Mirroring Fear in the Absence of a Functional Amygdala

To the Editor:

From an evolutionary perspective, facial expressions of fear convey highly recognizable emotional signals that serve adaptive functions by promoting survival and reproductive success (1). Current theories of how the brain interprets facial expressions of fear implicate the mirror neuron network (MNN) in echoing the emotional states of others by internal simulation (2,3). Originally discovered in the ventral premotor cortex of macaque monkeys (4,5), mirror neurons are defined as being responsive to observation and execution of the same actions. Evidence that facial emotion recognition in humans is impaired by damage to MNN-associated cortical regions or the amygdala (6) has stimulated the hypothesis that the amygdala forms an integral component of an emotion MNN (7,8). An alternative view holds that the amygdala plays only a supporting role (9), for example, by directing attention to the informative eye region (10), suggesting the emotional MNN could operate independently, without a functional amygdala. To test these hypotheses directly, we studied two 38-year-old female monozygotic twins (patient 1 and patient 2) with equivalent, selective bilateral amygdala calcification damage because of congenital Urbach-Wiethe disease (synonyms hyalinosis cutis et mucosae or lipoid proteinosis; Online Mendelian Inheritance in Man 247100) (11–15) and 16 healthy female control subjects (mean age $\pm$ SD = 35.8 $\pm$ 4.6 years). In previous experiments patient 1, but not patient 2, demonstrated preserved recognition of fearful faces and potentiated responses to them in MNN-associated regions suggesting that the MNN might functionally compensate her amygdala damage (14,15). We therefore predicted that patient 1, but not patient 2, would exhibit fear-specific supranormal MNN responses in a functional magnetic resonance imaging (fMRI) experiment (experiment 1) probing observation and imitation of dynamic face expressions and hand movements. Furthermore, we expected that if the amygdala forms an essential component of an emotional MNN, then patient 2 would exhibit impaired imitation of fearful faces (experiment 2). Both experiments were approved by the Institutional Review Board of the Medical Faculty of the University of Bonn, and all participants provided written informed consent.

In experiment 1, a 2 $\times$ 3 factorial design was used, with task (passively observe or imitate the depicted actions) and gesture (hand movement, fearful, or neutral facial expression) as factors. Stimuli were presented in 27 blocks (nine for each gesture) separated by a fixation cross (16-20 sec) that served as a low-level baseline. Each block contained three video clips (3 sec) of the same category separated by within block-interstimulus intervals of 1–2 sec. The fMRI using blood oxygenation level-dependent contrast was performed on a 3.0 Tesla Siemens Trio MRI System (Siemens, Erlangen, Germany). Standard preprocessing and first-level analyses were carried out using SPM8 (Wellcome Trust Centre for Neuroimaging, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) as described previously (14). The MNN can be mapped by identifying voxels that are active both while observing and executing the three categories of gestures, with the contrast, imitation minus observation, serving as a proxy for brain activity during execution, given that imitation trials required both the observation of the target stimulus and its execution. Therefore, a conjunction analysis using conjunction-null hypothesis for the contrasts, observe greater than baseline and imitate minus observe greater than baseline, was calculated for each gesture (16). To visualize MNN activity (Figure 1A), whole-brain analyses with a significance threshold of $p < .001$, as reported in the literature (17,18), were rendered and plotted on a standard single-subject brain. Two-sample Student t tests (patient 1 vs. controls, patient 2 vs. controls) with pooled estimates of the error variance were computed to compare MNN activity between the patients and controls. Each comparison

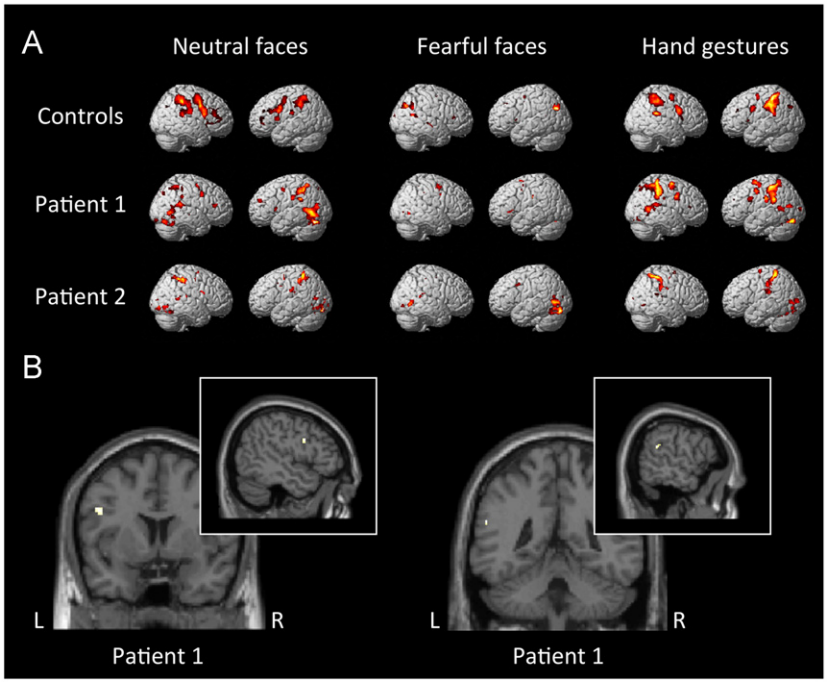

Figure 1. Results of the functional magnetic resonance imaging experiment. **(A)** The conjunction of the contrasts, observe greater than baseline and imitate minus observe greater than baseline, identified a widely distributed mirror neuron network (MNN) response to each gesture. For dynamic face stimuli but not hand movements, MNN activity was also found in the insula and anterior cingulum (whole-brain uncorrected analysis, $p < .001$). **(B)** During passive observation of fearful faces, patient 1 exhibited a larger MNN response than patient 2 in the left inferior frontal operculum (left panel; Montreal Neurological Institute x, y, z peak coordinates: –48, 6, 28, cluster size = 16 voxels, $p < .05$ family-wise error corrected) and superior temporal gyrus (right panel; Montreal Neurological Institute x, y, z peak coordinates: −58, –44, 22, cluster size = 5 voxels, $p < .05$ family-wise error corrected). L, left; R, right.

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Table 1. Compensatory Hyperactivity Measured in the Urbach-Wiethe Patients

Patient	Gesture	Condition	Region	x^T	y^T	z^T	Site	*k*	*p*	*t*	x	y	z
1	Hands	Observe	SPL L	−34	−50	50	IPL L	1	.047	3.9	−40	−46	52
	Fearful	Observe	IFG L	−48	6	28	FITri L	4	.011	4.89	−42	10	26
	Fearful	Observe	SPL L	−34	−50	50	SPL L	23	.009	5.06	−34	−54	58
							IPL L	9	.01	4.98	−40	−46	52
	Hands	Imitate	IFG L	−48	6	28	Prec L	3	.023	4.15	−40	0	34
	Neutral	Imitate	IPL L	−48	−32	38	IPL L	1	.021	4.3	−54	−34	48
	Neutral	Imitate	IPL R	40	−30	40	SMG R	10	.004	5.42	48	−36	44
							PCG R	4	.008	4.9	40	−24	36
			SPL L	−34	−50	50	SPL L	23	.011	4.71	−28	−54	60
2	Hands	Observe	IPL L	−48	−32	38	IPL L	1	3.92	.046	−44	−38	48
	Hands	Observe	SPL L	−34	−50	50	SPL L	9	4.85	.011	−26	−58	48
	Hands	Observe	SPL R	6	−64	56	Prec R	1	3.95	.044	10	−64	64
	Hands	Imitate	STG L	−50	−40	20	STG L	45	.003	5.49	−50	−36	22
	Neutral	limitate	SPL R	6	−64	56	Prec R	2	.048	3.74	10	−64	64
	Neutral	Imitate	STG L	−50	−40	20	STG L	28	.001	6.66	−52	−36	18
	Fearful	Imitate	STG L	−50	−40	20	STG L	5	.033	4.07	−56	−36	20

To compare mirror neuron network (MNN) activity between patients and controls, two-sample Student *t* tests were computed for the six following contrasts: gesture (hands, fearful faces, or neutral faces) observe greater than baseline or gesture (hands, fearful faces, or neutral faces) imitate greater than baseline. Each comparison was FWE corrected for a sphere with a radius of 10 mm at the coordinates of peak MNN activity as reported elsewhere (19). Region, anatomical structure corresponding to the center of the region of interest sphere defined by x^T, y^T, and z^T Talairach coordinates.

FITri, inferior frontal triangular gyrus; FWE; family-wise error corrected for the region of interest; IFG, inferior frontal gyrus; IPL, inferior parietal gyrus; *k*, cluster size; L, left; MNI, Montreal Neurological Institute; PCG, postcentral gyrus; Prec, precuneus; R, right; SMG, supramarginal gyrus; SPL, superior parietal gyrus; STG, superior temporal gyrus; Site, the region and the corresponding x, y, z MNI coordinates of the peak difference between neural response in the patients vs. controls.

was family-wise error (FWE) corrected for a volume of interest defined as a sphere with a radius of 10 mm centered around one of the coordinates of peak MNN activity reported in a recent meta-analysis (19) (Table 1). Our results identified widely distributed MNN responses to each gesture in both patients and controls (Figure 1A). An additional region of interest-based analysis restricted to the bilateral amygdala was performed to the contrast, observe-fearful, yielding bilateral amygdala activation during observation (MNI x, y, z peak coordinates: left amygdala, −20, −6, −12, cluster size = 5 voxels; right amygdala, 24, −4, −12, cluster size = 9; $p < .05$, FWE corrected for the amygdala region of interest), but not during imitation, of fearful faces in controls. None of the patients displayed extraamygdalar hypoactivations, as compared with controls. Patient 1 exhibited supranormal activity during passive viewing of fearful faces in frontoparietal MNN-associated regions (Figure 1A and Table 1). A direct comparison using the contrast, patient 1 (observe-fear greater than observe-neutral) greater than patient 2 (observe-fear greater than observe-neutral), corroborated this finding by identifying greater responses in the left inferior frontal operculum (MNI x, y, z peak coordinates: −48, 6, 28, cluster size = 16 voxels, $p < .05$ FWE corrected) and superior temporal gyrus (MNI x, y, z peak coordinates: −58, −44, 22, cluster size = 5 voxels, $p < .05$ FWE-corrected) of patient 1 (Figure 1B).

In experiment 2, all participants imitated a subset of the stimuli from the scanning session while being videotaped. Twenty-five independent judges (12 males, 13 females; mean age ± SD = 26.1 ± 2.8 years) rated imitation accuracy. Based on their assessments, patients and controls did not differ in their ability to imitate hand gestures and neutral or fearful facial expressions in general nor fear within the eye region specifically (all $p > .15$, one sided). In conclusion, both patients displayed intact fear imitation, showing that the amygdala is not required for an accurate motor imitation of fear. Likewise, the fMRI experiment yielded no differences in their MNN responses during fear imitation.

Taken together, our results suggest that the amygdala does not constitute an essential component of the human MNN. Our finding that patient 1, but not patient 2, displayed supranormal frontoparietal MNN activity while observing dynamic fearful faces is consistent with the hypothesis that the MNN can help compensate for fear recognition deficits because of bilateral amygdala damage (14,15) and provides further support for therapeutic approaches targeting the MNN to help alleviate impaired emotion processing (20).

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