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BOLD Signal Responses in Corticolimbic ‘Emotions’ Circuitry of Lactating Rats Facing Intruder Threat to Pups

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Abstract

Lactating rats must continuously maintain a critical balance between caring for pups and aggressively responding to nest threats. We tested the neural response of lactating females to the presentation of their own pups and novel intruder males using blood oxygen level dependent (BOLD) functional MRI at 7T. Dams were presented with a single sequence of a control stimulus, pups or a male intruder in one imaging session ($n = 7-9$). To further determine the selectivity of neural processing, dams were imaged for their response to a male intruder both in the absence and presence of their pups ($n = 6$). Several maternal cortical and limbic brain regions were significantly activated by intruder presentation, but not by pups or a control stimulus. These included the nucleus accumbens (NAcc), periaqueductal gray (PAG), anterior cingulate, anterior thalamus, basal nucleus of the amygdala, temporal cortex (TC), prelimbic/orbital area and insula. The NAcc, PAG, TC and mediodorsal thalamus still showed greater neural activity when compared to intruder presentation in the absence of pups. The present results suggest that the high emotional state generated by a threat to pups involves robust activation of classical limbic regions controlling emotional responses. This pattern of BOLD activity may precede behavioral states upon which lactating rats initiate attacks against a potential threat to offspring.

INTRODUCTION

Appropriate maternal behavior is crucial to offspring survival. Female rodents need to be able to provide warmth and nourishment, as well as protect the offspring from conspecific aggression (Wolff, 1985; Wolff, 1993). However, little is known about the neural mechanisms that balance the divergent expression of maternal care and maternal aggression. Female rodents often have to express both behaviors appropriately within a short time period. While the neural and hormonal control of maternal behavior has been extensively studied, the topic of maternal aggression has received less attention (Lonstein & Gammie, 2002). Upregulation of early response genes, such as *c-fos*, have provided some insight into the brain nuclei involved in aggression in rodents (Lonstein & Stern, 1997; Lonstein *et al.*, 1997; Gammie & Nelson, 2001). However, the temporal resolution of these markers render them incapable of easily distinguishing neural circuits mediating the initiation of aggression and those activated as a consequence of an aggressive display. Few studies investigate maternal care and aggression simultaneously (Johns *et al.*, 2005; Nephew & Bridges, 2008), and thus very little is known about potential interactions between aggression and offspring care. In the current study, conscious lactating rats were imaged while being presented with a novel male intruder to determine which nuclei are activated in response to a stimulus known

to induce maternal aggression. These imaging data were compared and contrasted to the neural responses to distal pup presentations.

Prior fMRI imaging studies in conscious lactating dams indicate that pup suckling activates the dopaminergic mesocorticolimbic system, which extends from the ventral tegmental area to the accumbens and prefrontal cortex (Ferris *et al.*, 2005). This system is a key component in the mechanism controlling the anticipatory and consummatory aspects of maternal behavior, as impaired dopamine activity in the accumbens disrupts multiple aspects of maternal behavior (Hansen *et al.*, 1991; Keer & Stern, 1999; Silva *et al.*, 2003). However, since there are several sensory inputs involved in suckling stimulus (auditory, visual, tactile, and olfactory), it is difficult to determine associations between specific nuclei and individual sensory components. Most notably, it is unclear which brain regions are involved in the acute response to physical suckling, and which are responsible for the initiation of maternal behavior upon pup presentation. While studies in humans indicate that the recorded cry of a mother's own infant activates the mesocorticolimbic system (Lorberbaum *et al.*, 2002), similar studies have not been done in rodents. The purpose of the present study was to determine which nuclei are acutely activated during the distal presentation of both pups and intruders. Our data indicate that multiple cortical association areas in addition to subcortical limbic sites involved in emotional responding show robust BOLD signal responses in response to an intruder, in the presence of pups.

MATERIALS AND METHODS

Subjects

Long-Evans female rats (225–275 g) were purchased from Charles River Laboratories (Wilmington, MA). Females were housed in pairs in a temperature and humidity controlled room and maintained on a 12L: 12D light-dark cycle (lights off at 1900 hr). Home cages consisted of hanging plastic microisolator cages of standard dimensions with woodchip bedding. Water and Purina rat chow were provided *ad libitum*. Following mating procedures, primiparous females were singly housed along with their litters for the remainder of the experiment. Rats were acquired and cared for in accordance with the guidelines published in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publications No. 85-23, Revised 1985) and adhere to the National Institutes of Health and the American Association for Laboratory Animal Science guidelines. The Institutional Animal Care and Use Committee at Northeastern University approved the protocols used for this study.

Acclimatization procedures and preparations for imaging

Detailed experimental procedures are available as online supporting material. All imaging experiments were done in fully awake, unanaesthetized primiparous dams. Anesthesia (2–4% isoflurane) was used only during rat setup, preceding the acclimatization procedures and experiments. In order to minimize physiological and gross motion during MR scanning, all rats were acclimatized to a head restraining unit and MRI sounds upon arrival and before mating as previously reported (King *et al.*, 2005). Before MR scanning, dams were again anesthetized with 2–4 % isoflurane. Details of the setup procedure have been previously reported (Ferris *et al.*, 2005). Briefly, a topical anesthetic of 5–10 % lidocaine cream was applied to the skin and soft tissue around the ear canals and over the bridge of the nose before the animal is placed inside a dual coil radiofrequency system under restraint (Ferris *et al.*, 2005). This procedure took 5–6 min, after which gaseous anesthesia flow was turned off and the entire unit was placed through the bore of the magnet for imaging. After the entire unit was placed in the magnet, scanning preparations controlled by Paravision 4.0 typically took 10–15 minutes and thereafter the entire imaging session including 1 anatomical scan

(ca. 6 minutes) and 3 functional scans (ca. 22 minutes total) lasted about 30 minutes. Thus the entire experiment per animal in an unanaesthetized state lasted 40–45 minutes.

Magnetic resonance imaging scanning parameters

Experiments were conducted in a 300 Mhz Bruker USR 7T/20 cm horizontal magnet (Bruker, Germany) equipped with a Paravision 4.0 console (Bruker, Billerica, MA U.S.A). Studies were performed with a multi-concentric dual-coil, small animal restrainer (Insight MRI, Worcester, MA). Radiofrequency signals are sent and received with dual coil electronics built into the animal restrainer (Ludwig *et al.*, 2004). Functional imaging was performed using a multi-segmented T2-weighted fast spin echo pulse sequence with the following parameters: repetition time TR = 1562msec, echo time TE = 7.5, effective echo time TE_{eff} = 45 msec and an echo train length ETL = 16. Geometry was setup as follows: 12 slices, field of view of 28 mm, 1.0 mm thick slices with no gaps, data matrix of 64² for functional scans and 256² for anatomical scans (Thus the in plane 2D pixel resolution was 438 μm² for functional and 117 μm² for anatomical scans). A full set of 12 coronal slices across the brain was collected at each effective repetition time and was completed every 6 seconds 24 msec.

Imaging the BOLD Response to Nest Intruder

Fourteen primiparous rats were imaged for the neural response to a control object (no pups), to object + pups (pups) and to nest intruder in the presence of pups (pups/intruder). Each rat imaged during 3 consecutive scanning sessions where an anatomical scan was first collected, immediately followed by a first fMRI scan of a control object (a custom-made delivery cradle alone without pups), a second fMRI scan of 4 pups (pups were presented on the delivery cradle), and a third and final fMRI scan of a male intruder in the presence of pups. For in vivo stimuli presentation, a custom made clear plastic cylindrical vivarium was used as previously reported (Ferris *et al.*, 2008). The outer diameter of the vivarium tube is just under 20 cm, thus permitting it to remain stable inside the magnet bore in front of the dual coil imaging setup during functional and anatomical imaging. The front end of the vivarium has a non-magnetic copper wire mesh that permits dams to smell, visualize and hear pups or intruder males. The outer enclosure contains air holes and the inner environment of divided in top and bottom levels. Maternal cage bedding is placed on the vivarium bottom floor to simulate the lactating rat's home environment. For cradle alone and cradle + pups, 60 repetitions (6 minutes) were collected and stimulus presented a repetition number 30 (3 minutes). For intruder presentation, 100 repetitions (total 10 minutes) were collected and stimulus male presented in the vivarium at repetition 50 (5 minutes). During intruder male presentation, pups were placed in the lower compartment of the vivarium along with their home cage bedding to protect them from any harm during scanning. In order to test the selectivity of maternal neural processing in response to the intruder, and whether it would depend on the presence of pups and nest odors, we tested a group of 7 lactating rats for their neural response to a male intruder using the same parameters but without pups or bedding in the presentation vivarium.

Statistical analysis

Full details for the MRI data analysis using in house software has been previously reported (Ferris *et al.*, 2005) and are available as online supporting materials. Animals showing an average displacement exceeding 25% of the total in plane (X-Y) pixel resolution (>109 μm out of 437 μm) or slice (Z) direction (>250 μm out of 1000 μm slice thickness) were excluded (n = 3). This a priori cutoff criterion was pre-established by stimulated studies showing false positive BOLD activation with movements corresponding to 6/10 of a single voxel (Ferris *et al.*, 2008). Also, scans with linear baseline drifts over 0.5% were corrected using in house software (Ferris *et al.*, 2008). Nine out of 14 studies survived the

preprocessing exclusion criteria and were included in the study. ROI-based statistical analysis was done using Medical Image Visualization and Analysis (MIVA) software (Ferris *et al.*, 2005).

Each subject was registered to a fully segmented electronic rat brain atlas (Paxinos & Watson, 1997; Swanson, 1999). Statistical *t* tests are performed on each subject within the original coordinate system. The baseline period used was 15 repetitions immediately preceding stimulus (object, pup, or intruder) presentation and the stimulation window was 15 repetitions. Statistical *t* tests used a 95 % confidence level, two-tailed distribution, and heteroscedastic variance assumptions. In order to provide a conservative estimate of significance, a false-positive detection-controlling algorithm is introduced into the analysis (Genovese *et al.*, 2002). This ensures that the false-positive detection rate is below our confidence level of 5 % (Ferris *et al.*, 2005). Statistically significant pixels were assigned their percentage change values (stimulus mean minus control mean) and all. Activated voxel numbers were exported to SPSS for statistical comparisons between groups. Unless otherwise noted in the results section, the number of voxels per region of interest and their corresponding average percent change values were statistically evaluated between groups using a multi-factorial analysis of variance ($p < 0.05$) with stimulus (no pups, pups, pups/intruder) as independent variables. A Bonferroni multiple comparison test was used for posthoc analysis. For statistical comparisons between intruder presentation, without and with pups, we used a single factor analysis of variance. All data were significant at $p < 0.05$ (two-tailed). Data were analyzed for both percent changes in BOLD signal intensity and volume of activation for both positive and negative BOLD responses.

RESULTS

The goal of the present experiment was to examine the maternal neural circuits that are activated by distal presentations of pups and intruders. Fourteen acclimatized primiparous rats were imaged for their BOLD response to a nest intruder in the presence of pups and nest bedding. This imaging paradigm was used in order to simulate key aspects of the home cage environmental conditions. The experiment also used a test/re-test type of fMRI design in which the each dam was tested during the same imaging session with varying stimuli. Stimulus order was: 1) pup presentation cradle alone (no pups), 2) cradle with pups and 3) intruder male in the presence of pups (see Supporting Online Materials Figure 1 for details). To control for non-specific general arousal, brain BOLD activation in response to the intruder male was statistically compared to the first 2 conditions, cradle and cradle with pups. An additional group of lactating rats were imaged in response to male rat in the absence of pups or bedding. Several statistical parameters were considered in order to remove functional scans containing severe motion. Only scans showing less than 110 μm shift from the center of mass were included. The final *n*'s were 9 for no pups, 9 for pups, 7 for the intruder scan and 6 for intruder scans with no pups.

Statistical significance for voxel activations was determined across 68 regions of interest for the 3 experimental conditions using in house software (Medical Image Visualization and Analysis). Figure 1 shows composite maps for positive BOLD activation for the three imaging sessions. Pairwise statistics revealed no significant differences in the number of activated voxels between cradle without and with pups. However, from the 2D composite images it may be appreciated that there was a general reduction in the number of activated voxels with the pup condition vs. the cradle alone.

Presentation of the intruder male in the presence of the pups and nest bedding activated several brain regions in dams that were significantly different from the first 2 conditions. As shown in Figure 1, increase BOLD activation was observed to occur in multiple cortical,

limbic and subcortical areas. The nucleus accumbens was conspicuously different between the three conditions, intruder presentation eliciting a dramatic increase in BOLD in this area (Figure 1 inset). Figure 2 shows that intruder stimulated BOLD activation was particularly robust for prefrontal cortical areas, the nucleus accumbens (Figure 2 inset) and septal-hippocampal circuitry.

Using a multi-factorial analysis of variance we observed statistically significant differences between the intruder male condition and the preceding stimuli. We made *a priori* assumptions about the systems that would become activated with male intruder condition; these included maternal circuits as well as limbic ‘emotional’ circuits. Data for known maternal circuits are shown in Figure 3. The nucleus accumbens and periaqueductal grey showed a significantly greater volume of activation with intruder presentation than the other conditions (statistical parameters summarized in Figure 3 legend). However, other regions known to play key roles in active maternal responding did not show significant differences between the groups or were not activation by any of the 3 stimuli. These included the ventral tegmental area, central and medial nuclei of the amygdala, bed nucleus of the stria terminalis, preoptic area and paraventricular nucleus of the hypothalamus (Figure 3). To corroborate whether limbic emotional circuitry become activated with intruder male presentation, we subdivided regions of interest according to well established models of limbic circuits, namely, the Papez proposed model for emotions (Papez, 1944) and the updated limbic model proposed by Maclean (Maclean, 1952). Using Papez’s model for emotion circuitry we observed several areas that showed increased BOLD activation with intruder male presentation. These included the anterior cingulate area, the anterior thalamus and hippocampal formation areas (statistical parameters summarized in Figure 4 legend). The only area using this model that did not show a significant difference was the mammillary nucleus. Using the Maclean limbic model we observed additional areas showing increased BOLD responses. These included limbic prefrontal areas such as the ventral orbital cortex, the prelimbic cortex and insular cortex, also the temporal cortex, the septum and basonucleus of the amygdala (statistical parameters summarized in Figure 5 legend). The lateral hypothalamus, lateral amygdala and infralimbic region did not show any significant differences between the groups or showed no significant BOLD activity. It is interesting to note that the dorsal striatum also showed a significant increase in BOLD in response to a male intruder compared to preceding stimuli (statistical parameters summarized in Figure 5 legend). We also assessed primary and secondary somatosensory region BOLD responses. We observed greater positive BOLD activation in both subareas when comparing the presentation of pups with and without the male intruder ($F_{3,27} = 5.5$, $p = 0.04$ for primary and $F_{3,27} = 5.9$, $p = 0.009$ for secondary somatosensory).

To test the selectivity of BOLD responses to the male intruder, separate groups of lactating rats were imaged in the absence or presence of pups and nest bedding. Figure 6 summarizes the results of this comparison. Shown are 2D composite maps of BOLD activation with no pups or pups. There are general similarities for the pattern of BOLD activation for several cortical areas, such as the supplementary somatosensory region, motor cortical and olfactory cortical areas. Figure 7 indicates the regions of interest that were significantly different for no pup and pup conditions. We specifically compared areas that were significantly different when comparing no pups, pups and intruder/pup presentations shown in Figures 3–5. Among the regions, the accumbens, basal amygdala, entorhinal cortex, periaqueductal grey and temporal cortex showed greater number of activated voxels for the intruder/pup condition than without the pups (statistical parameters summarized in figure legend). Thus, out of the 68 regions of interest screened for differences, only these were different between the conditions.

As indicated above, both volumes of activation and percent changes in BOLD for negative and positive changes were evaluated between the test conditions. For the number of negative BOLD voxels, only the anterior olfactory nucleus showed a significant difference, no pup condition showing greater number of negative BOLD voxels than with pups ($F_{1,12} = 14.6$, $p = 0.003$). A non-parametric Mann-Whitney U analysis by ranks was used to compare differences between no pup and pup conditions for percent changes in BOLD signal. For positive BOLD signal changes, we report that presenting intruder with pups elicited greater percent changes in BOLD in the CA1 ($p = 0.01$), dentate gyrus of the hippocampus ($p = 0.008$), temporal cortex ($p = 0.05$), midline thalamic nuclei ($p = 0.03$), lateral hypothalamus ($p = 0.05$), basal nucleus of the amygdala ($p = 0.05$), mediodorsal thalamus ($p = 0.001$), dorsal striatum ($p = 0.03$), insular ($p = 0.05$) and gustatory regions of the cortex ($p = 0.05$), accumbens ($p = 0.001$), orbital ($p = 0.008$) and prelimbic regions ($p = 0.005$). Time course data for the voxels in the mediodorsal thalamus, nucleus accumbens and prelimbic area of the medial prefrontal cortex are shown in Figure 8. For negative BOLD signal changes, we report that presenting intruder with pups elicited larger magnitude decreases in BOLD in the somatosensory cortex ($p = 0.03$), CA1 ($p = 0.02$), auditory cortex ($p = 0.05$), lateral hypothalamus ($p = 0.02$), substantia nigra ($p = 0.01$), motor cortex ($p = 0.01$), piriform cortex ($p = 0.008$), secondary somatosensory cortex ($p = 0.03$), insula ($p = 0.002$), substantia innominata ($p = 0.05$), gustatory ($p = 0.05$) and orbital cortex ($p = 0.005$).

DISCUSSION

The present study provides a novel fMRI model that can potentially be used to test BOLD signal changes during the initiation of maternal aggression. At minimum, the present imaging paradigm can be useful in determining brain activation during a state of high emotionality. Lactating rats showed a robust BOLD response to a male intruder when pups are present. The major areas that were activated upon presenting the intruder threat included regions that are involved in aggression (PAG), fear reactions (septum, anterior cingulate, anterior thalamus), emotional learning and memory (basal amygdala, CA1, entorhinal cortex), visceromotor responses (orbital, insular cortex), stereotypic behaviors (dorsal striatum) and surprisingly, the nucleus accumbens and medial prefrontal cortex, prelimbic area, regions implicated in searching for natural rewards. Excluding the latter 2 areas, the rest of the active brain regions are consistent with the notion that a high degree of emotionality was present at the time of intruder presentation. Perhaps a similar pattern of brain activity might occur during moments preceding an aggressive encounter with a nest intruder. We observed that presenting an intruder in the absence of pups still drives activity within most of these areas. However, some areas showed selectivity to the presence of pups, and these were the accumbens, PAG, entorhinal cortex, basal amygdala, and temporal cortex. These regions may comprise part of a neural circuit of maternal aggression and emotional processing.

An aggressive response to intruder males in Long-Evans rats is especially relevant, as males are likely to attack pups. Pup attacks have been observed in previous rodent studies (Lonstein & Stern, 1998; Weil *et al.*, 2006), as well as initial pilot studies for the present experiments. In contrast, the general lack of BOLD responses to a pup stimulus suggests that the distal presentation was not robust enough to increase BOLD, especially when compared to previous suckling induced BOLD activity (Febo *et al.*, 2005). Physical contact with pups may be required to see significant BOLD activation in maternal behavior associated regions.

Maternal Brain Circuit

There were two maternal brain areas activated by intruder presentation; the nucleus accumbens and the periaqueductal gray. The increased BOLD activity in the nucleus accumbens is likely to be involved in the switch from maternal responsiveness to aggressive

behavior. Maternal interaction with pups is associated with increased dopamine release in this region (Hansen *et al.*, 1993; Champagne *et al.*, 2004), and disruption of dopamine signaling in the nucleus accumbens disrupts maternal behavior (Hansen *et al.*, 1991; Keer & Stern, 1999; Silva *et al.*, 2003). Further study of the nucleus accumbens indicates that the medial preoptic area stimulates maternal behavior through inhibition of the nucleus accumbens, which then decreases inhibition at the ventral pallidum. Attenuating inhibition at the ventral pallidum facilitates the expression of maternal behavior (Numan *et al.*, 2005). It is postulated that the increased nucleus accumbens activity during intruder presentation inhibits the ability of the ventral pallidum to respond to pup stimuli. This insensitivity to pup stimuli decreases focus on pup care and allows for the initiation and expression of maternal aggression.

Although lesions to the periaqueductal gray have been shown to increase rat maternal aggression (Lonstein & Stern, 1998), in mice, neuronal activity in the periaqueductal gray increases following the display of maternal aggression (Gammie & Nelson, 2001). Furthermore, acute exposure of a maternal rat to a novel male disrupts maternal behavior and results in increased fos activity in the periaqueductal gray (Pavesi *et al.*, 2007). Studies suggest that 5-HT activity mediates the expression of maternal aggression through actions in this nucleus (De Almeida & Lucion, 1997; de Almeida *et al.*, 2005). Due to the fact that the periaqueductal gray is involved in active avoidance as well as maternal aggression responses, and acute behavioral responses are not possible during MRI imaging, it is possible that the recorded positive BOLD activity represents avoidance behavior. However, given the reliable expression of maternal aggression by Long-Evans mothers when presented with a novel male intruder, it is argued that the significant BOLD in the nucleus accumbens and periaqueductal gray represents aggression-associated responses. The current BOLD activity in the periaqueductal gray supports the hypothesis that this nucleus is involved in the initiation of maternal aggression (Lonstein & Stern, 1998). It is postulated that the lack of significant differences in BOLD in other nuclei associated with maternal behavior is due to the lack of direct mother-infant contact prevented significant increases in BOLD activity.

Papez Emotion Circuit

The intruder stimulus elicited increased BOLD in the anterior cingulate, hippocampal formation, and the anterior thalamic nucleus of the Papez circuit (Papez, 1944). The anterior cingulate is involved in the initiation of goal directed behaviors (Devinsky *et al.*, 1995), as well as the emotional control of visceral, skeletal, and endocrine functions (Vogt *et al.*, 1992). Activation of this region suggests that the sight, odor, and/or sound of a male intruder instigate aggressive behavior with the goal of driving the intruder away and protecting the pups. With respect to maternal behaviors, anterior cingulate lesions impair pup retrieval and the expression of typical full maternal behavior (Slotnick, 1968). The intruder specific activity in this region may be a key component in the initiation of maternal aggression.

The hippocampus is important in the processing of spatial memory (Teng & Squire, 1999). Primiparous females have improved reference and/or working memory compared to nulliparous females (Kinsley *et al.*, 1999; Pawluski *et al.*, 2006; Pawluski *et al.*, 2006), and these changes in memory performance may be related to dendritic remodeling of the CA1 and CA3 regions (Pawluski & Galea, 2006). It has been postulated that parity-induced dendritic remodeling (specifically decreased dendritic length and branching points) is a result of exposure to the elevated CORT levels during chronic stress, and this altered morphology improves learning and memory in primiparous females (Pawluski & Galea, 2006). Based on the current data, it is suggested that the parity-dependent dendritic remodeling in the CA1 region is involved in the aggressive response to an intruder male. Comparative studies with nulliparous females are needed to confirm this hypothesis.

The anterior thalamic nucleus is involved in the formation of spatial memory and acts in conjunction with the hippocampus. One hypothesis for the elevated BOLD activity in this region is that the female perceives that the male intruder is approaching the nest site, despite her location in the MRI.

Limbic System

In the limbic system, the male intruder stimulated BOLD activity in the basal amygdala, dorsal striatum, temporal cortex, prelimbic area, ventral orbital nucleus, and insula. The increased amygdalar BOLD recorded in the present study may be involved in the increased translational activity reported in previous studies. Previous research suggests that the medial, central, and basolateral amygdala are activated during maternal aggression, as early growth response factors increase following the display of aggression in rats (Lonstein & Stern, 1997), hamsters (Joppa *et al.*, 1995), and mice (Gammie & Nelson, 2001). Increased BOLD activity in the dorsal striatum is indicative of a heightened auditory response to the male intruder, as this region is activated in human mothers in response to general auditory stimuli (Lorberbaum *et al.*, 2002). Interestingly, the temporal cortex has been linked with Alzheimer's associated aggression. Decreased 5-HT_{1A} receptor density in the temporal cortex of Alzheimer's patients is associated with increased aggression. It is postulated that increased BOLD response to intruders in the temporal cortex may be linked to the initiation of attacks. Further investigation of this nucleus is needed to understand the function of increased BOLD activity in maternal aggression. Although the prelimbic area has not been extensively studied in female aggression, c-fos expression is increased in males following aggressive encounters (Halasz *et al.*, 2006). The present data indicate that it is not necessary for the expression of physical aggression for this nucleus to be stimulated. C-fos expression is also elevated in the ventral orbital nucleus following aggressive encounters of male mice bred for short attack latencies compared to mice bred for long attack latencies (Haller *et al.*, 2006). Recent studies have postulated that although 5-HT activity is typically negatively correlated with aggression, normal aggression is associated with short duration spikes in serotonergic activity (de Boer & Koolhaas, 2005). It is postulated, therefore, that the increased BOLD in the present study may represent acute 5-HT activation in the prelimbic and ventral orbital nuclei. Lastly, lesions to the insula decrease maternal aggression in rats, which suggests that olfactory stimuli are involved in the aggressive response to an intruder (Ferreira *et al.*, 1987). Our current data showing increased insula activity during the distal presentation of an intruder supports this hypothesis. Since the insula is involved in the processing of olfactory stimuli, it is suggested that increased BOLD in this nuclei is stimulated by intruder odor.

A remarkable but unanticipated finding of the present study was the enhanced BOLD signal in the somatosensory cortex during intruder presentation. The increased somatosensory BOLD signal response occurred in both primary and supplemental areas, but was not generalized to the overall cortical mantle. Furthermore, it did not occur with pup presentation. This finding is consistent with recent findings from our laboratory using the same pup-intruder model (Caffrey *et al.*, 2009). Both in the present work and the cited study, there is significant somatosensory activation in the presence of a male intruder. The remarkable aspect of this finding is that a somatosensory stimulus was not provided at the time of intruder or pup presentation, as in previous studies of lactation inputs (Febo *et al.*, 2005; Febo *et al.*, 2008). Although speculative at this moment, the somatosensory response may be related to the anxiety-evoking event instead of processing within a specific cortical representation of the body. Excitotoxic lesions of the substantia innominata in the nucleus basalis magnocellularis, which provides major cholinergic projections to the somatosensory cortex and prefrontal cortex, results in increased emotionality and deficits in working memory in rats (Wozniak *et al.*, 1989). Selective cholinergic lesions in the nucleus basalis

lead to reduced unconditioned fear-related freezing, a finding that supports a role for cholinergic inputs to the sensory cortex in anxiety and high emotionality (Knox et al., 2008). Recent work in our laboratory shows that both substantia innominata and somatosensory BOLD activation in response to male intruder is modulated by V1a receptors (Caffrey et al., 2009). Importantly, the somatosensory activation in the presence of a male intruder may hold significance for maternal aggression and anxiety related behavioral responses. If pups are separated from the mother for several hours or if the mother's ventral surface is anesthetized so that she cannot detect suckling/nuzzling pups, then maternal aggression wanes. In mice, thelectomy (nipple removal) prevents the occurrence of maternal aggression (Svare and Gandelman, 1976). We also observed differences for negative BOLD signal changes (signal decreases). This was observed across several areas (see Results section). In theory, signal decreases can occur when (i) oxygen extraction exceeds delivery, therefore increasing levels of paramagnetic deoxyhemoglobin, it can also occur with (ii) 'vascular steal' where upon blood from areas of low metabolic demand is re-routed to areas of high activity, and with (iii) neuronal inhibition. It is important to indicate that there is mounting evidence that signal decreases observed in fMRI studies largely correspond to neuronal inhibition (Shmuel et al., 2006).

Conclusion

Taken together, the fMRI data indicate that the presence of a novel male intruder is a potent stimulus of acute neural activity. Several nuclei previously associated with maternal behavior, maternal aggression and memory are activated in response to the presence of an intruder. The BOLD data support and connect several studies of maternal aggression, and also indicate that multiple male aggression associated nuclei are activated during the presentation of an intruder. It is likely that nuclei that did not show significant increases in BOLD activity in the present paradigm are nonetheless still significant in the tactile perception of and/or physical response to a male intruder. In contrast, the distal presentation of pups did not exert significant BOLD activity. When compared to previous data on the increased BOLD in response to pup suckling, the recent data indicate that actual physical contact is needed to stimulate significant changes in brain activity. Future studies of both maternal behavior and maternal aggression will improve the understanding of disorders of maternal behavior and/or aggression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 2.

Composite 3D volume brain maps showing positive BOLD signal changes in response to presentation cradle (no pups), cradle with pups and intruder in the presence of pups. Data are shown for the nucleus accumbens and limbic prefrontal cortical areas (upper row) and septal-hippocampal formation regions (lower row). Right column highlights the volume of BOLD activation in the nucleus accumbens in the pup/intruder condition.

Figure 3.

Number of positive BOLD voxels in several regions of interest implicated in maternal behavior in rats (Numan, 2007). The three stimulus conditions for each panel are the same as in Figure 1–3. Data are expressed as median (minimum-maximum). Statistical posthoc tests were done with Bonferroni multiple comparisons test. * indicates $p < 0.05$; † indicates $p < 0.01$ and ‡ indicates $p < 0.001$. Left panel black line under graph highlights differences in BOLD activation in the nucleus accumbens. Abbreviations: NAcc, nucleus accumbens; CeA, central amygdala; MeA, medial amygdala; BNST, bed nucleus stria terminalis; PVN, paraventricular nucleus; PAG, periaqueductal grey.

Figure 4.

Number of positive BOLD voxels in several regions of interest corresponding to the Papez circuitry (1944). The three stimulus conditions for each panel are the same as in Figure 1–3. Data are expressed as median (minimum-maximum). Statistical posthoc tests were done with Bonferroni multiple comparisons test. * indicates $p < 0.05$; † indicates $p < 0.01$ and + indicates $p < 0.001$. Abbreviations: ACing, anterior cingulate; EntCtx, entorhinal cortex; DG, dentate gyrus; SUB, subiculum; MB, mammillary bodies; AT, anterior thalamus.

Figure 5.

Number of positive BOLD voxels in several regions of interested corresponding to the additional limbic circuitry known for their roles in emotional responses (Maclean, 1952). The three stimulus conditions for each panel are the same as in Figure 1–3. Data are expressed as median (minimum-maximum). Statistical posthoc tests were done with Bonferroni multiple comparisons test. * indicates $p < 0.05$; † indicates $p < 0.01$ and + indicates $p < 0.001$. Abbreviations: BAmyg, basonuclei of amygdala; LAmygd, lateral amygdala; LH, lateral hypothalamus; Spt, septum; DS, dorsal striatum; TC, temporal cortex; Prl, prelimbic area; IL, infralimbic area; VO, ventral orbital area; INS, insular cortex.

Figure 6.

Composite 2D brain maps showing positive BOLD signal changes in response to presentation of a male intruder in the presence and absence of pups. Scale bar hue (orange-to-yellow) indicates percent increase in BOLD with a lower threshold cut-off of 2%. Various regions of interest are highlighted to the left of the figure.

Figure 7.

Number of positive BOLD voxels in several regions of interest compared in Figure 3 to 5. The stimulus conditions for each panel are the same as in Figure 6. Data are expressed as median (minimum- maximum). * indicates $p < 0.05$ and + indicates $p = 0.01$. Abbreviations: NAcc, nucleus accumbens; PAG, periaqueductal grey; BAmyg, basal nucleus of amygdala; Spt, septum; DS, dorsal striatum; TC, temporal cortex; Prl, prelimbic area; VO, ventral orbital area; INS, insular cortex; ACing, anterior cingulate; EntCtx, entorhinal cortex; DG, dentate gyrus; AT, anterior thalamus.

Figure 8. Positive BOLD signal changes over time for the prelimbic medial prefrontal cortex, nucleus accumbens and mediodorsal thalamus. The three stimulus conditions for each panel are the same as in Figure 6.