Combined Unilateral Lesions of the Amygdala and Orbital Prefrontal Cortex Impair Affective Processing in Rhesus Monkeys

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INTRODUCTION

The amygdala and the orbital prefrontal cortex (PFO) are known to be critical for various aspects of affective processing. Monkeys with either selective bilateral amygdala lesions (Malkova et al. 1997) or bilateral damage to orbital prefrontal cortex (Izquierdo and Murray 2000), although able to choose baited (i.e., rewarded) objects in favor of unbaited objects, are unable to update the value of a food reinforcer and to use that information to guide choice behavior. In addition, several studies have reported impairments in monkeys on another putative measure of stimulus-reward association, namely, object reversal learning, after aspiration or radiofrequency lesions that included the amygdala (Aggleton and Passingham 1981; Barrett 1969; Jones and Mishkin 1972; Schwartzbaum and Poulos 1965) or orbital prefrontal cortex (Dias et al. 1996; Iversen and Mishkin 1970; Jones and Mishkin 1972; Meunier et al. 1997). Similar findings have been reported in rats (Chudasama and Robbins 2003; Gallagher et al. 1999; Hatfield et al. 1996; Schoenbaum et al. 2003a).

The amygdala and orbital prefrontal cortex also play important roles in emotional behavior. Large removals of the temporal lobe produce alterations in emotional behavior (Kluver and Bucy 1939), alterations that Weiskrantz (1956) showed could be induced by aspiration lesions of the amygdala plus subjacent cortex. More recently, monkeys with selective amygdala damage produced by bilateral radiofrequency lesions (Aggleton and Passingham 1981; Zola-Morgan et al. 1991) or bilateral neurotoxic lesions (Kalnin et al. 2001; Meunier et al. 1999; Prather et al. 2001) of the amygdala have been found to display blunted emotional reactions to a variety of stimuli. For example, when confronted with a real or fake snake, monkeys with excitotoxic amygdala damage exhibit fewer defensive behaviors (e.g., freezing, piloerection, and head and eye aversion), reduced aggression (e.g., cage shaking, mouth threat), and an increase in submissive behaviors (e.g., lip smacking and presenting) relative to controls. Similarly, monkeys with bilateral orbital prefrontal cortex lesions exhibit reduced aggressive behaviors in several threatening situations such as the presence of a human observer, a big-eye doll, and a rubber snake (Butter and Snyder 1972; Butter et al. 1970).

The reciprocal anatomical connections between the PFO and the amygdala, together with the disruptions in reward processing and emotional behavior that result after damage to either region, suggest that these structures are part of a circuit critical for affective processing. Baxter et al. (2000) directly tested this idea by giving monkeys crossed disconnection lesions of the amygdala and PFO and testing them for responses to reinforcer devaluation. That is, monkeys in the experimental group sustained amygdala lesions in one hemisphere and orbital prefrontal cortex lesions in the other hemisphere combined with section of the forebrain commissures, an operation that prevents communication between the amygdala and orbital prefrontal cortex. Monkeys with the crossed disconnection lesion were impaired relative to controls, indicating that normal performance requires the functional interaction of the amygdala and orbital prefrontal cortex. Physiological evidence for the interaction between these two structures has also been observed in rats (Schoenbaum et al. 1999, 2003b).

What remains less clear, however, is whether these neural structures have the same importance for affective function in the right and left cerebral hemispheres. Studies in rats suggest a special role for the right hemisphere in the memory of aversive experience (Adamec et al. 1999; Coleman-Mesches and McGaugh 1995; Coleman-Mesches et al. 1996) as well as for the modulation of cortisol levels in times of stress (Sullivan and Gratton 1999). Neuropsychological (Funayama et al. 2001; Hartikainen et al. 2000; Ross et al. 1994) and functional

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imaging data (Morris et al. 1998; Whalen et al. 1998) in humans also suggest that the right hemisphere is more important than the left in the processing of emotion, particularly for mediating automatic, subconscious processing of negatively valenced affective stimuli.

Surprisingly few studies in nonhuman primates have directly investigated whether there might be a hemispheric specialization for affective processing. Some studies report hemispheric asymmetry using relatively indirect measures of emotion (Hatta and Koike 1991; Ifune et al. 1984; Kalin et al. 1998; Parr and Hopkins 2000). For example, Kalin et al. (1998) showed that higher baseline cortisol levels were associated with greater electrical activity in right prefrontal cortex and that these variables, in turn, were correlated with a more anxious temperament in rhesus monkeys.

To test for hemispheric functional specialization of emotion and reward processing, rhesus monkeys with unilateral, combined lesions of the amygdala and orbital prefrontal cortex were administered a battery of tasks that recruited such processing. The battery included two tasks that taxed reward processing, reinforcer devaluation and object reversal learning, and two tasks that recruited the processing of emotion, reactions to a rubber snake and reactions to an unfamiliar human, i.e., a human “intruder.” Monkeys in the present study received combined, two-stage lesions of the amygdala and PFo in one hemisphere (right or left). The decision to use a combined lesion was made, in part, because more extensive damage to the circuit outlined in the preceding text would presumably make any behavioral effect of a unilateral lesion easier to detect than that caused by single-region perturbation.

If the right hemisphere is important for processing raw emotion such as acute fear, perhaps the right hemisphere would be more important in recruiting appropriate emotional responses to the rubber snake. However, even if there was no evidence for hemispheric specialization, we might uncover evidence for a contribution of each hemisphere to affective processing. If so, we would expect effects of left and right lesions, but no left-right differences.

A preliminary report of this work has appeared elsewhere (Izquierdo and Murray 2002).

**EXPERIMENT 1**

**Methods**

The reinforcer devaluation task requires the monkey to link an object with the incentive value of a food reinforcer hidden underneath and to rapidly shift its responses when the value of the reinforcer changes. Intact monkeys perform this task quite readily. For example, having learned about a large number of objects and their associated foods, intact monkeys will avoid choosing objects overlying foods that have been devalued through satiation. This ability depends on the intrahemispheric interaction of orbital prefrontal cortex and the amygdala (Baxter et al. 2000).

In the present study, all monkeys were evaluated for their ability to choose between positive objects in the face of changes in value of the food reward hidden underneath. The reinforcer devaluation tests were carried out twice, once after the first stage surgery and again after the second stage. This experimental design was the same as that used by Baxter et al. (2000); thus the present results can be directly compared with those of the earlier study.

**SUBJECTS.** Twelve experimentally naïve rhesus monkeys (Macaca mulatta), all male, were used. They weighed between 4.8 and 9.1 kg at the beginning of the study, were housed individually in rooms with automatically regulated lighting (light/dark 12 h/12 h, lights on at 0700 h), and were maintained on primate chow (No. 5038, PMI Feeds, St. Louis, MO) supplemented with fresh fruit. Water was always available in the home cage.

**SURGICAL PROCEDURES.** Monkeys were randomly assigned to one of three groups: left, right, or unoperated controls. Lesions were counterbalanced for site of first surgery (PFo or amygdala). Accordingly, four monkeys received amygdala lesions first (n = 2 left, n = 2 right), four monkeys received PFo lesions first (n = 2 left, n = 2 right), and four were retained as unoperated controls. The first stage surgery took place after the monkeys had completed a food preference test but prior to object discrimination learning. The second stage surgery took place after the first test of reinforcer devaluation (description of methods to follow) but before the second test and subsequent experiments 2–4. The sequence of behavioral testing and surgical operations is illustrated in Fig. 1.

At the time of surgery, anesthesia was induced with ketamine hydrochloride (10 mg/kg im) and maintained with isoflurane (1.0–3.0%, to effect). The animals received 0.45% sodium chloride plus 5% dextrose via an intravenous drip. Aseptic procedures were employed. Heart rate, respiration rate, blood pressure, expired CO2, and body temperature were monitored throughout the procedure. After the aspiration removal (orbital prefrontal cortex) or injections of excitotoxins (amygdala) were completed, the wound was closed in anatomical layers.

All monkeys received a pre- and postoperative treatment regimen consisting of dexamethasone sodium phosphate (0.4 mg/kg) and Cefazolin antibiotic (15 mg/kg) for 1 d before surgery and 1 wk after surgery.

**Food preference test** → **Surgery 1** → **Discrimination learning**

**Devaluation Test 1** → **Expt 1** → **Surgery 2** → **Relearn discriminations**

**Devaluation Test 2** → **Expt 2** → **Emotional responses** → **Expt 3** → **Object reversal learning** → **Expt 4** → **Human Intruder** → **Food preference retest**

FIG. 1. Sequence of experiments.
surgery to reduce swelling and prevent infection, respectively. At the end of surgery, and for two additional days, the monkeys received the analgesic ketoprofen (10–15 mg); ibuprofen (100 mg) was provided for five additional days.

**Amygdala lesion by ibotenic acid injection.** We used the same method previously described by Malkova et al. (1997) and Baxter et al. (2000). After induction of anesthesia, monkeys were placed in a stereotaxic frame. A bone flap extending over the midline was made in the appropriate portion of the cranium, and a final reading taken on the position of the sagittal sinus, which served as the landmark for calculation of stereotaxic coordinates in the mediolateral dimension. Slits were cut in the dura to allow passage of the injection needle. Injections of ibotenic acid were placed stereotaxically throughout the amygdala in one hemisphere with coordinates determined from magnetic resonance imaging (MRI) scans performed an average of 5.1 days prior to each surgery. Eighteen to 22 injections, each consisting of 0.6–1.0 μl ibotenic acid (10 mg/ml, Biosearch Technologies), were made into the amygdala via the 30-gauge needle of a Hamilton syringe held in a micromanipulator. The injection sites were roughly 2 mm apart in each plane. Each injection was made at the rate of 0.2 μl/min, and the needle was left in place 2–3 min after each injection to limit diffusion of the toxin up the needle track. The intended lesion (see Fig. 2) included the entire amygdala, including both the basolateral nuclear group as well as the central, medial, and cortical nuclei.

**Orbital prefrontal cortex lesion by aspiration.** For the orbital prefrontal cortex lesions, general surgical procedures were identical to those used for the amygdala lesion except that MRI scans were not required and a regular (not stereotaxic) head holder was used. Preoperative scans were not needed because tissue removal was guided by sulcal landmarks.

A half-moon shaped craniotomy was performed over the region of the prefrontal cortex. The dura mater was cut near the dorsal edge of the bone opening and reflected ventrally. Using a combination of suction and electrocautery, the orbital prefrontal cortex was removed by subpial aspiration through a fine-gauge metal sucker, insulated except at the tip. The intended lesion (see Fig. 2) extended from the fundus of the lateral orbital sulcus, which marked the lateral boundary of the lesion, to the fundus of the rostral sulcus, medially. The rostral limit of the lesion was a line joining the anterior tips of the lateral and medial orbital sulci, and the caudal limit of the lesion was ~5 mm from the interaural plane (0). Note that the ventral views (top) have been reversed (i.e., left hemisphere is shown on the left) to aid in matching to the coronal sections.
rostral to the junction of the frontal and temporal lobes. Thus the lesion included Walker’s areas 11, 13, and 14 and the caudal part of area 10 (Walker 1940).

Although regions of the prefrontal cortex outside the intended removal have been reported to receive a direct projection from the amygdala (e.g., anterior cingulate cortex), the prefrontal cortical fields removed here appear to receive the bulk of the amygdalar projections (Amaral et al. 1992; Carmichael and Price 1995; Porrino et al. 1981). The extent of the orbital prefrontal cortex lesions was intended to be the same as in the monkeys studied by Baxter et al. (2000).

ASSESSMENT OF THE LESIONS. The lesions in all eight operated monkeys were quantitatively assessed from postoperative MRI scans. The extent of amygdala damage was evaluated from T2-weighted scans obtained within 10 days of surgery and the extent of orbital prefrontal cortex damage from T1-weighted scans obtained at a variety of surgery-scan intervals. For one monkey in the left group (L2), the amygdala damage could not be assessed because a T1-weighted scan was administered instead of a T2-weighted scan. In addition, after completing the test battery, one monkey (L4) was euthanized due to chronic illness that did not respond to treatment. Thus histological verification of the lesion is available for this one case. Figure 2 shows MR images from two representative cases, L1 and R4, and Fig. 3 shows photomicrographs through the lesions in monkey L4.

For each operated animal, MR scan slices were matched to drawings of a standard rhesus monkey brain at 1-mm intervals. Each lesion was subsequently plotted onto the standard sections. For amygdala lesions, the region of hypersignal evident in the T2-weighted MR scan was plotted onto the standard sections. The extent of hypersignal has been reported to accurately reflect the extent of neuronal cell loss, at least in the hippocampus (Malkova et al. 2001; Nemanic et al. 2002). For the orbital prefrontal cortex lesions, the extent of the lesion visible in the T1-weighted scan was plotted. We then measured the volume of the lesion as a function of the total volume of the structure (either amygdala or orbital prefrontal cortex) in the standard.

In all eight operated subjects, damage to the orbital prefrontal cortex was essentially as intended. The extent of the lesions relative to the total volume of each structure was as follows: left PFo, 82.1% (range: 75.3–87.4); right PFo, 82.2% (range: 79.5–84.9). The removals systematically spared cortex immediately ventral to the rostral sulcus, a region classified as infralimbic cortex by Preuss and Goldman-Rakic (1991). As for inadvertent damage, monkey L3 sustained an infarction involving cortex on the ventral bank of the principal sulcus and on the inferior frontal convexity for much of the anterio-posterior extent of the lesion. For the seven monkeys in which damage to the amygdala could be assessed, the damage was essentially as intended: left amygdala, 98.4% (range: 96.2–100); right amygdala, 99.0% (range: 97.2–100). Unintended damage to the rostral hippocampus, 2–3 mm in anterior-posterior extent, was evident in three monkeys in each operated group. In addition, two monkeys with right hemisphere lesions (R3 and R4) sustained substantial damage to the

FIG. 3. Photomicrographs of Nissl-stained coronal sections through the lesions in case L4. A, top to bottom: low-power photographs of sections approximately +36, +30, +18, +16, and +14 mm anterior to the interaural plane (0). The (left) orbital prefrontal cortex lesion is shown between black arrows at levels +36 and +30 and the left amygdala lesion is visible at levels +18, +16, and +14. B, top to bottom: higher-power photomicrographs of the medial temporal lobes in the most caudal 3 sections shown in the left column (+18, +16, and +14). There is extensive cell loss and atrophy in the left amygdala, together with expansion of the temporal horn of the lateral ventricle relative to the corresponding intact region in the right hemisphere. Note also the inadvertent involvement of the anterior tip of the left hippocampus (level +14). Scale bar = 3 mm.
entorhinal cortex, limited to its most medial portion, at the level of the amygdala.

**APPARATUS.** Monkeys were trained in a modified Wisconsin General Testing Apparatus (WGTA), which consists of a large monkey compartment that holds the test cage plus monkey together with a smaller test compartment, which contains the test tray. The test compartment was illuminated with two 60-W bulbs, whereas the monkey’s compartment was unlit. During test sessions, the room was unlit as well. An opaque screen separated the monkey compartment from the test compartment during intertrial intervals. In addition, a one-way vision screen, located between the experimenter and the test compartment, allowed the experimenter to view the monkey during intertrial intervals. In addition, a smaller test compartment was unlit. During test sessions, the room was illuminated with two 60-W bulbs, whereas the smaller test compartment, which contains the test tray. The test general Testing Apparatus (WGTA), which consists of a large monkey compartment, was limited to its most medial portion, at the level of the entorhinal cortex, and the test tray, measuring 19.2 cm (width) × 72.7 cm (length) × 1.9 cm (height), contained two food wells 290 mm apart, center to center, on the midline of the tray. The wells were 38 mm in diameter and 6 mm deep. Several dark gray matboard plaques measuring 76 mm on each side and three junk objects were used for pretraining only. For experiment 1, a set of 120 “junk” objects that varied in color, shape, and size were used. Food rewards for each monkey were two of the following: a single banana-flavored pellet (P. J. Noyes, Lancaster, NH), a half peanut, a raisin, a sweetened dried cranberry (Craisins, Ocean Spray, Lakeville-Middleboro, MA), a “fruit snack” (Giant Food, Landover, MD), or a chocolate candy (M&Ms, Mars Candies, Hackettstown, NJ).

**TESTING PROCEDURE.**

**Pretraining.** Monkeys were first accommodated to the WGTA by allowing them to take food freely from the test tray. They were then trained by successive approximation to displace gray plaques overlying the food wells to obtain a food reward hidden underneath. This procedure was repeated with the three objects dedicated to this phase. Before proceeding to the next stage, each monkey was required to complete a single session consisting of 10 plaque and 40 object trials. Items were presented one at a time and always covered a baited well. The purpose of this last step was to ensure that the monkeys would complete a 50-trial session.

**Food preference testing.** Monkeys were assessed for their preferences for six different foods. On each trial, monkeys were presented with two different foods, one in each food well. At the onset of each trial, the opaque screen was raised for the monkey to make its choice. Monkeys were allowed to choose only one of the two foods, and preference was noted by the experimenter. All food types were encountered 10 times during each session; each food was paired with each of the other foods twice, with the left-right positions balanced within a session. The different food pairs were presented in pseudorandom order each day. Each session comprised 30 trials with a 10-s intertrial interval. Monkeys were tested for a total of 15 days.

The data for each monkey were tabulated in terms of total number of choices of each food across the last 5 days of testing after food preferences had stabilized. In addition, we tabulated choices for each possible pairing of two foods as this was a more specific indication of relative palatability. Two foods that were roughly equally preferred were selected for each monkey; these were designated as food 1 and food 2.

**Visual discrimination learning.** To refamiliarize the monkey to the testing situation, each monkey was again given a single 50-trial pretraining session in which it was required to displace a plaque or one of the three pretraining objects to obtain the food reward hidden underneath. Monkeys were then trained to discriminate 60 pairs of objects. The 120 objects were assigned to 60 fixed pairs. For each pair, one object was arbitrarily designated as positive (i.e., S+, baited with a food reward) and the other negative (i.e., S−, unbaited). Half of the positive objects were assigned to be baited with food 1 and the other half, with food 2. On the first trial, the two objects comprising pair one were presented for choice, each overlying one of the two food wells on the test tray. If the monkey displaced the S+, it was allowed to take the food reward hidden underneath. If the monkey chose the S−, the trial was terminated without correction. After a 20-s intertrial interval, this procedure was repeated with the next pair of objects and so on until all 60 pairs had been used. The positive and negative assignment of objects, the presentation order of the object pairs, and the food reward assignments remained constant across sessions; the left-right position of positive objects followed a pseudorandom order. Monkeys were tested at the rate of one session per day for 5 days/wk. Criterion was set at a mean of 90% correct over five consecutive days (i.e., 270 correct responses in 300 trials).

**Reinforcer devaluation test 1.** After the monkeys attained criterion, their choices of objects were assessed in four critical test sessions. In these sessions, the negative objects were set aside and only the positive objects were used. Thirty pairs of objects, each consisting of one food-1 object and one food-2 object, were presented to the monkey for choice. Both objects were baited with the same foods used during acquisition. On each trial, the monkeys were allowed to choose only one of the objects in each pair to obtain the food reward hidden underneath, and the choice was scored by the experimenter. Two of the four critical test sessions were preceded by a selective satiation procedure intended to devalue one of the two foods, and the other two sessions were preceded by no satiation procedure and served as baseline measures. At least 2 days of rest followed each session that was preceded by selective satiation. In addition, between critical test sessions, the monkeys were given one regular training session with the original 60 pairs of objects presented in the same manner as during initial learning. This procedure ensured that any long-lasting effects of selective satiation, which might affect the outcome of later critical test sessions, could be detected. Objects were paired anew for each critical session, and sessions were administered in the following order for each monkey: first baseline session, session preceded by selective satiation with food 1, second baseline session, and session preceded by selective satiation with food 2. The unit of analysis, the “difference score,” is defined as the change in choices of object type (food-1- and food-2-associated objects) in the sessions preceded by selective satiation as compared with the mean of the two baseline sessions. The difference scores derived from each of the two critical sessions preceded by selective satiation were calculated separately, against the mean of the two baseline sessions, and then summed to provide the final difference score for test 1.

**Selective satiation.** For the selective satiation procedure, a food box measuring 7.7 cm (width) × 10.3 cm (length) × 7.7 cm (height) and attached to the monkey’s home cage was filled with a known quantity of either food 1 or food 2 while the monkey was in its home cage. The monkey was then left to eat unobserved for 15 min at which point the experimenter checked to see if it had eaten all the food. If it had, the food box was refilled. Whether additional food was given or not, the experimenter started observing the monkey through a window outside the animal housing room 30 min after initiation of the satiation procedure. Observation continued until the monkey failed to take food for five consecutive minutes. If the monkey emptied the food box again, the box was refilled, and observation continued until the monkey refrained from taking food for five minutes. When this criterion was fulfilled, the test session in the WGTA was initiated within 10 min. For baseline sessions, the monkey was simply taken from its home cage to the WGTA without undergoing a selective satiation procedure. For each instance of selective satiation, the amount of food eaten (in g) and the elapsed time spent in the satiation procedure (in min) were noted. At the end of the satiation procedure, any food remaining in the food box or dropped on the floor of the cage was taken into account when estimating the total amount of food eaten.

**Reinforcer devaluation test 2.** After recovering from the second surgery (or an equivalent period of rest for the controls), the monkeys were given a single session in which they were required to displace the three objects dedicated to pretraining. They were then retrained on the original set of object discriminations in the same manner and to
and stage by group interaction. In addition, there was no significant effect of group on stage (learning and relearning) revealed a marginally significant interaction between group and test \( F(2,9) = 8.253, P = 0.009 \); control > right and left and a within-subject effect of test \( F(1,9) = 5.753, P = 0.040 \). There was no significant interaction between group and test \( F(2,9) = 0.232, P = 0.798 \). Further analysis of reinforcer devaluation scores revealed a significant effect of group for test 1 \( F(2,9) = 7.80, P = 0.011 \) but the difference for test 2 fell short \( F(2,9) = 3.256, P = 0.086 \). Both right and left groups scored significantly lower than controls on test 1, but left and right groups did not differ significantly from each other (post hoc Bonferroni tests: control vs. left, \( P = 0.017 \); control vs. right, \( P = 0.032 \); left versus right, \( P = 1.00 \)). When left and right groups were collapsed into one unilateral group, both test 1 and test 2 difference scores were now significantly lower in this group than in the control group [test 1: \( F(1,10) = 16.876, P = 0.002 \]; test 2: \( F(1,10) = 7.236, P = 0.023 \]. There was no significant interaction between group and test [test 1: \( F(1,10) = 0.423, P = 0.530 \]. Finally, a Pearson correlation of objects “stolen” during initial learning and relearning of 60 object pairs

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Learning</th>
<th>Objects Stolen</th>
<th>Relearning</th>
<th>Objects Stolen</th>
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<tbody>
<tr>
<td></td>
<td>Trials</td>
<td>Errors</td>
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<tr>
<td>L1</td>
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<td>L2</td>
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</tr>
<tr>
<td>L3</td>
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<td>172</td>
<td>173</td>
<td>360</td>
</tr>
<tr>
<td>L4</td>
<td>660</td>
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<td>93.8</td>
<td>240</td>
</tr>
<tr>
<td>R1</td>
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<td>0</td>
</tr>
<tr>
<td>R2</td>
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<td>122</td>
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<td>0</td>
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<tr>
<td>R3</td>
<td>1020</td>
<td>336</td>
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<td>0</td>
</tr>
<tr>
<td>R4</td>
<td>780</td>
<td>231</td>
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<tr>
<td>Mean</td>
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</tr>
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<td>Con2</td>
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The number of objects stolen during learning and relearning is provided in Table 1. An ANOVA with repeated measures on stage (learning and relearning) revealed a marginally significant effect of group \( [F(2,9) = 3.457, P = 0.077] \) and nonsignificant stage and stage by group interaction. In addition, there was no significant effect of site of first surgery (amygdala or PFo) on the numbers of objects stolen. Using Pearson correlation matrices, it was found that the number of objects stolen during the initial learning of the 60 pairs was not correlated with learning scores (trials: \( r = -0.235, P = 0.463 \); errors: \( r = -0.208, P = 0.517 \). By contrast, the number of objects stolen during relearning was found to be highly correlated with relearning scores (trials: \( r = 0.658, P = 0.02 \); errors: \( r = 0.768, P = 0.004 \). Thus the higher average relearning scores for monkeys with left hemisphere lesions may be due to their disruption of trials by stealing objects.

REINFORCER DEVALUATION. As indicated earlier, the unit of analysis was a “difference score.” This was the change in choices of object type (food-1 and food-2-associated objects) in the sessions preceded by selective satiation as compared with baseline sessions. The greater the shift in responses away from objects overlying the sated food, a shift reflecting sensitivity to reinforcer devaluation, the higher the difference score. As shown in Fig. 4, the mean difference scores for test 1 and test 2, respectively, were as follows: 7.0 and 12.8 (left group), 8.3 and 12.8 (right group), and 18.5 and 21.3 (control group). A 2 × 3 ANOVA with repeated measures on the difference scores obtained in reinforcer devaluation tests 1 and 2 revealed a main effect of group \( [F(2,9) = 8.253, P = 0.009] \); control > right and left and a within-subject effect of test \( [F(1,9) = 5.753, P = 0.040] \). There was no significant interaction between group and test \( [F(2,9) = 0.232, P = 0.798] \). Further analysis of reinforcer devaluation scores revealed a significant effect of group for test 1 \( [F(2,9) = 7.80, P = 0.011] \) but the difference for test 2 fell short \( [F(2,9) = 3.256, P = 0.086] \). Both right and left groups scored significantly lower than controls on test 1, but left and right groups did not differ significantly from each other (post hoc Bonferroni tests: control vs. left, \( P = 0.017 \); control vs. right, \( P = 0.032 \); left versus right, \( P = 1.00 \)). When left and right groups were collapsed into one unilateral group, both test 1 and test 2 difference scores were now significantly lower in this group than in the control group [test 1: \( F(1,10) = 16.876, P = 0.002 \]; test 2: \( F(1,10) = 7.236, P = 0.023 \]. There was no significant interaction between group and test [test 1: \( F(1,10) = 0.423, P = 0.530 \]. Finally, a Pearson correlation

![Fig. 4](http://jn.physiology.org/DownloadedFrom)
revealed that there was high within-subject reliability of the scores obtained on test 1 and test 2 (r = 0.615, P = 0.033).

Each critical test session consisted of trials that required monkeys to choose between two rewarded objects (i.e., the food-1 and food-2-associated objects). Interestingly, after selective satiation, when monkeys displaced the objects overlying the sated food, they often refused to take the food. Monkeys refused the sated food on ~47% of the trials on which they had the opportunity to take it (left mean percent refusals = 30.0; right mean = 56.0; control mean = 55.3). Groups did not differ on this measure [F(2,9) = 2.346, P = 0.151].

Whereas 11 of the 12 monkeys refused the sated food on at least some occasions, only 1 of 12 monkeys ever refused the nonsated food, and the few occasions precluded statistical analysis.

Site of first surgery. Because monkeys differed not only with respect to which hemisphere was operated but also by which structure was first removed, we also analyzed the data by site of first surgery. A 2 × 3 ANOVA with between-subjects factor of treatment (amygdala lesion first, PFo lesion first, unoperated control) with repeated measures on the difference scores obtained on reinforcer devaluation test 1 and test 2 revealed a significant effect of treatment [F(2,9) = 8.687, P = 0.008; control > amygdala first and PFo first] and a within-subjects effect of test [F(1,9) = 6.531, P = 0.031], but no interaction between the two [F(2,9) = 0.872; P = 0.451]. Further analysis of test 1 revealed a significant effect of treatment [F(2,9) = 7.668, P = 0.011]; both monkeys with amygdala lesions and those with PFo lesions had significantly lower scores than control monkeys (post hoc Bonferroni test, P = 0.029 amygdala vs. control; P = 0.020 PFo vs. control). Monkeys with amygdala lesions and monkeys with PFo lesions, however, did not differ from each other (P = 1.0, amygdala vs. PFo as site of first surgery). Further analysis of test 2 revealed that the treatment was still significant [F(2,9) = 4.316, P = 0.049]. Monkeys that received amygdala lesions first were marginally significantly different from the control group (post hoc Bonferroni tests, P = 0.052 amygdala vs. controls; P = 0.315 PFo vs. controls; P = 0.889 amygdala vs. PFo).

Amount of food consumed during selective satiation. All monkeys considered together ate an average of 142.7 g during the two satiation procedures for test 1 and 145.4 g for test 2. The mean number of grams eaten in satiation procedures were analyzed using an ANOVA with repeated measures on the two tests and did not differ by group [F(2,9) = 0.577, P = 0.581]. There was no significant within-subjects effect of test [F(1,9) = 0.037, P = 0.852] or test by group interaction [F(2,9) = 1.204, P = 0.344].

LEARNING AND RELEARNING SCORES IN RELATION TO DEVALUATION SCORES. Learning and relearning trials and errors were analyzed using Pearson correlation matrices to assess if they were related to devaluation scores. Difference scores on tests 1 and 2 were not significantly correlated with learning and relearning scores, respectively, nor were they correlated with number of objects stolen.

Discussion

The idea that the right hemisphere is more important than the left for affective processing was not supported by the results of experiment 1. Instead, both groups of monkeys with unilateral lesions, left and right, displayed a significant impairment on the reinforcer devaluation task and the two operated groups were equally impaired. In addition, at the time test 1 was administered, the monkeys had received only the first-stage surgery involving a single structure; analysis by site of first surgery revealed that unilateral damage to either the amygdala alone or PFo alone is sufficient to produce a significant deficit on this task. Whether this deficit would have remained stable over time we cannot say; examination of groups of monkeys with unilateral lesions involving a single structure would be required to answer this question. Whereas the difference between operated and control groups was large on reinforcer devaluation test 1, this gap narrowed slightly with test 2 despite the fact that monkeys had just received their second stage surgeries. Nevertheless, when operated monkeys were considered as one unilateral group, their scores on test 2 were still significantly lower than those of the controls, and there was no group difference in the amount of improvement between tests 1 and 2.

The deficit cannot be explained by poor visual discrimination abilities in the operated groups because there were no group differences in the rate of acquisition of the 60 object pairs. Nor can it be explained by differences in the amount of food consumed during the selective satiation procedure because these amounts did not differ by group. In addition, all groups often refused sated foods in the critical test sessions that followed selective satiation and did so in equal measure. Thus it appears that the effects of the satiation procedure generalized from the home cage to the test apparatus and that the lesions in the operated groups did not interfere with satiety mechanisms. Moreover, changes in the ability to appreciate or discriminate foods cannot account for the impairment; a food preference test administered after all experiments were concluded (reported later) revealed no group differences relative to monkeys’ pretest food choices. Finally, it seems unlikely that the deficit could be accounted for by global changes in level of motivation; monkeys with crossed lesions of the amygdala and orbital prefrontal cortex (Baxter et al. 2000) or monkeys with either bilateral orbital prefrontal cortex lesions (unpublished observations) or bilateral amygdala lesions (Aggleton and Passingham 1982) are unimpairred on a progressive ratio task, a measure of monkeys’ willingness to work for food rewards.

The impairment resulting from ablation of a single structure seen in the present study (reinforcer devaluation test 1) was not observed in the earlier study by Baxter et al. (2000) even though the methods used in that study were virtually identical to those used here and even though that study, like the present one, involved either unilateral amygdala or unilateral orbital prefrontal cortex lesions as a first-stage surgery. There are at least two factors that may have led to this apparent discrepancy. First, the controls in the present study exhibited higher devaluation scores on test 1 than did the controls in the crossed-lesion experiment by Baxter at al., perhaps making it easier to see an impairment in the operated monkeys. Indeed, one of the controls in the study by Baxter et al. obtained a difference score of 1, the lowest such score attained by the 11 intact rhesus monkeys tested to date in the same manner. In addition, the present study had more power because of the larger sample size (n = 12 in this study versus n = 8 in Baxter et al.). The present results, however, do not alter the main finding of Baxter et al. Unlike controls, monkeys with crossed disconnection of the amygdala and orbital prefrontal cortex scored on average worse on test 2 relative to test 1 (Baxter et al. 2000), yielding a significant interaction of group and test; by contrast, monkeys in the present study with within-hemisphere lesions of the same structures, like the controls, scored higher on test 2 relative to test 1 (Fig. 3), yielding no significant interaction of group and test.
Methods

After completing reinforcer devaluation tests 1 and 2, monkeys were assessed for their emotional responses to a rubber snake. Fake and real snakes produce significant behavioral reactions in both snake-naive and -experienced rhesus monkeys (Mineka 1987; Nelson et al. 2003). In addition, Meunier et al. (1999) and Kalin et al. (2001) found that selective amygdala lesions significantly attenuated the reactions to a rubber snake in adult monkeys. Because Meunier et al. (1999) found that intact monkeys reacted more strongly to the rubber snake than to any other item in their test battery, the present study used a rubber snake. For comparison, we also assessed responses to neutral objects and to another potentially emotionally charged stimulus, a rubber spider. Because our monkeys had extensive experience with junk objects, our novel neutral objects might be considered generically familiar or even positive. Nevertheless, they serve as a baseline against which responses to the other stimuli can be gauged. The rubber spider perhaps better controls for degree of strangeness, as it was unlike most neutral junk objects in having long legs and a hairy appearance. The method was adapted from Mineka and her colleagues (Mineka 1987; Mineka et al. 1980). We collected two measures. First, we recorded latencies to retrieve a food reward located on top of a clear Plexiglas box containing two items within 30 s, the trial was terminated. Monkeys were tested for a total of five sessions, each consisting of 10 trials separated by 20-s intervals at a rate of one session every other day.

Experimenter. The experimenter raised the opaque screen at the beginning of the experiment. In addition, we used three novel objects (located to one side of the WGTA) and only her profile was visible to the monkey. If the monkey took the food, the screen was immediately lowered, terminating the trial. If the monkey failed to take the food within 30 s, the trial was terminated. Monkeys were tested for a total of five sessions, each consisting of 10 trials separated by 20-s intervals at a rate of one session every other day.

Videotape analysis. Videotaped fruit-retrieval latencies and emotional behavior were scored independently by two viewers. Latencies were scored to the nearest tenth of a second. Timing for the latency measure was initiated when the WGTA screen was raised above a given point, located at cm above the test tray, which was marked on the front of the cage and visible in the frontal camera view. The response was considered completed when the monkey grasped the food reward, just before it withdrew its arm. If no response was made within the 30-s time limit, a score of 30 s was given.

With minor modification—the inclusion of teeth gnashing as an “other” behavior—behavioral scoring methods were the same as those used by Meunier et al. (1999). A list of the behavioral categories, together with a brief description of the constituent behaviors, is provided in Table 2.

Results

Food-retrieval latencies. Food-retrieval latencies for the three trial types are shown in Fig. 5. A $3 \times 3 \times 5$ ANOVA of the latencies by group (control, left, and right) with repeated measures on trial type (snake, spider, and neutral object trial types) and session (1–5) revealed a significant main effect of group $F(2,9) = 8.818, P = 0.008$, a significant within-subjects effect of trial type $F(2,18) = 15.665, P < 0.001$, and a significant interaction between group and trial type $F(4,18) = 3.735, P = 0.022$. In addition, there was a significant within-subject interaction between trial type and session $F(8,72) = 7.092, P < 0.001$, and a significant within-subject interaction between trial type and session $F(4,36) = 32.426, P < 0.001$ and a significant within-subject interaction between trial type and session $F(8,36) = 0.603, P = 0.769$, and trial type by session by group $F(16,72) = 0.812, P = 0.668$.

Because of the significant results for the repeated-measures ANOVA, separate ANOVAs for each of the trial types were subsequently conducted. Only snake trials revealed a signifi-
TABLE 2. Behaviors analyzed during experiment 2 (snake task) and experiment 4 (human intruder task)

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild aggression</td>
<td>Wrinkles or moves eyebrows up and down</td>
</tr>
<tr>
<td>Frown</td>
<td>Wrinkles or moves eyebrows up and down</td>
</tr>
<tr>
<td>Ears back</td>
<td>Flattens ears against head</td>
</tr>
<tr>
<td>Yawn</td>
<td>Opens mouth wide, baring upper teeth</td>
</tr>
<tr>
<td>High aggression</td>
<td>Thrusts head or body forward</td>
</tr>
<tr>
<td>Head/body lunge</td>
<td>Shakes cage</td>
</tr>
<tr>
<td>Cage shake</td>
<td>Opens mouth slightly, exposing lower teeth</td>
</tr>
<tr>
<td>Mouth threat</td>
<td></td>
</tr>
<tr>
<td>Defense</td>
<td>Motionless for $\geq 3$ s</td>
</tr>
<tr>
<td>Freezing</td>
<td>Jerks suddenly</td>
</tr>
<tr>
<td>Startle</td>
<td>Avoids eye contact, shifts gaze or whole head</td>
</tr>
<tr>
<td>Eye/head aversion</td>
<td>Hair stands on end</td>
</tr>
<tr>
<td>Piloerection</td>
<td>Retreats from the stimulus</td>
</tr>
<tr>
<td>Move away</td>
<td></td>
</tr>
<tr>
<td>Submission</td>
<td>Purses, and alternatively closes and opens lips</td>
</tr>
<tr>
<td>Lip smack</td>
<td></td>
</tr>
<tr>
<td>Grimace</td>
<td>Mouth closed, pulls lips backward exposing teeth</td>
</tr>
<tr>
<td>Presentation</td>
<td>Presents its hindquarters with tail up</td>
</tr>
<tr>
<td>Approach</td>
<td>Makes eye contact</td>
</tr>
<tr>
<td>Look at</td>
<td></td>
</tr>
<tr>
<td>Move toward</td>
<td>Shifts body forward, closer to stimulus</td>
</tr>
<tr>
<td>Touch</td>
<td>Handles with hand or foot</td>
</tr>
<tr>
<td>Take/eat reward</td>
<td>Picks up or mouths the food reward</td>
</tr>
<tr>
<td>Other behaviors (not directed towards the stimulus)</td>
<td>Handles any part of its surrounding</td>
</tr>
<tr>
<td>Manual exploration</td>
<td></td>
</tr>
<tr>
<td>Oral exploration</td>
<td>Licks or mouths any part of its surrounding</td>
</tr>
<tr>
<td>Locomotor stereotypies</td>
<td>Activities, such as circling, hopping, repeated 3 or more times</td>
</tr>
<tr>
<td>Self-directed activities</td>
<td>Scratches, groans, holds, etc. any part of its body</td>
</tr>
<tr>
<td>Look away</td>
<td>Looks away while engaged in behavior not directed towards stimulus</td>
</tr>
<tr>
<td>Teeth gnashing</td>
<td>Chewing motion without food in mouth</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>Engages in any peculiar activity not described above</td>
</tr>
</tbody>
</table>

Significant main effect of group [snake: $F(2,9) = 11.193, P = 0.004$; control > left and right food-retrieval latencies; spider: $F(2,9) = 0.553, P = 0.594$; neutral object: $F(2,9) = 0.280, P = 0.762$]. All three trial types showed significant effects of session [snake: $F(4,36) = 8.718, P < 0.001$; spider: $F(4,36) = 27.392, P < 0.001$; neutral objects: $F(4,36) = 11.351, P < 0.001$]; there were no significant interactions of session and group [snake: $F(8,36) = 1.189, P = 0.333$; spider $F(8,36) = 0.485, P = 0.859$; neutral objects: $F(8,36) = 1.611, P = 0.156$].

Individual ANOVAs conducted on each session revealed that latencies differed significantly by group only on sessions 3 and 5 [session 3: $F(2,9) = 13.948, P = 0.002$; session 5: $F(2,9) = 13.005, P = 0.002$]. For both of these sessions, the control group spent significantly more time in the snake trials than both left and right groups (Bonferroni tests, session 3: $P = 0.004$ control vs. left; $P = 0.004$ control vs. right; session 5: $P = 0.004$ control vs. left; $P = 0.006$ control vs. right). Left and right groups, however, did not differ from each other (session 3: $P = 1.0$ left vs. right; session 5: $P = 1.0$ left vs. right).

BEHAVIOR DURING SNAKE TRIALS. For each monkey, behaviors during each of the five snake trials were analyzed for mean cumulative duration and mean cumulative frequency (Meunier et al. 1999). Subsequently, means for each group were obtained. Behaviors observed during the snake trials were grouped as either defensive (e.g., move away, freezing, and eye/head aversion) or approach (move toward and take reward). Because the “look at” behavior was observed in monkeys that never reached over the rubber snake as well as in monkeys that reached quickly, this behavior was excluded as an approach behavior. In addition, because the duration of the snake trial varied across monkeys, for purposes of analysis, each trial was prorated to a 30-s interval, which was the maximum trial length observed. The frequencies were kept as raw scores; an analysis of frequencies for only the first 5 s, which was the minimum trial length observed, was also conducted.

Videotapes were first scored by an observer who was aware of the group assignments. A subset of sessions was subsequently scored independently by a second observer, one who was unaware of group assignments. Interobserver reliability was calculated using Pearson correlation matrices and averaged across the five sessions per monkey in a sample of six monkeys (2 monkeys from each group). Interobserver reliability for each behavior is given in Table 3. In general, there was
good agreement between the two scorers, suggesting that behaviors exhibited by the monkeys in response to the rubber snake could be reliably identified and distinguished from one another. More importantly, however, high reliability between a scorer blind to the group assignments and one aware of the group assignments strongly argues against the possibility that any group differences observed are due to experimenter bias.

**Defensive behavior.** Fig. 6 shows the mean cumulative durations of defense and approach behaviors exhibited by each group during rubber snake exposure. A repeated-measures ANOVA on the cumulative duration of defensive behavior in the snake trials (referred to herein as “sessions” for consistency with food-retrieval latency data) revealed a significant main effect of group \(F(2,9) = 5.379, P = 0.029\). Individual ANOVAs revealed that groups differed significantly only in sessions 1 and 3 \([session 1: F(2,9) = 6.189, P = 0.020; session 3: F(2,9) = 6.701, P = 0.017]\). For session 1, only the left group was significantly different from the controls \((P = 0.021)\), and for session 3, both left and right groups were significantly different from controls \((P = 0.02 and P = 0.05, respectively)\, all Bonferroni adjusted comparisons). For both sessions, the left and right groups did not differ from each other \((session 1, P = 0.177; session 3, P = 1.0)\). An analysis of the frequency of defensive behaviors during the first 5 s of each trial yielded no significant differences between groups \(F(2,9) = 3.019, P = 0.099\).

**Freezing.** Because of the significant findings in the repeated-measures ANOVA on cumulative defensive behaviors, further analysis was done on the individual constituent behaviors. Freezing alone emerged as the behavioral feature that differed significantly in duration between operated and unoperated groups. A repeated-measures ANOVA on freezing revealed a robust main effect of group \(F(2,9) = 37.55, P < 0.001\); control > left and right). Post hoc Bonferroni tests revealed that left and right groups, though different from the control group, did not differ significantly from each other in any of the five sessions \((0.359 < P < 1.0)\).

**Approach behavior.** A repeated-measures ANOVA across the five snake trials was performed on approach behaviors. The mean cumulative duration of approach behavior did not differ significantly by group \(F(2,9) = 2.437; P = 0.143\). The frequency of approach behavior in the first 5 s of trials, however, did differ significantly by group \(F(2,9) = 15.633, P = 0.001\). Individual ANOVAs revealed significant group differences on sessions 2, 3, and 5 \([session 2, F(2,9) = 4.5, P = 0.04; session 3, F(2,9) = 57.000, P < 0.001; session 5, F(2,9) = 13.286, P = 0.002]\). Post hoc Bonferroni tests show that both left and right groups differed from the control group for sessions 3 \((left: P < 0.001; right: P < 0.001)\) and session 5 \((left: P = 0.003; right: P = 0.009)\). Left and right groups, however, did not differ from each other.

**Discussion**

The method we used, which was first described by Mineka et al. (1980), is particularly informative because it sets approach behavior (toward the food reward) directly against avoidance or defensive behaviors (from the snake). According to Mineka, monkeys show behavioral avoidance such as that seen in the reluctance to reach over the snake, this provides strong evidence that the monkeys are showing fear of the snake.

As expected, intact monkeys in the present study showed robust emotional reactions to the rubber snake. Whereas intact monkeys quickly reached over neutral objects to obtain the food reward, they hesitated or failed to reach altogether when given the opportunity to reach over the rubber snake. The facial expressions and movements made in the presence of the snake were mainly defensive, including moving to the back of the cage, eye and head aversion, freezing, and piloerection. The intensity of the defensive behaviors matched closely the description of the snake-naive monkeys studied by Nelson et al. (2003) in that the monkeys displayed a range of behaviors interpreted by human observers as orienting responses, wariness, and fear. These defensive behaviors are the same type of disturbance behaviors reported by Mineka and colleagues (1980), fully consistent with the idea that snakes induce an innate fear response.

Both operated groups, left and right, exhibited reduced emotional responses to the rubber snake. This was evidenced by the markedly shorter latencies relative to controls to reach over the rubber snake as well as increased approach responses and decreased defensive behaviors in the presence of the snake. We infer, therefore that monkeys with unilateral lesions, left or right, showed reduced fear of the snake. As already indicated,
Meunier et al. (1999) and Kalin et al. (2001) reported a blunted freezing response in young adult macaque monkeys following selective bilateral amygdala lesions, and therefore it seems likely that amygdala damage is contributing to the impairment observed in the operated monkeys in the present study. Direct support for this idea is provided by Kalin et al. (2001) who used a task much like that used in experiment 3 of the present study. Their preliminary findings indicate that monkeys with bilateral amygdala damage show markedly shorter food-retrieval latencies relative to controls on snake trials but not on trials with neutral objects. These findings are in accord with our own data (unpublished observations). In addition, preliminary evidence suggests that monkeys with bilateral Pfo damage likewise exhibit altered behavioral responses to fake snakes (Suda et al. 2002 abstract). Thus even though the results of the present study cannot speak to this issue, the foregoing findings suggest that damage to each structure in the combined lesion is contributing to the deficit. The present study is the first to show that unilateral (combined amygdala and Pfo) lesions are sufficient to disrupt this type of emotional response.

One possible interpretation of the deficit in food-retrieval latencies on snake trials is that the operated monkeys, rather than exhibiting reduced emotional responses, simply showed faster habituation than the controls. Data from the spider trials, however, suggest this explanation is insufficient. On spider trials, as on snake trials, all monkeys exhibited long food-retrieval latencies on session 1; furthermore, all monkeys, operated and controls alike, exhibited progressively shorter latencies over sessions on spider trials only. Thus the controls and operated monkeys showed equally rapid habituation to one novel, potentially feared stimulus, namely, the jumping spider. Given that group differences were restricted to snake trials, rapid “habituation” fails to provide a satisfactory explanation for the deficit. In addition, control monkeys spent a great deal more time in the presence of the snake than did operated monkeys and therefore had a greater opportunity, if anything, to habituate to it. This is because trials in which monkeys did not reach for the food were carried out to a maximum of 30 s. Two control monkeys never reached for the food, whereas all the monkeys in the left and right groups did.

For reasons elaborated earlier with respect to experiment 1, group differences were also probably not due to differences in motivation. Intact monkeys are just as willing to work for food as monkeys with bilateral lesions of the amygdala (Aggleton and Passingham 1982) or orbital prefrontal cortex (unpublished observations) or crossed disconnection lesions of the two structures (Baxter et al. 2000). In addition, in the present study daily food rations for each monkey were determined during pretraining for each experiment and not changed thereafter; presumably this precaution would prevent any systematic, between-groups bias in levels of motivation.

Experiment 3

Experiment 3 assessed object reversal learning abilities. Like experiment 1, experiment 3 was intended to measure aspects of reward processing. Whereas experiment 1 evaluated the monkeys’ abilities to choose between positive objects after changes in the value of a food reward, experiment 3 evaluated monkeys’ abilities to choose between two objects when the reinforcement contingencies were reversed but food value remained unchanged. In experiment 3, monkeys learned through trial and error that a previously nonrewarded object was now rewarded and a previously rewarded object was no longer rewarded.

Methods

SUBJECTS AND APPARATUS. The subjects, WGTA, and test tray were the same as those in experiments 1 and 2. The same three objects used during pretraining in experiment 1 were also used for pretraining in this experiment. A novel object pair was used for the initial discrimination task and the nine reversals that followed.

PROCEDURE. Pretraining. Before proceeding to the main task, monkeys were required to complete a single 40-trial session in which they displaced one of the three pretraining objects, presented singly, over one of the two available food wells. Objects were presented in random order, with each object baited on presentation, and with the location of the object (left or right) following a pseudorandom order. The purpose of this pretraining phase was to ensure that the monkeys would work for food rewards throughout a complete session. All monkeys completed pretraining in one day.

Main task. Each monkey was trained on a single visual-discrimination problem and its reversal. We used the same method as Jones and Mishkin (1972) and Murray et al. (1998) with the exception that training was continued for nine serial reversals instead of seven. On the first trial of initial learning, both objects were either baited (for half the monkeys in each group) or unbaired (remaining monkeys), and the object chosen designated as either the S+ (if it had been baited) or the S− (if it had been unbaired). Thus the monkeys’ choices on trial 1 determined the designation of the S+ and S− for initial learning, a procedure intended to prevent response biases due to object preferences. On each trial thereafter, the monkey was presented with the two objects, one baited and one unbaired, one each overlying the two food wells. The monkey was allowed to displace only one of the two objects and, if correct, to retrieve the food reward underneath. A noncorrection procedure was employed. Monkeys were tested at the rate of 30 trials per daily test session. Criterion was set at 28 correct responses out of 30 trials (93%) on 1 day, followed by ≥24 correct out of 30 trials (80%) the next day. The intertrial interval was 10 s and the left-right position of the correct object followed a pseudorandom order. After monkeys attained criterion on the original problem, the reward contingencies were reversed (starting the next day) and each monkey was trained to the same criterion as before. This procedure was repeated until a total of nine reversals had been completed.

Results

The number of errors scored in acquisition of the initial discrimination and on the subsequent reversals is illustrated in Fig. 7. Groups did not differ in the number of trials and errors to criterion scored in initial learning (trials: \(X^2 = 0.369, P = 0.832\); errors: \(X^2 = 0.289, P = 0.865\)). An ANOVA with repeated measures on errors to criterion for reversals 1–9 resulted in a nonsignificant effect of group \([F(2,9) = 2.861, P = 0.109]\) but a significant within-subject effect of reversal \([F(8,72) = 5.248, P < 0.001]\). There was no significant rever-
an ANOVA with repeated measures on trials to criterion for reversals 1–9 resulted in a marginally significant effect of group \( F(2,9) = 3.847, P = 0.062 \). There was no significant within-subject effect of reversal \( IF(8,72) = 1.574, P = 0.148 \) or reversal by group interaction \( F(16,72) = 1.010, P = 0.457 \).

Reversals were also analyzed according to stage of reversal learning (Aggleton and Passingham 1981; Jones and Mishkin 1972; Murray et al. 1998). Stages are categories of error type intended to identify when, during reversal, errors are scored. For each session of each reversal, all errors scored within that session were assigned to stages according to the following criteria: stage 1, \( \leq 21 \) errors; stage 2, 10–20 errors; stage 3, 3–9 errors. Thus for this analysis all errors scored on all sessions of reversal learning, including sessions in the criterion run, are counted, and the data are collapsed across reversals. A preponderance of stage 1 errors relative to controls would reflect difficulty in suppressing (or unlearning) the previously learned response, stage 2 errors reflect near chance performance, and a preponderance of stage 3 errors would reflect difficulty in learning the new association. Individual ANOVAs on the errors scored in the three stages revealed no significant differences between groups [stage 1: \( F(2,9) = 1.648, P = 0.246 \); stage 2: \( F(2,9) = 1.828, P = 0.216 \); stage 3: \( F(2,9) = 2.860, P = 0.109 \)].

When reversal learning was analyzed for total errors and total trials to criterion across all reversals, left and right groups were found to be no different from each other (errors: left vs. right, \( P = 1.0 \); trials: left vs. right, \( P = 1.0 \)). For this reason and because of the marginal effect of group on trials to criterion during reversal learning, both left and right groups were subsequently considered together as an unilateral group. As before, there was no significant difference in initial learning between the unilateral group and controls [errors: \( F(1,10) = 0.137, P = 0.719 \); trials: \( F(1,10) = 0.090, P = 0.770 \)]. Now, however, an ANOVA with repeated-measures on the nine reversals revealed a significant main effect of group on both errors \( [F(1,10) = 6.344, P = 0.03] \) and trials \( [F(1,10) = 7.477, P = 0.021] \) to criterion (unilateral group > control). There was a significant within-subjects effect of reversal for errors only \( [F(8,80) = 4.912, P < 0.01] \). There was no significant interaction of group by reversal for either trials or errors. For errors to criterion, reversals 3, 8, and 9 differed significantly by group \( [F(1,10) = 5.326, P = 0.044] \), reversal 3; \( F(1,10) = 5.183, P = 0.046 \), reversal 8; \( F(1,10) = 7.404, P = 0.022 \), reversal 9]. For trials to criterion, only reversals 8 and 9 differed significantly by group \( [F(1,10) = 7.282, P = 0.022] \), reversal 8; \( F(1,10) = 8.582, P = 0.015 \), reversal 9]. An analysis of stages revealed a marginal effect of group only on stage 2 errors \( [F(1,10) = 4.054, P = 0.072] \).

**Discussion**

When left and right groups were considered together, there was a significant main effect of the unilateral combined amygdala and PFo lesions on object reversal learning. Monkeys with unilateral lesions, although unimpaired in the acquisition of the initial discrimination, required more trials and errors to reach criterion than controls over the course of the nine reversals.

In the object-reversal task, monkeys must learn to associate a reward with an object and switch their responses when the reinforcement contingencies reverse. Earlier work found that monkeys with bilateral aspiration lesions that included the amygdala (Barrett 1969; Jones and Mishkin 1972; Schwartzbaum and Poulos 1965) and those with aspiration lesions of the orbital prefrontal cortex (Iversen and Mishkin 1970; Jones and Mishkin 1972; Meunier et al. 1997) were impaired in object-reversal learning. Because the present study involved damage to both structures, we cannot determine whether damage to the amygdala or to the orbital prefrontal cortex or both was critical in producing the deficit. However, preliminary data (Izquierdo et al. 2003) suggest that monkeys with selective amygdala lesions made with the excitotoxin ibotenic acid, unlike monkeys with aspiration lesions, are unimpaired on object-reversal learning. Thus it seems likely that the deficit in our operated monkeys is due primarily to the unilateral orbital prefrontal cortex damage they sustained.

**EXPERIMENT 4**

The response of intact rhesus monkeys to the stare of an unfamiliar human “intruder” produces emotional behavior characterized by defensive, submissive, and aggressive behaviors (Kalnin and Shelton 1989). Although Kalnin et al. (2001) found no effect of bilateral amygdala lesions on this measure, we included this task in our battery for three reasons. First, it provided a measure of emotional responses to a social stimulus, which would complement our evaluation in experiment 2 of reactions to a potential predator. Second, even if the behavior exhibited in response to a human intruder was unaffected by the lesions, it would provide a potential control for the production of emotional responses in the snake test. Third, the task might inform our question regarding hemispheric specialization.

**Methods**

**SUBJECTS AND APPARATUS.** Subjects were the same as those in experiments 1–3. In this experiment, monkeys were not tested in the WGTA but in the open space of an unfamiliar room. We also used the same videotaping apparatus as in experiment 2 but only the frontal camera view was used. A single male human, one never before seen by the monkeys, served as the intruder for all monkeys.
PROCEDURE. We used the same test procedure as Kalin and Shelton (1989) with the exception that the duration of each condition was shorter in our version of the task. In addition, our scoring of the monkeys’ behavioral responses followed the methods of Meunier et al. (1999). Monkeys were placed in their usual test cage, taken to a room they had never been in, and left alone for 5 min (alone condition, Alone). A human unfamiliar to the monkey then entered the room, sat ~2.5 m away from the cage, and presented his profile to the monkey for 5 min. The human never made eye contact (no eye contact condition, NEC) with the monkey during this time. After leaving the room for 3 min, the same human returned to the room, sat 2.5 m away from the monkey, and proceeded to stare at the monkey (stare condition, ST) for 5 min. In the ST condition, the human remained motionless and projected a neutral face toward the monkey. All conditions were videotaped and analyzed for frequency and duration of behavior in seconds, using the same scoring methods as in experiment 2 (see Table 2). As was the case for behavioral scoring in experiment 2, interobserver reliability was high (all Pearson correlations coefficients > 0.83; all P values < 0.04).

Results
CUMULATIVE DURATION. Mean cumulative durations of the different categories of behavior exhibited in the different conditions are shown in Fig. 8. A repeated-measures ANOVA on all three conditions yielded no significant group differences and no significant group by condition interactions. Furthermore, an analysis of cumulative duration with left and right groups collapsed into one unilateral group yielded the same result. Significant results included the effect of condition on high aggression [F(2,20) = 5.408, P = 0.013], submission [F(2,20) = 14.816, P < 0.001], defense [F(2,20) = 49.169, P < 0.001], approach [F(2,20) = 15.314, P < 0.001], and “other” categories [F(2,20) = 10.295, P = 0.001]. There were no submissive and approach behaviors observed in the Alone condition.

CUMULATIVE FREQUENCIES. A repeated-measures ANOVA on all three conditions yielded no significant group differences and no significant group by condition interactions. The pattern of results was identical to that obtained for cumulative duration.

Discussion
Experiment 4 shows that unilateral combined removals of the amygdala and PFo are insufficient to alter monkeys’ emotional responses to an unfamiliar human. More importantly, however, the data indicate that our monkeys were capable of exhibiting appropriate emotional responses under some circumstances. Thus there appears to be a difference in the neural substrates supporting snake fear versus emotional reactions to an unfamiliar human. We note that these same operated monkeys did obtain scores significantly different from controls on at least one cognitive test administered after experiment 4 (unpublished observations), so it cannot simply be the case that the lesions are no longer effective.

Kalin et al. (2001) found that bilateral excitotoxic lesions of the amygdala had no influence on rhesus monkeys’ responses to a human intruder but did blunt emotional reactions to a snake. These authors characterize the difference between responses to a snake and a human intruder as one of acute fear versus trait-like anxiety, suggesting the possibility that the snake and human intruder tasks may elicit a fight/flight response and risk-assessment behavior, respectively. However, there are alternative possibilities, none of which are mutually exclusive with the first. For example, perhaps the neural circuitry mediating the responses to a potential predator is different from that mediating responses to a social stimulus, a point taken up later in the DISCUSSION. Another possibility is that the amygdala is important for stimuli that require fast processing over events that can be discriminated for their potential threat over time (Wright et al. 2001). Or perhaps the critical difference is that one type of behavior is innate whereas the other is learned. Finally, yet another possibility is that the conflicting (approach and withdrawal) responses that are inherent in the design of the snake trials, but not the human-intruder task, might be the source of the different effects of the lesions on the two tasks. These possibilities invite future empirical investigation.
**FOOD PREFERENCE RETEST**

**Methods**

**PROCEDURE.** After all other experiments were concluded, monkeys were evaluated for their food preferences a second time to examine whether rankings of the six familiar foods changed over time or differed by group. The method was the same as that employed preoperatively.

**Results**

Choices from the final 5 days of the food preference test and retest were subjected to an ANOVA (data not illustrated). Although there was a significant effect of stage (before and after surgery), there were no significant group differences or group by stage (pre- and post-test) interactions. All monkeys, on average, increased in their preference for fruit snacks ($P < .01$) and decreased in their preference for craisins and banana pellets ($P < 0.02$).

**DISCUSSION**

**Hemispheric specialization**

The results of the present study provide no support for a functional hemispheric specialization for affective processing in the rhesus monkey, insofar as the amygdala and orbital prefrontal cortex are involved. Instead, although unilateral lesions (either left or right) yielded deficits on reinforcer devaluation, emotional reactions to a rubber snake, and object reversal learning, there were no differences between left and right groups. Thus both amygdalae or orbital prefrontal cortical regions or the combination need to be intact to achieve normal affective processing in monkeys. Consistent with our findings, Malkova et al. (2003) reported that social behavior in macaques is disrupted by unilateral (left or right) administration of GABA antagonists into the basolateral amygdala, indicating that both hemispheres are contributing to social behavior between conspecífics.

Our results are surprising given the lack of effect of unilateral lesions on visual discrimination learning and memory abilities in macaques (e.g., Ettlinger et al. 1968; Gaffan and Harrison 1988; Parker and Gaffan 1998; Z. Liu, B. J. Richmond, E. A. Murray, R. C. Saunders, S. Steenrod, B. K. Stubblefield, D. M. Montague, and E. I. Ginns, unpublished observations; cf. Bussey et al. 2002). Presumably, it is the affective processing or rule-related components of the tasks, rather than the visual processing components, that are disrupted by the unilateral lesions in the present study.

Given the limited number of tests we conducted, the present results in no way rule out the possibility of a hemispheric specialization for some aspects of affective processing not yet examined. For example, perhaps acquisition of fear behaviors (e.g., fear conditioning) would be lateralized even if innate fear responses are not. This idea seems unlikely, however, as humans with unilateral temporal lobectomies (LaBar et al. 1995) and rats with unilateral amygdala lesions (LaBar and LeDoux 1996) exhibit impaired fear conditioning, irrespective of the hemisphere damaged. Alternatively, perhaps a right hemisphere specialization for processing of emotionally charged “naturalistic” stimuli, reported by Ifune et al. (1984) in split-brain monkeys but not observed in the present study (experiment 2) in monkeys with unilateral lesions, is secondary to hemispheric asymmetries in autonomic activity (e.g., Wittling 1995).

Although the groups with left and right hemisphere lesions did not differ statistically, there were differences that failed to reach statistical significance. Monkeys with left hemisphere lesions “stole” more objects and retrieved more of the sated foods on average than monkeys in either of the other groups. These behaviors are reminiscent of certain Klüver-Bucy signs observed after temporal lobe damage (Klüver and Bucy 1939), which can now be attributed mainly to amygdala damage (e.g., Meunier et al. 1999), and of utilization behavior in humans, which has been linked to orbital and medial frontal cortex damage (Lhermitte 1983; Schall et al. 1989). These trends in the data, although they did not reach significance in the present study, deserve further investigation.

**Reward processing**

The present study found that monkeys with unilateral lesions were impaired in their responses to reinforcer devaluation; monkeys with either left or right hemisphere lesions were impaired on test 1, after removal of a single structure, and the left and right groups considered together were still impaired on test 2, after removal of the second structure in the same hemisphere. There was also an effect of the unilateral lesion on object reversal learning. For both tasks, the effects of unilateral lesions appeared to be milder than those observed after bilaterally symmetrical lesions. For example, monkeys with bilateral amygdala lesions studied by Malkova et al. (1997) had lower devaluation scores (i.e., a greater deficit) than the present monkeys on test 1, when monkeys had sustained damage to only a single structure, as well as on test 2, when monkeys had received the combined unilateral lesion. Monkeys with either bilateral removals of orbital prefrontal cortex or temporal pole plus amygdala studied by Jones and Mishkin (1972) likewise were more severely impaired in object reversal learning than the operated monkeys in the present study. In both cases, control groups had comparable scores. Thus unilateral combined damage to the amygdala-PF circuit yields a modest though significant impairment in these measures of reward processing, relative to bilaterally symmetrical damage to these brain areas, and, in addition, unilateral damage to a single structure (either the amygdala or orbital prefrontal cortex) yields a significant deficit on at least one measure. Because each hemisphere contributes roughly one-half of both types of behavior, it appears that neural signals from the left and right hemispheres converge, perhaps in a third region downstream, to produce the full complement of the response in intact monkeys.

Our data indicate that reward processing, broadly construed, is disrupted following unilateral damage to the amygdala-PF circuit. Although operated monkeys displayed difficulty in linking reward value with objects (experiment 1) and in responding to changes in reward contingencies (experiment 3), they were able to acquire visual discrimination problems at the same rate as controls (experiment 1, acquisition of the 60 pairs; experiment 3, initial learning). Thus on this measure of stimulus-reward association, the operated monkeys were unaffected. The dissociation between intact acquisition of approach responses to rewarded objects and the impaired ability to
respond appropriately in the face of changing reward value or contingency has been noted by others (Balleine et al. 2003; Blundell et al. 2001, 2003; Malkova et al. 1997; Schoenbaum et al. 2003a). Presumably, this pattern of results reflects intact stimulus-response (S-R) learning, which can support the instrumental responses to rewarded objects but deficient abilities to link objects with the value of food rewards (amygdala damage) (for review see Baxter and Murray 2002) or deficient abilities to use those associations to guide response selection (PFo damage) (e.g., Schoenbaum et al. 2003b).

Studies of reinforcer devaluation in rats (Gallagher et al. 1999; Hatfield et al. 1996) and monkeys (Baxter et al. 2000; Izquierdo and Murray 2000; Malkova et al. 1997) converge in suggesting that both the amygdala and orbital cortex contribute crucially to this function, and a recent functional imaging study in humans is consistent with this idea (Gottfried et al. 2003). By contrast, studies of odor reversal learning in rats (Schoenbaum et al. 2003a) and preliminary data on object reversal learning in monkeys (Izquierdo et al. 2003) yield a different pattern of results: on this task, bilateral damage to the orbital cortex produces substantial impairments, whereas bilateral damage to the amygdala produces little or no impairment. Ventromedial prefrontal cortex, including orbital cortex, is also critical for object reversal learning in humans (Fellows and Farah 2003; Rolls et al. 1994). Taken together, the results suggest that whereas the orbital cortex is critical for responding appropriately to changes in either reward value or contingency, of these two processes, the amygdala is critical only for responding appropriately to changes in reward value. Although the design of the present study precludes us from drawing inferences about the independent contributions of the amygdala and orbital prefrontal cortex to these processes, our data are consistent with the view that the basolateral amygdala is important for establishing a link between environmental stimuli and sensory properties of food reward (Blundell et al. 2001), whereas the orbital prefrontal cortex is important for response selection, whether based on values of food to be obtained (Schoenbaum et al. 2003b; Tremblay and Schultz 2000; Wallis and Miller 2003) or the rules governing the procurement of such foods (Dias et al. 1996; Wise et al. 1996). Consistent with this idea, recent work has demonstrated dissociable roles of the amygdala and orbital frontal cortex of rats in reinforcer devaluation; once light cues have been linked to the incentive value of food, the orbital frontal cortex but not amygdala is necessary for responding appropriately in the face of devaluation of the food (Pickens et al. 2003). In a similar vein, recent functional imaging work has provided evidence that amygdala activity is correlated with human subjects’ rating of the incentive value of restaurant menus (Arana et al. 2003), whereas activity of medial and orbital frontal cortex reflects both incentive judgments and goal selection.

Emotional processing

Monkeys with combined lesions of the amygdala and orbital prefrontal cortex in one hemisphere showed blunted emotional reactions to a rubber snake. Both left- and right-operated groups had shorter latencies to reach over the snake to obtain food, and both displayed less pronounced defensive behaviors and more approach behaviors relative to controls during snake trials. Thus as was the case for reward processing, both left and right amygdala-PFo circuits are required for normal emotional reactions in the presence of a rubber snake. A comparison of the data from the present groups with groups receiving bilaterally symmetrical lesions of the amygdala or orbital prefrontal cortex will be required to discern any selective amygdala and prefrontal contributions to snake fear. Nonetheless, as indicated in the DISCUSSION section following experiment 3, it appears that both structures are contributing to rhesus monkeys’ emotional responses to snakes.

The distinction observed between the attenuation of monkeys’ fear responses to a snake versus the lack of such an effect to an unfamiliar human is of interest for several reasons. First, it shows that monkeys with either bilateral amygdala damage (Kalin et al. 2001) or unilateral amygdala plus orbital prefrontal cortex lesions (present study) are capable of expressing appropriate emotional responses in some contexts. Meunier et al. (1999) found that monkeys with either excitotoxic or aspirative lesions of the amygdala were capable of displaying emotional responses, albeit often ones inappropriate to the situation. Thus monkeys in the present study with unilateral lesions, like those with bilateral excitotoxic amygdala lesions studied by Meunier et al. (1999), cannot be characterized as hypoemotional, and therefore their lack of emotional reactions to the rubber snake cannot be ascribed to an inability to generate an emotional response. Instead, it appears that the snake stimulus no longer elicits the appropriate emotional response. Second, it suggests a potential neurobiological distinction between the mechanisms underlying the defensive responses to the fake snake versus unfamiliar human. As discussed earlier, additional studies are needed to address this issue. One intriguing possibility is that the pattern of results observed might be due to the amygdala playing a critical role in marshalling defensive responses to stimuli that represent a threat to survival, especially those representing recurrent survival threats through evolutionary history (Öhman and Mineka 2002; Seligman 1971). On this view, the fake snake might constitute a phylogenetic fear-relevant stimulus (Öhman and Mineka 2002), whereas the human intruder would not. Although Öhman and Mineka (2002) proposed a specific role for the amygdala as part of a “fear module,” there is now ample evidence that the amygdala’s contribution to affective processing goes well beyond fear learning to include appetitive learning (e.g., Parkinson et al. 2000) and recognition of conspecifics (e.g., Fergusson et al. 2001) among other things (for review, see Baxter and Murray 2002; Holland and Gallagher 1999). Nevertheless, the extent to which snake fear, in particular, and other behaviors involving the amygdala might be genetically encoded and epigenetically expressed deserves additional study.

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