2013-08-27

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Proceedings of the National Academy of Sciences, Washington, v.110, n.35, p.14438-14443, 2013
http://www.producao.usp.br/handle/BDPI/44379

Ventral premammillary nucleus as a critical sensory relay to the maternal aggression network

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Edited* by Larry W. Swanson, University of Southern California, Los Angeles, CA, and approved July 15, 2013 (received for review March 26, 2013)

Maternal aggression is under the control of a wide variety of factors that prime the females for aggression or trigger the aggressive event. Maternal attacks are triggered by the perception of sensory cues from the intruder, and here we have identified a site in the hypothalamus of lactating rats that is highly responsive to the male intruder—the ventral premammillary nucleus (PMv). The PMv is heavily targeted by the medial amygdalar nucleus, and we used lesion and immediate-early gene studies to test our working hypothesis that the PMv signals the presence of a male intruder and transfers this information to the network organizing maternal aggression. PMv-lesioned dams exhibit significantly reduced maternal aggression, without affecting maternal care. The Fos analysis revealed that PMv influences the activation of hypothalamic and septal sites shown to be mobilized during maternal aggression, including the medial preoptic nucleus (likely to represent an important locus to integrate priming stimuli critical for maternal aggression), the caudal two-thirds of the hypothalamic attack area (comprising the ventrolateral part of the ventromedial hypothalamic nucleus and the adjacent tuberal region of the lateral hypothalamic area, critical for the expression of maternal aggression), and the ventral part of the anterior bed nuclei of the stria terminalis (presently discussed as being involved in controlling neuroendocrine and autonomic responses accompanying maternal aggression). These findings reveal an important role for the PMv in detecting the male intruder and how this nucleus modulates the network controlling maternal aggression.

Postpartum rats are highly aggressive toward other animals that enter the vicinity of their nest and pups, with the presumed function of protecting their offspring from harm (1). Maternal aggression is under the control of a wide variety of factors that prime the females for aggression or trigger the aggressive event. The priming of maternal aggression in laboratory rats relies first on the hormonal changes associated with late pregnancy and parturition, and then on exteroceptive stimulation from the pups (2–6). Maternal attacks are triggered by the perception of polymodal sensory cues from the intruder (7). Information from the main olfactory pathway is crucial for high maternal aggression, although any importance for accessory olfactory inputs is less clear (5, 6). Furthermore, somatosensory signaling from the perioral region of the dams' face while they investigate an intruder is critical for attacks (8).

Considering the overlap of sensory modalities involved in how offspring cues prime maternal aggression and those involved in how intruders trigger aggressive behavior, experimental approaches that permanently destroy a sensory system are difficult to interpret because the result observed could be due to altered priming or altered triggering of the behavior. For that reason, identifying a brain area that specifically responds to the intruder as a threat would clarify what sensory information is important for triggering maternal attacks and thus shed light on the neural organization of maternal aggression.

The research on the neurobiology of maternal aggression has traditionally investigated individual brain sites and not often conceptualized the behavior as the outcome of activity of a much larger neural network. A region in the mediobasal hypothalamus, encompassing both the ventrolateral part of the ventromedial nucleus and adjacent regions of the subfornical lateral hypothalamic area, is critical for the expression of maternal aggression because electrolytic lesions therein practically abolished dam's attacks on male intruders (9). It is notable that this part of the mediobasal hypothalamus corresponds to the caudal two-thirds of the so-called hypothalamic attack area (HAA), a region defined on functional grounds as the hypothalamic site with the lowest threshold to evoke aggressive responses (10). At this point, it is unclear how the sensory inputs priming or triggering maternal aggression influence this particular region of the HAA essential for the expression of aggressive behavior in lactating rats.

To begin addressing what brain sites are involved in triggering maternal attacks, we have identified a site in the hypothalamus of lactating rats that is highly responsive to the male intruder—the ventral premammillary nucleus (PMv). We here used lesion and immediate-early gene studies to test our working hypothesis that the PMv signals the presence of a male intruder and transfers this information to the network organizing maternal aggression. Overall, the results help to unravel the basic neural organization of underlying maternal aggression, suggesting sites potentially involved in integrating priming and triggering events, and how those sites are related to the regions involved in the expression of aggressive behavior or its accompanying endocrine and autonomic responses.

Results

Fos Expression in the PMv in Response to a Male Intruder. First, we compared the Fos expression in the PMv between dams left undisturbed in their home cage with their pups ($n = 5$) and unlesioned dams tested for maternal aggression ($n = 5$). When exposed to an intruder, unlesioned dams tested for maternal aggression started attacking 73.16 ± 6.97 s after males had been placed in the cage. During a 5-min observation period, these dams attacked 6.4 ± 0.75 times, with a total duration of attacks of 42.95 ± 6.44 s. Other aggressive responses such as aggressive grooming, sideways postures, kicking the male, and boxing were also observed.

Compared with dams left undisturbed with their pups, unlesioned dams exposed to intruder males presented a striking increase in Fos expression in the PMv (846 ± 132 cells per square millimeter for exposed dams vs. 14 ± 3 cells per square millimeter

Author contributions: S.C.M. and N.S.C. designed research; S.C.M., C.C.G., I.C.F., and N.S.C. performed research; S.C.M., C.C.G., M.H.S., M.V.C.B., J.S.L., and N.S.C. analyzed data; and S.C.M., J.S.L., and N.S.C. wrote the paper.

The authors declare no conflict of interest.

*This Direct Submission article had a prearranged editor.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1305581110/-/DCSupplemental.

for undisturbed dams; Fig. 1). Univariate ANOVA revealed that the difference between groups was highly significant ($F_{1,8} = 39.46$; $P = 0.0002$).

Role of the PMv in the Expression of Maternal Aggression. Next, we examined the putative roles of the PMv in maternal aggression in dams bearing bilateral cytotoxic lesions of the nucleus. In five animals, we obtained relatively symmetric bilateral lesions mostly restricted to the PMv. These lesions included almost the entire extent of the PMv with only a minimal spread over neighboring sites, such as the posterior periventricular nucleus and adjacent parts of the lateral hypothalamic area (Fig. 2). Another four animals sustained either unilateral ($n = 3$) or asymmetric bilateral lesions with one side sustaining only minimal damage to the PMv ($n = 1$). These animals were removed from the present analysis.

Bilateral cytotoxic PMv lesions did not affect any measure of maternal behaviors examined (Mann–Whitney u tests; $P > 0.531$ for all four variables). The lesions did, however, produce a clear reduction in maternal aggression (Fig. 3). Both the number ($Z = 2.611$, $P = 0.009$) and the total duration ($Z = 2.611$, $P = 0.009$) of the attacks were significantly reduced in PMv-lesioned animals. The latency to begin sniffing the intruder did not differ between the groups ($Z = 0.522$, $P = 0.601$), suggesting that both unlesioned and PMv-lesioned dams equally noticed the introduction of the intruder to the cage. However, the latency to start attacking the intruder tended to be longer for PMv-lesioned dams ($Z = 1.776$, $P = 0.075$). Likewise, the time spent in sideway postures and boxing tended to be lower in PMv-lesioned animals (for both sideway postures and boxing; $Z = 1.776$, $P = 0.075$). Duration of aggressive grooming was similar between the groups ($Z = 0.104$, $P = 0.92$). In the relative absence of aggressive behaviors, PMv-lesioned dams instead spent more time exploring the cage ($Z = 2.611$, $P = 0.009$) and tended to sniff the intruder more ($Z = 1.776$, $P = 0.075$).

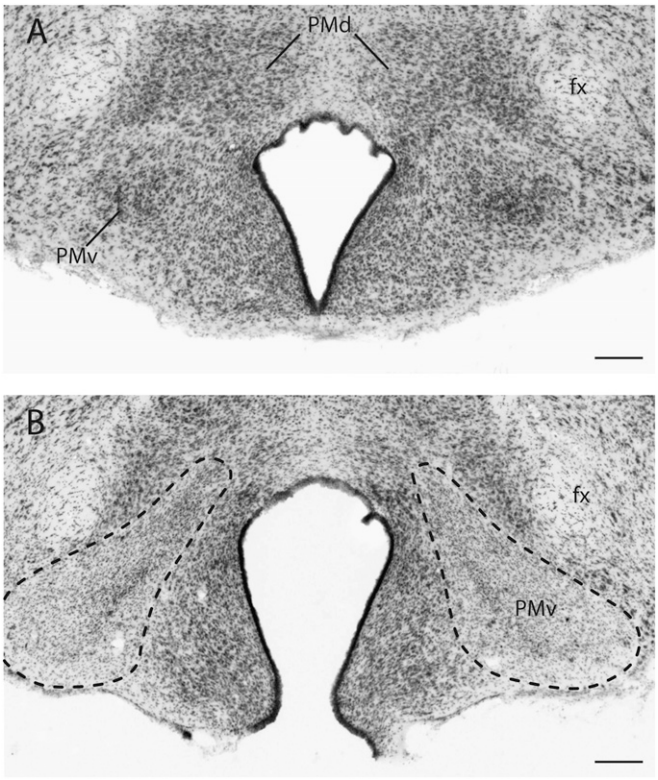

Fig. 2. Photomicrograph of a transverse thionin-stained section from an unlesioned dam depicting the intact PMv (*A*) and a representative lesioned dam illustrating the extent and appearance of the PMv lesion (*B*). The broken lines delineate the lesion extent. (Scale bars, 200 μm.)

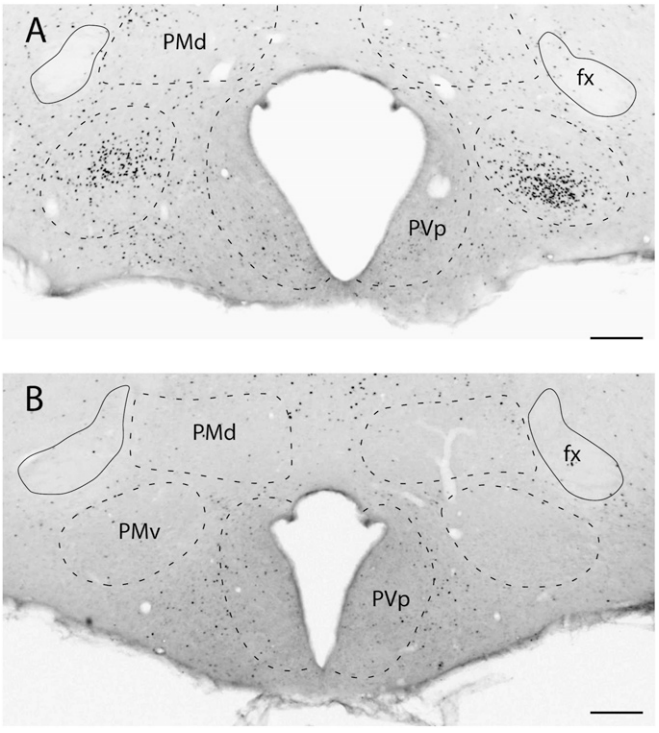

Fig. 1. Photomicrographs of transverse sections containing Fos-immunoreactive cells at the level of the PMv from an unlesioned dam exposed to a male intruder (*A*) and a lactating rat left undisturbed with her pups (*B*). (Scale bars, 200 μm); fx, Fornix; PMd, dorsal premammillary nucleus; PVp, periventricular hypothalamic nucleus, posterior part.

Brain Fos Expression in Unlesioned and Bilateral PMv-Lesioned Dams Exposed to an Intruder Male and Dams in the Home Cage with Pups. We performed a comparative analysis among the three groups (i.e., unlesioned and PMv-lesioned dams tested for maternal aggression and dams left undisturbed in their home cage with the litter) of the Fos expression in selected nuclei of the amygdalar and septal region, and in a comprehensive number of hypothalamic sites. Univariate ANOVA revealed a significant group effect for the posterodorsal ($F_{2,12} = 12.46$, $P = 0.0012$) and posteroventral ($F_{2,12} = 14.86$, $P = 0.0006$) parts of the medial amygdalar nucleus, the posterior amygdalar nucleus ($F_{2,12} = 54.88$, $P < 0.0001$), the ventral part of the anterior bed nuclei of the stria terminalis ($F_{2,12} = 18.38$, $P = 0.0002$), the medial preoptic nucleus ($F_{2,12} = 33.35$, $P < 0.0001$), the tuberal region of the lateral hypothalamic area ($F_{2,12} > 15.47$, $P < 0.0005$), and the ventrolateral part of the ventromedial hypothalamic nucleus ($F_{2,12} > 14.97$, $P < 0.0005$). See Table S1 for a complete account on the density of Fos-labeled cells in the brain regions of unlesioned and PMv-lesioned dams tested for maternal aggression and dams left undisturbed with the pups.

Compared with dams left undisturbed in their home cages with their litter, post hoc pairwise comparisons revealed that unlesioned dams that expressed maternal aggression had significantly higher Fos expression in amygdalar sites processing intruder's olfactory cues [i.e., posteroventral and posterodorsal parts of the medial amygdalar nucleus (MEApv and MEApd); $P < 0.0011$] and in the posterior amygdalar nucleus (PA; $P = 0.0002$). In the septal area, unlesioned aggressive dams showed significantly more Fos-labeled cells in the ventral part of the anterior division of the

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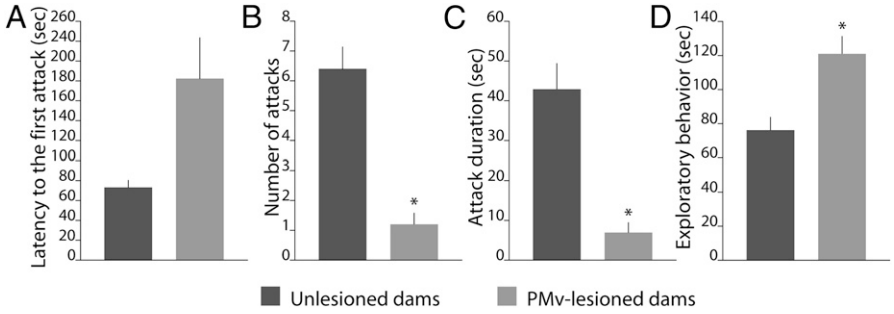

Fig. 3. Histograms of the behavior of unlesioned ($n = 5$) and PMv-lesioned ($n = 5$) dams tested for maternal aggression, showing (*A*) latency to the first attack, (*B*) number of attacks, (*C*) total duration of attacks, and (*D*) exploratory behavior. Data are expressed as mean ± SEM; *$P < 0.05$ vs. unlesioned dams (Mann–Whitney *u* test).

bed nuclei of the stria terminalis (BSTv; Fig. 4*C*; $P = 0.0032$), which encompasses the dorsomedial, anteromedial, magnocellular, and ventral nuclei (11–13). In the hypothalamus, in addition to the PMv, unlesioned dams displaying maternal aggression had a significantly greater number of Fos-immunoreactive cells in the medial preoptic nucleus (MPN; Fig. 4*C*; $P = 0.0066$), the ventrolateral part of the ventromedial hypothalamic nucleus (VMHvl; Fig. 4*D*; $P < 0.0006$), and the tuberal region of the lateral hypothalamic area (LHAtu; Fig. 4*D*; $P < 0.0005$).

Compared with what was found for unlesioned dams expressing maternal aggression, bilateral PMv lesions that reduced maternal aggression resulted in significant less Fos expression in the BSTv ($P = 0.0004$), the MPN ($P = 0.0002$), and the entire rostrocaudal extent of the VMHvl ($P < 0.0068$) and the LHAtu ($P < 0.0185$). It is interesting to note that unlesioned and PMv-lesioned dams exposed to a male intruder had comparable levels of Fos expression in the posterodorsal and posteroventral MEA ($P > 0.2077$) and the PA ($P = 0.0613$).

Correlations Between Maternal Aggression and Septal and Hypothalamic Fos Expression in PMv-Lesioned Dams. Considering that PMv-lesioned dams showed a significant reduction in maternal aggression, and at the same time, failed to exhibit increased Fos expression in specific septal and hypothalamic sites, we tried to understand whether the activation of these brain areas influenced by the PMv would have any relationship to the expression of maternal aggression. To this end, we collapsed unlesioned ($n = 5$), partial-lesioned ($n = 4$), and bilateral-lesioned dams ($n = 5$) tested for maternal aggression into a single group and tested the linear Spearman correlation between the total duration of attacking and brain Fos expression. A significant positive correlation was found between duration of attacking and Fos expression in the medial preoptic nucleus ($r = 0.77$, $P = 0.0012$), caudal level of the VMHvl ($r = 0.68$, $P = 0.0077$), BSTv ($r = 0.66$, $P = 0.0102$), and caudal level of the LHAtu ($r = 0.54$, $P = 0.0445$).

Discussion

We observed here that the PMv is highly responsive to a male intruder placed in the home cage and critical for high maternal aggression. Comparing the pattern of Fos expression in unlesioned and PMv-lesioned dams exposed to a male intruder, we have been able to outline a network likely to underlie maternal aggression and study how the PMv modulates activation of this network. Fos protein expression has been widely used as a sensitive cellular marker for neuronal activation induced by a variety of stimuli. An important caveat, however, is that there are several examples in which neuronal activation occurs without induction of Fos, so the absence of neuronal Fos expression cannot be interpreted as a lack of influence on neuronal activity (14). In line with this view, Fos expression is not induced by the opening of ionotropic channels that do not increase the intracellular levels of second messengers, and it provides only a partial view of the mobilized brain systems (14). Despite the limitations, the visualization of Fos and other immediate-early genes as markers of neuronal modulation will continue to be an extremely powerful tool.

Compared with lactating rats left undisturbed with their pups, dams exposed to a male intruder displayed maternal aggression and had increased Fos expression in numerous amygdalar, septal, and hypothalamic sites. In the amygdala, significantly increased Fos was observed in posterodorsal and posteroventral medial amygdalar nucleus (MEApd and MEApv, respectively). This is consistent with the increased Fos immunoreactivity in the MEApd and MEApv of maternally aggressive mice (15, 16). The MEA receives accessory and main olfactory bulb inputs (17, 18) and projects to medial hypothalamic sites shown here to upregulate Fos during maternal aggression (18). Because the MEA is critical for social recognition in rodents (19, 20), it may exert a key role in processing olfactory information critical for detecting a male intruder. Olfactory inputs are necessary for maternal aggression, as dams bearing surgical interruption between the olfactory bulbs and the peripheral sensory organs (i.e., the olfactory mucosa) do not attack (5, 6). Although the role for pheromonal information in maternal aggression in laboratory rats is debatable (5, 6), in mice, loss of the *Trpc2* gene that codes for an ion channel in the vomeronasal organ severely diminishes maternal aggression (15).

The PA also had increased Fos expression in dams displaying maternal aggression. It receives projections from the MEA and projects to medial hypothalamic sites mobilized during maternal aggression (21). The PA expresses very high levels of mineralocorticoid receptors (21), and in the present context, it is particularly noteworthy that blockade of mineralocorticoid receptors inhibits aggressive behavior in male rats (22). Therefore, the PA may work as a potential target of mineralocorticoids to influence maternal aggression.

In the septal region, dams expressing maternal aggression had significantly more Fos-labeled cells than controls in the BSTv, a region encompassing the dorsomedial, anteromedial, magnocellular, and ventral nuclei (11–13). This is consistent with the high Fos expression in the BSTv in maternally aggressive mice (23). The BSTv receives projections from regions of the medial hypothalamus here shown to be mobilized during maternal aggression, including the VMHvl and the LHAtu (24). The BSTv also contains the densest noradrenergic terminal field in the forebrain, arising mostly from collaterals of A1 and A2 cells that collateralize to the paraventricular nucleus (25), compatible with a role in visceral and neuroendocrine control. In line with this view, on the efferent side, the BSTv projects to hypothalamic regions controlling the autonomic (i.e., the descending autonomic parts of the paraventricular nucleus) and neuroendocrine system (i.e., neurons containing oxytocin, corticotropin-

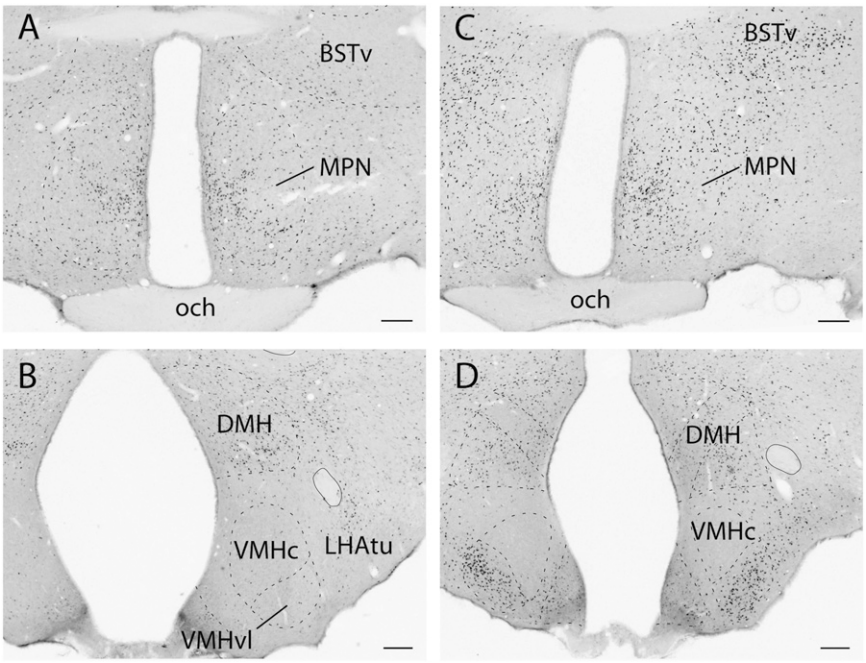

Fig. 4. Photomicrographs of transverse Fos-stained sections of the MPN and BSTv (*A* and *C*) and the VMHvl and LHAtu (*B* and *D*) of dams left undisturbed with their pups (*A* and *B*) and intact dams tested for maternal aggression (*C* and *D*). (Scale bars, 200 μm.) DMH, dorsomedial hypothalamic nucleus; och, optic chiasm; VMHc, ventromedial hypothalamic nucleus, central part.

releasing hormone, thyroid stimulating hormone (TSH)-releasing hormone, somatostatin, and dopamine) (11–13). The noradrenergic innervation of the BSTv is also involved in stress-induced anxiety (26), which may interfere with maternal aggression (1). Last, the BSTv strongly projects back to the LHAtu (11–13) a component of HAA, which as we shall discuss below, is involved in the expression of maternal aggression. Therefore, the BSTv is in a position to influence behavioral, as well as neuroendocrine and vegetative responses, related to maternal aggression. However, further studies are needed to understand the role of the BSTv in the context of maternal aggression.

Among the hypothalamic nuclei mobilized during maternal aggression, the PMv presented one of the highest increases in Fos expression. The PMv is heavily targeted by the MEA (18) and likely conveys olfactory information about the male intruder. In fact, the PMv contains neurons particularly responsive to animals of the opposite sex (27, 28). Moreover, PMv neurons can produce nitric oxide (29), which enhances incoming glutamatergic neurotransmission (30), and, in the present context, may serve to amplify responses to the male intruder and help trigger maternal attacks. In line with this view, deletion of the nitric oxide synthase gene in mice reduces maternal aggression (31).

We here show PMV lesions significantly reduced maternal aggression and this was not the result of general behavioral debilitation because lesioned dams normally approached and began sniffing the intruder and showed significantly more time exploring the home cage. Also important was that PMv lesions did not appear to affect maternal care, so the deficit in aggression is not secondary to a lack of sensory inputs from pups. We suggest that the PMv-lesioned dams did not consider the intruder presence as a threat. This may be mediated through the strong ascending projections from the PMv to other hypothalamic sites (32) shown here to up-regulate Fos expression during maternal aggression. This included the medial preoptic nucleus (MPN), VMHvl, and LHAtu. During maternal aggression, PMv lesions reduced Fos in these hypothalamic targets, compatible with the idea the PMv signals intruder presence and transfers this information to these sites for the expression of maternal aggression. Moreover, compared with unlesioned animals, PMv lesions also prevented the increased Fos expression in the BSTv seen during maternal aggression. As previously mentioned, the BSTv receives strong inputs from the VMHvl and LHAtu (24) likely to mediate this influence from the PMv.

A significant positive correlation was found between the duration of maternal attacking and Fos expression in all brain sites influenced by PMv lesions (MPN, VMHvl, LHAtu, and BSTv). The medial preoptic area is critical for the hormonal onset and maintenance of maternal behaviors (33), but as such its separate role in maternal aggression can be difficult to assess. It does have increased Fos expression after maternal aggression in mice (23). Postpartum aggression in rats is stimulated by hormonal factors associated with late pregnancy, but later supported by sensory stimulation from pups (4). Similar to maternal behavior (33), the MPN may integrate the hormonal and pup-related sensory cues critical for the onset and maintenance of maternal aggression. The MPN can receive pup olfactory inputs directly from the MEA and possibly their tactile inputs via the subparafascicular nucleus (34). Moreover, the MPN did respond to the presence of a male intruder, and this response was impaired in PMv-lesioned dams.

The MPN may influence maternal aggression via its descending projections to the VMHvl and adjacent LHAtu (35), which are in the caudal two-thirds of the so-called hypothalamic attack area. The HAA has been defined on functional grounds and is the hypothalamic region with the lowest threshold to evoke aggressive responses (see references in ref. 10). It begins at the level of the anterior hypothalamic nucleus occupying the intermediate hypothalamic area just below and medial to the fornix, and continues caudally through the entire rostrocaudal extent of the VMHvl and adjacent LHAtu (10). Of particular interest, maternal aggression was associated with a significant increase in Fos expression in the caudal two-thirds, but not in the rostral third, of the HAA. This has also been found in lactating mice (23). Optogenetic activation of the VMHvl neurons rapidly induces aggression, confirming that VMHvl activity is sufficient to induce aggressive responses (36).

Confirming the role of the caudal part of the HAA in maternal aggression, electrolytic lesions encompassing the VMHvl and LHAtu practically abolished dam's attacks on male intruders, but did not affect maternal care (9). Therefore, the VMHvl and LHAtu may be the key hypothalamic sites for the expression of maternal aggression. Functional studies revealed that attacking responses induced by ventromedial hypothalamus stimulation depend on a descending pathway through the ventral supraoptic commissures to the subparafascicular nucleus and peripeduncular area (37). Indeed, lesions of the peripeduncular area reduce maternal aggression (38), but further studies are necessary to clarify how these target areas of the ventromedial hypothalamus would be able organize the aggressive responses.

The relationship between anxiety and aggression in postpartum rats is also unclear—some early reports suggest negative relationship and more recently others indicate a positive relationship (1). Of particular interest, neither intact nor lesioned dams exposed to intruder males showed clear defensive or submissive postures. In line with this view and contrasting to what has been found in subordinate rats exposed to dominant males, aggressive dams do not increase Fos expression in the dorsomedial part of the dorsal premammillary nucleus, a key hypothalamic region associated with social defense (39).

In sum, the results of the present studies help clarify the neural substrates involved in maternal aggression (Fig. 5). We provide evidence that the PMv is involved in maternal aggression, by showing it contains high immediate-early gene activity in response to maternal aggression and that lesioning it reduces dams' aggression as well as the activation of key hypothalamic and septal sites related to maternal aggression. We were able to outline a broader network regulating the maternal detection of intruders and triggering of postpartum aggression. The present findings add an important piece of information to understand the maternal aggression network and open interesting perspectives for future studies.

Materials and Methods

Animals. Long–Evans female rats ~3 mo old at the beginning of the experiments were used. They were kept in groups of two or three females with an adult Long–Evans male rat for breeding. About one week before labor, pregnant females were individualized in a Plexiglas home cage (45 × 45 × 30 cm) where the behavioral tests were conducted. On day 1 postpartum, the litter was standardized to contain four males and four females. Naive Wistar male rats (3 mo old) were used as intruders in the maternal aggression tests.

All animals were obtained from the Universidade de São Paulo breeding facility, kept under 12/12 h light–dark cycle, food and water available ad libitum and controlled temperature (23 °C), according to the guidelines of the Committee on Animals of the Colégio Brasileiro de Experimentação Animal and the Committee on Care and Use of Laboratory Animal Resources, National Research Council. All experimental procedures had been previously approved by the Committee on Care and Use of Laboratory Animals of the Institute of Biomedical Sciences, Universidade de São Paulo (Protocol CEEA 030.09).

In the present experiments, we have used the following groups: PMv-lesioned dams tested for maternal aggression, unlesioned dams tested for maternal aggression, and a third group of dams left undisturbed with their pups.

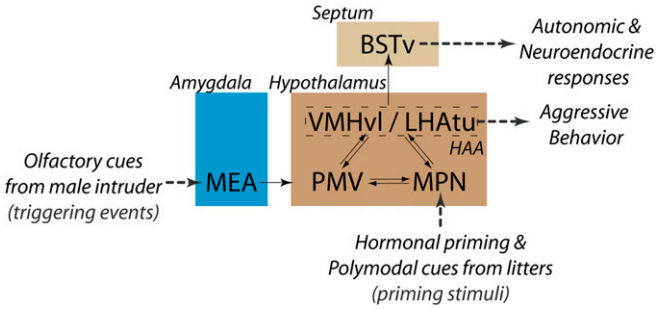

Fig. 5. Schematic diagram showing the putative brain network underlying maternal aggression. Olfactory input from the MEA to the PMv may be required for dams' ability to detect the intruder, and this information may then be transmitted to the MPN as a locus for the hormonal and sensory integration critical for priming maternal aggression. Descending projections from the PMv and primed MPN to the caudal two-thirds of the HAA (including the VMHvl and LHAtu) may then trigger aggressive responses, and HAA projections to the BSTv may control accompanying neuroendocrine and visceral responses.

PMv NMDA Lesions. For the lesion procedure, we iontophoretically applied the neurotoxin, *N*-Methyl-D-aspartate (NMDA), bilaterally in the PMv ($n = 12$). Considering that PMv damage may lead to estrous cycle disturbance (40), the lesions were performed between days 14 and 17 of pregnancy. The animals were anesthetized with an injection of sodium pentobarbital (Cristália; 40 mg/kg, i.p.) and placed in a stereotaxic frame. Bilateral iontophoretic deposits of a 0.15 M NMDA solution (Sigma) were centered in the PMv through a glass micropipette (40-μm tip diameter) for a 10 min using a constant-current device (model CS3; Midgard Electronics) set to deliver −15 mA, with 7-s pulse and interpulse durations.

Behavior Observations. ***Maternal behavior.*** Maternal care toward the pups was determined in unlesioned and PMv-lesioned dams tested for maternal aggression as previously described (41). After giving birth, dams were left with their litters, culled to eight pups on day 1 of lactation (four males and four females). Maternal behavior was assessed from postpartum day 1 to day 4 at the beginning of the light period. During a 50-min session, dams' behaviors were observed 10 times for 5 s each at 5-min intervals, and classified in one of the following four parameters: nursing, licking or grooming one of the pups, in contact with at least half of her pups, and nonmaternal behavior; the frequency of each parameter was compared between groups (41).

Maternal aggressive behavior. Unlesioned and PMv-lesioned dams were tested for maternal aggression on day 5 or day 6 of lactation, in the first hour of the dark period, by placing an unfamiliar intruder male rat into her home cage with the pups. The tests lasted for 5 min and were video recorded for later analysis using the software The Observer (Noldus). The attacks were characterized by lunging motion toward the male followed by wresting bout, biting, and pinning. The scored behaviors were: latency to the first attack, aggressive grooming, sideways postures, boxing, attacks (jump-attack or side-attack, including the subsequent pinning of the intruder), sniffing the intruder, exploratory behaviors, and maternal behaviors. Each behavior was compared between groups.

Fos Immunohistochemistry. The group of dams left undisturbed with their pups was perfused on day 6 of lactation, whereas unlesioned and PMv-lesioned dams tested for maternal aggression were perfused 90 min after the intruder had been removed from the cage. For the perfusion procedure, dams were deeply anesthetized with sodium pentobarbital (Cristália; 40 mg/kg, i.p.) and transcardially perfused with saline (0.9%) followed by 4% (mass/vol) paraformaldehyde in 0.1 M phosphate buffer at pH 7.4; the brains were removed and left overnight in a solution of 20% sucrose in 0.1 M phosphate buffer at 4 °C. The brains were then frozen and five series of 40-μm-thick sections were cut with a sliding microtome. One series was processed immunohistochemically to detect Fos using a rabbit anti-Fos antiserum (Ab-5; Calbiochem) for 72 h at 4 °C at a dilution of 1:20,000. The primary antiserum was localized using a variation of the avidin–biotin complex procedure. In brief, sections were incubated for 90 min at room temperature in a solution of biotinylated goat anti-rabbit IgG (Vector Laboratories) and then placed in the mixed avidin–biotin HRP complex solution (ABC Elite Kit; Vector Laboratories) for 90 min. The peroxidase complex was visualized by a 10-min exposure to a chromogen solution containing 0.02% 3,3′-diaminobenzidine tetrahydrochloride (Sigma) with 0.3% nickel-ammonium sulfate in 0.05M Tris buffer (pH 7.6), followed by incubation for 10 min in chromogen solution with hydrogen peroxide (1:3,000) to produce a blue-black product. The reaction was stopped by extensive washing in potassium PBS (pH 7.4). Sections were mounted on gelatin-coated slides and then dehydrated and coverslipped with DPX neutral mounting medium (Sigma). One adjacent series of sections was stained by the Nissl method with thionin for cytoarchitecture reference and to delineate the NMDA lesion site.

Quantification of Fos-Labeled Cells. Fos immunoreactivity was quantified in selected amygdalar, septal, and hypothalamic areas (Fig. S1 and Table S1 give a more complete account on the brain sites studied). The number of Fos immunoreactive neurons was evaluated by an observer without knowledge of the animal's experimental status and were quantified by using the 10× objective of a Nikon Eclipse 80i (Nikon Corporation) microscope equipped

with a Nikon Digital Camera DXM1200F (Nikon Corporation). For the quantification of Fos labeling, we first delineated in a given section the borders of a region of interest, as defined in adjoining Nissl-stained sections, and Fos-labeled cells were counted therein. Only darkly labeled oval nuclei that fell within the borders of a region of interest were counted. The density of Fos labeling was determined bilaterally, at a particular level of selected brain regions, by dividing the number of Fos immunoreactive cells by the area of the region of interest (Fig. S1 and Table S1). Both cell counting and area measurements were done with the aid of a computer program (Image-Pro Plus, version 4.5.1; Media Cybernetics). For the presently examined brain regions, the quantification of Fos labeling represents an average of the density of Fos labeling found on each side of the brain. Rat neuroanatomical parceling and mapping followed Swanson's atlas of the rat brain (42).

Statistical Analyses. The data on the density of Fos-labeled cells were analyzed using a univariate ANOVA for each dependent variable, followed by post hoc analysis (Tukey's honestly significant difference post hoc test) to isolate the respective effect, when appropriate. To keep the overall type I error at 5%, the significance level used in the univariate ANOVA was adjusted downward according to the number of dependent variables ($\alpha = 0.002$, Sidak's correction).

In both unlesioned and PMv-lesioned dams tested for maternal aggression, maternal and aggressive behaviors were analyzed using Mann–Whitney *u* tests with the significance level set at 5%. Correlations between the duration of maternal attacking and the density of Fos-labeled cells in selected brain sites were performed with Spearman's rank correlation coefficient and a significance level at 5%.

ACKNOWLEDGMENTS. This research was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) Grants 05/59286-4 and 11/02425-3, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (awarded to N.S.C., S.C.M., I.C.F.. and M.H.S.) were supported by FAPESP fellowships, and C.C.G. received Coordenação de Aperfeiçoamento de Pessoal de Nível Superior scholarship.

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