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

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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, geometric (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 responsibility (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.

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.


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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. 1B), 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. 1B): (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. 1C 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-

![Diagram](https://www.pnas.org/cgi/doi/10.1073/pnas.1218406109)
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. 4A). 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. 4B). 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
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-

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**Table 1. Extent of amygdala sparing**

<table>
<thead>
<tr>
<th>Case</th>
<th>Left hemisphere (%)</th>
<th>Right hemisphere (%)</th>
</tr>
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<tbody>
<tr>
<td>Monkey C</td>
<td>5</td>
<td>35</td>
</tr>
<tr>
<td>Monkey P</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Monkey M</td>
<td>100</td>
<td>6</td>
</tr>
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</table>

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

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**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 $\geq +19$ 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.
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 to the hippocampus, suggesting 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.

Table 2. Extent of amygdala activation

<table>
<thead>
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<th>Right hemisphere (mm$^3$)</th>
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<td>Monkey K</td>
<td>46</td>
<td>44</td>
</tr>
<tr>
<td>Monkey I</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>Monkey T</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Monkey M</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>38 ± 3</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Ablated hemispheres</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey C</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Monkey P</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Monkey M</td>
<td>—</td>
<td>4</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>4 ± 0</td>
<td>10 ± 3</td>
</tr>
</tbody>
</table>

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–grim 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.

![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.](image-url)
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 subjects and any difference in fixation time for any

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.

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 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.
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. 1A): 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 nanocollod (MION)) was injected into the saphenous vein to increase the contrast-to-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 with a 4.7-T vertical scanner. Images were normalized previously to images from the Saleem and Logothetis stereotactic atlas (31). Thus, our normalization procedure allowed us to project functional images from the T1-weighted scans (Fig. 3). We then delineated ROIs within face-selective regions by identifying the peak of activation in the amygdala. We then 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 the 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. They measured the volume of spared amygdala tissue using 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 template atlas for the Rhesus macaque (68). These MRI-based template images were normalized previously to images from the Saleem and Logothetis stereotactic 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 (99, 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 and two lateral 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. We then 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 pairwise t tests.

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