Neural Basis of Maternal Communication and Emotional Expression Processing during Infant Preverbal Stage

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During the first year of life, exchanges and communication between a mother and her infant are exclusively preverbal and are based on the mother's ability to understand her infant's needs and feelings (i.e., empathy) and on imitation of the infant's facial expressions; this promotes a social dialog that influences the development of the infant self. Sixteen mothers underwent functional magnetic resonance imaging while observing and imitating faces of their own child and those of someone else's child. We found that the mirror neuron system, the insula and amygdala were more active during emotional expressions, that this circuit is engaged to a greater extent when interacting with one's own child, and that it is correlated with maternal reflective function (a measure of empathy). We also found, by comparing single emotions with each other, that joy expressions evoked a response mainly in right limbic and paralimbic areas; by contrast, ambiguous expressions elicited a response in left high order cognitive and motor areas, which might reflect cognitive effort.

Keywords: attachment, emotional expressions, empathy, mirror system, mother-child

Introduction

During the first year of life, human infants establish their first affective bond with their mothers. At this time, an infant's basic need is to be protected and cared for by a sensitive caregiver. This experience, involving a relationship to a significant other, is believed to be critical for the development of a child's personality and normal development into adulthood (Bowlby 1958). This relationship is based exclusively on preverbal behavior. A mother's ability to share emotions with the infant and understand the infant's needs and feelings (i.e., empathy) are essential for the building of the attachment bond, which, according to the attachment theory (Bowlby 1958), subsequently determines the nature of the infant's future relationships. According to this theory (Bowlby 1958), maternal empathic ability is closely correlated with *reflective functioning* (Slade et al. 2005): this competence, which is linked to the quality of the mother's attachment experiences (i.e., when she herself was an infant), allows the mother to ascribe the baby mental states (intentions, motivations and feelings) and to interpret them. The infant's attachment behavior develops according to the mother's protection and responsiveness, which are based on the correct interpretation (mediated by the reflective function) of the infant's emotional cues, which are in turn conveyed primarily by affective facial expressions (Bowlby 1988). The infant's facial expressions of emotion,

which are the first regulators of the mother–infant relationship, direct the appropriateness of the mother's responses. Infants are sensitive, from a very early age, to the timing and quality of the mother's affective expressions, a mechanism that mediates reciprocal affective exchanges between mother and child (Tronick and Weinberg 1997). During face-to-face interaction, a highly sensitive mother is inclined to imitate her infant's facial expressions and gestures, thereby demonstrating that she is able to read the infant's feeling state from overt behavior, and promoting a social dialog that shapes the infant's self-development (Emde 1992). The newborn also displays imitative capabilities (Meltzoff and Moore 1977) from the very first days of life, thereby making mother-child reciprocal imitation a very common behavior that facilitates dyadic interactive exchanges (Beebe and Lachmann 1988).

In the last decade, neurophysiological studies have discovered a system associated with action understanding and imitation (Iacoboni et al. 1999). This group of neurons, called *mirror neurons* (MNs), have the capacity to discharge both when performing an action with the hand or the mouth and when observing the same action performed by another individual (Rizzolatti and Craighero 2004). Functional magnetic resonance imaging (fMRI) studies in humans have identified areas with mirror-like properties in the ventral premotor cortex (vPMC)-inferior frontal gyrus (IFG) and the posterior parietal cortex (Iacoboni et al. 1999). This system may, by interacting with the limbic system through the anterior insula, also be also critical for empathy (Carr et al. 2003). Activation of the vPMC-IFG-insula-limbic system (MN-I-Ls) is detected by fMRI in normal volunteers imitating and observing emotional facial expressions (Carr et al. 2003). Activation in MN areas also correlate with empathy in healthy volunteers (Kaplan and Iacoboni 2006). An fMRI study found that this system (especially the IFG) fails to activate in children with autism spectrum disorder, who typically display deficits in both imitation and social communication (Dapretto et al. 2006). The role of the MN-I-Ls in the mother-child relationship has not yet been explored.

Bearing in mind all these findings, we hypothesized that 1) as the MN-I-Ls is critical for the imitation of faces and empathy, emotional expressions, which are facial actions with a high communicative content, should activate this system to a greater extent than neutral ones; 2) according to the attachment theory, a healthy mother should activate the MN-I-Ls greatly when observing or imitating her own child than someone else's child, and 3) as empathy hinges on the reflective function, the MN-I-Ls should be activated to a greater extent in mothers with a higher reflective function.

Moreover, these differential activations are presumably located preponderantly in the right hemisphere, in accordance with the idea that the right hemisphere is involved in emotional processing more than the left hemisphere (Dalgleish 2004).

To test these ideas, we explored brain activity in 16 healthy mothers with a child between 6 and 12 months of age. The mothers were tested during both the imitation and observation of pictures of their own child and of someone else's child with different facial expressions. The variety of expressions that an infant of 6–12 months makes is not wide but is surprisingly well defined. There are several archetypal patterns, including joy, distress, ambiguity and neutrality, that allow a range of appropriate stimuli to be designed (Izard et al. 1983). Affective neuroscience has proposed different theories on anatomical substrates of emotions, the leading one being that discrete emotions are underpinned by relatively separable systems in the brain (Damasio et al. 2000). Neuroimaging studies conducted so far have explored this issue by subtracting one single emotion from another single emotion (almost always a neutral emotion) or within a priori areas (Dalgleish 2004). To better define single emotion substrates, we decided to explore this model using a whole-brain approach, comparing a single emotion with 3 other emotions.

Methods

Subjects
We recruited 16 right-handed mothers, aged from 23 to 42 years (mean 33.7 years) without any psychopathological symptoms and with an adequate ability to represent a child's affective experience (as assessed by the psychodiagnostic screening). The mothers' children were all first-born, nonadopted infants aged between 6 and 12 months (mean 9.5 months; 10 males, 6 females).

Exclusion criteria were 1) history of major medical illnesses, 2) ongoing medical therapy, 3) pregnancy, 4) MRI contraindications. All the participants gave their informed consent and the study was approved by the local Ethics Committee.

Psychodiagnostic Screening and Psychological Testing
The following tests were administered: Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) Axis I Disorder (First et al. 1997), Centre for Epidemiological Studies Depression Scale (Radloff 1997), Symptom Checklist-90-Revised (Derogatis 1977).

To evaluate the quality of their relationship with the child, the mothers underwent the Adult Attachment Interview (AAI) (Main and Goldwyn, 1997), in order to assess maternal reflective function (Fonagy et al. 1998) (see Supplementary materials).

Stimuli
Each baby was videotaped during a face-to-face interaction with the mother and 36 full-face, color pictures, with eye gaze on the center, were selected. Videos were analyzed to define specific affective configurations, according to precise, coded combinations of changes observed in the forehead, nose, and mouth. Four expressions (joy, distress, ambiguous, and neutral) were identified according to the following criteria (Fig. 1) (Oster et al. 1992):

- Joy (J): narrowed eyes, arched eyebrow, widened mouth with corners raised.
- Distress (D): brows drawn together and lowered to create a mid-brow bulge; a deepened naso-labial furrow; tight squeezing of the eye orbit muscles, resulting in a strong squint; widened mouth with corners lowered (Oster et al. 1992).
- Ambiguous (A): blended expression, co-presence of different facial mimic patterns in the upper and lower areas of the face (Izard et al. 1983; Sullivan and Lewis 2003).
- Neutral (N): brows raised slightly and eyes wide open; the mouth is relaxed with semi-opened lips, or (rarely) closed lips; naso-labial folds are absent (Izard et al. 1983; Sullivan and Lewis 2003).

A total of 8 different conditions were thus assessed (8 stimuli or conditions, 8 pictures for each condition): distress own child (Dow), ambiguous own child (Aow), joy own child (Jow), neutral own child (Now), distress other child (Doth), ambiguous other child (Aoth), joy other child (Joth), neutral other child (Noth).

Activation Paradigm
Before scanning, subjects were shown pictures of the unknown child to overcome the novelty effect associated with someone else's child. During scanning, the mothers were asked to perform 2 different tasks, one per session. During each session, they were instructed either to imitate or to observe and empathize with the children (2 sessions per task, counterbalanced within the group: that is, 8 mothers started the experiment with an imitation session, whereas the other 8 mothers started with an observation session).

During each session, 72 pictures were presented in blocks (18 active blocks and 3 rest blocks per session). Each block comprised 4 images of the same child type and expression (one block = one condition) which made 8 conditions in all: Dow, Aow, Jow, Now, Doth, Aoth, Joth, and Not. During each session emotional expression blocks were repeated twice (Dow, Aow, Jow, Doth, Aoth, and Joth) and neutral and rest blocks 3 times (Now, Noth, and R). Both the stimuli and blocks were
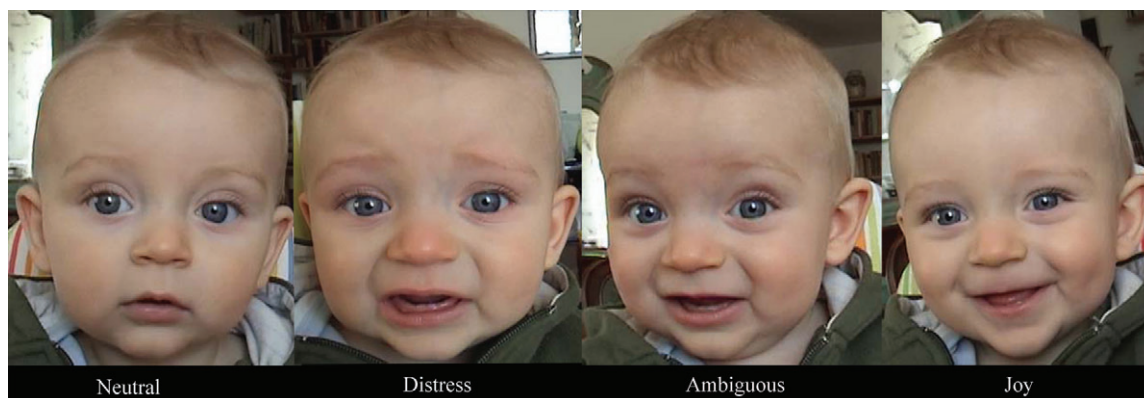
Figure 1. Example of a set of expressions shown to the mothers. Expressions were selected according to Sullivan and Lewis (Oster et al. 1992; Sullivan and Lewis 2003).

randomized in each session. During the rest condition, subjects saw a blank screen and were instructed to keep their eyes open. Stimulus duration was 2000 ms, whereas the interstimulus interval was 2500 ms.

fMRI Data Acquisition

Data were acquired on a Siemens Allegra 3 Tesla head-only scanner. Blood oxygenated level–dependent contrast was obtained using echo planar T_2^*-weighted imaging (WI) according to the following parameters: time repetition (TR) = 2080 ms, time echo (TE) = 30 ms, 32 axial slices, slice thickness = 2.5 mm, gap = 0, voxel size = 3 mm × 3 mm, matrix size 64 × 64, field of view (FOV) = 192 mm. For each run, 190 whole-brain volumes were collected. A high-resolution 3D T_1 WI was also acquired (TR = 2000 ms, TE = 4.38 ms, 128 axial slices, slice thickness = 1 mm, voxel size =1 mm × 1 mm, matrix size = 256 × 256, FOV = 256 mm).

Image Analysis and Statistics

fMRI data were analyzed using SPM2 (Statistical Parametrical Mapping, http://www.fil.ion.ucl.ac.uk). Pre-processing consisted of rigid-body transformation (realignment) and slice timing. The images were normalized to the Montreal Neurological Institute space (affine regularization to the International Consortium for Brain Mapping space template), using the mean of the functional volumes, and then smoothed (Gaussian filter of 8 mm full-width at half maximum). The statistical inference was based on a random effect approach (Holmes and Friston 1998) .

Individual data were then modeled using a boxcar design, convolved with SPM hemodynamic response function. The movement parameters (translation and rotation) resulting from motion correction were included as regressors in our statistical model to account for any residual effects of head motion.

For each subject and task, we calculated the following contrasts: all expressions (all 8 facial expressions vs. rest), emotional expressions versus neutral expression (D\A\J > N) and own child versus other child. Contrasts for each expression for each child were also obtained, that is, Dow, Aow, Jow, Now, Doth, Aoth, Joth, and Noth. All these contrasts were then submitted to a second-level random effect analysis. For each task separately, the single-subject all-expressions contrast images were entered in a one-sample t-test to explore the areas of cerebral activation common to all subjects.

The 8 conditions (Dow, Aow, Jow, Now, Doth, Aoth, Joth, Noth) were entered into an ANOVA (one for each task, within-subjects, SPM2) to obtain the following contrasts: 1) emotional expressions versus neutral expression (D\A\J > N) to explore the structures within the predicted MN-I-Ls related to the processing of emotions and to rule out the effects of viewing faces; 2) own child expressions versus other child expressions [(D\A\J\N)own > (D/A/J/N)other], to investigate any specific effect within the MN-I-Ls of viewing one's own child as opposed to someone else's child; 3) single expression versus all other expressions (D > A\J\N, A > D\J\N, J > D\A\N, N > D\A\J), to investigate the network specifically related to each single expression; 4) interaction on single expression and on emotional expressions by the child type (for example, [D > A\J\N] own > [D > A\J\N] other), to determine whether brain activation could be influenced by the maternal bond.

Second-level data analyses were performed using 2 approaches: 1) an a priori analysis to evaluate activity within the MN-I-Ls in relation to the emotional content of facial expressions and, more importantly, to assess whether this system is influenced by maternal empathy (small volume correction [SVC], regions with the same coordinates as Carr et al.; see methods and Supplementary Material, Table 1) (Carr et al. 2003); 2) a whole-brain analysis to investigate whether different emotions rely on different neural substrates (not an a priori anatomical hypothesis).

Peaks of the a priori analysis (sphere of 8 mm, SVC, SPM2) were positioned on the coordinates reported by Carr et al. (vPMC, IFG-pars opercularis, posterior superior temporal sulcus [STS], inferior parietal lobule [IPL], anterior insula and amygdale; see Supplementary Materials, Table 1) (Carr et al. 2003).

Simple regression analyses were performed between activity during observation and imitation of all expressions (vs. rest) and of emotions

Table 1
Emotions and own child

Contrast	Side	Imitation				Observation			
		x	y	z	t Values	x	y	z	t Values
Emo–neutral									
vPMC	R	52	3	28	3.35	n.s.	n.s.	n.s.	n.s.
	R	48	2	29	2.94	n.s.	n.s.	n.s.	n.s.
IPL	R	n.s.	n.s.	n.s.	n.s.	34	−42	46	3.20
	L	n.s.	n.s.	n.s.	n.s.	−32	−46	43	4.01
Ant insula	R	n.s.	n.s.	n.s.	n.s.	32	26	4	3.34
	L	n.s.	n.s.	n.s.	n.s.	−36	25	2	3.30
STS	R	46	−40	11	3.62	n.s.	n.s.	n.s.	n.s.
	R	50	−42	10	3.33	n.s.	n.s.	n.s.	n.s.
	L	−42	−42	8	2.95	−48	−52	6	2.98
	L	−48	−54	8	2.81	n.s.	n.s.	n.s.	n.s.
Amygdala	R	26	1	−14	5.45	18	−2	−7	4.28
	L	−28	4	−7	5.15	−20	−4	−7	4.04
Own–other									
vPMC	R	n.s.	n.s.	n.s.	n.s.	48	4	33	5.27
	R	n.s.	n.s.	n.s.	n.s.	48	9	22	3.28
	L	n.s.	n.s.	n.s.	n.s.	−46	3	29	3.22
IFG	R	n.s.	n.s.	n.s.	n.s.	50	9	18	3.18
IPL	R	n.s.	n.s.	n.s.	n.s.	34	−48	50	3.59
Ant insula	R	n.s.	n.s.	n.s.	n.s.	40	35	9	2.99
STS	R	n.s.	n.s.	n.s.	n.s.	44	−48	10	2.88

Note: The table shows the significant peaks and t values of areas predicted a priori according to our hypothesis. The results of emotional expressions versus neutral expressions (DAJ > N) and of one's own child versus someone else's child are shown for each task. Peaks are shown in Tailarach coordinates (voxel level corrected, $P < 0.05$ FWE, SVC) (Fig. 2). n.s., not significant; R, right; L, left.

(emotional expressions vs. neutral expression) and the subjects' reflective function scores.

For all the analyses, voxels corrected for multiple comparisons ($P < 0.05$, family-wise error [FWE]) were considered to be significant. Peaks of activity were converted to Talairach space (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach).

Results

Psychological Testing

Psychological testing showed that none of the mothers had any psychiatric disorders. The mean reflective function score was 4 ± 1.02 (range: 3–6), which shows that the mothers had a sufficiently articulated comprehension of their own and of their children's mental states (Ammaniti et al. 1999) (see Methods and Supplementary Materials).

Neuroimaging

fMRI was acquired during imitation and observation of different facial expressions (Joy [J], Distress [D], Ambiguous [A], and Neutral [N]) of the mother's own child and of someone else's child. As described in the Methods, 2 approaches were used: in a priori areas for the exploration of activity within the MN-I-L, and a whole-brain approach for the study of single emotions.

The MN-I-Ls (A Priori Hypothesis)

One-sample t-test revealed that when observing and imitating facial expressions of both children (vs. rest), subjects activated the predicted regions belonging to the MN-I-Ls. These data are consistent with the expectations that the MN-I-Ls is crucial for imitation and empathy (Fig. 2, Supplementary Material, Table 2). ANOVA was then performed, separately for the 2 tasks (imitation and observation), on 8 conditions (2 × 4), that is, 4 different expressions for each child.
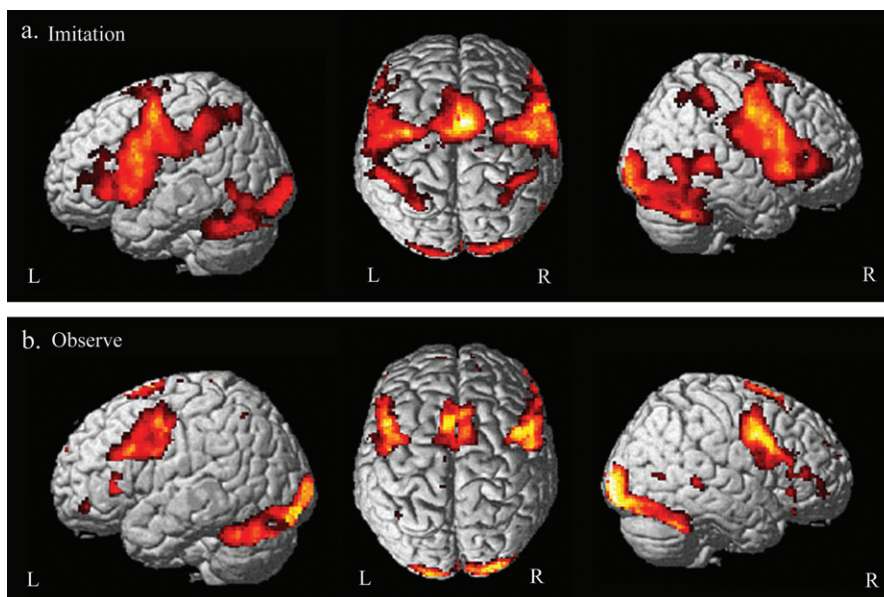
Figure 2. Imitation and observation versus rest. Imitation (*a*): areas activated by the mothers during imitation of all expressions (own child and other child) versus rest (*t*-test). Observation (*b*): areas activated in mothers during observation of all expressions (own child and other child) versus rest (*t*-test). For display purposes, activity (*a*, *b*) is shown at the corrected cluster level ($P < 0.0001$). R, right; L, left.

Table 2
Joyous expression

Region	Hemisphere	Talairach coordinates (*x, y, z*)			*t* Values
STG	R	59	−12	−1	4.96
MTG	R	48	−9	−15	5.20
ITG	R	55	−21	−21	4.91
	L	−52	−17	−19	5.49
TP	L	−38	14	−28	6.67
	R	44	14	−37	5.88
Fusiform gyrus	R	38	−4	−27	5.88
Insula	R	28	−25	14	5.31
Hippocampus	L	−35	−32	−2	6.06
	R	38	−24	−11	5.31
Amygdala	L	−30	−5	−18	5.14
	R	20	−2	−7	5.13
Thalamus	R	22	−33	9	5.51
Putamen	L	−22	−9	12	5.86
Caudate	R	22	−1	15	5.26

Note: Imitation: joyous stimuli. The table shows areas that reveal greater activation during happy faces than during other expressions (J > D/A/N) (whole-brain analysis, voxel level corrected, $P < 0.05$ FWE) (Fig. 5). R, right; L, left.

The results show that imitation of emotional expressions (J/D/A > N) significantly activated the MN-I-Ls bilaterally (prevalently the right): the R vPMC, and the STS and amygdala bilaterally (Table 1). No differences in brain activity were detected either between imitation of the mothers' own child and that of someone else's child, or in the interaction between child type and emotional expressions.

As we had predicted, the MN-I-Ls was also activated to a greater extent when mothers observed emotional expressions as opposed to neutral expressions (J/D/A > N). In particular, we found increased activity in the L STS and in the IPL and amygdala bilaterally (Table 1).

Furthermore, observation of one's owns child as opposed to someone else's child evoked a greater lateralized response in the bilateral vPMC and in the R IFG, R IPL, R STS, and R anterior insula (Table 1, Fig. 3). No interaction was found between activity during emotional expressions and child type.

It is noteworthy that simple regression analysis revealed a positive correlation between activity in the R anterior insula during observation of all faces (vs. rest) and reflective function ($P < 0.003$, Fig. 4), which supports the hypothesis that the MN-I-Ls is activated to a greater extent in mothers with greater reflective function (empathy).

Single Emotions (Whole-Brain Analysis)

ANOVA revealed that imitation of joyous (J > D\A\N) expressions activated a network that is distinct from that associated with other facial expressions. Mothers imitating children with a happy expression prevalently activated the right hemisphere, particularly the temporal cortex (temporal pole [TP], anterior part of the inferior, middle, and superior temporal gyrus [ITG, MTG, STG], hippocampus, insula, and fusiform gyrus), as well as the R thalamus, and the amygdala and basal ganglia bilaterally (Table 2, Fig. 5). The interaction analysis showed that this effect was independent of the child type. Imitation of each of the other expressions, when compared with all the others (D > J/N/A, A > J/N/A), did not reveal any significant cerebral activation.

ANOVA also yielded a significant pattern of cerebral activation during observation of ambiguous expressions (A > D/J/N) when compared with all the others. Observation of ambiguous faces significantly activated brain areas located in the frontal (L anterior middle frontal gyrus [MFG], L presupplementary motor area [pre-SMA], and R anterior cingulum) and parietal (L precuneus) cortices (Table 3, Fig. 6). This effect also was independent of the child type, as demonstrated by the absence of any interaction between child type and ambiguous facial expressions. Observation of each of the other expressions, when compared with all the others (D > J/N/A, J > N/A/D), did not reveal any significant cerebral activation.

R Premotor cortex (48, 0, 36)
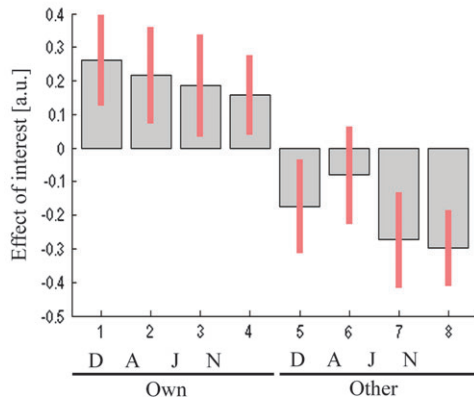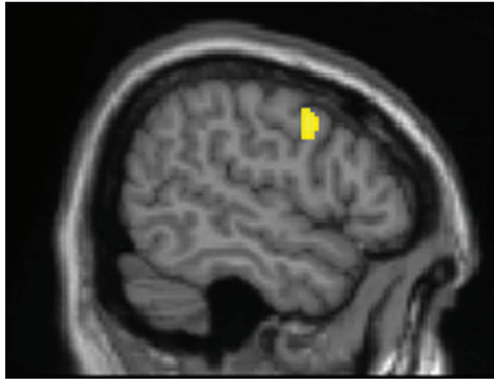
Figure 3. Own child versus other child. Observing, own child > other child: the figure shows the right vPMC, which is one of the areas activated more during observation of the mother's own child than during that of someone else's child (Dow/Aow/Jow/Now > Dot/Aot/Jot/Not) (SVC, voxel level corrected, FWE, $t > 5.68$). The plots show the mean effects in all 8 conditions. Tailarach coordinates are shown in brackets. See Results and Table 1 for other areas that are significantly more active during observation of the mother's own child. a.u. = arbitrary units, 90% confidence interval; D = distress; A = ambiguous; J = Joy; N = neutral; R = right.
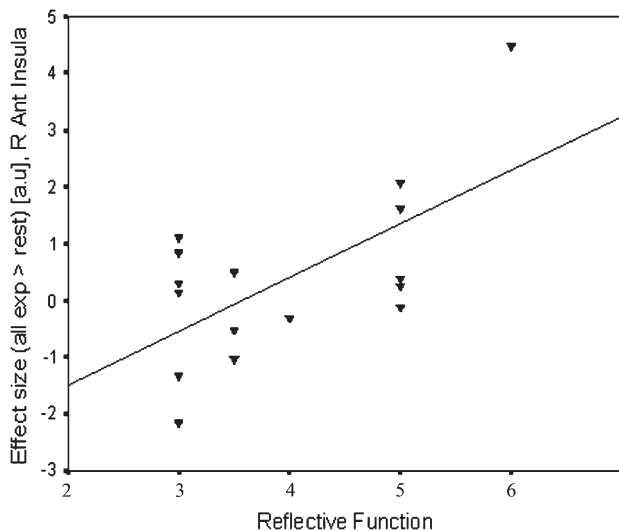
Figure 4. Correlation with reflective function. Positive correlation was found between activity in the R anterior insula during observation of all expressions (vs. rest) and scores on the Reflective Functioning Scale (x, y, z: 30, 30, 12; SVC, FWE corrected $P < 0.003$; Fig. 4). a.u. = arbitrary units, 90% confidence interval.

Discussion

This study addressed the issue of maternal imitation and empathy in the preverbal period, before language becomes a major communicative tool. The activation of the maternal MN-I-Ls by emotional expressions revealed by our results supports our hypothesis that, as this system is critical for action representation and understanding, it is activated to a greater extent by expressions demanding mimicry which serve a social goal (i.e., the transmission of an emotion). Furthermore, this system is also more active when a mother observes and empathizes with her own (as opposed to someone else's) child and is a function of maternal reflective function. This finding may be explained by the greater effort a mother makes to understand her own child's emotions, a proposal that is perfectly in keeping with the attachment theory (Bowlby 1958). Single emotion exploration also yielded clearly separate neural substrates for the joyous and ambiguous expressions. Nicely, imitation of happiness prevalently activated right

subcortical and paralimbic temporal areas, whereas the observation of ambiguous ones activated high order cognitive and motor areas. These findings support the theory that different emotions, which arouse very different feelings, must have, at least in part, a separate neural basis.

The MN-I-Ls

As predicted, imitation and observation of facial expressions elicited activation of fronto-parietal mirror areas (vPMC-IFG-pars opercularis and IPL), STS, anterior insula, and amygdala. According to a model based on anatomical and functional studies on imitation and empathy, the MNs codes the goal of the action (Iacoboni et al. 1999; Rizzolatti et al. 2001) and reproduces it, whereas the anterior insula sends this information to the limbic system to give the action (in our case an expression) an emotional content (Gallese et al. 2004). Therefore, our results are in keeping with the *simulation theory* (or motor theory of empathy), according to which empathy is generated by inner imitation of actions of others (Gallese and Goldman 1998).

Our first goal was to evaluate whether the MN-I-Ls is more active during emotional expressions than neutral expressions. Data show that emotional expressions markedly activate the large-scale network composed of the MNs, anterior insula and the amygdala. Imitation, in particular, elicited activity in the R vPMC and in the bilateral STS and amygdala. Observation of emotional stimuli as opposed to neutral ones also elicited greater activity in the system we had predicted: L STS, R IPL, bilateral anterior insula and amygdala. The greater activity observed in the MNs and insula in the imitation task may also be explained by the fact that emotional expressions require active imitation whereas neutral expression do not (or at least to a far lesser extent). Moreover, results were similar in the observation task. This partial overlapping of activations (both in the task requiring imitation and in the task requiring no movement) suggests that emotional expressions tend to activate the MNs-I-Ls more than neutral expressions. This increased activity may be explained by the goal contained in the emotional expressions, that is, "the action" required to create an expression is aimed at conveying emotional content to obtain a reaction from the external world.
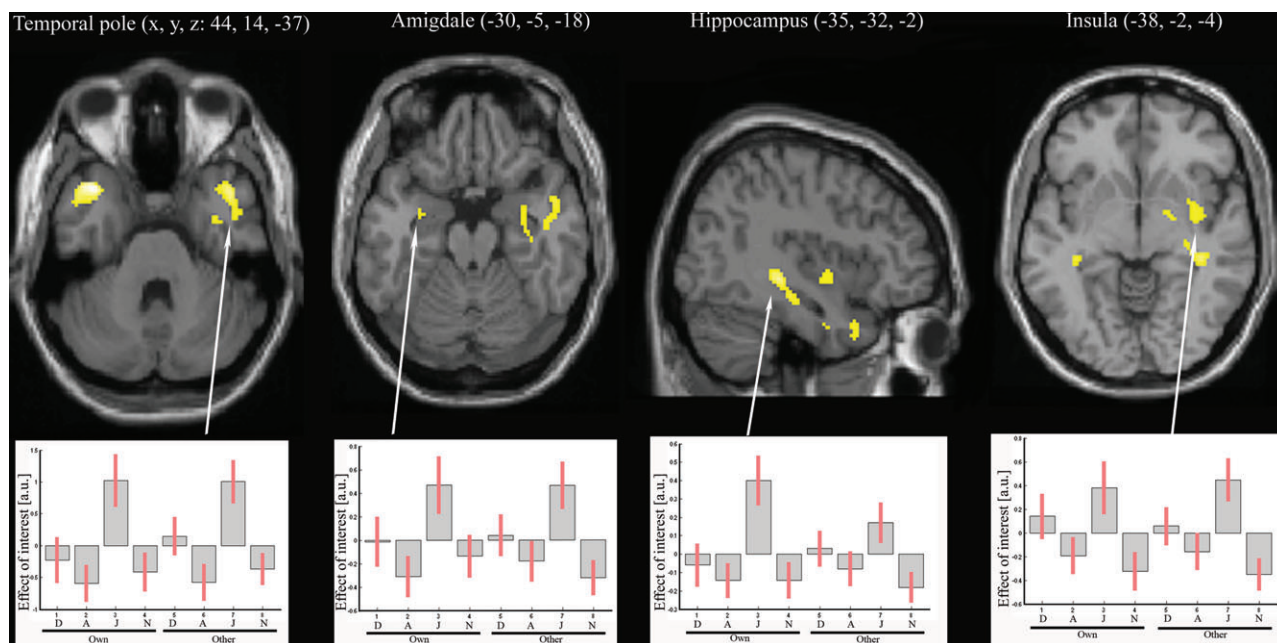
Figure 5. Joyous expression. Imitation, joyous stimuli: the figures show areas more active during joyous expressions than during the other expressions (J > D/J/N) (whole-brain analysis, voxel level corrected, FWE): bilateral polar temporal lobe; bilateral amygdale; right hippocampus; right insula. The plots of the effects of interests of these areas are also shown: imitating joyous expressions increased activations in these areas both in imitation of the mother's own child and in that of someone else's child (whole-brain analysis, voxel level corrected, FWE). Activation maps are shown at the cluster level (*P* uncorrected = 0.0001) for display purposes. Tailarach coordinates are shown in brackets. a.u. = arbitrary units, 90% confidence interval; D = distress; A = ambiguous; J = Joy; N = neutral.

Table 3
Ambiguous expression

Region	Hemisphere	Talairach coordinates (*x*, *y*, *z*)			*t* Values
Precuneus	L	−8	−62	53	5.21
Anterior Cingulum	R	8	27	34	5.16
MFG	L	−28	51	1	5.04
Pre-SMA	L	−2	18	41	4.93

Note: Observation: ambiguous stimuli. The table shows areas that reveal greater activity during the observation of ambiguous stimuli than during the other expressions (A > J/D/N) (whole-brain analysis, voxel level corrected, *P* < 0.05 FWE) (Fig. 6). R, right; L, left.

When looking at differences in brain activity between observation of one's own child and that of someone else's child, we found that the right MN-I-Ls is considerably more activated by the former. Observing pictures of one's own child elicited greater activity in the bilateral (R > L) vPMC, and in the R IFG pars opercularis, R IPL, R STS, and R anterior insula (see Table 1 and Fig. 3).

These data show how healthy mothers, according to the attachment theory, make a greater effort to try to understand the emotions of their own child, as opposed to someone else's, to be able to successfully respond to the child's needs and to promote their survival. Increased activity in the MNs may also be due to an effect of motor familiarity of the observed expression. A study by Calvo-Merino and colleagues recently found that we understand actions not only by visual recognition but also motorically, through the MNs (Calvo-Merino et al. 2006). Gobbini et al. (2004), in another study, focused on the effect of face familiarity during observation of adult neutral expressions. They found activity in the cingulated and paracingulate cortex, though not in regions belonging to the MN-I-Ls.

During the imitation task, we found no differences in brain activity between a mother's own child and someone else's child. This finding suggests that because imitating saturates the neural systems of action representation and emotional processing, no differences between these 2 conditions emerge from the analysis (a ceiling effect). Imitation may be a preferential and more natural way through which areas related to emotional processing are activated. This is suggested by the significance of mirror and limbic activations during imitation compared with observation (Supplementary Table 2; see also Carr et al. 2003).

Other fMRI studies have addressed the issue of maternal attachment during observation of photos/videos of their own child and of someone else's child (Bartels and Zeki 2004; Leibenluft et al. 2004; Nitschke et al. 2004; Ranote et al. 2004). Leibenluft and colleagues (Leibenluft et al. 2004) studied 7 mothers during a one-back repetition task and also found that viewing one's own child was associated with greater activity in different areas, including the PMC, IFG, STS, insula, and amygdala. Nitschke et al. (2004), who studied 6 mothers with children of approximately the same age as ours during the viewing of happy facial expressions, found orbitofrontal activations; however, as their data were analyzed using a different approach (fixed effect), their results cannot be extended to the general population .

Lastly, we found that activity within the MN-I-Ls, that is, the system we explored, is function of maternal reflective function. As hypothesized, we found that activity in the R anterior insula during the observation of child expressions is significantly positively correlated with the mother's capacity to ascribe the baby emotions and to interpret them. This finding strongly suggests that the circuit we explored is critical for empathy (Fig. 5), just as the anterior insula is, according to functional
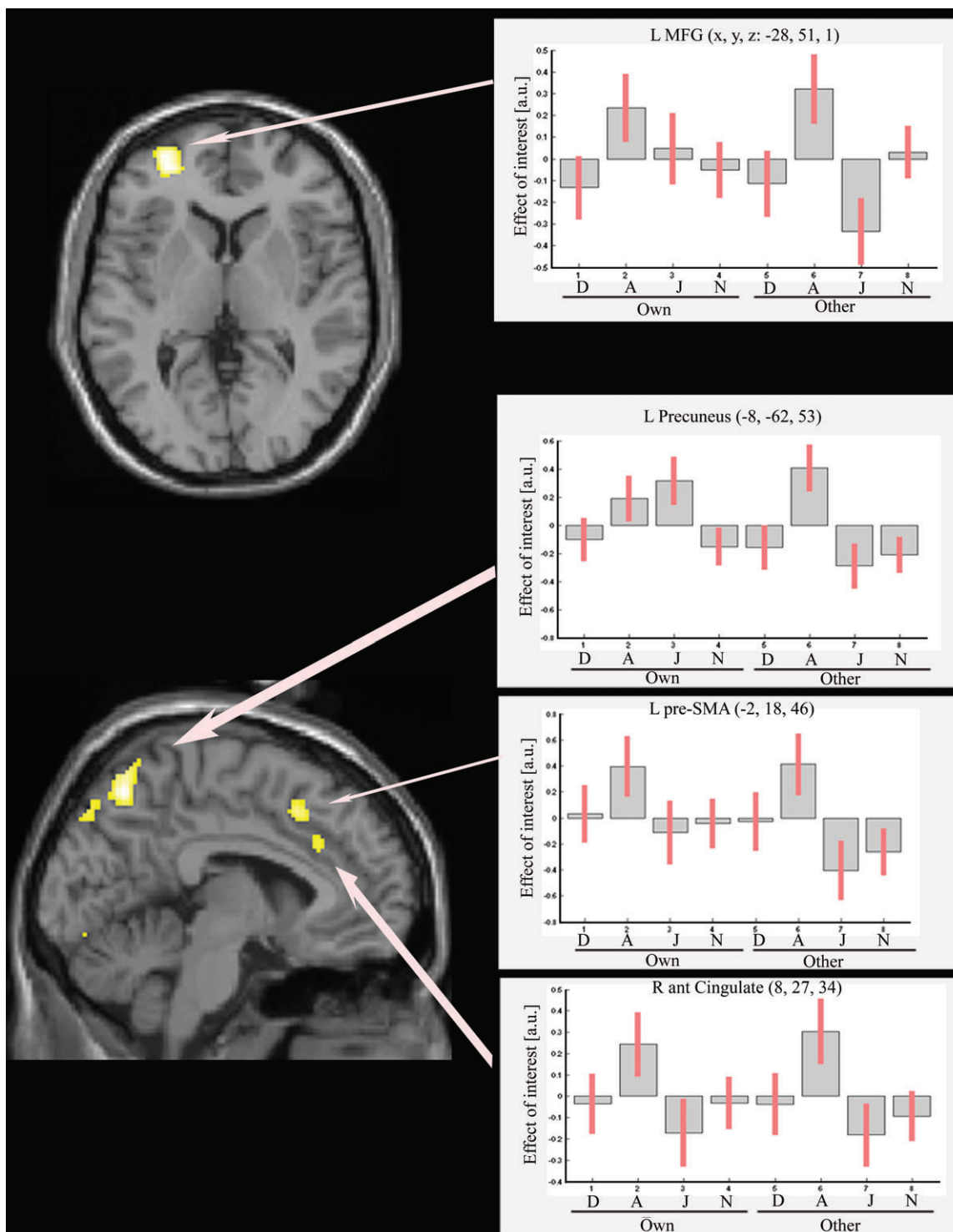
Figure 6. Ambiguous expression. Observation, ambiguous stimuli: areas with increased activity during ambiguous stimuli than during the other expressions (A > JDN) and their corresponding plots in all the 8 conditions. Observation of ambiguous expressions increased activations in the areas shown both in imitation of the mother's own child and in that of someone else's child (whole-brain analysis, voxel level corrected, FWE). Activation maps are shown at the cluster level (P uncorrected = 0.0001) for display purposes. Tailarach coordinates are shown in brackets. a.u. = arbitrary units, 90% confidence interval; D = distress; A = ambiguous; J = Joy; N = neutral; ant = anterior; R = right; L = left.

and anatomical data (Carr et al. 2003), considered to be the relay between action representation (MNs) and emotion processing (limbic system). Moreover, the anterior insula is a center of viscero-motor integration, and is considered to be

the primary cortical area for the interceptive state of the body (Gallese et al. 2004). The increased activity of the anterior insula in more empathic mothers may therefore also represent a greater ability to bodily feel the emotions of others.

Single Emotions

The neural substrate related to the processing of specific emotions was evaluated using a whole-brain approach because no anatomical a priori hypothesis had been formulated.

Our results show that imitation of joyous expressions compared with all other expressions activated certain right subcortical and cortical areas, supporting the theory that the right hemisphere is more involved than the left hemisphere in emotional processing and, thus, mothering (the right-hemisphere hypothesis) (Dalgleish 2004). Subcortical activity was located in the striatum and amygdala bilaterally (R > L) and in the R thalamus.

Given its rich innervation of mesolimbic dopaminergic neurons, the striatum is well positioned to respond to incentive reward motivation and to pre-goal attainment of positive effects arising from progression toward a desired goal (Davidson and Irwin 1999). Activation of the bilateral amygdala may instead be related to a mechanism of maternal reward. Studies have reported amygdala activation during positively-valence and rewarding stimuli (Zald 2003). Smiling back to a child is one of the most positive and common maternal imitative behaviors and is also very rewarding for the mother: when a mother is smiling back to her child, not only is she communicating that she knows what the child is feeling (happiness), but she also feels happy because the child is happy, that is, the process of mothering has been successful.

The thalamus is also part of the limbic system and is closely connected with the amygdala and insula (Morgane et al. 2005). Thalamic activation during emotional stimuli has also frequently been reported to be correlated with positive stimuli (Phan et al. 2002) and seems to be related to visual salience.

At the cortical level, joyous expressions activated numerous temporal areas: the R anterior temporal lobe, TPs bilaterally, R insula, R hippocampus and R fusiform gyrus.

The increased TP (a paralimbic region) activation during the imitation of joyous stimuli may be related to the social and maternal value of imitating a child's smile. Studies on primate and humans have shown that TP pathology causes maternal aberrant behavior and lack of empathy (Carr et al. 2003; Olson et al. 2007).The anterior part of the temporal lobe and the hippocampus (which receives a number of subcortical inputs from the amygdala and thalamus) are critical for mood stability (Phillips et al. 2003), socially appropriate behavior and personality (Glosser et al. 2000). Therefore, these patterns of activity during imitation of joyous facial expressions compared with all others may be due to the great social and maternal worth of the stimuli as well as to the feeling of well-being and reward they engender. All the afore-mentioned cortical and subcortical areas have connections with the anterior insula (Augustine 1996), which can receive information from the MNs (not significantly activated here because they are in common with all other expressions, see Fig. 1), as described by Carr et al. (2003). We found no interaction of child type and joyous expression. We believe this is due to a ceiling effect during imitation and to the mother's increased sensitivity toward children in general, particularly those of the same age as her own child (Henninghausen and Lyons-Ruth 2005).

Studies have investigated the neural circuit of imitation (Lee et al. 2006) and observation (Damasio et al. 2000; Murphy et al. 2003) of happiness by contrasting versus passive viewing of the same stimuli or versus neutral stimuli, and found partly overlapping areas. Differences between results may be accounted for by the fact that we compared imitation of happy stimuli versus imitation of 3 other expressions.

During observation, we found areas that were significantly more active during ambiguous expressions than during other expressions. Activation was located in fronto-parietal areas, prevalently in the left hemisphere, as if the processing of such stimuli depended on activation of emotion-related areas to a lesser extent. Activations were located in the L anterior MFG, the L precuneus, the L pre-SMA, and the R anterior cingulum (Fig. 5).

The anterior MFG (lateral Brodmann area 10 [BA]) and the precuneus (BA 7) have strong reciprocal connections and are activated during higher order cognitive tasks, for example, in response to conflict paradigms (the former) (Badre and Wagner 2004; Gilbert et al. 2006) and during the execution of complex movements and during shifting attention between different locations in space (the latter) (Cavanna and Trimble 2006). The pre-SMA is connected to the precuneus and is involved in high order aspects of motor control, such as internal selection of an aspect of movement (Picard and Strick 1996). The anterior cingulum is related to accurate performance of complex tasks likely to elicit conflict (Kerns et al. 2005).

Greater activity in these areas during ambiguous stimuli may therefore represent cognitive effort. Difficulty in decoding and reproducing complex or conflicting expressions, unlike the cardinal ones of joy, distress or neutral expressions, may explain this result. Activity in the precuneus, in particular, may be due to the repetitive shifting of attention across informative parts of the face (i.e., mouth, nose, and eye) to decode ambiguous expressions.

To our knowledge, this is the first study that has identified areas specific to the processing of infant ambiguous faces. The fact that Nomura and colleagues (Nomura et al. 2003) reported the activation of some of these regions (MFG, pre-SMA, and anterior cingulum) in 8 subjects viewing ambiguous adult facial expressions suggests that the processing of infant and adult ambiguous faces share the same neural substrate.

Conclusions

We addressed the issue of maternal empathy and communicative skills during an infant's first year of life before language develops. We used a study design based on the imitation and observation of the different facial expressions of children. We found that the MNs, the anterior insula and the limbic system are the basis of these mother–infant interactions. This system is activated to a greater extent when the mother is observing her own child than someone else's child, and its activity is function of the mother's capacity to interpret the child's internal states. Furthermore, we found that imitating the joyous expression of an infant greatly activates right limbic and temporal areas, whereas observing ambiguous expressions greatly activates areas in the left hemisphere, which is involved in higher cognitive and motor control. This study sheds light on a large, fascinating mosaic that constitutes the neural basis of the maternal circuit of empathy. Further studies are warranted to investigate and clarify all the physiological and pathological aspects of this circuit that are characterized by reduced mimicry and empathy–like, for example, maternal depression (Psychiatric Association 2000).

References

Ammaniti M, Tambelli R, Zavattini GC, Vismara L, Volpi B. 1999. Attaccamento e funzione riflessiva in adolescenza. Tras: attachment and reflective function in adolescence. Psicol Clin Sviluppo. 1:155-175.

Augustine JR. 1996. Circuitry and functional aspects of the insular lobe in primates including humans. Brain Res Brain Res Rev. 22:229-244.

Badre D, Wagner AD. 2004. Selection, integration, and conflict monitoring; assessing the nature and generality of prefrontal cognitive control mechanisms. Neuron. 41:473-487.

Bartels A, Zeki S. 2004. The neural correlates of maternal and romantic love. Neuroimage. 21:1155-1166.

Beebe B, Lachmann FM. 1988. The contribution of mother-infant mutual influence to the origins of self- and object representations. Psychoanal Psychol. 5:305-337.

Bowlby J. 1958. The nature of a child's tie to his mother. Int J Psychoanal. 39:350-373.

Bowlby J. 1988. A secure base. London: Routledge.

Calvo-Merino B, Grezes J, Glaser DE, Passingham RE, Haggard P. 2006. Seeing or doing? Influence of visual and motor familiarity in action observation. Curr Biol. 16:1905-1910.

Carr L, Iacoboni M, Dubeau MC, Mazziotta JC, Lenzi GL. 2003. Neural mechanisms of empathy in humans: a relay from neural systems for imitation to limbic areas. Proc Natl Acad Sci USA. 100:5497-5502.

Cavanna AE, Trimble MR. 2006. The precuneus: a review of its functional anatomy and behavioural correlates. Brain. 129:564-583.

Dalgleish T. 2004. The emotional brain. Nat Rev Neurosci. 5:583-589.

Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LL, Parvizi J, Hichwa RD. 2000. Subcortical and cortical brain activity during the feeling of self-generated emotions. Nat Neurosci. 3:1049-1056.

Dapretto M, Davies MS, Pfeifer JH, Scott AA, Sigman M, Bookheimer SY, Iacoboni M. 2006. Understanding emotions in others: mirror neuron dysfunction in children with autism spectrum disorders. Nat Neurosci. 9:28-30.

Davidson RJ, Irwin W. 1999. The functional neuroanatomy of emotion and affective style. Trends Cogn Sci. 3:11-21.

Derogatis LR. 1977. SCL-90-R: administration, scoring and procedures manual.

Emde RN. 1992. Social referencing research: Uncertainty, self, and the search for meaning. In: Fainman S, editor. Social Referencing and the social construction of reality in infants. New York: Plenum Press.

First MB, Spitzer RL, Gibbon M, Williams JBW. 1997. SCID-I. Structured Clinical Interview for DSM-IV Axis I Disorders. Washington (DC): American Psychiatric Press.

Fonagy P, Steele M, Steele H, Target M. 1998. Reflective-functioning manual. Version 4.1. For application to adult attachment interviews. London: University College London.

Gallese V, Goldman A. 1998. Mirror neurons and the simulation theory of mind reading. Trends Cogn Sci. 12:493-501.

Gallese V, Keysers C, Rizzolatti G. 2004. A unifying view of the basis of social cognition. Trends Cogn Sci. 8:396-403.

Gilbert SJ, Spengler S, Simons JS, Steele JD, Lawrie SM, Frith CD, Burgess PW. 2006. Functional specialization within rostral prefrontal cortex. Area 10: a meta-analysis. J Cogn Neurosci. 18:932-948.

Glosser G, Zwil AS, Glosser DS, O'Connor MJ, Sperling MR. 2000. Psychiatric aspects of temporal lobe epilepsy before and after anterior temporal lobectomy. J Neurol Neurosurg Psychiatry. 68:53-58.

Gobbini MI, Leibenluft E, Santiago N, Haxby JV. 2004. Social and emotional attachment in the neural representation of faces. Neuroimage. 22:1628-1635.

Henninghausen K, Lyons-Ruth K. 2005. Disorganization of behavioral and attentional strategies toward primary attachment figures: from biologic to dialogic processes. In: Attachment and bonding: a new synthesis. Cambridge (MA): MIT Press. p. 269-301.

Holmes AP, Friston KJ. 1998. Generalisability, random effects & population inference. Neuroimage. S754.

Iacoboni M, Woods RP, Brass M, Bekkering H, Mazziotta JC, Rizzolatti G. 1999. Cortical mechanisms of human imitation. Science. 286:2526-2528.

Izard CE, Doughery LM, Hembree EA. 1989; Revised 1995. A system for identifying affect expressions by holistic judgements (AFFEX). Newark: University of Delaware.

Kaplan J, Iacoboni M. 2006. Getting a grip on other minds: mirror neurons, intention understanding and cognitive empathy. Soc Neurosci. 1:175-183.

Kerns JG, Cohen JD, MacDonald AW, 3rd, Johnson MK, Stenger VA, Aizenstein H, Carter CS. 2005. Decreased conflict- and error-related activity in the anterior cingulate cortex in subjects with schizophrenia. Am J Psychiatry. 162:1833-1839.

Lee TW, Josephs O, Dolan RJ, Critchley HD. 2006. Imitating expressions: emotion-specific neural substrates in facial mimicry. Soc Cogn Affect Neurosci. 1:122-135.

Leibenluft E, Gobbini MI, Harrison T, Haxby JV. 2004. Mothers' neural activation in response to pictures of their children and other children. Biol Psychiatry. 56:225-232.

Main M, Goldwyn R. 1997. Adult attachment scoring and classification systems. Unpublished manual. Berkeley (CA): University of California at Berkeley.

Meltzoff AN, Moore MK. 1977. Imitation of facial and manual gestures by human neonates. Science. 198:74-78.

Morgane PJ, Galler JR, Mokler DJ. 2005. A review of systems and networks of the limbic forebrain/limbic midbrain. Prog Neurobiol. 75:143-160.

Murphy FC, Nimmo-Smith I, Lawrence AD. 2003. Functional neuroanatomy of emotions: a meta-analysis. Cogn Affect Behav Neurosci. 3:207-233.

Nitschke JB, Nelson EE, Rusch BD, Fox AS, Oakes TR, Davidson RJ. 2004. Orbitofrontal cortex tracks positive mood in mothers viewing pictures of their newborn infants. Neuroimage. 21:583-592.

Nomura M, Iidaka T, Kakehi K, Tsukiura T, Hasegawa T, Maeda Y, Matsue Y. 2003. Frontal lobe networks for effective processing of ambiguously expressed emotions in humans. Neurosci Lett. 348:113-116.

Olson IR, Plotzker A, Ezzyat Y. 2007. The enigmatic temporal pole: a review of findings on social and emotional processing. Brain. 130:1718-1731.

Oster H, Hegely D, Nagel L. 1992. Adult judgements and fine-grained analysis of infant facial expressions: Testing the validity of a priori coding formulas. Dev Psychol. 28:1115-1131.

Phan KL, Wager T, Taylor SF, Liberzon I. 2002. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. Neuroimage. 16:331-348.

Phillips ML, Drevets WC, Rauch SL, Lane R. 2003. Neurobiology of emotion perception I: the neural basis of normal emotion perception. Biol Psychiatry. 54:504-514.

Picard N, Strick PL. 1996. Motor areas of the medial wall: a review of their location and functional activation. Cereb Cortex. 6:342-353.

Psychiatric Association. 1994. Diagnostic and statistical manual of mental disorders, text revised. DSM-IV-TR. Washington (DC): Psychiatric Association.

Radloff LS. 1997. The CES-D scale. Appl Psychol Meas. 1:385–401.

Ranote S, Elliott R, Abel KM, Mitchell R, Deakin JF, Appleby L. 2004. The neural basis of maternal responsiveness to infants: an fMRI study. Neuroreport. 15:1825–1829.

Rizzolatti G, Craighero L. 2004. The mirror-neuron system. Annu Rev Neurosci. 27:169–192.

Rizzolatti G, Fogassi L, Gallese V. 2001. Neurophysiological mechanisms underlying the understanding and imitation of action. Nat Rev Neurosci. 2:661–670.

Slade A, Grienenberger J, Bernbach E, Levy D, Locker A. 2005. Maternal reflective functioning, attachment, and the transmission gap: a preliminary study. Attach Hum Dev. 7:283–298.

Sullivan MW, Lewis M. 2003. Emotional expressions of young infants and children. A practitioner's primer. Infants Young Child. 16:120–142.

Tronick EZ, Weinberg MK. 1997. Depressed mothers and infants: failure to form dyadic states of consciousness. In: Murray L, Cooper PJ, editors. Postpartum depression and child development. New York: Guilford Press. p. 54–81.

Zald DH. 2003. The human amygdala and the emotional evaluation of sensory stimuli. Brain Res Brain Res Rev. 41:88–123.