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Research report

Intracellular preoptic and striatal monoamines in pregnant and lactating rats: possible role in maternal behavior

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Abstract

In many mammals, hormonal fluctuations during pregnancy and parturition produce neurochemical events that are necessary for the transition from a non-maternal state to a maternal state that occurs when infants are born. However, the nature of these events is mostly unknown. We investigated whether changes in dopamine (DA) and serotonin (5-HT) activity within the preoptic area (POA) and striatum, neural sites important for some maternal behaviors, could be part of this process. Female rats were sacrificed as either diestrus virgins, on pregnancy day 10 or 20, on the day of parturition, or on day 7 or 17 of lactation. Bilateral tissue punches from the POA, dorsolateral striatum (ST_{dl}), and nucleus accumbens (NA) were obtained and levels of intracellular DA and 5-HT analyzed with high-performance liquid chromatography with electrochemical detection (HPLC–EC). In the POA, DA was high in virgins and during early pregnancy, lowest on the day of parturition, and very high during lactation. Although there were no changes in the DOPAC to DA ratio (i.e., turnover), DOPAC levels also followed this pattern. 5-HT turnover in the POA was lower in virgins compared to other groups. In the ST_{dl}, DA turnover was highest during late pregnancy and on the day of parturition, while no changes in 5-HT measures were found. No significant effects were found in the NA. Therefore, decreased DAergic activity in the POA and increased DAergic activity in the ST_{dl} occurs around parturition, the time when maternal behavior emerges, and may influence its onset.

Theme: Neural basis of behavior

Topic: Monoamines and behavior

Keywords: Dopamine; Lactation; Maternal behavior; Postpartum estrus; Pregnancy; Preoptic area; Serotonin; Striatum; Suckling

1. Introduction

The dramatic transition in maternal responsiveness that occurs between insemination and parturition in female rats is one of the most remarkable behavioral modifications that occurs in adult animals. Indeed, the vast majority of virgin and even late-pregnant rats either ignore or attack pups and only within a few hours of birth does the peri-parturient female begin to display nurturant behaviors [40]. This behavioral change is thought to critically depend on fluctuations in ovarian and pituitary hormones that occur during pregnancy and parturition [6]. Once mothering is well established after parturition, however, hormones are thought to have little influence on the maintenance of the dam's maternal behavior, and sensory cues from pups instead sustain her maternal responsiveness.

Numerous areas of the brain likely respond to ovarian and pituitary hormones to prepare the dam to act maternally and coordinate her maternal behavior, and the preoptic area (POA) is thought to be particularly important [44]. Lesions of the POA severely and selectively impair retrieval of pups and nest building [45], as well reduce the motivation of dams to work for physical access to pups [32]. Administration of estradiol directly into the POA promotes maternal responding in virgin female rats [48], suggesting that the onset of maternal behavior around the time of parturition is produced, at least in part, by estrogen-sensitive neurons in the POA. Although the POA

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is an extensively studied component of the neural circuit necessary for some maternal behaviors, relatively little is known about the neurochemical events that occur here in response to estradiol and other hormones that might be necessary to produce the general 'maternal state' that allows female rats to act parentally at parturition or maintain the behavior through lactation. In fact, the role of any neurotransmitter acting specifically within the POA for the onset or maintenance of maternal behavior in rats has never been investigated.

The POA contains and is sensitive to a variety of neurotransmitters, including GABA [22,35], glutamate [16,38], norepinephrine [14], dopamine (DA) [1,5], and serotonin (5-HT) [15,58]. Changes in the POA activity of any of these neurotransmitters during pregnancy or lactation may influence the dam's maternal behavior. However, it appears that POA norepineprine [6] and serotonin [2,7] are not absolutely critical for this behavior. In contrast, systemic or widespread central manipulations of DAergic activity severely impair many aspects of maternal behavior in rats [17–21,46,65,66], but it remains to be seen if the POA is one of the necessary sites of action. This possibility is supported by the fact that DA release increases in the POA of recently parturient sheep [30], and that elevated DA release in the POA during copulation in rats is critical for their sexual motivation and performance [24,39], which is in some ways similar to maternal motivation and performance (e.g., Ref. [63]).

We herein measured intracellular stores of DA in the POA during different stages of pregnancy and lactation with the hypothesis that basal DAergic activity would be lower in non-maternal (virgin and pregnant) females than maternal (parturient and lactating) females. Because a change in intracellular levels of DA could either reflect altered DA release or altered synthesis, we also measured intracellular levels of the DA metabolite DOPAC as an indicator of DA turnover. Levels of intracellular 5-HT and its 5-HIAA metabolite were also measured with the expectation that no changes would be found in the POA across the reproductive cycle.

In addition, we examined DA and 5-HT activity in the dorsal and ventral striatum. Although there has been no study of striatal 5-HT release and its effects on maternal care, the function of DA release in this structure has been widely studied. Postpartum interactions with pups increases dopamine release in the nucleus accumbens (NA) [19], and lesions of it impair active maternal behaviors such as retrieval and licking [20,32], as does antagonism of its D1 and D2 receptors [29]. In light of these results, increases in DA activity in the NA might occur through the reproductive cycle to prepare the dam for her maternal role. Studies that have manipulated the dorsal striatum in lactating rats have focused on medial areas of this region and have found very few effects on maternal behavior [20,29,71]. However, we have instead sampled an area of the dorsolateral striatum that is known to show electrophysiological responses and increased 2-DG uptake after perioral stimulation [9] and during licking [41] in rats. Considering that perioral somatosensory stimulation is critical for retrieval and licking of pups [64], increased DAergic activity in the ST_{dl} may influence the onset and maintenance of oral maternal behaviors.

2. Materials and methods

2.1. Subjects

Subjects were 60 adult female Sprague–Dawley rats (Taconic, Germantown, NY, USA) purchased at 65–75 days of age. Subjects were housed in groups of two to three animals/cage in clear polypropylene pan cages (48×28×16 cm) with wood shaving for bedding for 1 week prior to any manipulation. Non-mated females were then rehoused individually and mated females were housed individually after mating and detection of a sperm plug, with this day designated as day 1 of pregnancy. Food and water were available ad lib, lights were on between 0800 and 2000 h daily, and the ambient temperature was ~22 °C. For females with pups, litters were culled to contain eight pups (four males, four females) within 24 h after parturition. Dams remained with their pups continuously until sacrifice.

2.2. Groups and sacrifice

Subjects were randomly assigned to one of six groups. Five of the groups were mated with sexually experienced Sprague–Dawley males from our colony and one group of females was not. Mated females were sacrificed between 1000 and 1600 h on either day 10 of pregnancy ($n=9$), day 20 of pregnancy ($n=11$), the day of parturition ($n=13$), 7 days after parturition ($n=10$), or 17 days after parturition ($n=9$). Eight of the females sacrificed on the day of parturition were decapitated at a mean of 3.75 h after the first pup was born, all prior to 1600 h. For the remaining five females in this group, parturition was either not observed or it was not complete by 1600 h, and these females were decapitated at 1000 h the next morning. Non-mated females ($n=8$) had their estrus cycles monitored each morning for at least 1 week by inspection of vaginal smears and sacrificed on a day of diestrus between 1200 and 1400 h, 3–4 h after vaginal smearing. For sacrifice, females were carried in their home cage a short distance from the colony room to the sacrifice room, removed from their home cage and quickly decapitated without prior anesthetization. Brains were removed and flash frozen in cold 2-methylbutane. The entire process, from initial disturbance of the home cage to freezing the brain, was typically completed within 90 s. Brains were stored at −70 °C until further processing. After sacrifice,

vaginal smears were taken from lactating females to verify that they were in lactational diestrus, which all were.

2.3. Tissue processing and HPLC–EC analysis

Tissue sampling and analysis was performed as previously described [13]. One millimeter thick sections through the POA and striatum were cut on a cryostat and the tissue punched bilaterally with an 18-gauge stainless-steel tube. The rostral aspect of the POA punches began at approximately −0.26 mm from bregma where the decussation of the anterior commissure is first visible (Fig. 1); samples included the middle of the POA, including portions of the medial preoptic nucleus and anteroventral preoptic area, as well as small portions of the lateral POA. The rostral aspects of the NA and ST_{dl} punches began approximately +1.5 mm from bregma where the corpus callosum decussates (Fig. 1). The NA samples included both the shell and core regions of the NA, as well as small portions of the ventral pallidum and bed nucleus of the stria terminalis at the most caudal levels of the punches. The tissue was weighed and immediately placed in 500 μl of 0.1 M perchloric acid. Samples were homogenized and centrifuged in self-filtering centrifuge tubes at 10,000 rpm for 30 min. The supernatant was removed and analyzed with high-performance liquid chromatography with electrochemical detection (HPLC–EC) for DA and its DOPAC metabolite, as well as 5-HT and its 5-HIAA metabolite.

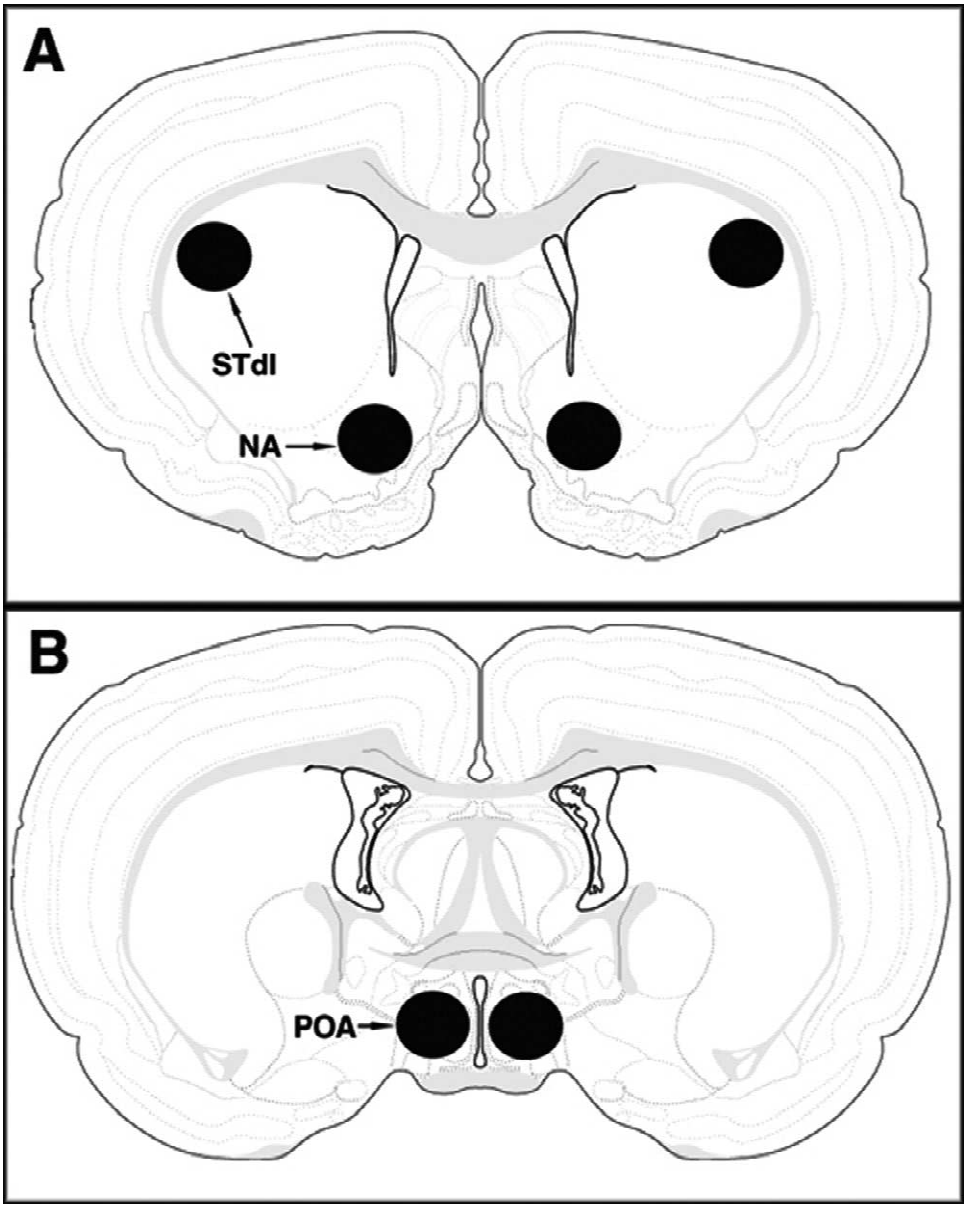

Fig. 1. Location of tissue sampling in the (A) ST_{dl} and NA and (B) POA as indicated by black circles. Levels shown represent the most rostral starting point of each sample. Figure modified from Ref. [67].

The chromatography system consisted of a Valco (Houston, TX, USA) injector with a 500 nl sample loop, and an Antec (Leiden, the Netherlands) microelectrochemical detector, equipped with a microflow cell (11 nl cell volume), with a glassy carbon working electrode and a Ag/AgCl reference electrode. The analytical column was an LC Packings Fusica reversed-phase capillary column (300 mm inner diameter, 5 cm long, packed with 3 mm C-18 particles). The working electrode was maintained at an applied potential of 0.8 V relative to the reference electrode. The mobile phase [32 mM citric acid, 54.3 mM sodium acetate, 0.074 mM ETDA, 0.215 mM octyl sulfonic acid (Fluka, Milwaukee, WI, USA) and 4% methanol (v/v); pH 3.45] was delivered through the system at 0.5 ml/min; however, after the tee splitter, flow through the analytical column was ~7 μl/min.

All standard curves and POA samples were analyzed in duplicate and the data are expressed as the mean of these two runs. NA and ST_{dl} samples were analyzed once. Amounts of DA and 5-HT from all three brain areas were determined by the height of their peaks on the chromatograms and are expressed as pg/mg of tissue. Amounts of DOPAC within the ST_{dl} and NA are also expressed in pg/mg by the height of their peaks on the chromatograms. Because the necessary standard curves were not obtained due to experimenter error, DOPAC levels in the POA and levels of 5-HIAA in all three sites are expressed as the total areas under the appropriate chromatogram curves. Turnover ratios in all cases were calculated using the total areas under the appropriate curves. HPLC analysis was completed over the course of several days, and new standard curves for each substance were obtained each day; tissue samples run on a given day were analyzed using that day's standard curves.

2.4. Statistical analyses

Data were analyzed with analysis of variance (ANOVA) followed by Fisher's least significant difference post-hoc tests. There were no significant differences on any measure between the females in the P1 group whose parturition was observed and those whose parturition was not observed (all $P>0.5$), and all females were therefore included in a single group for comparisons with other groups. In a few cases, samples from a given brain were lost and these subjects were removed from the statistical analyses. This resulted in slightly reduced group sizes for some analyses, but in no case did a group lose more than one or two subjects for any analysis. Statistical significance was indicated by $P<0.05$.

3. Results

In the POA, levels of intracellular DA decreased during pregnancy and reached their nadir on the day of parturition, rose to pre-pregnancy levels by day 7 postpartum, and remained there by day 17 postpartum ($F(5,45)=2.63$, $P<0.05$; Fig. 2a); significant post-hoc differences were found between dams sacrificed on the day of parturition and dams sacrificed on either day 7 or 17 postpartum ($P<0.05$). DOPAC levels followed a similar pattern ($F(5,45)=2.5$, $P<0.05$; Fig. 2b), and were significantly lower on gestation day 20 and the day of parturition compared to females on gestational day 10 and postpartum day 17 ($P<0.05$), but the ratio of DOPAC/DA did not differ between groups ($F(5,43)=0.8$, $P>0.5$; Fig. 2c). Levels of 5-HT ($F(5,45)=0.9$, $P<0.4$; Table 1) and 5-HIAA ($F(5,44)=1.2$, $P>0.3$) did not differ between groups, but the ratio of 5-HIAA/5-HT was greater in early pregnant (G10) and lactating females compared to virgins

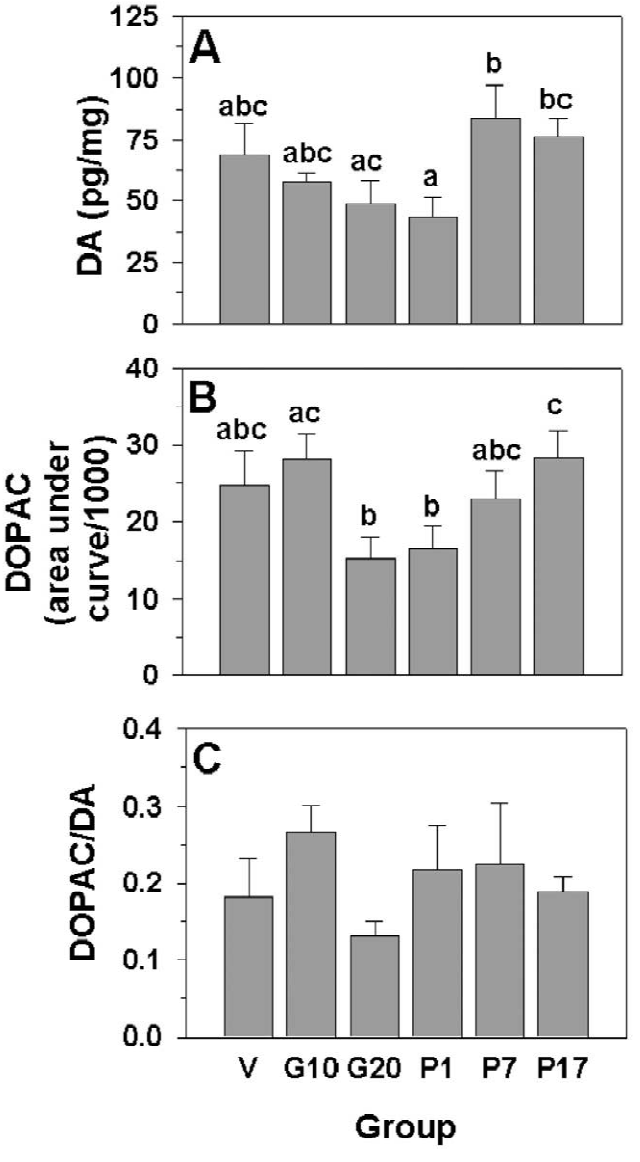

Fig. 2. Measures (mean±S.E.M.) of intracellular (A) dopamine, (B) DOPAC, and (C) DA turnover in the POA of virgin, pregnant and lactating rats. Bars with the same letter(s) are not significantly different.

Table 1
Dopamine and 5-HT activity (mean±S.E.M.) in the POA, ST_{dl} and NA of virgin, pregnant, and lactating rats

	Virgin	G10	G20	P1	P7	P17	*F*
POA							
5-HT (pg/mg)	77±11	56±7	56±10	56±7	59±7	64±5	0.9
5-HIAA (area/1000)	218±44	186±27	146±25	199±23	214±28	242±22	1.2
Turnover	0.73±0.04[a]	0.99±0.08[b]	0.87±0.06[ab]	1.07±0.09[b]	1.11±0.10[b]	1.07±0.10[b]	3.3
ST_{dl}							
5-HT (pg/mg)	51±11	30±6	30±5	57±15	41±6	35±6	1.4
5-HIAA (area/1000)	156±14	133±22	152±23	169±38	208±32	185±38	0.6
Turnover	0.90±0.05	1.6±0.34	1.35±0.41	1.79±0.31	1.13±0.08	1.18±0.07	1.3
NA							
DA (pg/mg)	1790±189	1358±109	1570±199	1530±255	1670±269	1622±272	0.3
DOPAC (pg/mg)	479±76	490±62	383±46	404±49	366±38	484±54	1.1
Turnover	0.29±0.06	0.38±0.06	0.27±0.06	0.37±0.07	0.28±0.06	0.34±0.05	0.6
5-HT (pg/mg)	73±8	55±8	60±10	83±14	56±10	71±15	0.9
5-HIAA (area/1000)	183±18	152±22	128±15	215±28	145±20	186±35	1.9
Turnover	0.67±0.10	0.68±0.5	0.97±0.28	1.29±0.21	0.88±0.23	0.69±0.04	1.8

Significant post-hoc differences between groups indicated by different superscript letters ($P<0.05$).

($F(5,44) = 3.3$, $P<0.02$); this ratio in late-pregnant females was intermediate between virgin and other groups and not significantly different from any group ($P>0.05$). A representative chromatogram from the POA of a virgin subject is provided in Fig. 3.

In the ST_{dl}, levels of intracellular DA were similar between groups ($F(5,49) = 1.1$, $P>0.3$; Fig. 4a), as were levels of DOPAC ($F(5,50) = 1.8$, $P>0.1$; Fig. 4b), although the ratio of DOPAC/DA was higher for females on the day of parturition compared with females sacrificed as virgins or during lactation ($F(5,49) = 3.6$, $P<0.01$; Fig. 4c). The amount of 5-HT was also similar between groups ($F(5,47) = 1.4$, $P>0.2$), as were the levels of 5HIAA ($F(5,49) = 0.7$, $P>0.6$) and the 5HIAA/5-HT ratio ($F(5,47) = 1.4$, $P>0.2$; Table 1).

In the NA, groups did not differ in the levels of DA

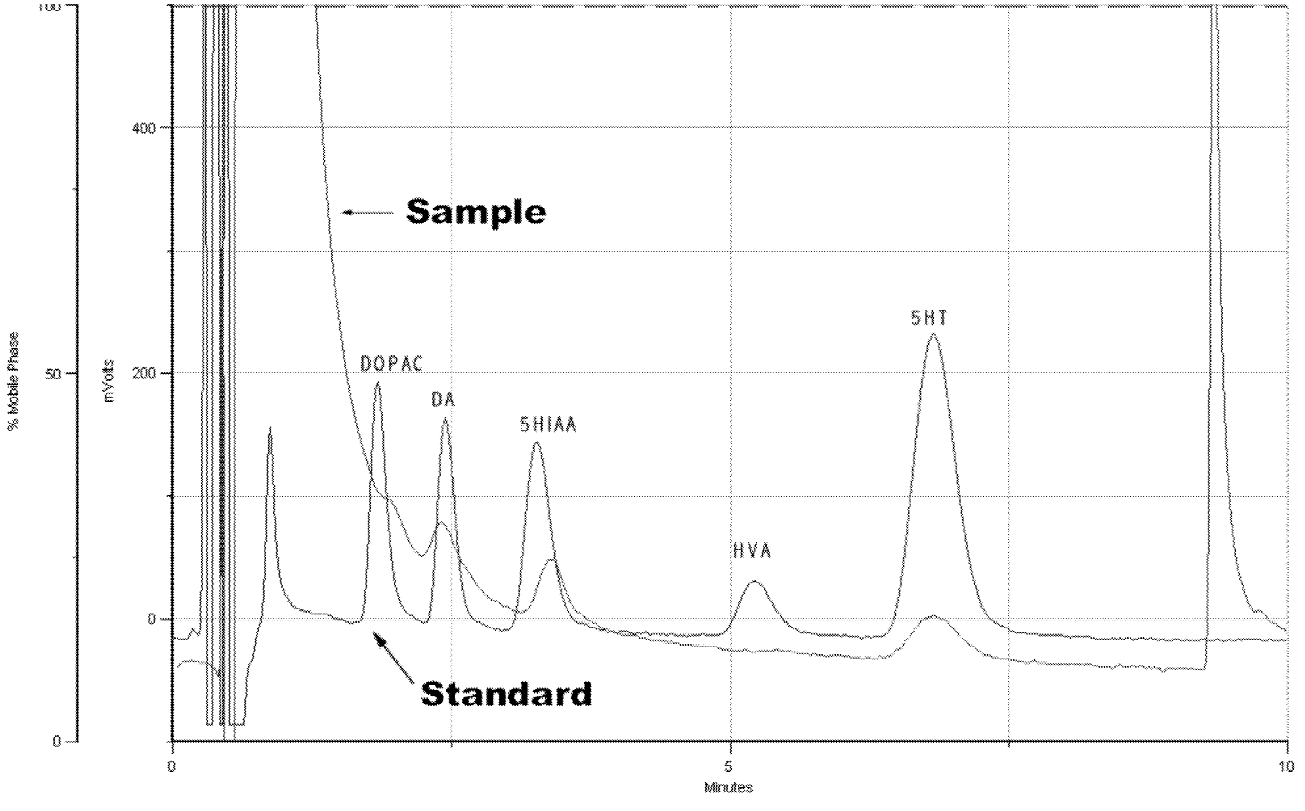

Fig. 3. Chromatogram of the POA of a representative virgin female rat showing sample curve with standard curve superimposed to show the times that monoamine and metabolite peaks eluded. HVA, homovanillic acid (non-detectable in our samples).

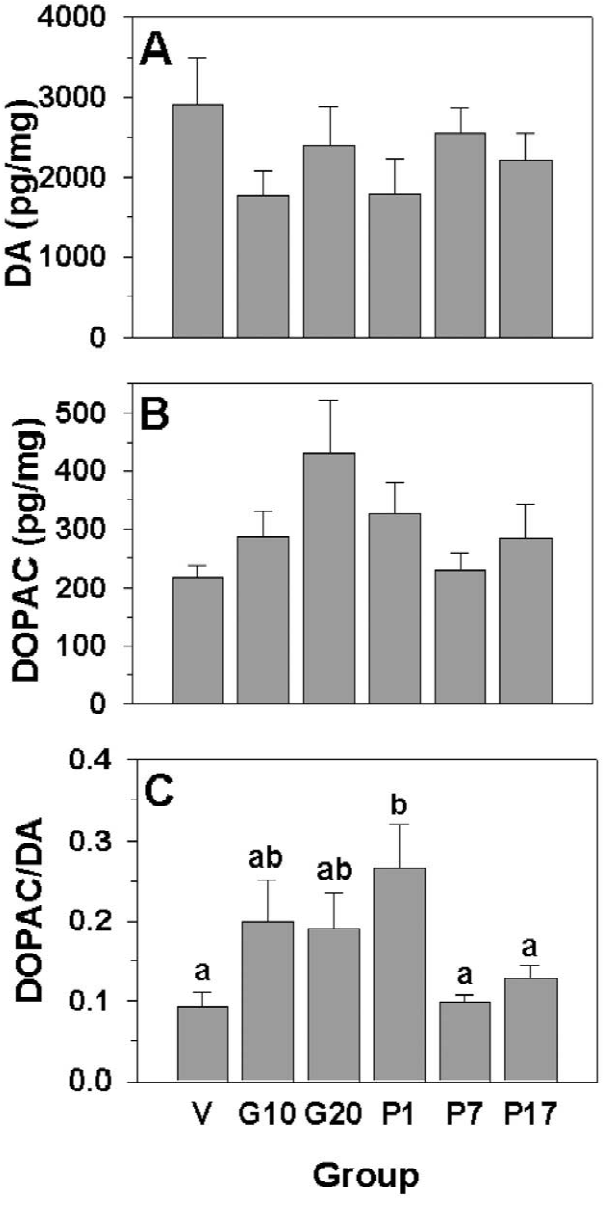

Fig. 4. Measures (mean±S.E.M.) of intracellular (A) dopamine, (B) DOPAC, and (C) DA turnover in the ST_{dl} of virgin, pregnant and lactating rats. Bars with the same letter(s) are not significantly different.

($F(5,52) = 0.3$, $P > 0.8$), DOPAC ($F(5,53) = 1.0$, $P > 0.3$), DOPAC/DA ratio ($F(5,52) = 0.6$, $P > 0.7$), levels of 5-HT ($F(5,50) = 0.9$, $P > 0.5$), 5-HIAA ($F(5,53) = 1.9$, $P > 0.05$) or the 5-HIAA/5-HT ratio ($F(5,50) = 1.8$, $P > 0.1$) (Table 1).

4. Discussion

The present results demonstrate that: (1) intracellular levels of DA in the POA were significantly lower on the day of parturition compared to early or late lactation; (2) DOPAC levels in the POA were significantly lower during late pregnancy and on the day of parturition compared to early pregnancy and late lactation; (3) 5-HT turnover in the POA was lower in virgin females compared to early pregnant and parturient/lactating females; (4) no significant changes in DA or 5-HT activity were found across groups in the NA, and (5) DA turnover in the ST_{dl} was higher on the day of parturition compared with virgin females or during early and late lactation.

Female rats are typically not maternal until undergoing the hormonal fluctuations associated with late pregnancy and parturition, and the full expression of this behavior is not exhibited until just a few hours before the pups are born [40]. It can be assumed that changes in circulating gonadal and pituitary hormones chronically alter neurochemistry to produce a 'maternal state' that produces nurturant responding towards pups. Basal DAergic activity changed in the POA and ST_{dl} around the same time that maternal behavior emerges, and these changes may be important for the onset of the dam's nurturant behavior.

4.1. Methodological considerations

Studies of the POA of castrated male rats demonstrate that basal intracellular DA is higher than intact controls, while extracellular levels are lower [13]. It would be logical, then, to assume that DA release would be greatest when intracellular stores are lowest. Our results from the POA suggest that this is not necessarily the case, because not only were intracellular stores of DA lowest around the time of parturition, but levels of DOPAC were also low. Tissue levels of DOPAC can be used alone or in relation to levels of intracellular DA to indicate postsynaptic DA deactivation, and therefore, the amount of DA released and metabolized. Even though we found no group differences in DA turnover as indicated by the ratio of DOPAC to DA, the fact that both measures decreased around the time of parturition strongly indicates a decrease in both DA synthesis and release, as has been argued previously [73]. In vivo microdialysis will be necessary to determine whether this is indeed the case, as well as whether DA release changes acutely in the POA during the performance of specific maternal behaviors.

Levels of intracellular DA in the POA found in the present study were lower than levels sometimes reported for lactating [49] and cycling or ovariectomized female rats (e.g. Refs. [36,73,74]), but similar to at least one other study [54]. An extremely wide range can be found in reported intracellular POA DA levels—from 820 pg/mg [13] to over 20,000 pg/mg [55,59]. We do not think that our relatively low levels are due to problems with the HPLC analysis because levels of DA found in the ST_{dl} and NA were in a similar range to those previously reported for lactating rats [20,21,49]. It may instead be related to the method of tissue collection. Most DAergic perikarya in the POA lie close to the third ventricle [5,70]. Our tissue punches were in the center of the POA and did not include

this periventricular tissue, and probably only included fibers from these and other DA projection cells. In addition, there are fewer DA-containing neurons in the anterior POA than in the most posterior POA [70], and portions of the latter were not included in our samples. Complete dissection of the POA or obtaining tissue punches that were more posteromedial would likely lead to substantially increased levels of DA in the samples.

Given its role in motivated behavior and reward-seeking [26], the NA has been investigated many times with regards to maternal behavior. Microdialysis has shown that extracellular DA increases in the NA if dams are separated from pups overnight and then reintroduced to soiled pups that elicit profuse licking [19]. Although this may indicate that changes in DAergic activity in the NA are difficult to observe in lactating rats, and that unnatural circumstances (i.e., long separation from the litter followed by a manipulation of pups that elicits unusually high levels of licking in the dam) are required, it was nonetheless surprising that we did not find any changes in intracellular DA activity in the NA. A similar example can be found in the POA of estrogen-primed female rats, where in vivo microdialysis reveals that progesterone increases extracellular DA release [39], whereas progesterone does not alter DA turnover when measured in microdissected POA tissue punches [55]. There is not necessarily a one-to-one relationship between intracellular and extracellular DA, and in some cases, intracellular stores of striatal DA need to be severely reduced before extracellular DA release and postsynaptic firing are significantly modified [50,51]. This explains why ingestive and motor behaviors are not affected in rats with even up to a 50–90% depletion of striatal DA [50]. It is also known that the shell and core regions of the NA differ in their importance for maternal behavior, with the former more critical than the latter [29,34,61,62] and differences between pregnant and lactating groups may appear if it was feasible to sample just the NA shell.

4.2. DA activity in the POA and maternal behavior

Prior to the present experiment, there has been no investigation of neurotransmitter activity specifically in the POA that may be important for maternal behavior, and previous studies that investigated intracellular monoamine activity in pregnant and lactating rats or mice did not examine areas of the brain known to be necessary for maternal behavior [12,42,60]. Although our results can only correlate monoamine activity with changes in maternal behavior, they suggest that a reduction in DAergic activity in the POA around the time of parturition followed by an increase during lactation may be related to the onset and maintenance of maternal responsiveness. Because virgin and lactating rats show dramatic differences in their maternal responsiveness, but both had high DAergic activity in the POA compared to late-pregnant females and females on the day of parturition, it may be possible that a withdrawal of DAergic tone in the POA during parturition followed by a return to pre-parturient levels soon after is a necessary pattern. Other studies taking a similar correlational approach have examined the concentration of receptors for estrogens [72], progestins [47], prolactin [37], and dopamine [1] in the POA of pregnant and lactating rats. Another recent study examining monoamine activity in the maternal rat brain also found that virgin and lactating (sacrificed on day 4 postpartum) rats have similar intracellular DAergic activity in the POA, but this study did not examine recently parturient dams. However, virgins induced to be maternal through continuous exposure to pups (i.e., sensitization) had higher POA monoamine activity than non-sensitized virgin females [49], indicating that there are differences in the neural regulation of natural and sensitized maternal responding.

Many dopamine-producing cells in the rat brain contain receptors for estradiol and progesterone [31,75] and exogenous hormones or naturally occurring changes in ovarian hormones during the estrus cycle has often been reported to alter intracellular and extracellular measures of DA activity in the POA. If hormone-induced changes in DA release in the POA are involved in the onset of maternal behavior around the time of parturition, there are many ways that it could influence this behavior. Cells in the POA contain both D1 and D2 receptors [1], and changes in DA activity could directly influence those cells necessary for maternal behavior. In fact, elevated DAergic activity in the POA by infusion of cocaine impairs maternal behavior in lactating rats [71]. Hormones probably do not need to grossly alter neurotransmitter release to exert an influence on maternal behavior, but can also influence neurotransmitter receptor number in sites necessary for parental responding [1], as well as otherwise modify the sensitivity of cells in the POA to a given amount of DA [25].

It may have been somewhat surprising that a decrease, rather than increase, in DAergic activity was found in the POA when maternal behavior emerges. A periparturitional reduction of DAergic activity in the POA, however, could release the tonic inhibition on maternal responding that exists prior to parturition. DA inhibits many hypothalamic neurons by increasing GABAergic tone, as well as reducing excitatory neurotransmitter activity [4]. More than half of the cells in the POA that show *c-fos* expression after the performance of maternal behavior are GABAergic [35], and many GABAergic cells in the POA are contacted by DAergic terminals [23,33]. A reduction of DAergic input could theoretically reduce GABAergic tone and increase excitatory influences in the POA, thereby allowing for the expression of maternal behavior.

We acknowledge that, in addition to the onset of maternal behavior, numerous other behavioral and physiological functions occur during late pregnancy and at the time of parturition, and one could argue that these are

associated with changes in DAergic activity in the POA. However, DA in the POA is probably not related to milk production or release because levels would have been similar on the day of parturition to those during the rest of lactation. Furthermore, suckling does *not* affect DA levels in the POA [57], and pre- or postpartum lesions of the POA do not eliminate milk production and letdown if pups are able to suckle the dam [10,27,28,68]. A second possibility is that the observed changes in DA activity in the POA is related to the ovulation and sexual receptivity that occurs during the postpartum estrus. This is not likely because most of the females in the current study were sacrificed many hours prior to these events that peak approximately 9 h after parturition [11]. A third possibility is that changes in DA in the POA could be related to the process of parturition itself or the stress involved with parturition, but this also appears unlikely, considering that even large prepartum lesions of the POA do not prevent the delivery of pups [10,28] and that restraint stress increases, not decreases, intracellular DA in the POA [56].

We do not know why 5-HT turnover was greater in most groups of pregnant and lactating females compared to virgins; neither the elevated progesterone levels associated with pregnancy [69], nor suckling by the pups [8], alter 5-HT activity in this area of the brain.

4.3. DA activity in the ST_{dl} and maternal behavior

In contrast to the lack of effects in the NA and the reduction of DAergic activity in the POA, DA turnover increased in the ST_{dl} during late pregnancy and parturition. There is no information about any function for the area of the ST_{dl} that we sampled in maternal behavior and, as noted above, studies that have investigated the dorsal striatum in lactating rats have focused on more medial areas and found very few effects [20,29,71]. Gonadal hormones alter DAergic activity in the striatum [3], probably by acting locally, as well as by acting on steroid hormone receptors on DA cells of the ventral tegmental area and substantia nigra that project to the striatum [31]. Considering the sensitivity of the ST_{dl} cells to perioral stimulation [9,41], and that perioral somatosensory stimulation is needed for retrieval and licking of pups [64], increased DAergic activity here during late pregnancy and around the time of parturition may be necessary for the onset of oral maternal behaviors. Similar to the POA, it may act indirectly via changes in GABAergic neurotransmission in ways that alter motor output necessary for the behavior [43,53]. Also possibly relevant to maternal retrieving, licking, and handling of pups is the finding that food-deprived rats with lesions of the same region of the ST_{dl} show impairments in their ability to use their paws to retrieve food pellets from a confined space, and also show transient deficits in licking [52].

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