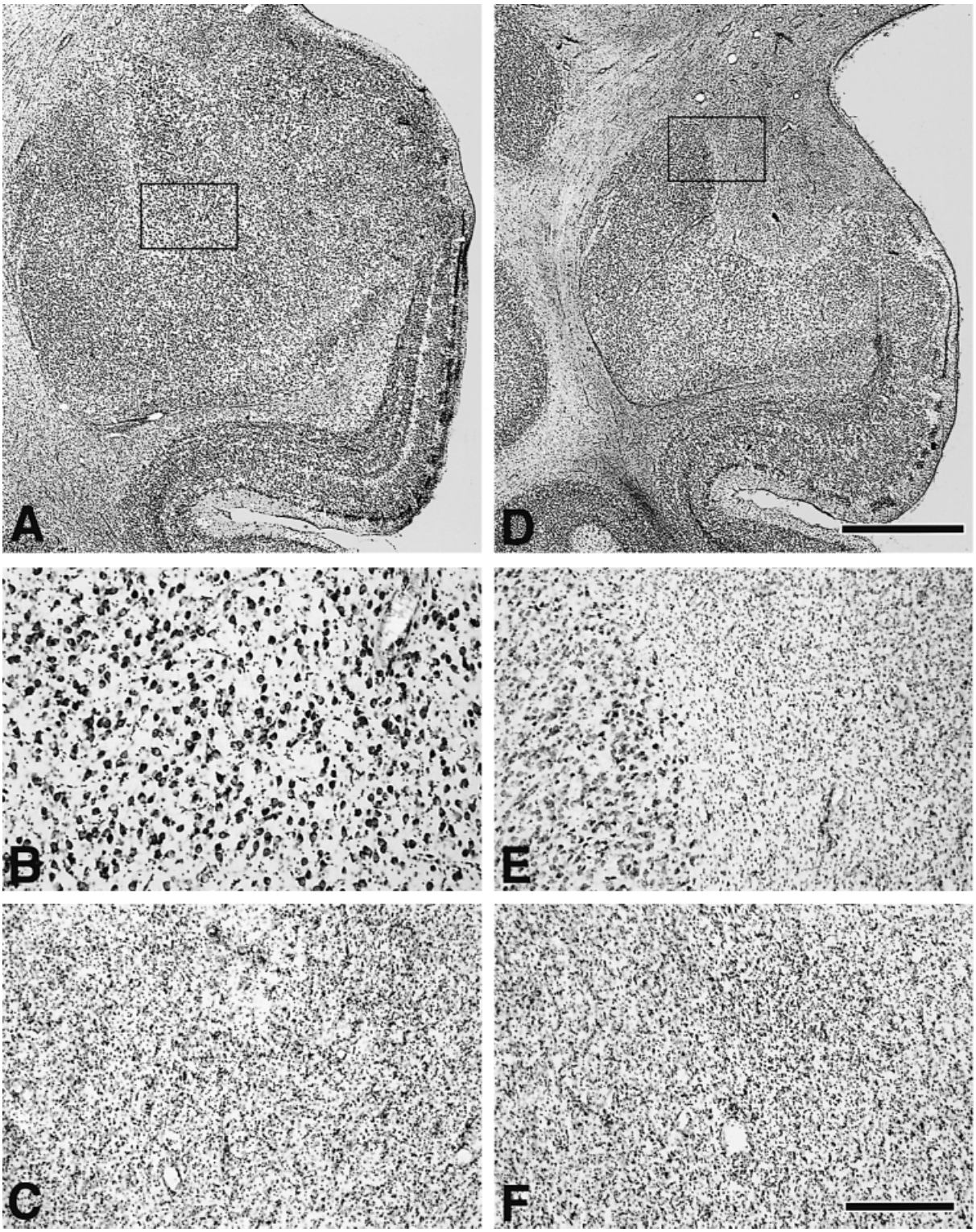

FIG. 2. Matching low- and high-power photomicrographs of the left amygdala region from a control brain (A and B) and a brain with a partial excitotoxic lesion of the amygdala (D and E), together with a set of high-power photomicrographs from a brain with a complete excitotoxic lesion of the amygdala (C and F). (A and D) Rectangles within the amygdala indicate approximate boundaries of photographs shown in B and E, respectively. (B) Large, deeply stained neurons in the lateral basal nucleus in the control brain. (E) Gliosis and cell loss in the lateral basal nucleus (right side) together with intact neurons in the lateral nucleus (left side) in case A_{IBOp}-3. (C and F) Extensive cell loss and gliosis in the left and right amygdala (same region as depicted in D) in case A_{IBOc}-1. Magnification in A as in D (scale bar, 2 mm), and in B, C and E as in F (scale bar, 300 μm).

in a Kopf manipulator (David Kopf Instruments, Tujunga, CA, USA) to be lowered to the proper coordinates. Each animal received 23–31 injections of 0.6–1.0 µL of ibotenic acid (10–15 mg/mL; Regis Chemical, Morton Grove, IL, USA, or Sigma, St Louis, MO, USA) into each amygdala at a rate of 0.2 µL per minute. Because of the length of time required to make the injections and the potentially lethal effect of administering large amounts of ibotenic acid in a single stage, the neurotoxic lesions were carried out in two stages, left hemisphere followed by right, separated by 2–3 weeks. The neurotoxic lesions were intended to produce complete cell loss in the amygdala, but to spare adjacent medial temporal cortex and fibres running nearby or through the amygdala.

Lesion assessment

At the conclusion of behavioural testing, the monkeys were given a lethal dose of pentobarbital sodium and were perfused intracardially with normal saline followed by aldehyde fixatives. The brains were then removed, and either embedded in celloidin and cut at 25 µm, or frozen and cut at 50 µm in the coronal plane. A series of sections from each brain was stained with thionin. The extent of the lesion was plotted onto drawings of coronal sections, at 1-mm intervals, of a standard rhesus monkey brain. The volume of direct damage to the amygdala and to the entorhinal and perirhinal cortices was estimated for each case in the manner described by Meunier *et al*., (1993).

Amygdala aspiration lesions

In one of the three cases (A_{ASP}-1), damage to the amygdaloid complex was extensive (89.5% of its total volume; see X% column in Table 1), but there was slight sparing of the left lateral nucleus; in this case, the entorhinal cortex was essentially intact. The two remaining monkeys (A_{ASP}-2 and 3) had virtually complete ablations of the amygdala (98 and 96.5% damage, respectively) together with substantial damage to the anterior portion of the entorhinal cortex, bilaterally (Figs 1A and 3A). Cases A_{ASP}-1 and 3 sustained in addition some damage to the tail of the caudate nucleus. The hippocampus and the perirhinal cortex were preserved in all cases.

Amygdala neurotoxic lesions

Among the six animals with A_{IBO} lesions, the extent of damage to the amygdala ranged from 51.5 to 99.5% (see X% column in Table 1). For the purpose of data analysis, the animals were divided into two groups on the basis of the extent of this damage. One group (A_{IBOc}; Figs 1B, 2C and F, and 3B) consisted of three animals with virtually complete damage to the amygdala (average, 97.3%), whereas the other group (A_{IBOp}; Figs 1C, 2D and E, and

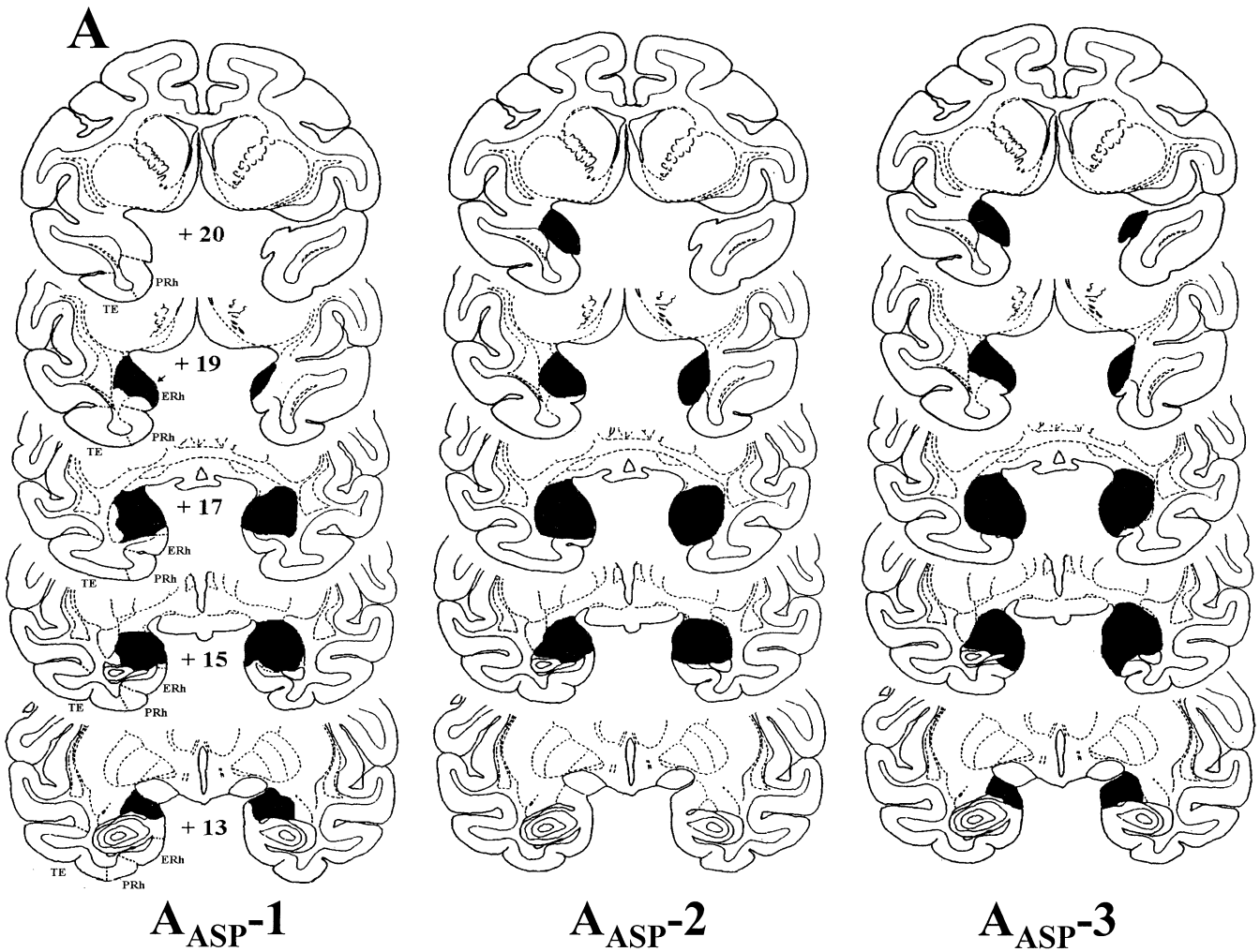

FIG. 3A. For legend see over (page 4409).

3C) consisted of three animals with partial damage to the amygdala (average, 65.3%). Sparing in group A_{IBOp} was mostly unilateral (see W% column in Table 1) and located in the ventral portion of the amygdala. In both groups, a variable extent of cell loss was evident in the anterior portion of the entorhinal cortex and the lesion encroached slightly on the rostral tip of the hippocampus, bilaterally, but the perirhinal cortex remained virtually intact. The basal nucleus of Meynert was intact except for slight, unilateral (left) cell loss in cases A_{IBOc}-1 and 2. Small infarcts were noted in addition in the right pallidum in case A_{IBOc}-1, and in the left putamen in case A_{IBOp}-2.

Evaluation of emotional responses

Apparatus

Testing was conducted inside a sound-shielded room; additional sound masking was provided by a white-noise generator. The animal's transport cage was placed in a Wisconsin General Testing Apparatus (WGTA). A test tray equipped with food wells was used for presentation of one of the stimuli (object, see section on Procedure). Except when indicated (see section on Procedure), the opaque screen of the WGTA, which separated the animal from the testing area, and the one-way vision screen, which separated the testing area from the experimenter, were both raised to allow recording of the animal's behaviour with a video camera placed in front of the apparatus. The animals were first adapted to these unfamiliar testing conditions during four daily 10-min simulated recording sessions without stimuli.

Selection of the stimuli

Our objective was to use a small set of stimuli to generate a large variety of reactions in normal animals (not only fear, but also approach, submission and aggression), and thereby potentially unveil many different symptoms of the Klüver–Bucy syndrome in amygdalectomized monkeys (loss of fear, hypermetamorphosis, hyperorality, reduced aggressivity, etc.). Another objective was to maintain constant testing conditions for different animals that would become available for this study at unpredictable times over a long period. Accordingly, we used a stimulus set consisting of the following four items: an unfamiliar human (Human Face), a conspecific stimulus (Monkey Head), a negative item (Snake) and a positive item (Object). The unfamiliar human was the experimenter wearing a white laboratory coat and a rubber face mask representing a human female. The conspecific stimulus was a taxidermic monkey head attached to a 50-cm wooden rod. The negative item was an 80-cm toy rubber snake (see Mineka *et al.*, 1980). Finally, the positive item was an object covering a well containing a 300 mg banana pellet (P.J. Noyes, Lancaster, NH, USA) or a half-peanut (all animals in this

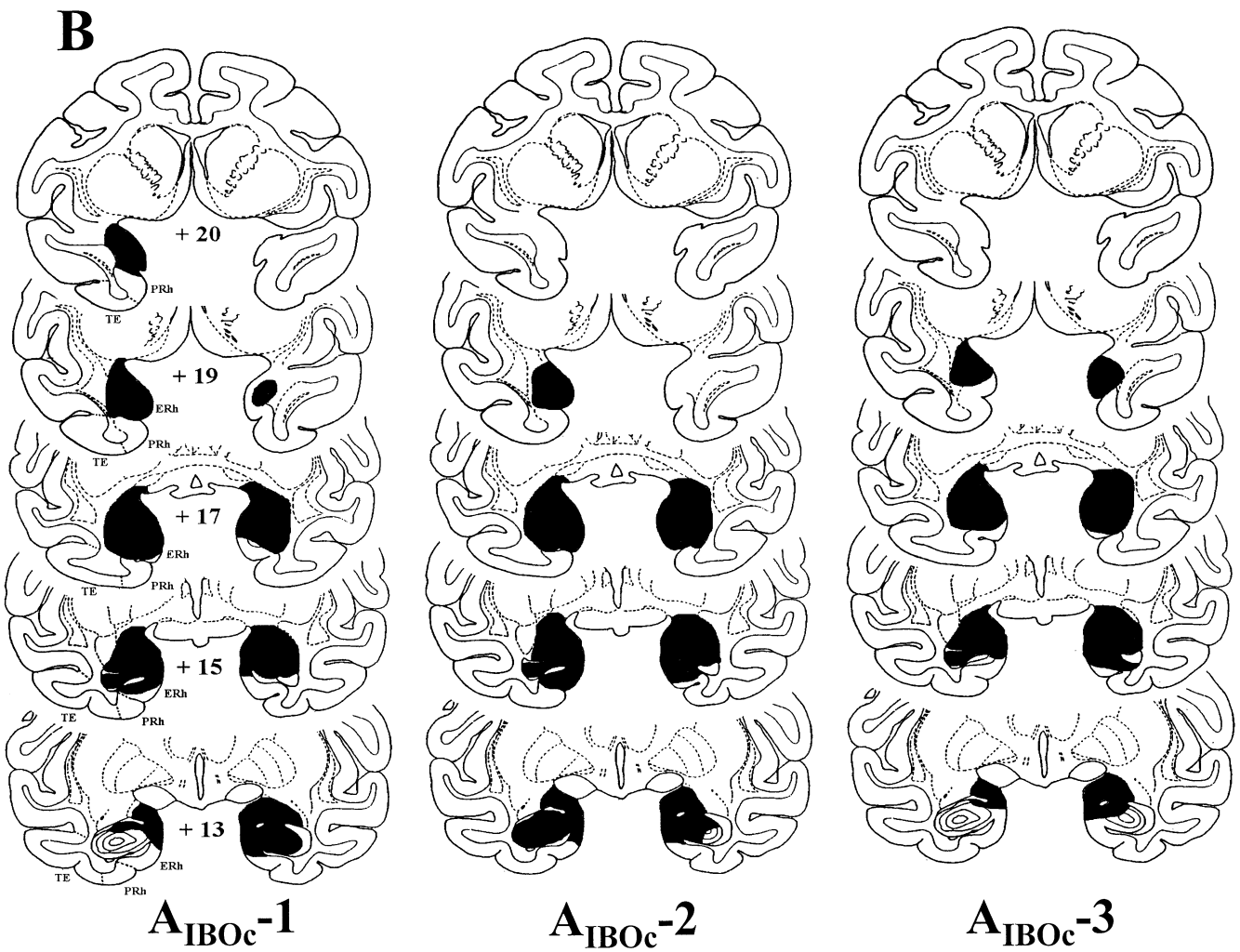

FIG. 3B. For legend see page opposite (4409).

study having previously been trained to respond to visual stimuli to obtain food rewards).

Procedure

Each daily session was divided into a fixed sequence of non-recorded and recorded episodes as illustrated in Fig. 4. Within the recorded episodes, five segments, each lasting either 1 min (OFF 1, 2 and 5) or 20 s (OFF 3 and 4), provided samples of the monkey's behaviour before (OFF 1, 2 and 3) and after (OFF 4 and 5) the presentation of a stimulus. A single stimulus was presented per session during the middle portion of the third recorded episode. All stimuli except the Object were presented for a duration of 20 s. For the Object, there were four successive presentations of 20 s each, separated by lowering of the opaque screen for rebaiting of the well. Consequently, unlike each daily session with Human Face, Monkey Head and Snake, which lasted 9 min (as shown in the figure), each session with the Object lasted 10 min.

The four stimuli were each presented in a separate session once a week for 3 weeks. They appeared in a different sequence each week but in the same overall sequence for all subjects. For the Human Face presentation, the experimenter, dressed in a lab coat and the human face mask described above, sat in a chair ~80 cm

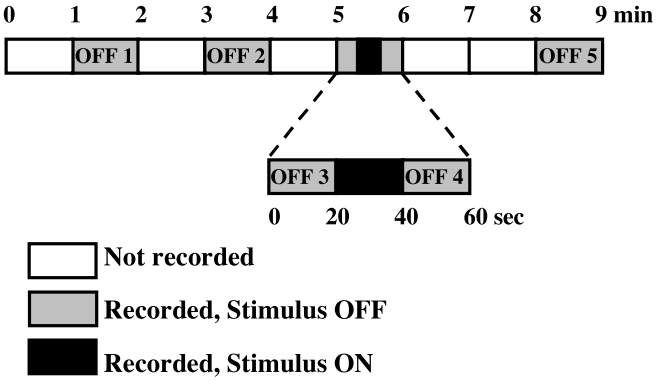

FIG. 4. Daily recording session. One stimulus was presented per session during the third recorded episode, for a duration of 20 s (or 4 × 20 s for the Object; see section on Procedure). The general behaviour of the animal in the absence of the stimulus was sampled at intervals both before and after the stimulus presentation for a total of three 1-min segments (OFF 1, 2 and 5) and two 20-s segments (OFF 3 and 4).

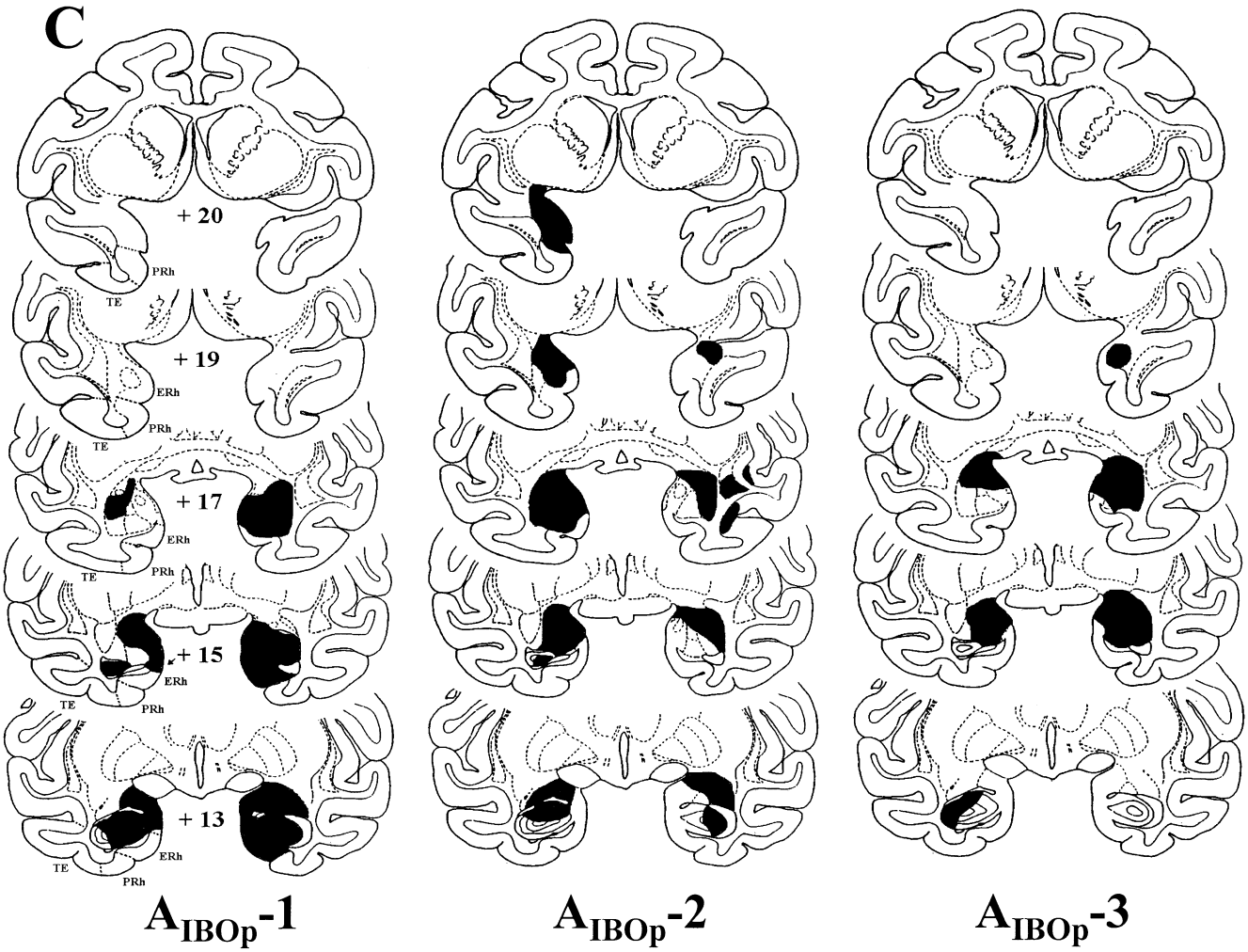

FIG. 3. (A–C) Aspiration and neurotoxic lesions of the amygdala (black areas) plotted on coronal sections of a normal rhesus monkey brain for each of the nine experimental animals. Numerals indicate the distance in mm from the interaural plane. Conventions as in Fig. 1. Abbreviations: amt, anterior middle temporal sulcus; ERh, entorhinal cortex (area 28); ot, occipitotemporal sulcus; PRh, perirhinal cortex (areas 35 and 36); rh, rhinal sulcus; TE, cytoarchitectonic field of von Bonin & Bailey, 1947).

TABLE 2. Behavioural activities in the absence of a stimulus

Behaviour	Description	Interobserver reliability
Passive	Remains motionless for any length of time (still, or with only eye/head slow movements)	0.98
Move	Locomotes, or moves at least one limb, without engaging in any of the four activities below	0.97
Manipulate	Explores any part of the surrounding with hands (but not mouth)	0.95
Mouth	Explores any part of the surrounding with hands and mouth, or mouth only (e.g. licks or chews)	0.96
Locomotor stereotypies	Activities, such as circling and hopping, repeated three or more times	0.99
Self-directed activities	Scratches, grooms, holds, etc. any part of the body	0.79

Interobserver reliability was calculated for the duration of each of the six behavioural activities, over a sample of 55 observations, using Pearson correlation coefficients (all $P < 0.001$).

TABLE 3. Behavioural activities during stimulus presentation

		Interobserver reliability	
Behaviour	Description	Frequency	Duration
Mild aggression			
Frown	Wrinkles or moves eyebrows up and down	0.99	0.94
Ears back	Flattens ears against the head	0.72	0.90
Yawn	Opens mouth wide, baring upper teeth	NA	NA
High aggression			
Head/body lunge	Thrusts head or body forwards	0.95	0.98
Cage shake	Shakes transport cage	0.80	0.74
Mouth threat	Opens mouth slightly, exposing lower teeth	1.00	1.00
Striking attack	Attempts to, or delivers a blow using hand or foot	1.00	1.00
Biting attack	Attempts to, or bites the stimulus	NA	NA
Defence			
Freezing	Remains motionless for at least 3 s	0.97	0.94
Startle	Jerks suddenly	0.86	0.73
Eye/head aversion	Avoids eye contact, shifting gaze or whole head	0.83	0.93
Piloerection	Hair stands on end	0.80	0.85
Move away	Retreats from the stimulus	0.90	0.97
Submission			
Lip smack	Purses, and alternatively closes and opens lips	0.95	0.92
Grimace	Mouth closed, pulls lips backward exposing teeth	1.00	1.00
Presentation	Presents its hindquarters with tail up	1.00	1.00
Approach			
Look at	Makes eye contact	0.57	0.89
Move toward	Shifts body forward, close to stimulus	0.86	0.97
Touch	Handles with hand or foot	0.93	0.99
Mouth	Licks, chews or mouths	0.98	1.00
Smell	Sniffs either the stimulus, or own fingers after having touched the stimulus	1.00	0.94
Take/eat reward	Picks up and/or mouths the food reward hidden underneath the Object	1.00	0.98
Other behaviours (not directed towards the stimulus)			
Manipulate	Handles any part of its surrounding	0.98	0.97
Locomotor stereotypies	Activities, such as circling and hopping, repeated three or more times	NA	NA
Self-directed activities	Scratches, grooms, holds, etc. any part of the body	1.00	1.00
Miscellaneous	Engages in any peculiar activity not described above	NA	NA
Look away	Looks away from stimulus while engaged in one of the four above activities	0.80	0.94

Interobserver reliability was calculated for frequency and duration of each activity in a sample of 24 sessions, using Pearson correlation coefficients (all $P \leqslant 0.003$); NA, activity was not observed within the sample. The 27 activities were grouped into six composite categories as shown.

from the animal's cage. During the first 10 s, the experimenter refrained from making eye contact, but during the last 10 s of the presentation, the experimenter looked straight at the animal's face. For Monkey Head, the stimulus was first placed gently on the testing area and maintained immobile for 10 s, ~40 cm from the animal's cage. Then, it was thrust towards the cage and held there for an additional 10 s. For Snake, the stimulus was introduced suddenly within the testing area of the WGTA and held there for 20 s. For Object, the opaque screen of the WGTA was lowered and the stimulus placed over the central well of the test tray; then, the screen was raised for 20 s. The Object concealed a food reward during the first three presentations, but not during the fourth; this arrangement was designed to evaluate the monkey's reactions not only to rewarding events, but also to an unexpected, and presumably frustrating, event. During all stimulus presentations, except Human Face, the experimenter remained out of the animal's view by standing to one side of the WGTA.

Behavioural scoring

All videotapes were first rated by one observer. Subsequently, samples of the videotapes, distributed over the course of the study, were scored by a second observer. The first observer had been involved in the testing phase of the study and therefore knew which treatment each monkey had received (N, A_{ASP} or A_{IBO}); however, this observer did not know which individuals were assigned to the

two A_{IBO} subgroups (see section on Lesion assessment, above). The second observer was blind to the animals' group category. Inter-observer reliability is provided in Tables 2 and 3.

Evaluation of behaviour in the absence of a stimulus

Behaviours videotaped during the five OFF segments were classified into six different activities defined to be mutually exclusive: passive, move, manipulate, mouth, locomotor stereotypies and self-directed activities (Table 2). Because most of these activities did not occur as discrete events, only their duration was measured.

Evaluation of behaviour during stimulus presentation

Behaviours videotaped during stimulus presentation were classified into 27 activities (Table 3), defined to be exhaustive but not mutually exclusive (e.g. move toward and lip smack can co-occur). Both duration and frequency were measured. The 27 activities were assigned to six non-overlapping, composite categories: mild aggression, high aggression, defence, submission, approach and other behaviours. Definition of the activities and their grouping into behavioural categories was based on preliminary observations of monkeys with medial temporal damage (Meunier *et al*., 1991), as well as on information in the literature. The resulting behavioural scale is congruent with rating scales used in earlier ablation studies in laboratory rhesus monkeys (e.g. Butter *et al*., 1967; Horel *et al*., 1975; Aggleton & Passingham, 1981), but draws in addition from ethological studies characterizing gestures and postures of intact animals living either in social settings (e.g. Hinde & Rowell, 1962; Chevalier-Skolnikoff, 1973; Sade, 1973; Kenney *et al*., 1979; De Waal, 1989; Kalin *et al*., 1991; Maestripieri & Wallen, 1997) or in isolation (e.g. Goosen, 1981; Suomi, 1982; Capitano, 1986).

Data analysis

Individual behavioural scores were expressed either as the percentage of time dedicated to each of the six behaviours observed in the absence of stimuli, or as the cumulative duration and frequency of the different activities comprising each of the six composite categories observed in the presence of the stimuli. All scores (except those for high aggression) were analysed by parametric analyses of variance (ANOVAs) with the Huynh–Feldt (H–F) correction for factors that included repeated measures, and *post hoc* comparisons were performed using the Tukey test. Because several animals received a score of zero in the composite category 'high aggression', group differences on this measure were evaluated by non-parametric tests (Kruskal–Wallis, Friedman, or Mann–Whitney *U*-tests as appropriate).

Results

Behaviour of unoperated controls

Behaviour in the absence of a stimulus

During the five OFF segments, control monkeys generally remained passive (average proportion of time per segment: passive, 68.0%) or displayed non-specific motor activity (move, 19.6%). Only small proportions of time were dedicated to the other four activities: manipulate, 2.4%; mouth, 1.3%; stereotypies, 7.4%; and self-directed activities, 1.3%.

Behaviour during stimulus presentation

Analyses of the behavioural scores summed over the three weeks, using one-way ANOVAs with repeated measures for stimuli, revealed three different patterns of responses in control animals: one for Human Face and Monkey Head and the two others for Snake and Object, respectively (Fig. 5).

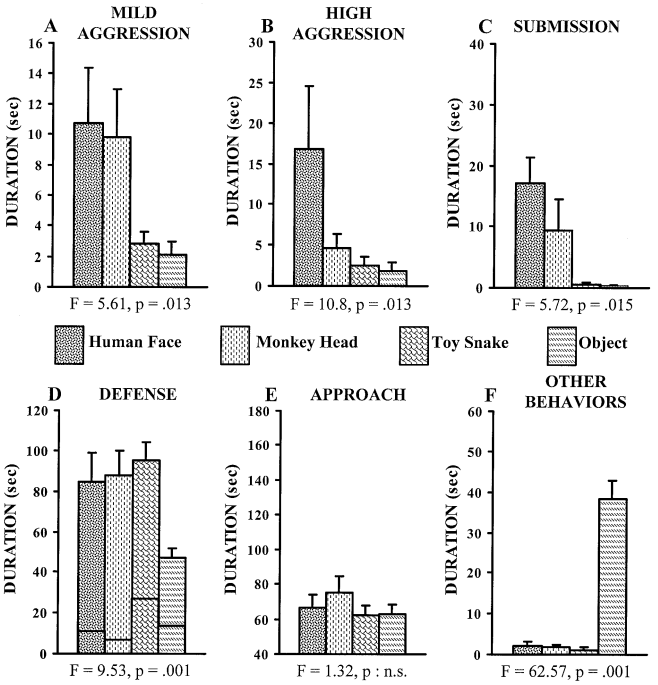

FIG. 5. Reactions of normal controls towards the four different stimuli. For each composite behavioural category (A–F), scores for each stimulus are durations cumulated over the 3 weeks. Scores for the four Object presentations per session were averaged to allow direct comparisons with scores for the other stimuli, which were presented only once per session. (D) The lines inside each bar indicate the amount of freezing elicited by each stimulus. Parametric one-way ANOVAs with the H–F correction for the repeated measure (stimulus) were used for comparisons in each behavioural category, except high aggression, which was analysed using a non-parametric Friedman two-way ANOVA. Analyses of frequencies yielded similar results.

'Human Face' and 'Monkey Head'. These two stimuli were the most effective in triggering mild and high aggression, as well as submission (Fig. 5A–C). For both stimuli, mild aggression consisted primarily of ears back, and submission was expressed almost exclusively by lip smack and grimace gestures. The Human Face evoked the largest amount of high aggression responses (consisting of head/body lunges, mouth threats and cage shakes), but the qualitatively most aggressive responses (viz. striking attacks) were elicited by the Monkey Head.

'Snake'. Freezing, one of the defence activities (Fig. 5D), emerged as the hallmark reaction to the Snake [$F_{2,12}$(H–F) = 7.06, $P = 0.007$, for duration], lasting two to three times longer than to any other stimulus.

'Object'. The Object elicited considerably fewer defence reactions than the three other stimuli (Fig. 5D), prompting, e.g. few eye/head aversions and move away responses. As a corollary, in its presence, animals more readily engaged in other behaviours (e.g. manipulate parts of the cage and WGTA other than the stimulus; Fig. 5F). The total amount of approach did not vary across stimuli (Fig. 5E); nevertheless, only the Object consistently induced touch responses (except for a single brief instance, mouth never occurred in controls). Reward withdrawal did not reliably alter approach responses, but affected both the defence and other behaviours categories (see Fig. 9D–F). Defence reactions increased, whereas other behaviours decreased on unrewarded

TABLE 4. Effects of complete amygdala damage on 'manipulate' and 'mouth' activities in the absence of a stimulus

Case	Manipulate	Mouth
N-1	4.52	7.87
N-2	2.79	2.64
N-3	0.25	0.00
N-4	8.11	0.00
N-5	0.78	0.08
N-6	1.16	0.12
Mean (controls)	2.94	1.79
A_{ASP}-1	23.70	8.32
A_{ASP}-2	6.97	3.03
A_{ASP}-3	11.48	8.53
Mean (A_{ASP})	14.05*	6.63†
A_{IBOc}-1	21.07	1.52
A_{IBOc}-2	43.33	0.57
A_{IBOc}-3	0.08	0.00
Mean (A_{IBOc})	21.49†	0.70

Individual scores are expressed as the average percentage time dedicated to each activity during the three 1-min-long, OFF segments (OFF 1, 2, and 5; see Fig. 4). $*P \leq 0.05$ and $\dagger P \leq 0.07$, ANOVAs in comparison with the control (N) group.

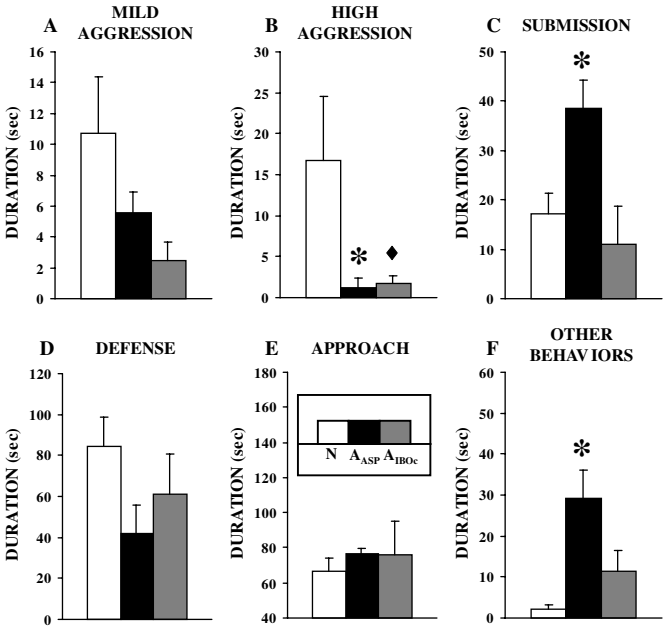

FIG. 6. Effects of complete, aspiration (A_{ASP}) versus neurotoxic (A_{IBOc}), lesions of the amygdala on behavioural reactions towards an unfamiliar Human Face. For each composite behavioural category (A–F), scores are expressed as durations cumulated across the three weekly presentations of the stimulus. Symbols indicate differences ($*P \leq 0.05$; ◆ $P \leq 0.07$) between an operated group and the control group (N) as revealed by Mann–Whitney *U*-tests (for high aggression) or Tukey HSD pairwise comparisons (for all other categories).

relative to rewarded presentations of the Object during the first 2 weeks of testing [$F_{1,5} = 12.83$, $P = 0.016$ and $F_{1,5}$(H–F) = 8.26, $P = 0.035$, respectively, for duration].

Effects of complete amygdala damage: aspiration versus neurotoxic lesions

Behaviour in the absence of a stimulus

Scores of group A_{ASP} and group A_{IBOc} were compared with those of group N using group × OFF segment ANOVAs with repeated measures for the last factor. Neither passive and move behaviours, nor stereotypic and self-directed activities differed among groups. By contrast, the lesions altered the manipulate and mouth scores recorded for the three, 1-min-long, OFF segments (Table 4). As shown in the table, all animals in both lesion groups except case A_{IBOc}-3 showed an exaggerated tendency to manipulate parts of the environment (careful histological examination failed to reveal any explanation for the difference in behaviour of case A_{IBOc}-3, a difference which was also seen in three other instances; see Snake and Object sections below). In addition, the A_{ASP} but not the A_{IBOc} group tended to show enhanced oral exploration. This hyperorality in group A_{ASP} was particularly salient for segment OFF 5 (A_{ASP} versus N, $P = 0.033$; A_{ASP} versus A_{IBOc}, $P = 0.045$).

Behaviour during stimulus presentation

Scores for each composite category other than high aggression were subjected to group × week ANOVAs with repeated measures for weeks (for Human Face, Monkey Head and Snake) or to group × week × reward condition ANOVAs with repeated measures for the last two factors (for Object).

Human Face

The time factor had no influence on the animals' reactions, except on defence [$F_{2,18}$(H–F) = 4.83, $P = 0.021$ for duration) which increased with weeks in operated and unoperated monkeys alike. The following analyses will therefore focus on the group factor, which affected all behavioural categories but mild aggression (Fig. 6A) and approach (Fig. 6E).

High aggression (Fig. 6B). Unlike the normal controls, the animals in groups A_{ASP} and A_{IBOc} displayed little or no high aggression (Kruskal–Wallis: $H = 5.90$, $P = 0.052$ for duration; $H = 5.65$, $P = 0.059$ for frequency). The difference between the N and A_{ASP} groups was significant ($U = 1$, $P = 0.038$; $U = 1.5$, $P = 0.051$, for duration and frequency, respectively), but the difference between the N and A_{IBOc} groups fell short ($U = 2$, $P = 0.071$; $U = 2$, $P = 0.068$).

Submission (Fig. 6C). Animals with A_{ASP} lesions were the most submissive ($F_{2,9} = 5.30$, $P = 0.03$, for duration), tending to differ in that respect from both controls and monkeys with A_{IBOc} lesions ($P = 0.055$ and 0.035, respectively). All three monkeys in the A_{ASP} group expressed submission not only by the lip smack and grimace gestures commonly observed in normal animals, but also by repeated hindquarter presentations.

Defence (Fig. 6D). Both groups A_{ASP} and A_{IBOc} tended to exhibit fewer defence behaviours than group N ($F_{2,9} = 3.87$, $P = 0.061$, for frequency).

Other behaviours (Fig. 6F). Group A_{ASP} spent more time engaged in other behaviours., e.g. manual exploration than both groups N and A_{IBOc} ($F_{2,9} = 13.97$, $P = 0.002$; A_{ASP} versus N, $P = 0.001$; A_{ASP} versus A_{IBOc}, $P = 0.037$, for duration).

Monkey Head

These analyses yielded several group × week interactions, reflecting the tendency of both operated groups to habituate to the stimulus over repeated presentations. As a result, group differences did emerge, but, except for defence, were limited to either the first (week 1) or third (week 3) presentation of the stimulus (Fig. 7).

Aggression (Fig. 7A and B). Mild and high aggression did not differ among groups, nor across weeks. Interestingly, striking attacks,

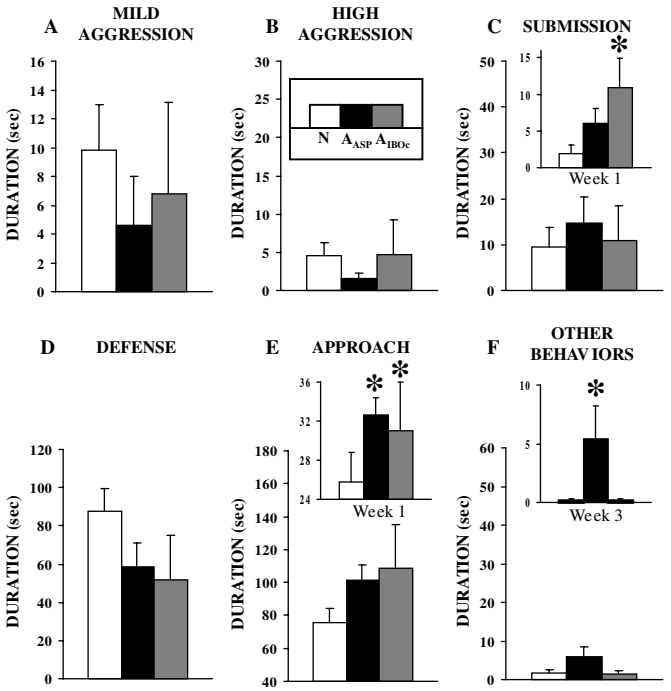

FIG. 7. Effects of complete, aspiration versus neurotoxic, lesions of the amygdala on behavioural reactions towards a taxidermic Monkey Head. Scores and conventions as in Fig. 6. Inserts (C, E and F) illustrate between-group differences that occurred for only one of the 3 weeks of testing.

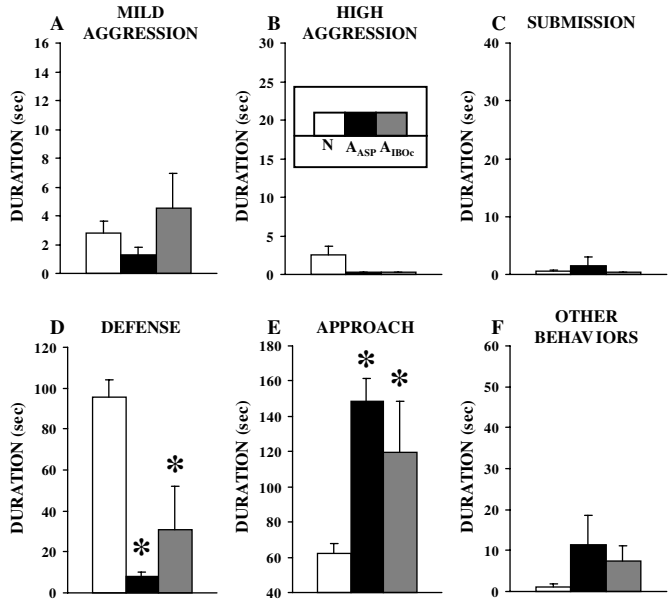

FIG. 8. Effects of complete, aspiration versus neurotoxic, lesions of the amygdala on behavioural reactions towards a toy snake. Scores and conventions as in Fig. 6.

the most aggressive responses observed in control animals over the entire course of the study, were also observed in one monkey of group A_{ASP} and one monkey of group A_{IBOc} (the latter animal, case A_{IBOc}-1, showed in addition the only instances of biting attacks recorded during the study).

Submission (Fig. 7C). Both lesion groups were more submissive than controls on week 1 [group × week interaction, $F_{4,17}$(H–F) = 5.24, $P = 0.006$ for duration, and $F_{3,15}$(H–F) = 8.36, $P = 0.001$ for frequency], although the pairwise comparisons reached significance only for group A_{IBOc} (A_{IBOc} versus N, $P = 0.035$ for duration). Animals in this experimental group showed excessive lip smack and grimace responses, and they displayed, in addition, hindquarter presentations, which were never observed in controls.

Defence (Fig. 7D). Both experimental groups tended to display fewer defence reactions than controls over the 3 weeks of testing (group effect, $F_{2,9} = 4.21$, $P = 0.051$, for frequency).

Approach (Fig 7E). Animals in both experimental groups displayed significantly more approach responses than controls on week 1 [group × week interaction, $F_{2,18}$(H–F) = 4.17, $P = 0.015$; A_{ASP} versus N, $P = 0.024$; A_{IBOc} versus N, $P = 0.002$, for frequency]. In particular, they occasionally touched or mouthed the stimulus, whereas the control monkeys did not.

Other behaviours (Fig. 7F). Other behaviours differed across groups only on week 3 [group × week interaction, $F_{2,18}$(H–F) = 4.83, $P = 0.008$ for duration; $F_{2,18}$(H–F) = 2.92, $P = 0.051$ for frequency], with group A_{ASP} exhibiting other behaviours, in particular manual exploration, more than controls ($P = 0.026$ for duration and $P = 0.044$ for frequency).

Snake

These analyses yielded no week effect, and resulted in group effects only for the defence and approach categories (Fig. 8).

Defence (Fig. 8D). Defence behaviours were markedly diminished in operated animals ($F_{2,9} = 16.04$, $P = 0.011$ for duration, and $F_{2,9} = 22.80$, $P < 0.001$ for frequency). Both the A_{ASP} and A_{IBOc} groups differed significantly from controls (A_{ASP} versus N, $P = 0.002$ and 0.001, and A_{IBOc} versus N, $P = 0.01$ and 0.003 for duration and frequency, respectively). The most striking change was the complete absence of freezing in all monkeys of both lesion groups except case A_{IBOc}-3.

Approach (Fig. 8E). Approach behaviours were significantly enhanced in both operated groups ($F_{2,9} = 10.32$, $P = 0.005$; A_{ASP} versus N, $P = 0.005$; A_{IBOc} versus N, $P = 0.047$, for duration). Unlike controls, all animals in both lesion groups (except case A_{IBOc}-3) engaged in extensive examination of the Snake, touching, mouthing and also smelling the stimulus.

Object

The major effects were related to the group and reward factors. Group effects, i.e. changes persisting over the 3 weeks and two reward conditions, were observed only for defence and approach behaviours. Reward effects occurred mainly during the first 2 weeks of testing, as reflected by several week–reward interactions, and altered defence and approach, as well as other behaviours and mild aggression (Fig. 9).

Group effects. Defence behaviours (Fig. 9D) were reduced in both groups A_{ASP} and A_{IBOc} ($F_{2,9} = 5.70$, $P = 0.025$; A_{ASP} versus N, $P = 0.028$; A_{IBOc} versus N, $P = 0.043$, for duration), particularly eye/head aversions and move away responses. Conversely, approach behaviours (Fig. 9E) were enhanced in the two experimental groups ($F_{2,9} = 4.27$, $P = 0.050$; A_{ASP} versus N, $P = 0.022$; A_{IBOc} versus N, $P = 0.028$, for duration). Whereas unoperated animals simply displaced the Object to retrieve the reward, all monkeys in both lesion groups (except case A_{IBOc}-3) engaged in excessive manual and oral investigation of the stimulus.

Reward condition effects. Both unoperated and operated animals presented a reliable increase in defence behaviours (Fig. 9D) on unrewarded relative to rewarded presentations of the Object, and this

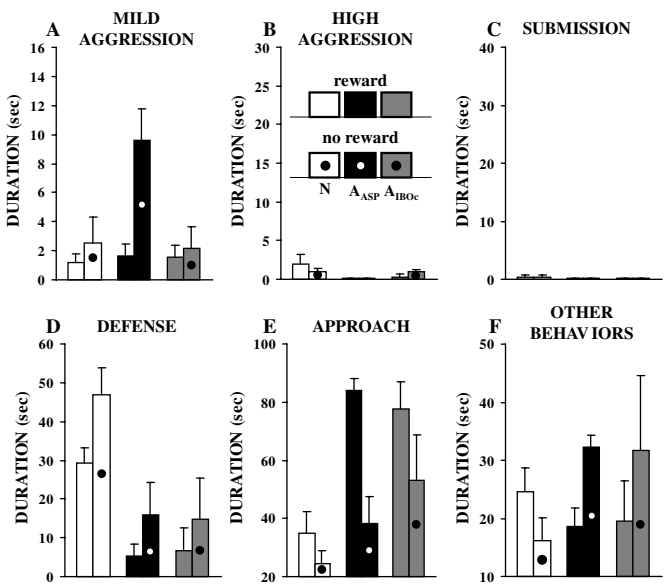

FIG. 9. Effects of complete, aspiration versus neurotoxic, lesions of the amygdala on behavioural reactions towards an Object. Scores are durations, averaged first over the three rewarded presentations per session (reward) to allow direct comparison with the single unrewarded presentation (no reward), and then cumulated across the first 2 weeks of testing. Scores for week 3 are not illustrated in order to show the effects of reward withdrawal which, in all groups, were most prominent during weeks 1 and 2. For statistical comparisons, see section on Object in Results. Other conventions as in Fig. 6.

effect was more salient for week 1 [week × reward interaction, $F_{2,18}$(H–F) = 3.67, $P = 0.046$, for duration]. Approach responses were reduced by reward withdrawal in all groups (Fig. 9E), but this change was greater in the lesion groups, particularly during weeks 1 and 2 [group × week × reward interaction, $F_{4,18}$(H–F) = 2.93, $P = 0.05$, for duration]. In contrast, other behaviours (e.g. manipulate; Fig. 9F) decreased in unoperated controls on unrewarded presentations, whereas they increased in both lesion groups (group × reward interaction, $F_{2,9} = 5.00$, $P = 0.035$, for duration). The only other effect of reward omission was an increase in mild aggression (Fig. 9A), which was most prominent in group A_{ASP} on week 1 [group × week × reward interaction, $F_{4,18}$(H–F) = 3.10, $P = 0.042$, for duration].

Effects of partial neurotoxic lesions of the amygdala

The behavioural changes induced by partial neurotoxic lesions were first determined by comparing group A_{IBOp} with group N using the same analyses as those described above. These changes were then compared with the effects of complete amygdala lesions.

In the absence of a stimulus, group A_{IBOp} did not differ from group N. Nor did the two groups differ in their responses to the Human Face, Monkey Head, or reward withdrawal during Object presentation. In particular, monkeys in group A_{IBOp} did not show enhanced submission. Rather, like controls, they displayed a large amount of high aggression towards the Human Face, differing in that respect from both the A_{IBOc} and A_{ASP} groups ($U = 0$, $P = 0.05$, and $U = 0$, $P = 0.05$, respectively, for duration).

As shown in Fig. 10A and B, however, exposure to the toy snake yielded differences between the A_{IBOp} and N groups in two behavioural categories: defence ($F_{1,7} = 10.18$, $P = 0.015$ for duration) and approach ($F_{1,7} = 11.21$, $P = 0.012$ for duration; and $F_{1,7} = 16.70$,

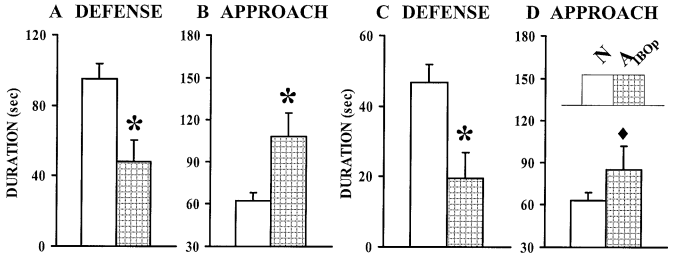

FIG. 10. Effects of partial neurotoxic lesions of the amygdala (A_{IBOp}). Only defence and approach responses towards the Snake (A and B) and Object (C and D) differed from the response of the control group (N). Scores are durations cumulated over the 3 weeks and, for Object, averaged over the four stimulus presentations. Conventions as in Fig. 6.

$P = 0.005$ for frequency). Also, exposure to the Object yielded differences in the same two behavioural categories and in the same direction (Fig. 10C and D); defence was decreased ($F_{1,7} = 10.24$, $P = 0.015$ for duration), whereas approach tended to increase ($F_{1,7} = 4.36$, $P = 0.075$ for frequency) compared with the controls.

As a result of these effects, the defence scores in group A_{IBOp} did not differ from those recorded in groups A_{IBOc} and A_{ASP} for either Snake or Object. All three monkeys with partial amygdala lesions, like monkeys with complete lesions, failed in particular to exhibit freezing in the presence of the Snake. For approach, however, a difference emerged when the touch and mouth responses elicited by Snake and Object were considered separately. Only touch responses were enhanced in group A_{IBOp} relative to controls ($U = 0$, $P = 0.02$ for duration), whereas both touch and mouth responses were increased in the six monkeys of groups A_{IBOc} and A_{ASP} ($U = 2$, $P = 0.01$, and $U = 3.5$, $P = 0.01$, respectively).

Both changes observed in the presence of the non-social stimuli, i.e. the reduction in defensive behaviours and enhancement of manual exploration, were evident in all three monkeys in group A_{IBOp}, although the loci of bilateral damage to the amygdala were different in each case. In other words, we failed to uncover any consistent relationship between bilateral damage to specific amygdaloid nuclei (see Fig. 3C) and the behavioural effects.

Discussion

Emotional responses in unoperated control monkeys

The behaviour of the control monkeys demonstrated that the four stimuli selected for this study were effective in evoking different patterns of emotional responses. Aggressive reactions (mouth threats, lunges and cage shakes) and submissive behaviours (lip smacks and grimaces) occurred primarily in response to the Human Face and Monkey Head. These gestures and postures are typically expressed by normal macaques exposed to social stimuli, e.g. another monkey or a human being (Hinde & Rowell, 1962; Chevalier-Skolnikoff, 1973; Sade, 1973; Kenney *et al.*, 1979; Kalin *et al.*, 1991; Maestripieri & Wallen, 1997). The Human Face and Monkey Head therefore appear to have been adequate (albeit limited) probes of the monkeys' emotional repertoire in social situations.

Responses to the two non-social stimuli also differed as expected. Freezing, a typical defensive fear reaction in infant (Kalin *et al.*, 1991) and adult (Kaufman & Rosenblum, 1966) macaques, was the hallmark response to the Snake. This reaction was observed in all unoperated animals, whether born in the wild or in domestic breeding colonies, a finding consistent with the well-known tendency of monkeys reared in social groups to fear snakes, real and toy alike (e.g. Mineka & Cook, 1988). By contrast, the Object stood out as a

TABLE 5. Summary of the effects of partial (A_{IBOp}) and complete (A_{ASP} and A_{IBOc}) amygdala lesions on responses towards the stimuli

	High aggression					Submission					Defence					Approach					Other behaviours				
	H	M	S	O	r–	H	M1	S	O	r–	H	M1	S	O	r–	H	M1	S	O	r–	H	M3	S	O	r–
A_{ASP}	– –					++					–	–	– –	– –			++	++	++	↓	++	++			↑
A_{IBOc}	–						++				–	–	– –	– –			++	++	++	↓					↑
A_{IBOp}													– –	– –				++	+						

For Human Face (H), Snake (S), and Object (O), the effects were observed on cumulative scores over the 3 weeks of testing. For Monkey Head (M), the effects were found only on week 1 (M1) or week 3 (M3). Minus signs indicate a decrease and plus signs an increase relative to controls (– and +, $P \leq 0.07$; – – and ++, $P \leq 0.05$). Arrows in columns labelled 'r–' denote direction of abnormal response change (decrease or increase) during unrewarded, compared with rewarded, presentation of the object.

non-aversive stimulus inasmuch as animals consistently touched it, and, in its presence, showed little defence as well as a greater readiness to explore their environment. Omission of food reward hindered these positive responses; indeed, defence was increased and environmental exploration reduced during unrewarded, compared with rewarded, presentations of the Object.

Similarities in the effects of aspiration and complete neurotoxic lesions of the amygdala

Because the effects of amygdala lesions have been found to attenuate during the first few months after surgery (Walker *et al*., 1953; Weiskrantz, 1956; Horel *et al*., 1975), the present experiment, carried out 7 months after surgery on average, provides an estimate of relatively enduring behavioural changes. Formal evaluation of the behaviour of animals in groups A_{ASP} and A_{IBOc} in the absence of stimuli revealed neither alterations in general motor activity nor an increase in abnormal, self-directed or stereotypic, behaviours commonly found in monkeys living in laboratory settings (Goosen, 1981). However, one change relative to the behaviour of the controls occurred in both experimental groups in the absence of stimuli, i.e. an increased tendency to manipulate parts of the environment (Table 4).

Evaluation of behavioural reactions during stimulus presentation revealed changes in all categories of emotional responses (Table 5), confirming and extending earlier reports of the effects of conventional amygdala lesions. Amygdala removal by aspiration or radio frequency (Klüver & Bucy, 1938, 1939; Weiskrantz, 1956; Horel *et al*., 1975; Aggleton & Passingham, 1981; Zola-Morgan *et al*., 1991; Kling & Brothers, 1992) typically results in reduced defence and increased approach responses. This pattern was evident in both groups A_{ASP} and A_{IBOc}, particularly in the presence of non-social items, and it occurred indiscriminately, whether the stimulus was positive (Object) or negative (Snake). The most striking changes were an absence of freezing during Snake presentation, and excessive manual and oral manipulation of all the stimuli within reach (Monkey Head, Snake, and Object). The two stimuli with a social component (Human Face and Monkey Head) revealed in addition decreased aggression in both experimental groups, a result consistent with the 'tameness' classically described in monkeys with amygdala damage (e.g. Klüver & Bucy, 1938, 1939; Aggleton & Passingham, 1981). This reduced aggression was accompanied by excessive submissive responses, a change that has occasionally been mentioned in the literature (Franzen & Myers, 1973; Horel *et al*., 1975; Aggleton & Passingham, 1981).

One interesting finding of this study was that both groups A_{ASP} and A_{IBOc} reacted more strongly than controls to unexpected omission of reward during Object presentation. Animals in both lesion groups showed an increase of both defence and mild aggression and, in addition, suppressed their typical, excessive investigation of the Object, exploring instead the transport cage and surrounding test apparatus (Fig. 9). This result suggests that operated animals not only reacted to the presumably frustrating withdrawal of reward, but were in fact unusually responsive to it. Although not previously reported in monkeys, an increased sensitivity to reduction in food reward has been described in rats with large amygdala neurotoxic lesions (Salinas *et al*., 1996).

More generally, the fact that reward omission evoked such strong reactions provides further evidence that amygdala damage does not suppress the incentive value of food. Indeed, amygdalectomized monkeys learn many food-motivated tasks normally (e.g. Málková *et al*., 1997), discriminate food from non-food items, and exhibit normal preferences for highly palatable foods (e.g. Horel *et al*., 1975; Aggleton & Passingham, 1982; Murray *et al*., 1996). Changes in food preferences after amygdala neurotoxic lesions appear to be limited to: (i) an exaggerated tendency to pick up and mouth inedible items; (ii) an increased willingness to eat unfamiliar food; and (iii) a reduced sensitivity to reinforcer devaluation (Murray *et al*., 1996; Málková *et al*., 1997). These abnormalities might reflect either inappropriately triggered emotional responses, or an impairment in associating current reward values with different stimuli or with the same stimulus in different contexts, but they cannot be attributed to a lack of appreciation (i.e. to a loss of the hedonic aspects) of the food reward itself.

Differences in the effects of complete aspiration and neurotoxic amygdala lesions

Although complete aspiration and neurotoxic amygdala lesions yielded the same pattern of changes, there were subtle differences in the intensity of some symptoms. First, the reduction in aggression was more reliable in group A_{ASP} than in group A_{IBOc} (Table 5, high aggression), and enhanced submission appeared more clear-cut in group A_{ASP} (occurring during three stimulus presentations [Human Face] in this group compared with just one presentation [Monkey Head] in the other; see Table 5, submission). Second, hyperorality was present in group A_{ASP} in both the absence and presence of stimuli, whereas it occurred only during stimulus presentation in group A_{IBOc} (Table 4). Third, during stimulus presentation, the excessive exploration in group A_{ASP} was aimed at both the stimuli (approach) and parts of the environment (other behaviours), whereas in group A_{IBOc} it was directed only towards the stimuli (Table 5).

The milder behavioural changes shown by monkeys in group A_{IBOc} might have resulted from the separation of their surgery into two stages, a procedure known to yield less detrimental effects than the one-stage procedure used for aspiration lesions (e.g. McIntyre & Stein, 1973). However, any amelioration of effects attributable to the two-stage operation was probably minimized in the present study by the short interval between the two surgical stages (2–3 weeks) as well

as by the absence of inter-operative exposure to the stimuli (see e.g. Corwin *et al.*, 1982). Perhaps a more important difference between groups A_{IBOc} and A_{ASP} is the difference in lesion type. Histological examination indicates that direct damage (within as well as outside of the amygdala; see Table 1) was similar in the two groups. However, removal of the amygdala by aspiration is known to produce additional damage due to transection of non-amygdaloid fibres originating in and probably projecting to temporal polar, entorhinal, perirhinal and area TE cortical fields (Baxter *et al.*, 1998; Goulet *et al.*, 1998). Indeed, radio frequency lesions of the amygdala in rats have long been known to produce such fibre transections (Dunn & Everitt, 1988) and, as a result, to yield impairment in behaviours that are dependent on structures other than the amygdala. In the case of monkeys given amygdala ablations, additional dysfunction of the temporal pole and area TE may be sufficient to explain the greater magnitude of the symptoms observed in group A_{ASP}, as it has been shown that ablations or disconnection of these two areas also yield Klüver–Bucy symptoms (Akert *et al.*, 1961; Myers & Swett, 1970; Meyer, 1972; Franzen & Myers, 1973; Horel & Misantone, 1974; Horel *et al.*, 1975; Raleigh & Steklis, 1981; Iwai *et al.*, 1986; Kling *et al.*, 1993). Indirect damage to entorhinal and perirhinal areas might contribute as well, but this seems less likely, inasmuch as preliminary data indicate that the emotional changes produced by rhinal cortex ablations take the form mainly of an increase in freezing behaviour, i.e. just the opposite of one of the Klüver–Bucy symptoms (Meunier *et al.*, 1991). Whatever the explanation, the present results suggest that although indirect damage to the medial temporal cortex is not primarily responsible for the emotional changes produced by conventional amygdalectomy in monkeys, this additional damage exacerbates them, particularly the classically described hypermetamorphosis and hyperorality. This conclusion converges with a recent analysis of clinical data (Aggleton, 1992) suggesting that these two symptoms occur most clearly in patients with both cortical and subcortical temporal lobe damage.

Differences in the effects of partial and complete neurotoxic amygdala lesions

Our study demonstrates that partial neurotoxic amygdala lesions are sufficient to produce the two major symptoms observed after complete lesions, i.e. reduced defence and enhanced approach in response to non-social items (Table 5). These changes were evident even in the two animals (cases A_{IBOp}-2 and 3) with virtually no damage to the temporal cortex, including the anterior entorhinal cortex, thereby demonstrating that cell loss located strictly within the amygdala is sufficient to disrupt emotional responses in monkeys. However, unlike the monkeys with complete neurotoxic lesions, those with partial lesions did not display hyperorality, and they reacted normally to the two stimuli with a social component. Milder behavioural effects of partial compared with complete amygdala removals have already been reported after radio frequency lesions in monkeys (Aggleton & Passingham, 1981; Zola-Morgan *et al.*, 1991) as well as after amygdalotomies in humans (see for review Aggleton, 1992). Results in both monkeys and humans therefore support the notion that extensive bilateral amygdala damage is necessary to produce all the changes in emotional responses classically associated with amygdalectomy.

Nevertheless, given the evidence in rodents that different amygdaloid nuclei have different roles in behaviour (e.g. Gallagher & Holland, 1994; Killcross *et al.*, 1997), and that the 'corticomedial' and central nuclei may have special roles in social and emotional behaviour (e.g. Potegal *et al.*, 1996; Killcross *et al.*, 1997; Stark *et al.*, 1998), we examined the possibility that damage to this particular region of the amygdala (containing the cortical, medial and central nuclei) was responsible for many of the emotionality changes we observed in our monkeys. For this purpose, we rated the extent of damage to the above region in each of the operated subjects and then ran correlations between the lesion ratings and each of the behavioural measures. None of the correlations was significant. However, inasmuch as there was relatively little variation in the damage to this region, the negative findings do not permit a strong conclusion. Rather, the question remains open as to whether the 'corticomedial', central or other specific amygdaloid nuclei make selective contributions to emotional responses in monkeys as they do in rodents.

Characterization of the syndrome produced by amygdala lesions

The effects of total amygdala damage have, since Klüver and Bucy's original description, often been viewed as 'a complete loss of emotional reactions' (Klüver & Bucy, 1938, p. 50), even though this characterization is actually one they had applied to the behaviour of monkeys with bilateral temporal lobectomy. The present, and earlier, results plead for a more conservative characterization of the effects of amygdalectomy alone. First, fear and aggression are markedly reduced, but they are not eliminated. This is consistent with human data, which indicate that patients with bilateral amygdalectomies show attenuated affect, but are not 'emotionless' (e.g. Aggleton, 1992). Second, opposite effects can also occur. Increased fearfulness was described in monkeys living in complex social settings (e.g. Kling & Brothers, 1992), and occasional bouts of hyperaggressivity were noted in the present study towards the Monkey Head, in particular, when attempts to mouth the stimulus were thwarted, as well as in earlier investigations carried out in either non-social (Horel *et al.*, 1975; Aggleton & Passingham, 1981) or social settings (Rosvold *et al.*, 1954; Franzen & Myers, 1973; Kling & Brothers, 1992). Third, some categories of affective behaviour, e.g. submission and approach, are consistently enhanced by amygdalectomy.

Increased submission in both groups A_{ASP} and A_{IBOc} results from both an indiscriminate use of responses displayed by unoperated controls and the emergence of a response, viz. hindquarter presentation, that was never shown by controls in the same situation, although it is a species-typical submissive response in normal social situations (Maestripieri & Wallen, 1997). Similarly, hypermetamorphosis and hyperorality, as well as enhanced responses to frustration (e.g. when food reward was unexpectedly omitted, or when attempts to mouth a stimulus were prevented), can be viewed as inappropriately elicited instances of what are otherwise normal emotional reactions. Thus, rather than hypoemotionality, the core deficit induced by complete amygdala damage might be better described as a combination of inadequate and inappropriate triggering of emotional responses by external stimuli. This characterization is needed to account for the full array of symptoms observed, including paradoxical effects, e.g. the co-occurrence of hypo- and hyperaggression, or the observation of either increased or decreased fearfulness depending on social contexts.

Acknowledgements

We thank Patricia Merjanian and Richard Richardson for their help in collecting and scoring behavioural data. This study was supported by NIMH-IRP, NIMH-58846 and NICHD-HD35471 (to J.B.), Foundation Simone & Cino Del Duca (to M.Me.), Human Frontiers Scientific Program Organization (to E.A.M.), and NATO International Scientific Exchange Programmes (to M.Me. and J.B.).

Abbreviations

A_{ASP}, aspiration lesion (group); A_{IBOc}, complete ibotenic acid lesion (group); A_{IBOp}, partial ibotenic acid lesion (group); H–F, Huynh–Feldt (correction); MRI, magnetic resonance imaging; N, control subjects; OFF 1–5, periods 1–5 without stimulation; WGTA, Wisconsin General Testing Apparatus.

References

Akert, K., Gruesen, R.A., Woolsey, C.N. & Meyer, D.R. (1961) Klüver–Bucy syndrome in monkeys with neocortical ablations of temporal lobe. *Brain*, **84**, 480–498.

Adolphs, R., Tranel, D., Damasio, H. & Damasio, A.R. (1994) Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature*, **372**, 669–672.

Aggleton, J.P. (1992) The functional effects of amygdala lesions in humans, a comparison with findings from monkeys. In Aggleton, J.P. (ed.), *The Amygdala. Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, New York, pp. 485–504.

Aggleton, J.P. & Passingham, R.E. (1981) Syndrome produced by lesions of the amygdala in monkeys (*Macaca mulatta*). *J. Comp. Physiol. Psychol.*, **95**, 961–977.

Aggleton, J.P. & Passingham, R.E. (1982) An assessment of the reinforcing properties of foods after amygdaloid lesions in rhesus monkeys. *J. Comp Physiol. Psychol.*, **95**, 961–977.

Baxter, M.G., Saunders, R.C. & Murray, E.A. (1998) Aspiration lesions of the amygdala interrupt connections between prefrontal cortex and temporal cortex in rhesus monkeys. *Soc. Neurosci. Abstr.*, **24**, 1905.

Breiter, H.C., Etcoff, N.L., Whalen, P.J., Kennedy, W.A., Rauch, S.L., Buckner, R.L., Strauss, M.M., Hyman, S.E. & Rosen, B.R. (1996) Response and habituation of the human amygdala during visual processing of facial expression. *Neuron*, **17**, 875–887.

Broks, P., Young, A.W., Maratos, E.J., Coffey, P.J., Calder, A.J., Isaac, C.L., Mayes, A.R., Hodges, J.R., Montaldi, D., Cezayirli, E., Roberts, N. & Hadley, D. (1998) Face processing impairments after encephalitis: amygdala damage and recognition of fear. *Neuropsychologia*, **36**, 59–70.

Butter, C.M., Mishkin, M. & Mirsky, A.F. (1967) Emotional responses toward humans in monkeys with selective frontal lesions. *Physiol. Behav.*, **3**, 213–215.

Cahill, L., Babinsky, R., Markowitsch, H.J. & McGaugh, J.L. (1995) The amygdala and emotional memory. *Nature*, **377**, 295–296.

Capitano, J.P. (1986) Behavioral pathology. In Mitchell, G. & Erwin, J. (eds) *Comparative Primate Biology Vol. 2A, Behavior, Conservation, and Ecology*. Allan R. Liss, New York, pp. 411–454.

Chevalier-Skolnikoff, S. (1973) Facial expression of emotion in primates. In Ekman, P. (ed.) *Darwin and Facial Expression: A Century of Research in Review*. Academic Press, New York, pp. 11–89.

Corwin, J.V., Vicedomini, J.P., Nonneman, A.J. & Valentino, L. (1982) Serial lesion effect in rat medial frontal cortex as a function of age. *Neurobiol. Aging*, **3**, 69–76.

Davis, M. (1992) The role of the amygdala in conditioned fear. In Aggleton, J.P. (ed.), *The Amygdala. Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, New York, pp. 255–305.

De Waal, F. (1989) *Peacemaking Among Primates*. Harvard University Press, Cambridge, MA, USA.

Dunn, L.T. & Everitt, B.J. (1988) Double dissociations of the effects of amygdala and insular cortex lesions on conditioned taste aversion, passive avoidance, and neophobia in the rat using the excitotoxin ibotenic acid. *Behav. Neurosci.*, **102**, 3–23.

Franzen, E.A. & Myers, R.E. (1973) Neural control of social behavior: prefrontal and anterior temporal cortex. *Neuropsychologia*, **11**, 141–157.

Fried, I., MacDonald, K.A. & Wilson, C.L. (1997) Single neuron activity in human hippocampus and amygdala during recognition of faces and objects. *Neuron*, **18**, 753–765.

Gaffan, E.A., Gaffan, D. & Harrison, S. (1988) Disconnection of the amygdala from visual association cortex impairs visual reward-association learning in monkeys. *J. Neurosci.*, **8**, 3144–3150.

Gallagher, M. & Chiba, A.A. (1996) The amygdala and emotion. *Curr. Opin. Neurobiol.*, **6**, 221–227.

Gallagher, M. & Holland, P.C. (1994) The amygdala complex: multiple roles in associative learning and attention. *Proc. Natl Acad. Sci., USA*, **91**, 11 771–11 776.

Goosen, C. (1981) Abnormal behavior patterns in rhesus monkeys: symptoms of mental disease? *Biol. Psychiatry*, **16**, 697–716.

Goulet, S., Doré, F.Y. & Murray, E.A. (1998) Aspiration lesions of the amygdala disrupt the rhinal corticothalamic projection system in rhesus monkeys. *Exp. Brain Res.*, **119**, 131–140.

Hinde, R.A. & Rowell, T.E. (1962) Communication by postures and facial expressions in the rhesus monkey (*Macaca mulatta*). *Proc. Zool. Soc. Lond.*, **138**, 1–21.

Hodos, W. & Bobko, P. (1984) A weighed index of bilateral brain lesions. *J. Neurosci. Meth*, **12**, 43–47.

Horel, J.A., Keating, E.G. & Misantone, L.J. (1975) Partial Klüver–Bucy syndrome produced by destroying temporal neocortex or amygdala. *Brain Res.*, **94**, 347–359.

Horel, J.A. & Misantone, L.J. (1974) The Klüver–Bucy syndrome produced by partial isolation of the temporal lobe. *Exp. Neurol.*, **42**, 101–112.

Iwai, E., Nishio, T. & Yamaguchi, K. (1986) Neuropsychological basis of a K-B sign in Klüver–Bucy syndrome produced following total removal of inferotemporal cortex of macaque monkeys. In Oomura, Y. (ed.), *Emotion: Neural and Chemical Control.* Japan Scientific Society Press, Tokyo, pp. 299–311.

Jones, B. & Mishkin, M. (1972) Limbic lesions and the problem of stimulus–reinforcement associations. *Exp. Neurol.*, **36**, 362–377.

Kalin, N.D., Shelton, S.E. & Takahashi, L.K. (1991) Defensive behaviors in infant rhesus monkeys: their ontogeny and context-dependent selective expression. *Child Dev.*, **62**, 1175–1183.

Kaufman, C. & Rosenblum, L.A. (1966) A behavioral taxonomy for *Macaca Nemestrina* and *Macaca Radiata* based on longitudinal observation of family groups in the laboratory. *Primates*, **7**, 205–254.

Kenney, M.D., Mason, W.A. & Hill, S.D. (1979) Effects of age, objects, and visual experience on affective responses of rhesus monkeys to strangers. *Develop. Psychol.*, **15**, 176–184.

Killcross, S., Robbins, T.W. & Everitt, B.J. (1997) Different types of fear-conditioned behavior mediated by separate nuclei within the amygdala. *Nature*, **388**, 377–380.

Kling, A.S. & Brothers, L.A. (1992) The amygdala and social behavior. In Aggleton, J.P. (ed.), *The Amygdala. Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, New York, pp. 353–378.

Kling, A.S., Tachiki, K. & Loyd, R. (1993) Neurochemical correlates of the Klüver–Bucy syndrome by *in vivo* microdialysis in monkey. *Behav. Brain Res.*, **56**, 161–170.

Klüver, H. & Bucy, P.C. (1938) An analysis of certain effects of bilateral temporal lobectomy in the rhesus monkey, with special reference to 'psychic blindness'. *J. Psychol.*, **5**, 33–54.

Klüver, H. & Bucy, P.C. (1939) Preliminary analysis of functions of the temporal lobes in monkeys. *Arch. Neurol. Psychiat.*, **42**, 979–1000.

LeDoux, J.E. (1992) Emotion and the amygdala. In Aggleton, J.P. (ed.), *The Amygdala. Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, New York, pp. 339–351.

Maestripieri, D. & Wallen, K. (1997) Affiliative and submissive communication in rhesus macaques. *Primates*, **38**, 127–138.

Málková, L., Gaffan, D. & Murray, E.A. (1997) Excitotoxic lesions of the amygdala fail to produce impairment in visual learning for auditory secondary reinforcement but interfere with reinforcer devaluation effects in rhesus monkeys. *J. Neurosci.*, **17**, 6011–6020.

McGaugh, J.L., Entroini-Collison, I.B., Cahill, L., Kim, M. & Liang, K.C. (1992) Involvement of the amygdala in neuromodulatory influences on memory storage. In Aggleton, J.P. (ed.) *The Amygdala. Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*. Wiley-Liss, New York, pp. 431–452.

McIntyre, M. & Stein, D.G. (1973) Differential effect of one-stage vs two-stage amygdaloid lesions on activity, exploratory, and avoidance behavior in the albino rat. *Exp. Neurol.*, **40**, 263–277.

Meunier, M., Bachevalier, J., Mishkin, M. & Murray, E.A. (1993) Effects on visual recognition of combined and separate ablations of the entorhinal and perirhinal cortex in rhesus monkeys. *J. Neurosci.*, **13**, 5418–5432.

Meunier, M., Bachevalier, J., Murray, E.A., Málková, L. & Mishkin, M. (1996) Effects of aspiration vs neurotoxic lesions of the amygdala on emotional reactivity in rhesus monkeys. *Soc. Neurosci. Abstr.,* **22**, 1867.

Meunier, M., Bachevalier, J., Murray, E.A., Merjanian, P.M. & Richardson, R. (1991) Effects of rhinal cortical or limbic lesions on fear reactions in rhesus monkeys. *Soc. Neurosci. Abstr.*, **17**, 337.

Meyer, D.R. (1972) Some features of the dorsolateral frontal and inferotemporal syndromes in monkeys. *Acta Neurobiol. Exp. (Warszawa)*, **32**, 235–260.

Mineka, S. & Cook, M. (1988) Social learning and the acquisition of snake fear in monkeys. In Zentall, T. & Galef, B. (eds), *Social Learning: Psychological and Biological Perspectives*. Erlbaum, Hillsdale, NJ, pp. 51–73.

Mineka, S., Keir, R. & Price, V. (1980) Fear of snakes in wild- and lab-reared rhesus monkeys. *Anim. Learn. Behav.*, **8**, 653–663.

Morris, J.S., Ohman, A. & Dolan, R.J. (1998) Conscious and unconscious emotional learning in the human amygdala. *Nature*, **393**, 467–470.

Murray, E.A. (1987) Normal learning set formation in monkeys with combined ablation of the amygdaloid complex and hippocampal formation. *Soc. Neurosci. Abstr.*, **13**, 206.

Murray, E.A. (1996) What have ablation studies told us about the neural substrates of stimulus memory. *Sem. Neurosci.*, **8**, 13–22.

Murray, E.A. & Gaffan, D. (1994) Removal of the amygdala plus subjacent cortex disrupts the retention of both intramodal and crossmodal associative memories in monkeys. *Behav. Neurosci.*, **108**, 494–500.

Murray, E.A., Gaffan, E.A. & Flint, R.W. Jr (1996) Anterior rhinal cortex and amygdala: dissociation of their contributions to memory and food preference in rhesus monkeys. *Behav. Neurosci.*, **110**, 30–42.

Murray, E.A., Gaffan, D. & Mishkin, M. (1993) Neural substrates of visual stimulus–stimulus association in rhesus monkeys. *J. Neurosci.*, **13**, 4549–4561.

Myers, R.E. & Swett, C. (1970) Social behavior deficits of free-ranging monkeys after anterior temporal cortex removal: a preliminary report. *Brain Res.*, **18**, 551–556.

Phelps, E.A., LaBar, K.S. & Spencer, D.D. (1997) Memory for emotional words following unilateral temporal lobectomy. *Brain Cogn.*, **35**, 85–109.

Potegal, M., Ferris, C.F., Hebert, M., Meyerhoff, J. & Skaredoff, L. (1996) Attack priming in female Syrian golden hamsters is associated with a c-fos-coupled process within the corticomedial amygdala. *Neuroscience*, **75**, 869–880.

Raleigh, M.J. & Steklis, H.D. (1981) Effects of orbitofrontal and temporal neocortical lesions on the affiliative behavior of Vervet monkeys (*Cercopithecus aethiops sabaeus*). *Exp. Neurol.*, **73**, 378–389.

Rosvold, H.E., Mirsky, A.F. & Pribram, K. (1954) Influence of amygdalectomy on social behavior in monkeys. *J. Comp. Physiol. Psychol.*, **47**, 173–178.

Sade, D.S. (1973) An ethogram for rhesus monkeys. I. Antithetical contrasts in posture and movement. *Am. J. Phys. Anthrop.*, **38**, 537–542.

Salinas, J.A., Parent, M.B. & McGaugh, J.L. (1996) Ibotenic acid lesions of the amygdala basolateral complex and central nucleus differentially effect the response to reductions in reward. *Brain Res.*, **742**, 283–293.

Scott, S.K., Young, A.W., Calder, A.J., Hellawell, D.J., Aggleton, J.P. & Johnson, M. (1997) Impaired auditory recognition of fear and anger following bilateral amygdala lesions. *Nature*, **385**, 254–257.

Spiegler, B.J. & Mishkin, M. (1981) Evidence for the sequential participation of inferior temporal cortex and amygdala in the acquisition of stimulus–reward associations. *Behav. Brain. Res.*, **3**, 303–317.

Stark, C.P., Alpern, H.P., Fuhrer, J., Trowbridge, M.G., Wimbish, H. & Smock, T. (1998) The medial amygdaloid nucleus modifies social behavior in male rats. *Physiol. Behav.*, **63**, 253–259.

Suomi, S.J. (1982) Abnormal behavior and primate models of psychopathology. In Fobes, J.L. & Kling, J.E. (eds) *Primate Behavior*. Academic Press, New York, pp. 171–215.

von Bonin, G. & Bailey, P. (1947) *The Neocortex of Macaca Mulatta*. University of Illinois, Urbana, IL, USA.

Walker, A.E., Thomson, A.F. & McQueen, J.D. (1953) Behavior and the temporal rhinencephalon in the monkeys. *Johns Hopkins Hosp. Bull.*, **49**, 381–391.

Weiskrantz, L. (1956) Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J. Comp. Physiol. Psychol.*, **49**, 381–391.

Whalen, P.J., Rauch, S.L., Etcoff, N.L., McInerney, S.C., Lee, M.B. & Jenike, M.A. (1998) Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J. Neurosci.*, **18**, 411–418.

Zald, D.H. & Pardo, J.V. (1997) Emotion, olfaction, and the human amygdala, amygdala activation during aversive olfactory stimulation. *Proc. Natl Acad. Sci. USA*, **94**, 119–124.

Zola-Morgan, S., Squire, L.R., Alvarez-Royo, P. & Clower, R.P. (1991) Independence of memory functions and emotional behavior: separate contributions of the hippocampal formation and the amygdala. *Hippocampus*, **1**, 207–220.