Amygdala lesions disrupt modulation of functional MRI activity evoked by facial expression in the monkey inferior temporal cortex

Fadila Hadj-Bouziane[a,1,2], Ning Liu[a], Andrew H. Bell[a,3], Katalin M. Gothard[b], Wen-Ming Luh[c], Roger B. H. Tootell[a,d], Elisabeth A. Murray[e], and Leslie G. Ungerleider[a,1]

Laboratories of [a]Brain and Cognition and [e]Neuropsychology and [c]Functional MRI Facility, National Institute of Mental Health, National Institutes of Health, Bethesda, MD 20892; [b]Department of Physiology, College of Medicine, University of Arizona, Tucson, AZ 85724; and [d]Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA 02129

Contributed by Leslie G. Ungerleider, October 23, 2012 (sent for review August 16, 2012)

We previously showed that facial expressions modulate functional MRI activity in the face-processing regions of the macaque monkey's amygdala and inferior temporal (IT) cortex. Specifically, we showed that faces expressing emotion yield greater activation than neutral faces; we term this difference the "valence effect." We hypothesized that amygdala lesions would disrupt the valence effect by eliminating the modulatory feedback from the amygdala to the IT cortex. We compared the valence effects within the IT cortex in monkeys with excitotoxic amygdala lesions (n = 3) with those in intact control animals (n = 3) using contrast agent-based functional MRI at 3 T. Images of four distinct monkey facial expressions—neutral, aggressive (open mouth threat), fearful (fear grin), and appeasing (lip smack)—were presented to the subjects in a blocked design. Our results showed that in monkeys with amygdala lesions the valence effects were strongly disrupted within the IT cortex, whereas face responsivity (neutral faces > scrambled faces) and face selectivity (neutral faces > non-face objects) were unaffected. Furthermore, sparing of the anterior amygdala led to intact valence effects in the anterior IT cortex (which included the anterior face-selective regions), whereas sparing of the posterior amygdala led to intact valence effects in the posterior IT cortex (which included the posterior face-selective regions). Overall, our data demonstrate that the feedback projections from the amygdala to the IT cortex mediate the valence effect found there. Moreover, these modulatory effects are consistent with an anterior-to-posterior gradient of projections, as suggested by classical tracer studies.

neuroimaging | nonhuman primate | social stimuli | temporal lobe

Evidence emerging from both the animal and human literature indicates that the perception of emotional signals is disrupted after damage to the amygdala (1–4). Animals with amygdala lesions are able to display emotional responses, but these responses often are inappropriate to the context (3, 5–10). For instance, normal macaque monkeys are slower to retrieve food in the presence of a snake stimulus than in the presence of neutral objects, but monkeys with amygdala lesions are not (7). Adolphs et al. (1, 2, 11) and Hamann et al. (12) have shown that recognition of fearful faces, in particular, is impaired in a patient with bilateral amygdala lesions (patient SM). Neuroimaging studies in humans (13–15) and, more recently, in monkeys (16, 17) have demonstrated valence effects in the amygdala [i.e., enhanced functional MRI (fMRI) responses when subjects view fearful faces compared with neutral faces]. Similar valence effects have been described in the inferior temporal (IT) and prefrontal cortex in both humans (13, 14, 18) and monkeys (16, 19).

The IT cortex sends highly processed visual information mainly to the lateral nucleus of the amygdala, which then projects to the basal nucleus (20–23). In turn, the basal nucleus of the amygdala sends extensive feedback projections to the entire ventral processing stream, extending from the anterior IT cortex (area TE) as far posterior as V1 (20). It is thought that the amygdala extracts the behavioral significance of visual information received from the IT cortex and, in turn, influences all stages of cortical visual processing (24, 25).

We know of only one study that has directly tested the hypothesis that the amygdala modulates the emotional responses observed in the visual cortex (26). In their fMRI study, Vuilleumier et al. (26) reported that patients with sclerotic damage to both the amygdala and hippocampus failed to show an enhanced response in the fusiform gyrus to emotional faces, whereas patients with damage to the hippocampus alone did show such an enhancement. However, the extent of the lesions in the patient groups was variable and not confined to the regions under investigation, as is common in patient studies.

To assess directly the amygdala's influence on emotional processing within the IT cortex, we scanned three monkeys with amygdala lesions and three intact controls while they viewed images of monkey faces with various facial expressions. We predicted that amygdala lesions would eliminate feedback influences on the visual cortex and thus would disrupt the valence effects normally found there.

Results

Responses to Neutral and Emotional Faces in Control Monkeys. As previously described (16), "face responses" (neutral faces >

Significance

Successful social interaction depends on the ability to recognize others, evaluate their mental states (e.g. intentions, desires, and beliefs), and "read" their emotional states. Here, we show that, in monkeys, damage to the amygdala, a brain structure that is central to the expression of emotion, significantly disrupts the processing of emotional facial expression in high-level visual cortical areas involved in face recognition. These findings suggest that the projections of the amygdala to visual cortical areas likely enhance the sensory processing of biologically important signals, including those related to potential environmental threats and social contexts.

Author contributions: F.H.-B., E.A.M., and L.G.U. designed research; F.H.-B., N.L., A.H.B., and W.-M.L. performed research; W.-M.L. contributed new reagents/analytic tools; F.H.-B. and K.M.G. analyzed data; and F.H.-B., N.L., A.H.B., K.M.G., R.B.H.T., E.A.M., and L.G.U. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

See Commentary on page 21180.

[1]Present address: Institut National de la Santé et de la Recherche Médicale U1028, Centre National de la Recherche Scientifique Unité Mixte de Recherche 5292, Lyon Neuroscience Research Center, ImpAct Team, 69500 Bron Cedex France.

[2]To whom correspondence may be addressed. E-mail: fadila.hadj-bouziane@inserm.fr or ungerlel@mail.nih.gov.

[3]Present address: Medical Research Council Cognition and Brain Sciences Unit, Cambridge CB2 7EF, United Kingdom.

scrambled faces) in control animals were widely distributed bilaterally within the IT cortex (including areas TE and TEO), within and along the superior temporal sulcus (STS), within the ventrolateral prefrontal cortex (Fig. 1*B*), and within the dorsal portion of the lateral and basal nuclei of the amygdala (Fig. 2). In addition, we identified two face-selective regions (neutral faces > non-face objects) bilaterally within the IT cortex (Fig. 1*B*): (*i*) an anterior face-selective region located within area TE (AP ~ +18 mm) and (*ii*) a posterior face-selective region within TEO (AP ~ +6 mm). These regions correspond to the previously described anterior and posterior face patches (27–29) originally referred to as the "anterior" and "middle" face patches, respectively (30).

In these control monkeys we also evaluated the responses to the various facial expressions within both the IT cortex and the amygdala. We found significantly increased activation for faces with expressions of emotion, as compared with neutral faces (main effect of valence found in both hemispheres in all three control monkeys, $P < 0.001$). Such valence effects were distributed throughout the IT cortex, including areas TE and TEO, and along the STS as well as in the amygdala. The response profiles observed in the three control monkeys are illustrated in Figs. 1*C* and 2 for the face-selective regions in the IT cortex and the face-responsive regions in the amygdala, respectively. As reported previously (16), the most consistent result was an enhanced response to fear-grin faces as compared with neutral faces ($P < 0.01$ in all six hemispheres in the three control monkeys). Images of faces with lip-smack expressions also yielded significantly enhanced responses compared with neutral faces in all six hemi-

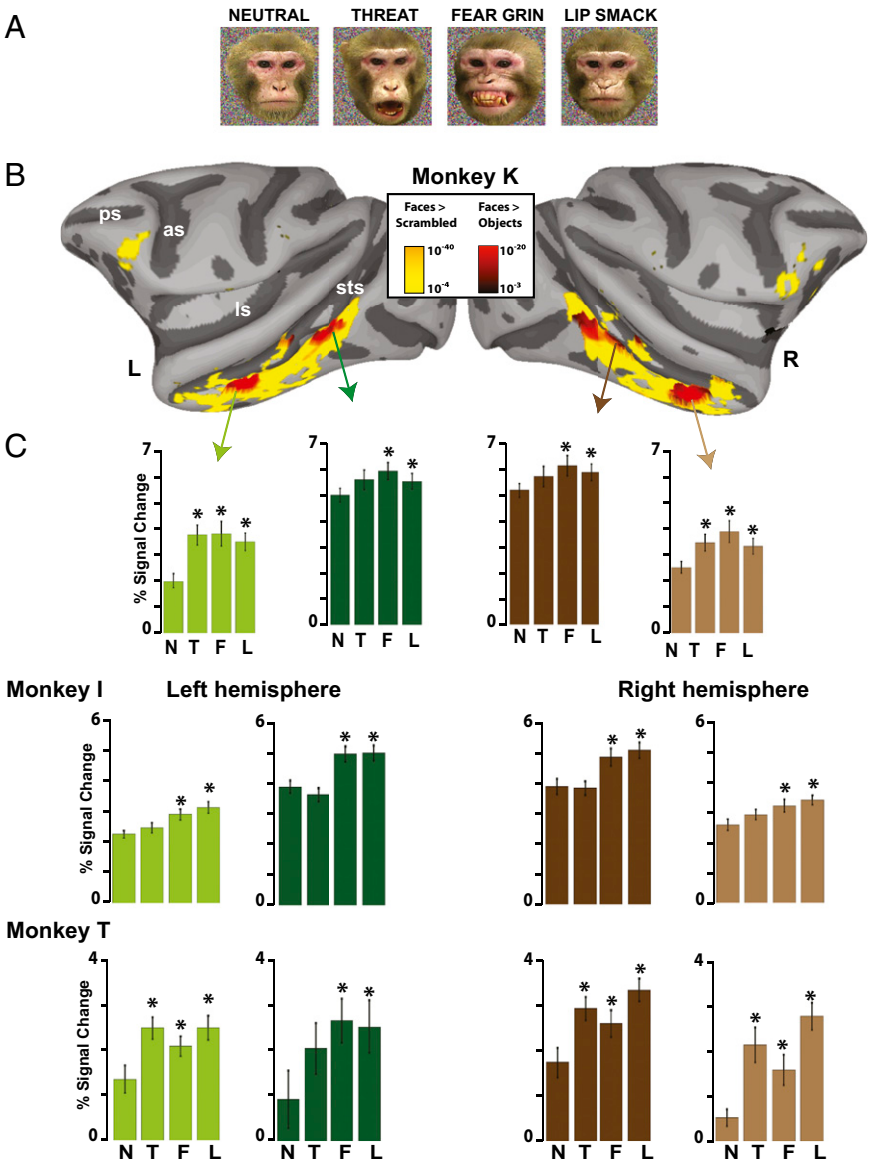

Fig. 1. Responses to neutral and emotional faces in the control animals. (*A*) Examples of monkey facial-expression stimuli used in the experiments. Animals were presented with 32-s–long blocks of various facial expressions consisting of exemplars of monkey faces (eight different identities). (*B*) Face-responsive (neutral faces > scrambled faces) regions (yellow) and face-selective (neutral faces > non-face objects) regions (red) are shown on inflated cortical surfaces for monkey K. as, arcuate sulcus; ls, lateral sulcus; ps, principal sulcus; rs, rhinal sulcus; sts, superior temporal sulcus. (*C*) Profile of responses to the various facial expressions (N, neutral; T, threat; F, fear grin; L, lip smack) within the anterior and posterior face-selective regions for the three control animals. As shown previously (16), facial expressions modulated face-related activations within the temporal cortical face-selective regions. Asterisks on histograms indicate a significant difference from neutral ($P < 0.05$).

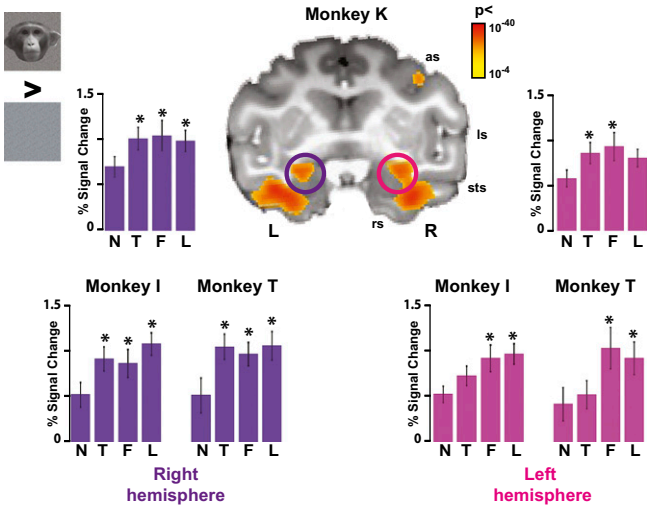

Fig. 2. Face-responsive regions in the amygdala illustrated on a coronal section for monkey K and profiles of responses to the various facial expressions for the three control animals. Asterisks on histograms indicate a significant difference from neutral ($P < 0.05$). N, neutral; T, threat; F, fear grin; L, lip smack.

spheres in the three controls ($P < 0.01$). In contrast, open-mouthed threat expressions elicited enhanced activation in two of three animals (monkeys K and T). Importantly, we observed no systematic difference between hemispheres in the response profile of any of the control monkeys (no interaction was found between hemispheres and valence effects). Although the fMRI signals were greater on average in the posterior face-selective regions than in the anterior regions, we found no difference in the magnitude of the valence effects in the posterior and anterior face-selective regions.

Evaluating the Extent of Amygdala Damage. Using both T1 and T2 scans for each monkey, we evaluated both the extent and the location of the lesions, particularly along the anterior-to-posterior axis (*Materials and Methods*), to help define the experimentally relevant subdivisions of the amygdala. We defined anterior and posterior parts of the amygdala corresponding to locations anterior or posterior to AP = +19 mm in a standard monkey brain atlas (31). Fig. 3 illustrates the contours of the estimated sparing along the anterior-to-posterior axis within each hemisphere of the three operated animals. As shown in this figure, the lesions were restricted to the amygdala. Importantly, this type of lesion leaves intact the fibers of passage, i.e., the axons traveling through and near the injection sites (32, 33). The lesions in monkey C were bilateral, albeit with more sparing in the right hemisphere than in the left (35% versus 5% spared); indeed, the lesion in the left hemisphere was almost complete. In monkey P, the amygdala lesions were largely symmetrical in the two hemispheres (~25% spared; Table 1). In monkey M, the amygdala in the left hemisphere was left intact intentionally, but the neuronal loss in the right amygdala was nearly complete (~6% spared).

We then compared the responses to neutral and emotional faces within the spared amygdala tissue of these operated animals with the responses of control animals. In the control group, the activation was found within the dorsal part of the lateral and basal nuclei of the amygdala, as shown earlier (16). Similarly, in all three operated animals the activation usually was located dorsally and laterally, presumably corresponding to the nuclei of the basolateral group (lateral, basal, and accessory basal nuclei). There, we found activation in response to neutral faces (Fig. 4*A*). However, not surprisingly, these activations were far smaller in these animals than in controls and varied between hemispheres in proportion to the amount of amygdala spared (Table 2). To quantify this result, we compared the percent of amygdala tissue activated by neutral faces (neutral > scrambled), expressed as a function of the total amygdala volume, in both groups. As shown in Table 2, the volume of amygdala-activated tissue differed significantly in the two groups ($P < 0.05$). The volume of activated tissue in the hemispheres of control monkeys was more than five times larger than in the hemispheres with amygdala lesions.

Despite the reduced activations in the monkeys with amygdala lesions, we found an interesting pattern across animals, consistent with our anatomical evaluation of spared amygdala tissue (Fig. 3). In monkey C we found bilateral activation in the anterior part of the amygdala (AP ≥ +19 mm), whereas in the posterior part of the amygdala (AP < +19 mm), we found activation only within the right hemisphere. In contrast, in monkey M we found activation only in the anterior part of the amygdala within the left hemisphere, whereas we found bilateral activation in the posterior part of the amygdala. Importantly, as in controls, the responses to faces within the spared amygdala in the operated animals were sensitive to emotional expressions; that is, emotional faces elicited greater activation than neutral faces. Of the emotional expressions, the fear grin consistently elicited the greatest response ($P < 0.05$, Fig. 4*B*). The other expressions did not modulate amygdala activity in any of the operated animals.

Amygdala Lesions Do Not Alter Face Responsivity or Face Selectivity Within the IT Cortex. We first evaluated the impact of amygdala lesions on face responsivity (neutral faces > scrambled faces) and face selectivity (neutral faces > non-face objects) within the IT cortex. Interestingly, the profile of activations found in control animals also was present in the monkeys with amygdala lesions, independent of the extent of the lesion. Face responses were distributed within the temporal cortex. As in control animals, all three operated monkeys also showed two face-selective regions (neutral faces > non-face objects) bilaterally within the IT cortex: an anterior face-selective region within area TE (AP ~ +18 mm) and a posterior face-selective region within TEO (AP ~ +6 mm). In monkey M, with a unilateral right amygdala lesion, the face-selective regions in the two hemispheres were comparable, both in extent and location, as shown in Fig. 5. Taken together, these data indicate that amygdala lesions did not alter face processing per se in the IT cortex.

Amygdala Lesions Alter the Processing of Facial Expressions Within the IT Cortex. To assess the impact of amygdala lesions on the processing of facial expressions within the IT cortex, we evaluated valence effects within each face-selective region (anterior and posterior) in each hemisphere for the three animals with amygdala lesions (Fig. 6). The goal here was twofold: (*i*) to compare the valence effects within the IT cortex of these animals relative to control animals, and (*ii*) to compare the valence effects in the two hemispheres of each monkey with different amounts of amygdala sparing.

When valence effects were observed in the operated group, as in control animals fear-grin expressions consistently elicited enhanced responses compared with neutral faces. The result was a significant difference between the effects of neutral and fear-grin expressions ($P < 0.001$). There were no group interactions; thus no difference in the magnitude of the response modulation was demonstrated between groups. One notable difference between groups was in the response to images of faces with lip-smack expressions. Although all six hemispheres in control animals showed an enhanced fMRI response to lip-smack as compared with neutral faces, none of the five hemispheres with amygdala lesions showed any significant difference in response to these facial expressions. Also, among the operated animals, in only one hemisphere (in monkey P; $P < 0.001$) was the activation in response to aggressive faces (open-mouthed

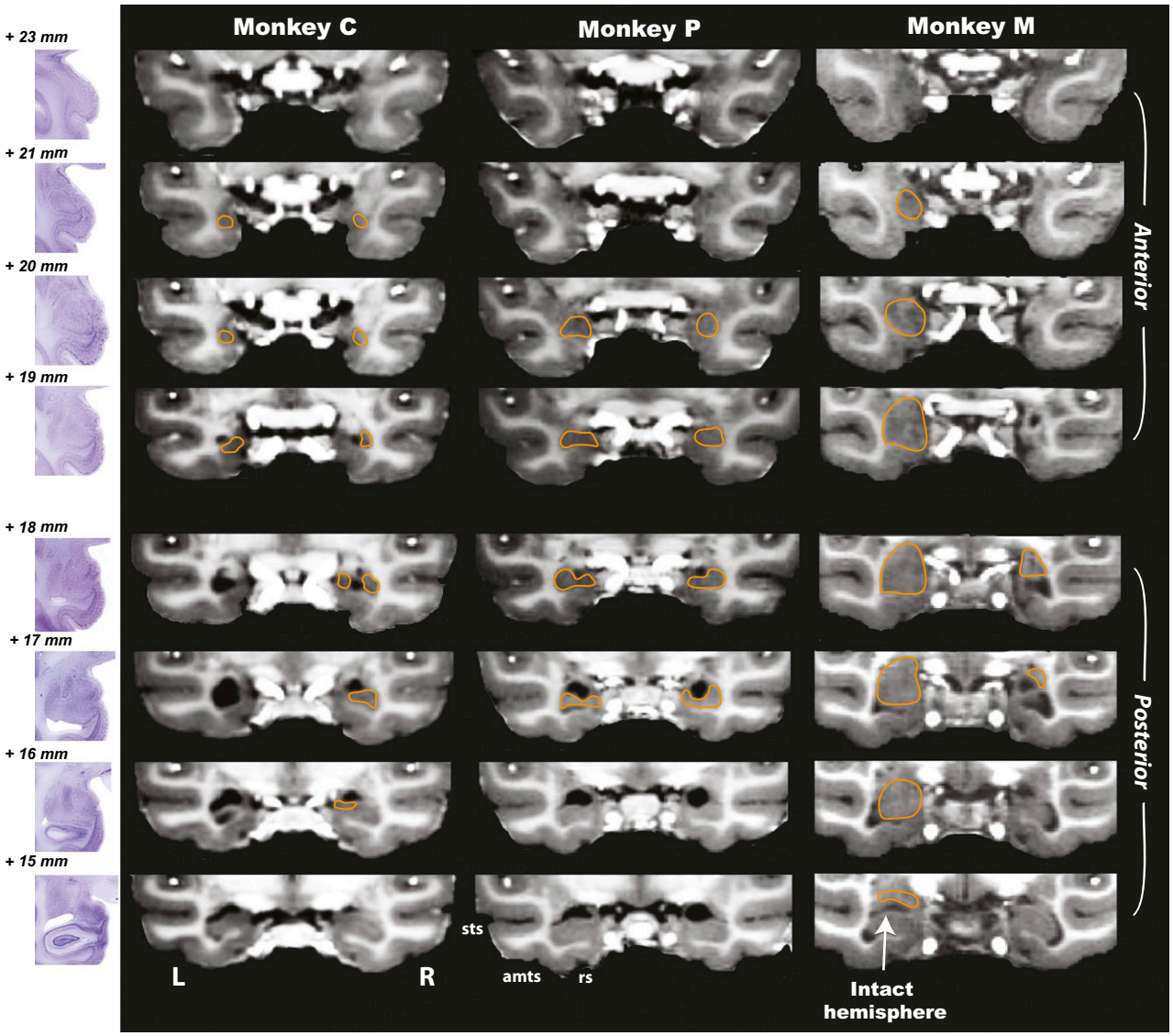

Fig. 3. The extent of amygdala lesions along the anterior-to-posterior axis is shown on coronal sections for the three operated animals. The orange outlines represent the estimated amount of sparing within each hemisphere based on the examination of MR scans performed postsurgery and used to calculate the volume of the spared tissue (also see Table 1 for the extent of spared amygdala tissue expressed as a function of the mean amygdala volume in controls). For illustrative purposes, we have reproduced the outlines using a heavier line weight. On the left are images of Nissl-stained histological sections through the monkey amygdala, along the anterior-to-posterior axis [+23 mm to +15 mm, according to a standard monkey brain atlas (31)]. In monkeys C and P, the lesions were bilateral, as intended. In monkey P, the lesions were symmetrical; in monkey C, there was bilateral sparing in the anterior portion of the amygdala (AP $\geq$ +19 mm) but unilateral sparing in the posterior portion of the amygdala (AP < +19 mm) in the right hemisphere only. In contrast, in monkey M, the left hemisphere was left intact, but in the right hemisphere only the posterior portion of the amygdala (AP < +19 mm) was spared. amts, anterior middle temporal sulcus; rs, rhinal sulcus; sts, superior temporal sulcus.

threat) significantly greater than the activation in response to neutral faces; in control monkeys, greater activation in response to aggressive faces was seen in four hemispheres.

Serendipitously, as a consequence of spared amygdala tissue in monkeys C and M, we were able to reveal an interesting spatial distribution for the effects. We ran a repeated-measures ANOVA using the selected regions of interest (ROIs), including the anterior and posterior face-selective regions for both hemispheres, with the various expressions and tested for any differences across ROIs and hemispheres. The results of this analysis yielded a significant interaction between hemispheres and valence effects for fear-grin expressions ($P < 0.001$). In monkey C, there was bilateral activation in response to faces in the anterior part of the amygdala, but the posterior part of the amygdala was activated only within the right hemisphere. As shown in Fig. 6, we found no difference in the response profile for the various facial expressions in the anterior face-selective regions in the two hemispheres, but in the posterior face-selective regions only the right hemisphere showed a significant valence effect. Within the left posterior face-selective region, no significant difference was ob-

Table 1. Extent of amygdala sparing

Case	Left hemisphere (%)	Right hemisphere (%)
Monkey C	5	35
Monkey P	27	25
Monkey M	100	6

Extent of spared amygdala tissue in the three monkeys with amygdala lesions, expressed as a function of the amygdala volume mean in controls.

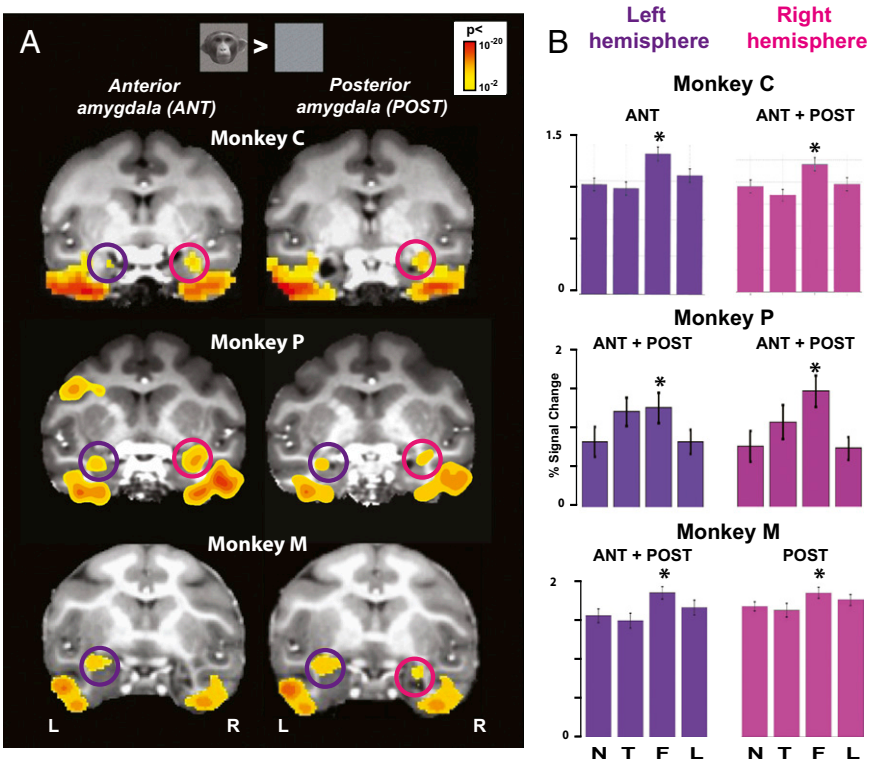

Fig. 4. Face-responsive regions within the spared amygdala tissue in three operated animals. (*A*) Coronal sections illustrating face responses (neutral faces > scrambled faces) through the anterior (AP ≥ +19 mm) and posterior amygdala (AP < +19 mm). In monkey C, we found face responses bilaterally within the anterior portion of the amygdala but in only the right of the posterior portion of the amygdala. In monkey P, we found face responses bilaterally within both the anterior and posterior portions of the amygdala. In contrast, in monkey M, we found face responses bilaterally within the posterior portion of the amygdala but in only the left of the anterior amygdala. (*B*) Profiles of responses to the various facial expressions within the right and left spared amygdala tissue in the three operated animals. In the spared amygdala tissue of the three animals, fearful faces consistently elicited significantly greater activation than neutral faces, as indicated by asterisks ($P < 0.05$). ANT, anterior; POST, posterior; N, neutral; T, threat; F, fear grin; L, lip smack.

served between the various facial expressions tested. In contrast, in monkey M there was unilateral activation in the anterior part of the amygdala in the left hemisphere, but the activation was bilateral in the posterior part of the amygdala. Here, we observed a significant valence effect bilaterally in the posterior face-selective regions, but in the left hemisphere the difference in valence effects was significant only within the anterior face-selective regions. Note that the amygdala tissue left intact in monkey P was almost symmetrical. Accordingly, we did not observe any difference in the valence effects for fear-grin expressions between hemispheres for this monkey. Nevertheless, in this monkey, we observed a marginal difference across hemispheres in the activation in response to aggressive faces ($P < 0.05$).

Taken together these results suggest an anterior-to-posterior organization of the amygdala's feedback projections to the visual cortex, so that valence effects are disrupted in the anterior IT cortex if the anterior amygdala is damaged and are disrupted in the posterior IT cortex if the posterior amygdala is damaged.

Discussion

We combined lesions and high-resolution fMRI in monkeys to demonstrate the role of the amygdala in modulating the processing of facial expressions in the IT cortex. We found that selective excitotoxic lesions of the amygdala do not affect face responsivity or face selectivity within the IT cortex but do disrupt the processing of facial expressions of emotion. This latter effect apparently is organized along an anterior-to-posterior axis, so that lesions to the anterior part of the amygdala disrupt valence effects in the anterior part of the IT cortex, and lesions of the posterior part of the amygdala disrupt valence effects in the posterior part of the IT cortex. Taken together, these data demonstrate that the amygdala is a source of the modulation of activation evoked by facial expressions in the IT cortex.

Disruption of the Valence Effects Within the IT Cortex Following Selective Lesion of the Amygdala. In our study, two groups of monkeys, normal controls and animals with selective amygdala lesions, passively fixated on images of monkey faces with various facial expressions. Face-responsive and face-selective maps (neutral faces > scrambled images and neutral faces > non-face objects) were similar in both groups of monkeys, suggesting that amygdala lesions did not affect the processing of neutral faces per se. Consistent with this result, previous studies have reported intact face-selective regions in various patients with amygdala dysfunction (26). Rather, it has been suggested that amygdala lesions specifically affect the processing and recognition of the facial expressions of emotion (2, 34–36). Here, we show that selective lesions of the amygdala greatly disrupt the modulation of activation by facial expressions of emotion, thus supporting a causal relationship between the amygdala and the modulation of activation evoked by facial expressions (valence effects) seen in areas of the IT cortex involved in face processing.

We previously have shown that the profile of responses to different facial expressions varies across animals, perhaps in relation to their position in the social hierarchy (16). However, we found that responses to fear-grin expressions were the most reliably enhanced responses in all monkeys. In the present study, fear-grin and lip-smack expressions yielded greater activation than neutral faces in all normal controls (12/12 IT ROIs; Fig. 1). Threat expressions modulated face-evoked responses in two animals

Table 2. Extent of amygdala activation

Case	Left hemisphere (mm^3)	Right hemisphere (mm^3)
Intact hemispheres		
Monkey K	46	44
Monkey I	40	43
Monkey T	36	33
Monkey M	31	—
Mean ± SEM	38 ± 3	40 ± 3
Ablated hemispheres		
Monkey C	4	15
Monkey P	4	10
Monkey M	—	4
Mean ± SEM	4 ± 0	10 ± 3

Volume of activated amygdala tissue in response to neutral faces ($P < 0.05$ corrected) in the three controls and the three monkeys with amygdala lesions, expressed as a function of the total amygdala volume (in mm^3).

(5/12 IT ROIs). In contrast, when a valence effect was found in animals with amygdala lesions, (10/12 IT ROIs; Fig. 6), only fear-grin expressions systematically modulated face-evoked responses (10/12 IT ROIs); lip-smack expressions did not modulate these responses (0/12 IT ROIs), and threat expressions modulated these responses in only one animal, monkey P (2/12 IT ROIs).

Based on these results, it is possible that there is a graded relationship between the extent of amygdala damage and the modulation of activation in the IT cortex in response to facial expression, such that restricted damage to the amygdala can disrupt the processing of threat and affiliative faces, but more extensive damage is required to disrupt the processing of fear expressions. This possibility is interesting, particularly given the literature suggesting a tight link between fear and the amygdala (37), a notion that later was extended to include any stimulus or situation that is unpredictable, novel, and/or ambiguous (34, 38–41). In line with the proposed graded relationship between the extent of amygdala damage and the modulation of activation in the IT cortex in response to facial expression, it is possible that the unilateral amygdala lesion in monkey M was sufficient to disrupt the processing of threat and affiliative faces in both hemispheres. However, this conclusion is not consistent with the results reported by Vuilleumier et al. (26), which showed that a unilateral lesion of the amygdala and the hippocampus affected the modulation of activity only in the temporal cortex of the ipsilateral hemisphere. It is equally possible that the difference between the intact hemisphere of monkey M and the intact hemispheres of all controls reflects individual variations. A comparison of the effects of facial expressions on activity in the IT cortex of the same animal before and after amygdala lesion could help clarify this issue.

As mentioned earlier, the only other study that to our knowledge directly tested the hypothesis that the amygdala modulates activations in the visual cortex in response to facial expressions of emotion was that of Vuilleumier et al. (26). They compared patients with sclerotic damage to both the amygdala and hippocampus (AH group) or with sclerotic damage limited to the hippocampus, sparing the amygdala, (H group) with normal volunteers. They used an event-related fMRI paradigm, in which the subjects were asked to judge whether two faces had the same or different emotional content. They showed that valence effects in the temporal cortex were disrupted only in the AH group. Based on these results, they concluded that the amygdala was the source of the modulation of activity in the temporal cortex by facial expressions. As in our study, those authors showed that the lesions affected responses to facial expressions. However, in their study, unlike in the current study, the lesions were not limited to the amygdala but also included the hippocampus. This difference is important, because the hippocampus has been shown to play a critical role in emotional processes (8, 9, 42–44). For instance, in monkeys, selective lesions of the hippocampus disrupt the expression of innate defensive behaviors. Both the amygdala and the hippocampus receive substantial inputs from the IT cortex, either directly or indirectly, and the hippocampus projects widely into the amygdala (23, 45). Therefore, the disruption of valence effects could have reflected the combination of amygdala and hippocampal damage. Although our data cannot rule out a direct or indirect role of the hippocampus in modulating valence effects in the IT cortex, they provide strong evidence for the key position of the amygdala in the processing of facial expressions. Future studies comparing the effects of selective lesions of the amygdala with those of the hippocampus in modulating valence effects in the IT cortex are necessary to evaluate further any specific contribution of the hippocampus to the processing of facial expressions.

Anterior-to-Posterior Influence of the Amygdala on the Modulation of Valence Effects in the IT Cortex in Response to Facial Expression. Given the asymmetrical pattern of amygdala sparing among our animals and between hemispheres, we were able to characterize further the influence of the amygdala on IT cortex responses. We found that the distribution of the amygdala modulation on the IT cortex followed an anterior-to-posterior gradient, so that damage to the anterior part of the amygdala disrupted modulation of activity in response to facial expression in the anterior part of the IT cortex (which includes the anterior face-selective regions), whereas damage to the posterior part of the amygdala disrupted modulation of activity in response to facial expression in the posterior part of the IT cortex (which includes the posterior face-selective regions).

Neuroanatomical studies in animals have revealed widespread, albeit spatially organized, feedback projections from the amygdala

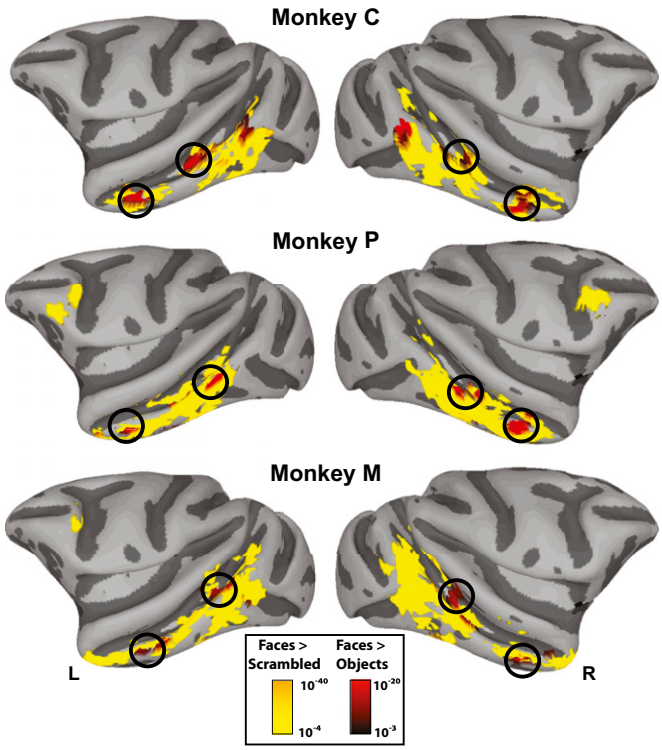

Fig. 5. Face-responsive (neutral faces > scrambled faces) regions (yellow) and face-selective (neutral faces > non-face) regions (red) in the animals with amygdala lesions, illustrated on inflated cortical surfaces. As in control animals, face-responsive regions were distributed mainly along the temporal cortex. We identified two bilateral face-selective regions (black circles) within the IT cortex in the three animals with amygdala lesions.

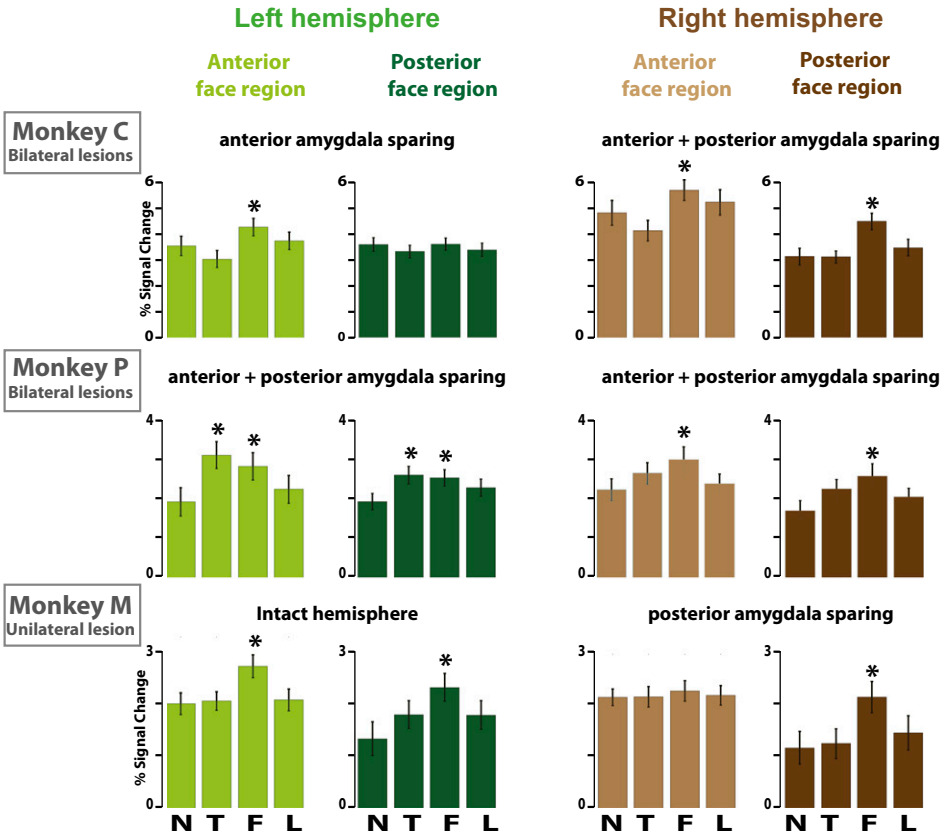

Fig. 6. Profile of responses to the various facial expressions in the anterior and posterior face-selective regions of the IT cortex in the right and left hemispheres of the three operated animals. These data indicate that the amygdala's influence on the responses within the IT cortex follows an anterior-to-posterior gradient. N, neutral; T, threat; F, fear grin, L, lip smack.

to the visual cortex (20–23). These projections follow an anterior-to-posterior gradient, with anterior regions of the amygdala projecting mainly to anterior parts of the IT cortex and posterior regions of the amygdala projecting to more posterior parts of the IT cortex (20, 21, 46). This anatomical organization of the amygdala feedback projection to the visual cortex thus supports our findings.

Based on neural architecture and connectivity, the nuclei in the amygdala can be divided into two main groups (47, 48). The nuclei in the basolateral group (lateral, basal, and accessory basal nuclei) are reciprocally connected with a broad array of cortical areas. The nuclei in the centromedial group (formed in large part by the central and medial nuclei) are reciprocally connected with subcortical structures, although they also receive cortically processed sensory input (49). Following the amygdala lesions, we observed that the temporal horn of the lateral ventricle invaded the space once occupied by the now atrophied amygdala (see also refs. 32 and 33). Based on the location of the amygdala activation in our group of control animals, it is likely that the disruption of valence effects within the IT cortex was a consequence of a lesion that included the basolateral group, which projects back to the entire visual cortex (47).

Despite the extensive damage to the amygdala (i.e., sparing only 5% of amygdala tissue in the left hemisphere in monkey C), the valence effects still were widely distributed within the ipsilateral IT cortex. We cannot rule out the possibility that the spared amygdala tissue influences visual processing indirectly, e.g., via its projections to the basal forebrain, which has widespread projections to the entire cortical mantle, including the visual cortex, (for a review, see ref. 36) or via its projections to the prefrontal cortex, in particular to the orbitofrontal and ventrolateral subdivisions that also project back to the IT cortex (21, 50–53) and are involved in the recognition of emotional expressions (e.g., refs. 54 and 55; for reviews see refs. 56 and 57). Furthermore, it is possible that direct reciprocal connections between the posterior orbitofrontal cortex and the IT cortex (21, 50, 51, 58–60) are responsible for some of the remaining valence effects found in the IT cortex. However, based on the dissociation of the effects of the amygdala lesions along the anterior-to-posterior axis, our data support the idea that direct feedback projections of the amygdala to the IT cortex play a key role in the processing of facial expressions.

In conclusion, we combined selective lesions of the amygdala and fMRI in monkeys and demonstrated a key role for the amygdala's feedback projections to the IT cortex, organized along an anterior-to-posterior gradient, in modulating the responses in the IT cortex to emotional facial expressions. These widespread feedback projections to higher-order visual-processing areas likely enhance sensory processing of biologically important signals, including those related to potential environmental threats and social contexts.

Materials and Methods

Subjects and General Procedures. Six male rhesus monkeys (*Macaca mulatta*; 6–8 kg; three intact controls and three with amygdala lesions) were used in this study. All procedures were in accordance with the *Guide for the Care and Use of Laboratory Animals* and were approved by the National Institute of Mental Health Animal Care and Use Committee (61). Each monkey was surgically implanted with a plastic head post while under anesthesia. After recovery, monkeys were trained to sit in a sphinx position in a plastic restraint barrel (Applied Prototype) with their heads restrained, facing a screen on which visual stimuli were presented. During MR scanning, gaze location was monitored using an infrared pupil-tracking system (ISCAN). Each stimulus was overlaid with a small (0.2°) central fixation point on which the monkeys were required to fixate to receive a liquid reward. To promote long periods of fixation, the frequency of reward delivery increased as the duration of fixation increased (62). We did not find any difference in fixation time for any

monkey across the various conditions tested, nor did we find differences in fixation time between the normal monkeys and those with amygdala lesions.

Stimuli and Task. Stimuli were presented using the Presentation program (Neurobehavioral Systems; www.neurobs.com) and were displayed via a LCD projector (Sharp NoteVision 3) onto a front-projection screen positioned within the magnet bore. All stimuli used in this experiment were identical to the ones used in Hadj-Bouziane et al. (16). Specifically, the stimuli were high-resolution color images of facial expressions displayed by eight unfamiliar macaque monkeys (11° in height and frontal view) (Fig. 1*A*): neutral, aggressive (open-mouthed threat), fearful (fear grin), and appeasing (lip smack) (63). Stimuli from each condition were presented in blocks of 32 s each, interleaved with 20-s blanks (gray background). Each stimulus was presented for 2 s and was viewed twice per block, for a total of 16 images presented per block. The order of blocks was randomized across runs. In separate scan sessions, we mapped the location of face-responsive and face-selective regions in these animals. During these sessions, each block was devoted to one of four visual stimulus categories: neutral faces, non-face objects, places, and Fourier-phase scrambled versions of these stimuli. Each block lasted 40 s, during which 20 images (11° in height) were presented for 2 s, and the blocks were interleaved with 20-s blanks. Using these sessions, we identified brain regions that responded to neutral faces more strongly than to scrambled faces (face-responsive regions) or non-face objects (face-selective regions).

Scanning. Before each scan session, 10–12 mg/kg of an exogenous contrast agent [monocrystalline iron oxide nanocolloid (MION)] was injected into the saphenous vein to increase the contrast/noise ratio and to optimize the localization of fMRI signals (62, 64, 65). Imaging data were collected using a 3T General Electric scanner (GE Healthcare) with an eight-channel receive-only RF coil (RAPID MR International). Functional data were obtained using gradient-recalled echo-planar imaging with sensitivity-encoding acceleration factor = 2, TR = 2 s, TE = 17.9 ms, flip angle = 90°, field of view = 100 mm, matrix = 64 × 64, slice thickness = 1.5 mm, and 27 contiguous coronal slices. The imaging slices covered most of the temporal lobe, including areas TE and TEO, the amygdala, and part of the prefrontal cortex.

In separate scan sessions, high-resolution anatomical scans were obtained from each monkey under anesthesia in a 4.7-T vertical scanner (Biospec 47/60; Bruker). We acquired both T1-weighted (3D modified driven-equilibrium Fourier transform, TR = 13.6 ms; TE = 4.9 ms; flip angle = 14°; voxel size = 0.5 mm isotropic) and T2-weighted scans [2D Rapid Acquisition with Refocused Echoes (RARE), TR = 1 s; TE = 124.8 ms; flip angle = 180°; voxel size = 0.3 × 0.3 × 0.5 mm]. These anatomical scans were used as an underlay for the functional data to create anatomical ROIs and/or to evaluate the extent of the amygdala lesions.

Excitotoxic Amygdala Lesions. A detailed description of the surgical procedure for the amygdala lesions is provided in Izquierdo et al. (7). The lesions were performed under aseptic conditions using stereotaxic injections of ibotenic acid within the amygdala. A total of 1.0 μL ibotenic acid (10–15 mg/mL) was injected at each site at a rate of 0.2 /min. Two animals (monkeys C and P) received bilateral injections into the amygdala in two or three stages. They received injections at a mean of 22 sites per hemisphere (range, 21–25 sites). A third animal, monkey M, received injections in 25 sites in a single stage in the right amygdala only. This third animal allowed us to compare the impact of an amygdala lesion on the processing of facial expressions in the two hemispheres of the same animal.

The extent of the lesion in the amygdala and surrounding tissue was evaluated by an anatomical expert blind to the fMRI results, based on both T1-weighted and T2-weighted scans, acquired 1–5 y after the surgery, at the time the animals were enrolled in the current fMRI experiment. At this time, tissue shrinkage/gliosis had set in fully. Relative to T2-weighted scans, the T1-weighted scans revealed more detailed anatomical landmarks for estimating the contours of spared amygdala tissue. Accordingly, we show representative images from the T1-weighted scans (Fig. 3).

Specifically, the contours of the remaining tissue within the amygdala were drawn, and the percentage of intact tissue (as visible in the scans) was determined relative to the total volume of the amygdala in control animals (Fig. 3 and Table 1). Note that variability in the size of the neurotoxic lesions has been reported in most studies that have used this technique in non-human primates (e.g., see refs. 6 and 66) and that lesion extent evaluated with MR scans is highly correlated with lesion extent as measured from microscopic examination of Nissl-stained material after standard histological processing of the brain (32, 33).

Data Analysis. Functional data were analyzed using Analysis of Functional Images (AFNI) (67). Images were realigned to the first volume of the first session for each subject and were smoothed spatially using a 2-mm full-width half-maximum Gaussian kernel. Signal intensity was normalized to the mean signal value within each run. For the facial expression experiment, an average of 4,224 functional volumes were collected for the three control animals (2,736 for monkey I, 4,674 for monkey K, and 5,264 for monkey T), and an average of 4,750 functional volumes were collected for the three animals with amygdala lesions (4,218 for monkey C, 4,674 for monkey M, and 5,358 for monkey P) across two or three scan sessions. In addition, an average of 2,000 functional volumes was collected for the face localizer for each animal. Data were analyzed using a general linear model and a MION kernel to model the hemodynamic response function (65). The different facial expression conditions were used as regressors of interest. Regressors of no interest included the baseline condition, movement parameters from realignment corrections, and signal drifts (linear as well as quadratic). Note that throughout this paper all fMRI signals have been inverted so that an increase in cerebral blood volume is represented by an increase in signal intensity. A false discovery rate-adjusted P value (or q-value) of $P < 0.001$ was used to create statistical maps for each animal. We then aligned these statistical maps onto a population-average MRI-based atlas for the Rhesus macaque (68). These MRI-based template images were normalized previously to images from the Saleem and Logothetis stereotaxic atlas (31). Thus, our normalization procedure allowed us to project our statistical results into this standardized coordinate space and thereby derive coordinates in a common space. We also were able to project our statistical results onto a rendered and inflated version of a single macaque cortical surface (F99, packaged with CARET), which also was normalized to the standard monkey brain space (31). We used this atlas to evaluate the amount of amygdala tissue spared along an anterior-to-posterior axis. We delineated two parts within the amygdala: anterior and posterior, corresponding to AP ≥ +19 mm and AP ≤ +18 mm, respectively.

Face-responsive and face-selective maps reflected brain regions that responded more strongly to neutral faces than to scrambled faces (face-responsive regions) or non-face objects (face-selective regions), respectively (for more details see ref. 16).

We then defined ROIs within face-selective regions by identifying the peak of activation and drawing a 2-mm sphere around this peak. In addition, we drew anatomical ROIs around the amygdala. Therein we identified the peak of activation using the face-responsive maps and drew a 2-mm sphere around this peak. We extracted the signal in response to the different facial expressions from these ROIs and performed an ANOVA within and across groups, testing for the effect of expression (neutral, threat, fear grin, and lip smack), followed by multiple paired t tests.

ACKNOWLEDGMENTS. We thank Shruti Japee, Ziad Saad, and Gang Chen for help with data analysis; Lucas Glover, Fern Baldwin, and James Ellis for assistance with animal training and scanning; Gary Griffiths for providing the contrast agent, MION; George Dold, David Ide, and Tom Talbot for technical assistance; and Kadharbatcha Saleem for preparing images of Nissl-stained histological sections. This study was supported by the National Institute of Mental Health Intramural Research Program (F.H.-B., N.L., A.H.B., E.A.M., and L.G.U.).

1. Adolphs R, Baron-Cohen S, Tranel D (2002) Impaired recognition of social emotions following amygdala damage. *J Cogn Neurosci* 14(8):1264–1274.
2. Adolphs R, et al. (2005) A mechanism for impaired fear recognition after amygdala damage. *Nature* 433(7021):68–72.
3. Meunier M, Bachevalier J, Murray EA, Málková L, Mishkin M (1999) Effects of aspiration versus neurotoxic lesions of the amygdala on emotional responses in monkeys. *Eur J Neurosci* 11(12):4403–4418.
4. Phelps EA, LeDoux JE (2005) Contributions of the amygdala to emotion processing: From animal models to human behavior. *Neuron* 48(2):175–187.
5. Amaral DG (2003) The amygdala, social behavior, and danger detection. *Ann N Y Acad Sci* 1000:337–347.
6. Emery NJ, et al. (2001) The effects of bilateral lesions of the amygdala on dyadic social interactions in rhesus monkeys (Macaca mulatta). *Behav Neurosci* 115(3):515–544.
7. Izquierdo A, Suda RK, Murray EA (2005) Comparison of the effects of bilateral orbital prefrontal cortex lesions and amygdala lesions on emotional responses in rhesus monkeys. *J Neurosci* 25(37):8534–8542.
8. Machado CJ, Bachevalier J (2006) The impact of selective amygdala, orbital frontal cortex, or hippocampal formation lesions on established social relationships in rhesus monkeys (Macaca mulatta). *Behav Neurosci* 120(4):761–786.
9. Machado CJ, Bachevalier J (2008) Behavioral and hormonal reactivity to threat: Effects of selective amygdala, hippocampal or orbital frontal lesions in monkeys. *Psychoneuroendocrinology* 33(7):926–941.

10. Machado CJ, Kazama AM, Bachevalier J (2009) Impact of amygdala, orbital frontal, or hippocampal lesions on threat avoidance and emotional reactivity in nonhuman primates. *Emotion* 9(2):147–163.
11. Adolphs R, Tranel D, Damasio H, Damasio A (1994) Impaired recognition of emotion in facial expressions following bilateral damage to the human amygdala. *Nature* 372 (6507):669–672.
12. Hamann SB, et al. (1996) Recognizing facial emotion. *Nature* 379(6565):497.
13. Ishai A, Schmidt CF, Boesiger P (2005) Face perception is mediated by a distributed cortical network. *Brain Res Bull* 67(1-2):87–93.
14. Pessoa L, McKenna M, Gutierrez E, Ungerleider LG (2002) Neural processing of emotional faces requires attention. *Proc Natl Acad Sci USA* 99(17):11458–11463.
15. Breiter HC, et al. (1996) Response and habituation of the human amygdala during visual processing of facial expression. *Neuron* 17(5):875–887.
16. Hadj-Bouziane F, Bell AH, Knusten TA, Ungerleider LG, Tootell RB (2008) Perception of emotional expressions is independent of face selectivity in monkey inferior temporal cortex. *Proc Natl Acad Sci USA* 105(14):5591–5596.
17. Hoffman KL, Gothard KM, Schmid MC, Logothetis NK (2007) Facial-expression and gaze-selective responses in the monkey amygdala. *Curr Biol* 17(9):766–772.
18. Vuilleumier P, Armony JL, Driver J, Dolan RJ (2001) Effects of attention and emotion on face processing in the human brain: An event-related fMRI study. *Neuron* 30(3): 829–841.
19. Hasselmo ME, Rolls ET, Baylis GC (1989) The role of expression and identity in the face-selective responses of neurons in the temporal visual cortex of the monkey. *Behav Brain Res* 32(3):203–218.
20. Amaral DG, Behniea H, Kelly JL (2003) Topographic organization of projections from the amygdala to the visual cortex in the macaque monkey. *Neuroscience* 118(4): 1099–1120.
21. Webster MJ, Bachevalier J, Ungerleider LG (1994) Connections of inferior temporal areas TEO and TE with parietal and frontal cortex in macaque monkeys. *Cereb Cortex* 4(5):470–483.
22. Webster MJ, Ungerleider LG, Bachevalier J (1991) Connections of inferior temporal areas TE and TEO with medial temporal-lobe structures in infant and adult monkeys. *J Neurosci* 11(4):1095–1116.
23. Price JL, Amaral DG (1981) An autoradiographic study of the projections of the central nucleus of the monkey amygdala. *J Neurosci* 1(11):1242–1259.
24. Adolphs R, Spezio M (2006) Role of the amygdala in processing visual social stimuli. *Prog Brain Res* 156:363–378.
25. Vuilleumier P, Pourtois G (2007) Distributed and interactive brain mechanisms during emotion face perception: Evidence from functional neuroimaging. *Neuropsychologia* 45(1):174–194.
26. Vuilleumier P, Richardson MP, Armony JL, Driver J, Dolan RJ (2004) Distant influences of amygdala lesion on visual cortical activation during emotional face processing. *Nat Neurosci* 7(11):1271–1278.
27. Bell AH, Hadj-Bouziane F, Frihauf JB, Tootell RB, Ungerleider LG (2009) Object representations in the temporal cortex of monkeys and humans as revealed by functional magnetic resonance imaging. *J Neurophysiol* 101(2):688–700.
28. Nasr S, et al. (2011) Scene-selective cortical regions in human and nonhuman primates. *J Neurosci* 31(39):13771–13785.
29. Pinsk MA, DeSimone K, Moore T, Gross CG, Kastner S (2005) Representations of faces and body parts in macaque temporal cortex: A functional MRI study. *Proc Natl Acad Sci USA* 102(19):6996–7001.
30. Tsao DY, Freiwald WA, Knutsen TA, Mandeville JB, Tootell RB (2003) Faces and objects in macaque cerebral cortex. *Nat Neurosci* 6(9):989–995.
31. Saleem KS, Logothetis NK (2007) *A Combined MRI and Histology Atlas of the Rhesus Monkey Brain in Stereotaxic Coordinates* (Elsevier/Academic Press, San Diego, CA).
32. Málková L, Lex CK, Mishkin M, Saunders RC (2001) MRI-Based evaluation of locus and extent of neurotoxic lesions in monkeys. *Hippocampus* 11(4):361–370.
33. Nemanic S, Alvarado MC, Price RE, Jackson EF, Bachevalier J (2002) Assessment of locus and extent of neurotoxic lesions in monkeys using neuroimaging techniques: A replication. *J Neurosci Methods* 121(2):199–209.
34. Adolphs R (2010) What does the amygdala contribute to social cognition? *Ann N Y Acad Sci* 1191:42–61.
35. Blair RJ (2003) Facial expressions, their communicatory functions and neuro-cognitive substrates. *Philos Trans R Soc Lond B Biol Sci* 358(1431):561–572.
36. Pessoa L (2011) Emotion and cognition and the amygdala: From "what is it?" to "what's to be done?" [reprint]. *Neuropsychologia* 49(4):681–694.
37. LeDoux J (1998) Fear and the brain: Where have we been, and where are we going? *Biol Psychiatry* 44(12):1229–1238.
38. Davis M, Whalen PJ (2001) The amygdala: Vigilance and emotion. *Mol Psychiatry* 6(1): 13–34.
39. Morrison SE, Salzman CD (2010) Re-valuing the amygdala. *Curr Opin Neurobiol* 20(2): 221–230.
40. Murray EA (2007) The amygdala, reward and emotion. *Trends Cogn Sci* 11(11): 489–497.
41. Whalen PJ (2007) The uncertainty of it all. *Trends Cogn Sci* 11(12):499–500.
42. Bachevalier J, Alvarado MC, Malkova L (1999) Memory and socioemotional behavior in monkeys after hippocampal damage incurred in infancy or in adulthood. *Biol Psychiatry* 46(3):329–339.
43. Chudasama Y, Izquierdo A, Murray EA (2009) Distinct contributions of the amygdala and hippocampus to fear expression. *Eur J Neurosci* 30(12):2327–2337.
44. Beauregard M, Malkova L, Bachevalier J (1995) Stereotypies and loss of social affiliation after early hippocampectomy in primates. *Neuroreport* 6(18):2521–2526.
45. Saunders RC, Rosene DL, Van Hoesen GW (1988) Comparison of the efferents of the amygdala and the hippocampal formation in the rhesus monkey: II. Reciprocal and non-reciprocal connections. *J Comp Neurol* 271(2):185–207.
46. Freese JL, Amaral DG (2005) The organization of projections from the amygdala to visual cortical areas TE and V1 in the macaque monkey. *J Comp Neurol* 486(4): 295–317.
47. Amaral DG, Price JL, Pitkänen A, Carmichael ST (1992) Anatomical organization of the primate amygdaloid complex. *The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction*, ed Aggleton JP (Wiley-Liss, New York), pp 1–66.
48. McDonald AJ (1992) Projection neurons of the basolateral amygdala: A correlative Golgi and retrograde tract tracing study. *Brain Res Bull* 28(2):179–185.
49. Amaral DG, Insausti R (1992) Retrograde transport of D-[3H]-aspartate injected into the monkey amygdaloid complex. *Exp Brain Res* 88(2):375–388.
50. Gerbella M, Belmalih A, Borra E, Rozzi S, Luppino G (2010) Cortical connections of the macaque caudal ventrolateral prefrontal areas 45A and 45B. *Cereb Cortex* 20(1): 141–168.
51. Ghashghaei HT, Hilgetag CC, Barbas H (2007) Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage* 34(3):905–923.
52. Petrides M, Pandya DN (2002) Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. *Eur J Neurosci* 16(2):291–310.
53. Amaral DG, Price JL (1984) Amygdalo-cortical projections in the monkey (Macaca fascicularis). *J Comp Neurol* 230(4):465–496.
54. Hornak J, Rolls ET, Wade D (1996) Face and voice expression identification in patients with emotional and behavioural changes following ventral frontal lobe damage. *Neuropsychologia* 34(4):247–261.
55. Shaw P, et al. (2005) Differential effects of lesions of the amygdala and prefrontal cortex on recognizing facial expressions of complex emotions. *J Cogn Neurosci* 17(9): 1410–1419.
56. Zald DH, Andreotti C (2010) Neuropsychological assessment of the orbital and ventromedial prefrontal cortex. *Neuropsychologia* 48(12):3377–3391.
57. Barbas H (2007) Specialized elements of orbitofrontal cortex in primates. *Ann N Y Acad Sci* 1121:10–32.
58. Barbas H, Zikopoulos B (2007) The prefrontal cortex and flexible behavior. *Neuroscientist* 13(5):532–545.
59. Murray EA, Izquierdo A (2007) Orbitofrontal cortex and amygdala contributions to affect and action in primates. *Ann N Y Acad Sci* 1121:273–296.
60. Saleem KS, Kondo H, Price JL (2008) Complementary circuits connecting the orbital and medial prefrontal networks with the temporal, insular, and opercular cortex in the macaque monkey. *J Comp Neurol* 506(4):659–693.
61. Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council (2011) *Guide for the Care and Use of Laboratory Animals, 8th Edition* (National Academies Press, Washington DC).
62. Vanduffel W, et al. (2001) Visual motion processing investigated using contrast agent-enhanced fMRI in awake behaving monkeys. *Neuron* 32(4):565–577.
63. Gothard KM, Erickson CA, Amaral DG (2004) How do rhesus monkeys (Macaca mulatta) scan faces in a visual paired comparison task? *Anim Cogn* 7(1):25–36.
64. Mandeville JB, Marota JJ (1999) Vascular filters of functional MRI: Spatial localization using BOLD and CBV contrast. *Magn Reson Med* 42(3):591–598.
65. Leite FP, et al. (2002) Repeated fMRI using iron oxide contrast agent in awake, behaving macaques at 3 Tesla. *Neuroimage* 16(2):283–294.
66. Murray EA, Mishkin M (1998) Object recognition and location memory in monkeys with excitotoxic lesions of the amygdala and hippocampus. *J Neurosci* 18(16):6568–6582.
67. Cox RW (1996) AFNI: Software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 29(3):162–173.
68. McLaren DG, et al. (2009) A population-average MRI-based atlas collection of the rhesus macaque. *Neuroimage* 45(1):52–59.