Maternal Prefrontal Cortex Activation by Newborn Infant Odors

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Accepted November 1, 2013

Abstract

Mothers are attracted by infant cues of a variety of different modalities. To clarify the possible neural mechanisms underlying maternal attraction to infant odor cues, we used near-infrared spectroscopy to examine prefrontal cortex (PFC) activity during odor detection tasks in which 19 mothers and 19 nulliparous females (nonmothers) were presented with infant or adult male odors. They were instructed to make a judgment about whether they smelled an odor during each task. We estimated the PFC activity by measuring the relative oxyhemoglobin (oxyHb) concentrations. The results showed that while detecting the infant odors, bilateral PFC activities were increased in mothers but not in nonmothers. In contrast, adult male odors activated the PFC similarly in mothers and nonmothers. These findings suggest that maternal activation of the PFC in response to infant odors explains a part of the neural mechanisms for maternal attraction to infant odors.

Key words: imaging, infant odor, maternal behavior, near-infrared spectroscopy, prefrontal cortex, reproduction

Introduction

Evidence from both human (Seifritz et al. 2003; Proverbio et al. 2006; Glynn 2010; Kuzawa et al. 2010; Kim et al. 2010; Nishitani et al. 2011; Barrett et al. 2013) and animal studies (Kinsley et al. 2008) suggests that when women become mothers, their behaviors change to enable them to fulfill the unique demands of their child. It is well known that in most experimental mammals, pup odors promote maternal behavior in mothers (Lévy et al. 2004; Lévy and Keller 2009) but are aversive for nulliparous females (nonmothers). Indeed, the nonmothers may bite and kill the pups (Fleming et al. 1979). In recent years, the neural mechanisms of the pup odor–induced maternal behavior have been explored (Numan and Numan 1994; Kinsley et al. 1995; Morgan et al. 1997; Brunton and Russell 2008) and have been found to involve neural circuits of reward systems. In humans, several studies of infant odor–induced maternal behavior such as mother’s identification of their infant’s odors have been reported (Schaal et al. 1980; Porter et al. 1983; Russell et al. 1983; Kaitz et al. 1987; Schaal and Marlier 1998). It is, however, important to note that these studies are focused exclusively on mothers and their own infants. In contrast, there is only one behavioral study that has examined the effect of infant odors on maternal behavior more generally. Fleming and colleagues (1993) measured mothers’ attraction to infant odors and then compared those results with results for nonmothers. They found that mothers were more attracted to infant odors than nonmothers. However, the possible neural basis underlying this phenomenon in humans has not been clarified.

Infant-related visual and auditory stimuli have been mostly used to elucidate the neural basis of human maternal behaviors because stimuli such as seeing an infant’s face and hearing an infant’s cry are readily considered to induce maternal affections. Functional magnetic resonance imaging (fMRI) studies have reported that the prefrontal cortex (PFC) containing the orbitofrontal cortex (OFC) is activated when mothers view a picture or video of their own infant (Bartels and Zeki 2004; Nitschke et al. 2004; Ranote et al. 2004; Strathern et al. 2008). When mothers were exposed...
to infant crying, the PFC was also activated (Lorberbaum et al. 1999; Lorberbaum et al. 2002). The PFC, especially the OFC, is considered to be the secondary and tertiary olfactory cortical area. It is also known to represent the reward or affective value of primary reinforcers including facial expression, touch, taste, and olfaction (Mak et al. 2009; Kida and Shinhara 2013). Taken together with the animal studies cited, these human studies indicate that the regulation of the infant cue–induced human maternal affections also involves neural circuits of reward systems.

Although recent neuroimaging studies support the notion that maternal PFC activations in response to infant cues could reflect reward activation, few studies have examined whether the PFC activations induced by infant-related cues are mother specific. In a previous study (Nishitani et al. 2011), we reported that right PFC activity was increased in mothers compared with that in nonmothers while discriminating infant facial expressions (visual cues). Furthermore, it has been reported by another research group that maternal left PFC activations in response to infant auditory cues were higher than those of nonmothers (Seifritz et al. 2003). These findings raise the possibility that, in addition to infant visual and auditory cues, infant olfactory cues also activate the PFC in mothers compared with nonmothers. It is also unknown whether hemispheric lateralization occurs in the activation of the PFC for the processing of infant olfactory cues. We predicted that lateralization occurs because the right hemisphere shows higher activity in the OFC when a subject is exposed to a pleasant smell (Zatorre et al. 2000; Gottfried et al. 2002; Brancucci et al. 2009). However, to date, there is no neuroimaging study investigating PFC activities when mothers and nonmothers are exposed to olfactory infant cues.

In this study, we used near-infrared spectroscopy (NIRS) to compare the PFC activity of mothers and nonmothers. NIRS is a noninvasive optical method based on the property of hemoglobin to absorb near-infrared light. NIRS relies on the principle that an increase in hemoglobin concentration represents an increase in blood flow, which in turn reflects neural activation. In NIRS, the subject wears a set of optodes (emitters/detectors of near-infrared light) on his/her head; the method does not require the subject to be confined to a scanner (Strangman et al. 2002). NIRS has been used to study cortical activity associated with visual and motor function (Kuboyama et al. 2004; Kashou et al. 2007), language (Watanabe et al. 1998), emotions (Herrmann et al. 2003), cognition (Kameyama et al. 2004; Schroeter et al. 2004), and, more recently, olfactory function (Bartocci et al. 2000; Ishimaru et al. 2004; Harada et al. 2006; Fujii et al. 2007; Kobayashi et al. 2007; Aoyama et al. 2010; Kokan et al. 2011).

The aim of this study was to determine whether infant olfactory cues activate the PFC in women in a parental state-dependent fashion. To achieve this goal, we compared PFC activity during the detection of infant odors between mothers and nonmothers by means of NIRS. Furthermore, we measured PFC activity during the detection of adult male odors in mothers and nonmothers to determine whether the maternal PFC response to human odors is infant specific.

### Materials and methods

#### Participants

Thirty-eight healthy females participated in this study (Table 1). There were 19 mothers who were biological parents and 19 nulliparous females (nonmothers). Mothers were recruited from the academic community and through the Nagasaki City Public Health Center and were married and living with their husbands. Nonmothers were recruited from the academic community and were required to meet the following criteria: 1) they had never been pregnant, and 2) were not cohabiting with a heterosexual partner. Mean ages were somewhat different between the groups [t (1, 36) = 3.22, P < 0.003], but we analyzed this confounding effect. The phase of menstrual cycle at testing in each group is shown in Table 1 and no significant difference was found (χ² = 3.69, P = 0.16). No significant difference in the prandial status was found (χ² = 0.11, P = 0.75). All participants identified themselves as nonsmokers and were righthanded as assessed by the Edinburgh Handedness Inventory (Oldfield 1971). All participants gave written informed consent after the purpose of the experiment was explained. The experimental protocol was in accordance with the Helsinki Declaration and was approved by the Ethics Committee of the Nagasaki University Graduate School of Biomedical Sciences.

#### Odor detection testing

Participants were seated on a comfortable chair in a quiet room and were given two series of odor detection tasks: infant odor and adult male odor tasks (shown in Figure 1). On the day of the experiment, only female experimenters (S.K., A.T., and technicians) who were not scented themselves, were permitted in the room. Participants were informed that “human body odors were included in the glass vials” but were not told what kinds of human body odors were included. We employed a single-blind test so that the experimenters knew the full facts, whereas participants did not. For each task, participants closed their eyes and were handed a 30-ml conical glass vial (Asahi Glass CO., LTD), which was warmed to 37°C in a water bath more than 30 min before the experiment. They were then asked to sniff the contents for 5 s. Participants randomly received one of the glass vials, which contained “worn” or “unworn” T-shirts. They were then asked to make a verbal judgment about whether or not they smelled a body odor. All decisions were to be made on the basis of a binary (yes–no) response (correct detection...
response being “yes” for worn, and “no” for unworn). This was repeated 6 times for each task (60 s). In each task, worn T-shirts were randomly presented 3 times (50%) and unworn ones were presented 3 times. The experimenter recorded the participant’s response to each task and later scored both the detection accuracy (worn and unworn) for each task. As a result, each participant responded correctly 0, 1, 2, or 3 times (0, 33, 66, or 100 %) for each subset of 3 worn or 3 unworn T-shirts. The two detection accuracy scores were then averaged for each individual. After that, the mean detection accuracy within groups was calculated and was compared using t-test, separately for each task. Before the start of each task, participants were asked to relax for 60 s, during which the baseline NIRS recordings were made.

**Stimuli**

The infant odor stimuli consisted of 3 T-shirts containing infant body odors from 10 different 4-day-old newborn infants (5 males and 5 females). To obtain the odor stimuli, the infant T-shirts (100% cotton swaddling clothes) were prepared in a standard way (Porter et al. 1983; Fleming et al. 1997; Fleming et al. 2002). Mothers at 3 days postpartum who did not participate in this experiment were asked to provide samples of odors from their newborn infants. They were instructed not to use perfumes or other odors on either themselves or their infants before providing the samples. Infants were clothed in clean swaddling clothes for 24 h. Control T-shirts were from the same stock, washed in

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**Table 1** General demographic information for each group

<table>
<thead>
<tr>
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<th>Mothers</th>
<th>Nonmothers</th>
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<tbody>
<tr>
<td>n</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Age of participants (year)</td>
<td>33.3 ± 3.4</td>
<td>28.6 ± 5.3</td>
</tr>
<tr>
<td>Age of youngest child (year)</td>
<td>3.16 ± 1.7</td>
<td>NA</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
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<tr>
<td>Primiparous</td>
<td>7 (36.8 %)</td>
<td>NA</td>
</tr>
<tr>
<td>Multiparous (two/three)</td>
<td>9 (47.4 %) / 3 (15.8 %)</td>
<td>NA</td>
</tr>
<tr>
<td>Breastfeeding at testing</td>
<td>3 (15.8 %)</td>
<td>NA</td>
</tr>
<tr>
<td>Menstrual cycle*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Follicular phase</td>
<td>5 (26.3 %)</td>
<td>8 (42.1 %)</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>11 (57.9 %)</td>
<td>11 (57.9 %)</td>
</tr>
<tr>
<td>Not regularly cycling</td>
<td>3 (15.8 %)</td>
<td>0 (0 %)</td>
</tr>
<tr>
<td>Prandial status (preprandially/postprandially)</td>
<td>10 (52.6 %)/9 (47.4 %)</td>
<td>9 (47.4 %)/10 (52.6 %)</td>
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</table>

Mean ± standard deviation. NA = Nonapplicable.
*The phases of menstrual cycle were divided into one of two categories. The follicular phase was defined as the period from the day of menstruation to the predicted day of ovulation and the luteal phase was defined as the period from the predicted day of ovulation to the day of menstruation.
*Participants’ approximate prandial status estimated at the start of the experiment.

**Figure 1** Illustration of the experimental procedure. Participants were exposed according to an alternating sequence of 5 s of odor stimulation and a 5-s break in each task period.
the same detergent “Skinabebe (Mochida Health Care CO., LTD.).” The adult male odor stimuli consisted of 3 T-shirts containing body odors from 3 different adult males (mean age: 25.7 ± 3.2 years). The T-shirts (100% cotton) were washed in the same detergent and worn twice overnight by adult males, who refrained from using scented products and used scent-free shampoo and soap provided by the experimenter (Lenochova et al. 2009). Control T-shirts were from the same stock, washed in the same detergent. On delivery, we carefully selected the back area of each T-shirt in the same way across groups. T-shirts were cut into pieces of 150 cm² (15 cm × 10 cm) and frozen at −80°C until use. These measures were to ensure that there would be no difference in odor intensity/quality between the cloth pieces. We used each piece only once to keep the odor fresh and avoid degradation from repeated freeze-thaw cycles. The time between collection and testing was about 1 month on average.

NIRS measurements
Relative oxyhemoglobin (oxyHb) and deoxyhemoglobin (deoxyHb) were measured with a two-channel NIRS (OM-220, Shimadzu Corp.). As described previously (Nishitani et al. 2011), two NIRS probes were placed over the prefrontal regions, so that the emitters were positioned at Fp1 and Fp2, with the detectors positioned 4 cm to the lateral side and placed along the T3–T4 line according to the international 10/20 system. The NIRS set-up recorded hemoglobin changes approximately 2–3 cm beneath the scalp, that is, the cortical surface area (Hock et al. 1997; Toronov et al. 2001). The rate of data sampling was 1 s. The baseline was determined as the mean level over a 10-s period just before the 60-s task period.

Dependent variables
Simultaneous NIRS and fMRI studies have correlated cerebral blood flow more strongly to oxyHb than to deoxyHb (Strangman et al. 2002). Therefore, oxyHb changes were adopted as a measure of cerebral activation in this analysis.

Statistical analyses
The oxyHb data were averaged for each participant during the baseline period and each task period. The oxyHb changes (task minus baseline) were then analyzed using two-way repeated measures analysis of variance (ANOVA) as two independent variables (group and cerebral hemisphere). In addition, the oxyHb changes were analyzed by using analysis of covariances (ANCOVAs) with the participants’ ages as covariates. All analyses were done with SPSS 15.0 (SPSS Inc.).

Results
The mean accuracy for infant odor detection was compared between mothers and nonmothers (Table 2). The accuracy of infant odor detection was significantly higher in mothers than nonmothers [t (1, 36) = 2.07, P < 0.05]. However, no significant differences were found with the adult male odor detection task [t (1, 36) = 0.52, P = 0.61].

In the infant odor detection task, two-way repeated measures ANOVA showed a significant main effect of “group” [F (1, 36) = 7.34, P < 0.05] in oxyHb changes (Figures 2a and 3a). There was no significant main effect of “cerebral hemisphere” [F (1, 1) = 0.25, P = 0.62]. There was no significant interaction between “group” and “cerebral hemisphere” [F (1, 36) = 0.01, P = 0.92]. However, the magnitude of the activation in both cerebral hemispheres in mothers was not significantly correlated with the accuracy of detecting the infants’ odors (Left: P = 0.24, Right: P = 0.48). Furthermore, ANCOVAs revealed that differential PFC responses were not confounded with participants’ age (P < 0.05).

In the adult male odor detection task, no significant differences were found both in “group” [F (1, 36) = 1.95, P = 0.28] and “cerebral hemisphere” [F (1, 1) = 1.07, P = 0.31] (Figures 2b and 3b). There were no significant interactions between “group” and “cerebral hemisphere” [F (1, 36) = 0.03, P = 0.86].

Discussion
This study investigated whether infant olfactory cues modulate PFC activity in mothers and nonmothers. We found that infant odor detection increases bilateral PFC activity significantly more in mothers than in nonmothers. This finding suggests that infant olfactory cues activate the maternal PFC and infant visual and auditory cues (Seifritz et al. 2003; Nishitani et al. 2011). In contrast, adult male odors increased PFC activity in nonmothers in a similar way to mothers. In humans, therefore, it is possible that the female brain is molded when women become mothers. This possibility was also supported by a recent neuroimaging study, which reported that the first months of motherhood are accompanied by structural changes in brain regions including the PFC (Kim et al. 2010). Taken together, our finding of increased activation in the PFC in mothers during infant odor detection suggests that the response of the PFC toward infant odors is changed when women become mothers. This change

<table>
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<th>Table 2</th>
<th>Behavioral data for each odor detection task</th>
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<tr>
<td></td>
<td>Detection accuracy (% correct)</td>
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<tr>
<td></td>
<td>Mothers</td>
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<tr>
<td>Infant odor</td>
<td>81.6 ± 5.4*</td>
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<tr>
<td>Adult male odor</td>
<td>77.2 ± 4.5</td>
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</table>

Mean ± standard error. *P < 0.05 (mothers vs. nonmothers).
of response to infant odors could be important for mothers caring and fulfilling the unique demands of their children.

The current findings raise the question of how enhanced PFC activity might functionally benefit mothers. One possibility is to assume that mothers become more skillful at detecting infant odors because of increased PFC activation. In this study, we examined whether detection accuracy for infant odors influences maternal PFC activity. The detection accuracy for infant odors was higher in mothers compared with nonmothers. This suggests that there was an enhanced maternal ability to detect infant odors. It is, therefore, possible that the greater PFC response to infant odors in mothers explains their detection ability of infant odors. However, this seems to be unlikely because there was no significant correlation between the detection accuracy and PFC activity. There may also be limitations in the general olfactory abilities of participants because this was not evaluated by testing prior to the study. Although behavioral parameter measurements are needed for more definitive conclusions, we tentatively propose that infant odors enhance mothers’ willingness to approach their infants, which then serves as an intrinsic reward.

It remains unclear whether hemispheric lateralization occurs in the activation of the PFC for the processing of infant olfactory cues. We had initially predicted that such lateralization favors the right PFC because a higher activity has been observed here when a subject is exposed to a pleasant smell (Zatorre et al. 2000; Gottfried et al. 2002; Brancucci et al. 2009). However, the data from this study showed unpredictable bilateral PFC activation in mothers. Further research may allow for a better understanding of hemispheric lateralization in the activation of the PFC for the processing of infant olfactory cues in different populations, or under conditions in which infant odor exposures are performed in a nostril-specific procedure (Zhou et al. 2012).

In our experiment, participants were instructed to sniff the contents of a glass vial and then make a verbal judgment regarding whether or not they smelled a body odor. There is a methodological limitation inherent in NIRS in that motor artifacts derived, for example, from the sniffing and verbal responses, may be included in the data. However, there was a significant difference in the PFC activity between mothers and nonmothers during infant odor detection but not in adult male odor detection. It is possible that even if
motor artifacts affect the NIRS data, such influences occur randomly. Facial electromyogram measurements may be required for future studies of this kind.

In conclusion, the results of this study demonstrated that PFC activity increased during the detection task of infant odors in mothers but not in nonmothers. This PFC activation increase did not differ between mothers and nonmothers during the detection task of adult male odors, suggesting that the PFC activation during the detection task of infant odors is specific to motherhood. This finding leads us to propose that maternal PFC activation results from dynamic reproductive hormonal changes during pregnancy and motherhood. However, it is also plausible that the maternal experience itself modulates brain function without any changes in reproductive hormones. Further studies are required to verify these hypotheses.

Funding

This work was supported by the Research Institute of Science and Technology for Society, Japan Science and Technology Agency, and by KAKENHI, Grant-in-Aid for Young Scientists (B), (19790180) and Grant-in-Aid for Scientific Research (C), (21590259).

Acknowledgments

We are grateful to Prof. Sumihisa Honda, PhD, for providing statistical help. The authors wish to acknowledge the participants and research assistants in the project, Kaoru Ideguchi, Hiromi Hosoyamada, Nahoko Mochinaga, Yuko Iwanaga, for their efforts in recruitment and data collection. We are also grateful to Chiharu Hensley and Joel Hensley for help in preparing the manuscript.

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