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# The content of dopamine, serotonin, and their metabolites in the neural circuit that mediates maternal behavior in juvenile and adult rats

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**Abstract**

Continuous exposure of non-parturient rats to pups can induce maternal behavior similar in most aspects to that found in the postpartum rat. Surprisingly, young juvenile rats (20–24 days of age) only require 1–3 days of exposure to pups, while adults require 4–8 days before maternal behavior emerges. Dopamine (DA) and possibly serotonin (5-HT) may mediate the expression of adult maternal behavior. We hypothesize that postnatal changes in DA and 5-HT within the neural circuit that supports maternal behavior including the medial preoptic area (MPOA), medial and cortical amygdala (MCA), and nucleus accumbens (NAC), may underlie these differences in responsiveness across juveniles and adults. We measured DA, 5-HT, and their metabolites in postmortem samples of these regions in maternal and non-maternal juvenile and adult females.

The only difference found across behavioral groups was that the MPOA of adults induced into maternal behavior by pup exposure had more DA than did that of isolated adult females or maternal juveniles. However, when adults versus juveniles were compared, the content of DA and 3,4-dihydroxyphenylacetic (DOPAC) was higher in the adult than in the juvenile NAC and MCA; the content of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in these structures did not vary across the age groups. In contrast, higher levels of 5-HT and 5-HIAA were found in the MPOA in juveniles compared to adults. We propose that these region-specific age differences in DA and 5HT may underlie differences in juvenile–adult responses to pups.


*Keywords:* Accumbens; Amygdala; MPOA; Neurochemistry; Pups

## 1. Introduction

Maternal behavior in the rat consists of maternal nest building, and pup directed behaviors including retrieving, nursing, grooming, and protection from intruders. Parturient females are immediately maternally responsive to their pups. Less generally known is that fact that female, male, or juvenile rats can be induced to express maternal behavior by continuous exposure to new born pups provided from parturient female foster mothers. The expression of pup-induced maternal behavior has been studied extensively; it is virtually indistinguishable from hormonally-induced maternal behavior, except that parturient females nurse their pups. [47,58]. Adult rats (>60 days of age) require 4–8 days of exposure to pups before maternal behavior emerges, while surprisingly young juvenile rats (20–26 days of age) only require 1–3 days of exposure to pups. This required exposure period increases as the juveniles approach puberty [6,7,43–45,65]. These data suggest an intriguing difference in behavioral responsiveness across young juveniles and adults.

Key components of the neural circuit that mediate maternal behavior include the medial preoptic area (MPOA), the medial and cortical amygdala (MCA), and the nucleus accumbens (NAC), along with other limbic and hypothalamic structures [48]. Most of our knowledge of the neural circuit that mediates maternal behavior has been gathered from studies using adult animals; much less has been done using the juvenile. The MPOA is necessary for both the expression of and motivation to perform maternal behavior [48]. In adults both large and very small lesions in the MPOA disrupt maternal behavior while in the juvenile or pubertal rat, only large lesions disrupt the behavior, small lesions do not [36,50,51]. Moreover, increased expression of

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immunoreactive c-fos in the MPOA and other components of this neural circuit occurs in adults during pup-induced maternal behavior, however, in juveniles c-fos induction depends upon the age of the juveniles and perhaps on the extent of pup exposure [24,37,51], suggesting that this is a maturing circuit component.

In the non-maternal adult female, neuronal activity in the MCA inhibits maternal behavior by a mechanism that involves processing of olfactory cues from the initial exposure to pups [20,47,48]. The hormones of the peripartum period are thought to alter or release this basal inhibition so that the expression of maternal behavior can occur. Prolonged pup exposure also reduces this basal inhibition allowing the onset of pup-induced maternal behavior. Lesions in the medial amygdala had similar effects in both older juveniles and adults, shortening the time of pup exposure needed to induce maternal behavior [51]. The NAC is necessary for retrieval components of maternal behavior, and for processing of pup related stimuli likely to be related to mechanisms of motivation in the adult, not yet studied in the juvenile [48].

The scope of our knowledge concerning the neurotransmitters that mediate maternal behavior is limited to a few transmitters and to the adult model [48]. The literature provides the clearest case for a role for dopamine (DA) in the mediation of maternal behavior [5,21,22,25,26,28,49,66]. Extensive lesions of the dopaminergic system [25–27] disrupt maternal behavior, and dopamine receptor antagonists infused locally into the NAC inhibited retrieval and licking components of maternal behavior [39]. These studies suggest that expression of the behavior requires dopamine. Increases in DA and DA metabolites, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), in the extracellular space in the ventral striatum after separated mother rats and pups were reunited [28] suggest a role in the motivational components of the behavioral response.

The exploration of a role for other neurotransmitters in the mediation of maternal behavior is very limited; however, there is modest evidence that serotonin (5-HT) may be involved. Barofsky et al. [2] found that serotonergic lesions in the median raphe nucleus caused short-term disruption in maternal behavior but not the prolactin response with suckling, however, the study does not establish functional specificity since locomotor impairment was not ruled out. Ferreira et al. [17] found that buspirone (a partial 5-$HT_{1A}$ agonist) inhibited maternal behavior; however, they did find reduced motor activity, limiting the specificity of the interpretation. Microdialysis also showed an increase in the concentration of the metabolite for 5-HT, 5-hydroxyindoleacetic (5-HIAA), in the ventral striatum when dams were reunited with pups [28]; perhaps motivational components of maternal behavior are supported by this system as well. Infusions of cocaine into the NAC or MPOA disrupt adult maternal behavior [74]; suggesting the exact level of DA and possibly 5-HT neurotransmitters is important. Thus, an excess of these transmitters could be as disruptive as their reduction or absence.

Our working hypothesis is that the brain regions mediating maternal behavior in the adult, also mediate maternal behavior in the juvenile, and that postnatal neurochemical changes within these regions may underlie the maturing time course of pup-induced maternal behavior. While it is formally possible that maternal behavior in the juvenile might be supported by different neural structures or transmitters than those that support maternal behavior in the adult, we know of no data to support this alternative hypothesis.

It has been hypothesized that adult rats, without benefit of the hormones of parturition, require longer exposure to pups to become maternal because their initial response to pups is a typical adult neophobic response to novel stimuli, while juveniles are generally less neophobic and affiliate readily with pups [18,19,43,45,55,63]. We hypothesize that postnatal changes within the neural circuit may add a level of inhibition to social stimuli, and that this is the fundamental difference between the juvenile and adult state. The comparison of juveniles (20–27 days old) to adults (>60 days) offers the intriguing view before and after these hypothesized developmental changes in the inhibitory components of the neural circuit occur, and to consider that these two neurotransmitters might be part of the mechanistic basis for this additional behavioral inhibition.

Our goal was to examine the content of dopamine and serotonin and their metabolites in juveniles and adults within the neural circuit that mediates the expression of maternal behavior. We choose postmortem sampling of tissue punches as our approach so that we could examine multiple brain areas in juveniles and adults. An alternative, microdialysis would allow measurements in only one area at a time. First, the behavioral state of each animal was assessed. Then postmortem tissue analysis was used to determine, in each individual animal, the amount of these neurotransmitters and their metabolites in three different brain regions, the NAC, MPOA, and MCA.

Two specific questions were addressed. Does the amount of these neurotransmitters and their metabolites vary with the maternal state of the rat? Is the amount of these substances in these specific regions different in adults compared with juveniles?

## 2. Material and methods

### 2.1. Animal care and subjects

The animals were from our colony maintained at the Rutgers University Laboratory Animal Facility in Newark which is accredited by the Association for the Accreditation and Assessment of Laboratory Animal Care. They were Sprague–Dawley strain rats, bred from animals originally purchased from Charles River Laboratories (Wilmington, DE). Additional breeding animals are systematically added to the colony. Animals are maintained under a 12-h light/12-h dark cycle at 22 °C with ad libitum access to

food and water. Wood shavings (BETA CHIP®, NEPCO™, North Eastern Products Corp., NY) were used to cover the floor of the cages. All procedures followed the standards approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Rats were housed in groups of two until used in the experiments. Then they were housed individually in cages measuring 53 cm × 36 cm × 25 cm with transparent Plexiglas walls. Females for the parturient adult experimental group or as donor mothers to supply foster pups were mated by our published procedures [36,37,50].

### 2.2. Behavioral paradigm

#### 2.2.1. Pup-induction of maternal behavior

Adult virgin or juvenile females were continuously exposed in their home cages to three newborn pups 2–7 days old. Every 24 h these pups were returned to donor mothers for care and feeding, while other freshly fed pups were supplied to the experimental animals. Donor mothers were separate from experimental groups [58]. The number of days of pup exposure needed to induce maternal behavior is also called the latency to express maternal behavior. This was calculated as the interval between the first day of pup exposure and the first of the two consecutive days in which the animals exhibited maternal behavior. Further, details of our operational definition of maternal behavior and our behavior testing paradigm were as previously published [36,37,50].

#### 2.2.2. Time of pup exposure and maternal behavior latencies

All groups exposed to pups were tested daily for maternal behavior. As expected, the average number of days of continuous pup exposure required for the emergence of maternal behavior was much longer in adults (6 ± 1 days, median 6 days) than in juveniles (1.5 ± 0.4 days, median 1 day). Maternal groups included only animals that showed all components of maternal behavior and were exposed to pups in this state for 4 days before sacrifice. Adults induced into the maternal state by pup exposure were, therefore, necessarily exposed to pups on average for 10 ± 1 day (median 10) total, whereas juveniles were exposed for 5.5±0.4 days (median 5) total. In order to produce groups of non-maternal juveniles, exposure to pups had to be limited to 1 day, whereas some adults could be exposed to pups 7 ± 1 days without being induced into the maternal state. For these two non-maternal pup exposed groups, this was a close match of pup exposure to the comparable maternal groups as was possible for the two age groups.

### 2.3. Animal groups

Twenty-eight adult females (virgin adult females or parturient females, 60–100 days old) and twenty seven juvenile females (19–21 days old), were used. Adult virgins were not classified as to stage of their cycle.

Three behavioral groups were comparable across the two ages.

1. Maternal by pup exposure
   These were adults or juveniles that became maternal by pup exposure which were exposed to pups, obtained from donor mothers, until they showed maternal behavior on four consecutive days, so that the fourth day of full maternal behavior was the day of sacrifice.
2. Non-maternal pup exposed
   These were adults or juveniles which did not become maternal with pup exposure. These differ from animals group 1 only in that they were non-maternal.
3. Social isolates
   These were adults or juveniles isolated 1–8 days, and not exposed to either pups or comparable age siblings.

Two additional groups specific to each age group included

4. Sibling exposed juveniles
   Juveniles that were exposed to sibling, age-matched females, sacrificed on the fourth day of such housing.
5. Maternal lactating females
   These were sacrificed on day 4 postpartum that is 4 days after the onset of maternal behavior at parturition.

### 2.4. Brain tissue sample preparation

Rats were decapitated with a guillotine, immediately after behavioral testing. Sacrifice was during the mid-portion of the light phase of the light/dark cycle. The brains were immediately removed from the skull, submerged in cold phosphate-buffered saline (PBS, pH 7.4; 4 °C) for 20 s, and placed ventral side up in a chilled brain mold designed for cross sectioning the brain. Sections were made at 1 mm intervals; five razor blades were used to cut the cross sections needed for further dissection of the NAC, the MPOA, and the MCA. The first blade was placed in the mold's division parallel to the rostral extent of the optic chiasm. A second blade was placed 1 mm caudal to it, and the section between blades 1 and 2 contained the MPOA sample. A third blade was placed 1 mm rostral to the optic chiasm, and the section between blades 1 and 3 was discarded. A fourth blade was placed 3 mm rostral to the optic chiasm to obtain a 2-mm-wide section, and this section between blades 3 and 4 was sampled for the NAC. The last blade was placed 3 mm caudal to the optic chiasm to obtain a 2 mm wide section, between blades 2 and 5, which was sampled for the MCA. Once all blades were in place, the sections were cut and then the blades were lifted out in sequence from rostral to caudal, with the sections adhering to blade.

The selected brain regions were further dissected on the blade in the order NAC, MPOA, and MCA [52]. A bilateral sample of the NAC was taken using a 12-gauge stainless steel tube. The tube was pushed into the NAC immediately medial and adjacent to the anterior commissure (Fig. 1A), and then withdrawn. When necessary, a thinner tube placed inside the

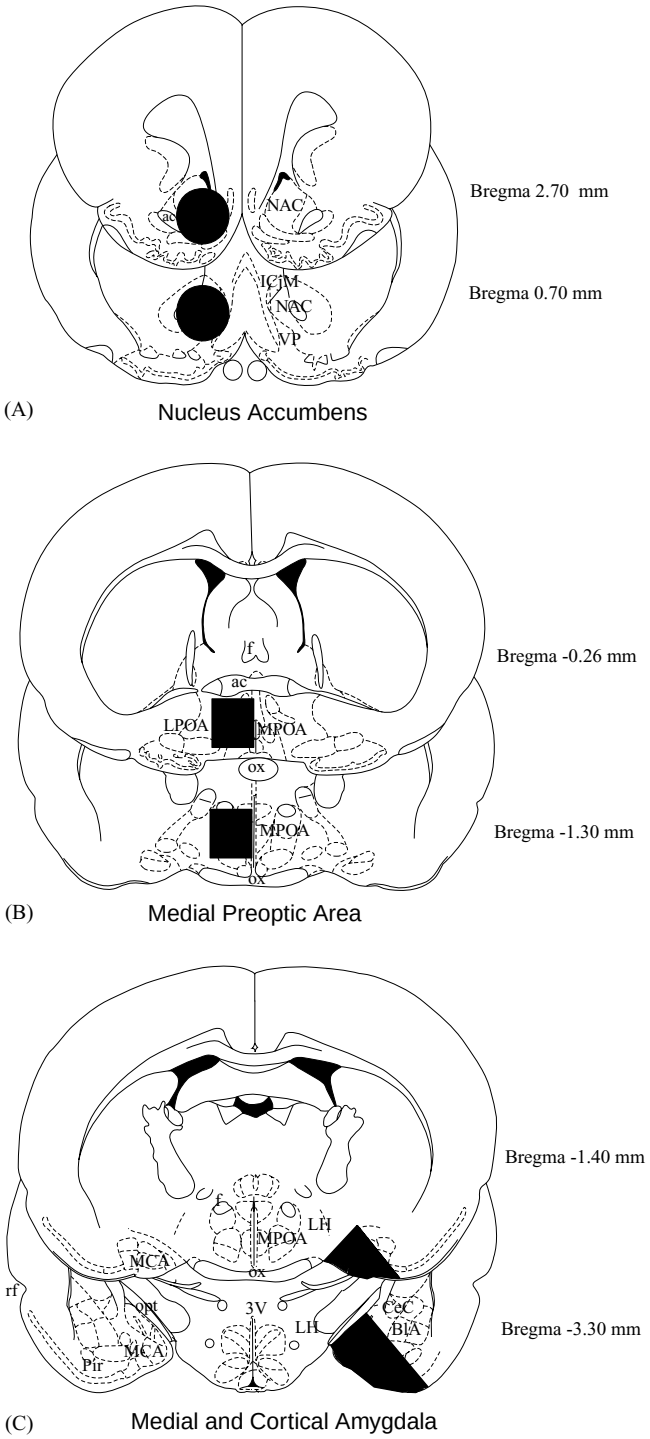


Fig. 1. Diagram showing the rostral–caudal extent, location, and relative size of the tissue samples taken from the NAC (A), MPOA (B), MCA (C). The black circle, rectangle, and triangle show the rostral and caudal extension of the area of the brain dissected. 3 V, third ventricle; ac, anterior commissure; BlA, basolateral amygdala; CeA, central amygdala; f, fornix; IcjM, islands of Calleja; LH, lateral hypothalamus; LPOA, lateral preoptic area; MCA, medial and cortical amygdala; MPOA, medial preoptic area; NAC, nucleus accumbens; opt, optic tract; ox, optic chiasm; Pir, piriform cortex; and VP, ventral pallidum.

12-gauge dissecting tube was used to eject the tissue punch. The MPOA and MCA were further dissected with a scalpel by cutting a square and a triangle of tissue, respectively (Fig. 1B and C). The samples were placed immediately in small cryogenic tubes, rapidly frozen and stored by emersion in liquid nitrogen. The complete dissection procedure took 4 min or less from sacrifice to sample freezing.

The total wet weight of the samples for adults and juveniles, respectively, was NAC $4.2 \pm 0.1$ mg and $4.2 \pm 0.2$ mg; MPOA $2.9 \pm 0.1$ mg and $2.6 \pm 0.2$ mg, and MCA $8.6 \pm 0.4$ mg and $7.6 \pm 0.4$ mg.

### 2.5. Tissue analysis and chromatographic conditions

To begin analysis, the samples were removed from liquid $N_2$ and kept in dry ice briefly. Samples were wet weighed, immediately placed in Eppendorf tubes, and homogenized in a solution of 0.1N $HClO_4$ and 100 μM EDTA (20 μl/mg of wet tissue). The homogenate was centrifuged at 15,000 rpm for 13 min. The supernatant from each sample was transferred to 0.4-ml tubes and stored at $-80\,^{\circ}C$. To measure DA, DOPAC, HVA, 5-HT, and 5-HIAA content in tissue, samples (20 μl) were injected into a high-performance liquid chromatography (HPLC) with electrochemical detection (Antec-Leyden, VT-03 flow cell). The potential of the working electrode was +600 mV. A reverse-phase column (Varian, Brownlee, RP-18, Velosep, 3 μm, C18, 100 Å) was used, and a filtered mobile phase containing 3.3 ml of 0.4 M sodium octyl sulfate, 1 ml of 0.1 M EDTA, 75 ml of MeOH, and NaAc 13.61 g (pH = 4.2) was pumped (LC-10AD VP Shimadzu) at a flow rate of 0.7 ml/min.

Before processing of the samples, the system was calibrated with 20 μl of a standard solution of known concentration. All neurotransmitters and metabolites were identified by retention time and quantified based on the peak height. The detection limit for DOPAC and 5-HIAA was ∼2 pg; for HVA, ∼50 pg; for DA, ∼4 pg; and for 5-HT, ∼12 pg. At the end of the working day a standard solution was again injected to confirm the stability of the system. Dynamax HPLC Method Manager MacIntegrator version 1.3 (Rainin Instrument Co. Inc., Oakland, CA) was used to process the data.

The HVA content in the MPOA and MCA was not detected in certain individual samples, which reduced the *n* for this metabolite's samples such that it was not appropriate to run statistical analysis across the four adult or juvenile groups. This was also true for DOPAC measurements in the MCA. These specific cases are indicated in Tables 1 and 2. Although the sensitivity of the HPLC was such that even low amounts of these metabolites could be detected in these two brain regions, other sources of variability in the method, including the tissue sampling procedures, may have contributed to this minor limitation on our data yield.

### 2.6. Data analysis

All neurotransmitter and metabolite amounts were calculated and expressed as pg/mg wet weight of tissue. When

Table 1
Concentrations of dopamine, serotonin, and metabolites in brain regions of adults

| | Treatment | DA | DOPAC | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|---|
| NAC | Isolated w/o pups ($n = 8$) | 7419 ± 936 | 1328 ± 187 | 408 ± 47 | 342 ± 84 | 153 ± 34 |
| | Non-maternal w/pups ($n = 7$) | 6781 ± 1375 | 1057 ± 146 | 436 ± 56 | 344 ± 120 | 92 ± 28 |
| | Maternal sensitized ($n = 6$) | 8542 ± 713 | 1221 ± 122 | 449 ± 39 | 632 ± 216 | 209 ± 63 |
| | Maternal lactating ($n = 7$) | 7598 ± 912 | 1187 ± 94 | 444 ± 73 | 375 ± 125 | 171 ± 58 |
| MPOA | Isolated w/o pups ($n = 5$) | 184 ± 26 | 65 ± 11 | 63 ±11 (1 n.d) | 228 ± 99 | 93 ± 36 |
| | Non-maternal w/pups ($n = 5$) | 248 ± 34 | 75 ± 15 | 38 ± 3 (3 n.d) | 100 ± 13 | 74 ± 9 |
| | Maternal sensitized ($n = 5$) | 300 ± 36* | 87 ± 12 | 62 ± 14 (2 n.d) | 245 ± 150 | 102 ± 57 |
| | Maternal lactating ($n = 8$) | 218 ± 28 | 63 ± 6 | 66 ± 19 (4 n.d) | 105 ± 22 | 65 ± 12 |
| MCA | Isolated w/o pups ($n = 7$) | 198 ± 40 | 46 ± 19 (5 n.d) | 159 (6 n.d) | 320 ± 93 | 102 ± 40 |
| | Non-maternal w/pups ($n = 6$) | 263 ± 96 | 74 ± 12 (3 n.d) | 33 ± 14 (4 n.d) | 408 ± 91 | 165 ± 50 |
| | Maternal sensitized ($n = 5$) | 247 ± 68 | 62 ± 27 | 187 ± 143 (3 n.d) | 223 ± 59 | 116 ± 27 |
| | Maternal lactating ($n = 9$) | 165 ± 24 | 66 ± 18 (1 n.d) | 136 (8 n.d) | 484 ± 127 | 175 ± 43 |

Data are expressed in pg/mg wet weight (means ± S.E.M.). n.d., non-detected values.
* $P < 0.03$ statistically significant with respect to isolated.

Table 2
Concentrations of dopamine, serotonin, and metabolites in brain regions of juveniles

| | Treatment | DA | DOPAC | HVA | 5-HT | 5-HIAA |
|---|---|---|---|---|---|---|
| NAC | Isolated w/o pups ($n = 4$) | 4307 ± 768 | 1011 ± 87 | 524 ± 68 | 105 ± 56 | 72 ± 39 |
| | Non-maternal w/pups ($n = 5$) | 6453 ± 1009 | 1007 ± 82 | 637 ± 112 | 224 ± 104 | 141 ± 68 |
| | Maternal sensitized ($n = 6$) | 6234 ± 657 | 936 ± 98 | 509 ± 33 | 438 ± 110 | 172 ± 35 |
| | Social ($n = 4$) | 6334 ± 798 | 991 ± 99 | 602 ± 55 | 303 ± 127 | 109 ± 31 |
| MPOA | Isolated w/o pups ($n = 3$) | 112 ± 15 | 32 ± 17 | 46 (2 n.d) | 248 ± 79 | 149 ± 18 |
| | Non-maternal w/pups ($n = 5$) | 445 ± 194 | 87 ± 22 | 81 ± 20 (1 n.d) | 260 ± 39 | 124 ± 12 |
| | Maternal sensitized ($n = 7$) | 146 ± 21 | 77 ± 17 | 70 ± 16 (2 n.d) | 266 ± 34 | 121 ± 13 |
| | Social ($n = 6$) | 117 ± 8 | 50 ± 6 | 57 ± 3 (2 n.d) | 239 ± 39 | 112 ± 20 |
| MCA | Isolated w/o pups ($n = 8$) | 158 ± 41 | 59 ± 16 (4 n.d) | 66 ± 18 (3 n.d) | 358 ± 33 | 140 ± 21 |
| | Non-maternal w/pups ($n = 5$) | 100 ± 16 | 37 ± 6 (2 n.d) | 69 ± 9 (1 n.d) | 332 ± 89 | 148 ± 42 |
| | Maternal sensitized ($n = 8$) | 125 ± 25 | 50 ± 18 (4 n.d) | 53 ± 3 (6 n.d) | 322 ± 66 | 115 ± 13 |
| | Social ($n = 6$) | 91±20 | 34±13 (2 n.d) | 44 ± 9 (4 n.d) | 297±62 | 103±19 |

Data are expressed in pg/mg wet weight (means ± S.E.M.). n.d., non-detected values.

the data passed Bartlett's test of homogeneity of variance, the parametric tests including ANOVA and the post hoc Fisher protected least significant difference (PLSD) test were applied. In only one case, the juvenile MPOA value for dopamine, the variance was not homogeneous and no correction was possible; these comparisons were made with non-parametric methods, specifically the Kruskal–Wallis test followed by the Mann–Whitney test. Adult and juvenile behavioral groups that were not statistically different from each other ($P > 0.05$) were combined within the age categories of adults or juveniles, and subsequently the two age groups were compared by *t*-test, after Bartlett's test verified that parametric testing was appropriate.

## 3. Results

### 3.1. Nucleus accumbens and medial cortical amygdala

There were no statistically significant differences across behavioral groups within each age group in samples from the NAC or the amygdala. Therefore, all the individuals in the behavioral groups were averaged for comparisons of adults versus juveniles.

In the NAC, higher amounts of DA (*t*-test, $P < 0.02$) and DOPAC (*t*-test, $P < 0.03$) were found in adults than in juveniles (Fig. 2). HVA content was substantial in all individuals in this brain region, and it was higher in juveniles than in adults (*t*-test, $P < 0.004$). No statistically significant difference was found in 5-HT and 5-HIAA within the accumbens between juvenile and adults (Fig. 2; Tables 1 and 2).

In the amygdala, higher amounts of DA were also found in adults than in juveniles (*t*-test, $P < 0.01$; Fig. 3). No statistically significant difference was found in DA metabolites or any 5-HT related values between juvenile and adult females in this region (Fig. 3).

### 3.2. Medial preoptic area

Adults induced into maternal state by pup exposure had higher DA content in their MPOA than did adult isolates (ANOVA $F = 3.286$, d.f. $= 2$, $P < 0.07$; post hoc Fisher's

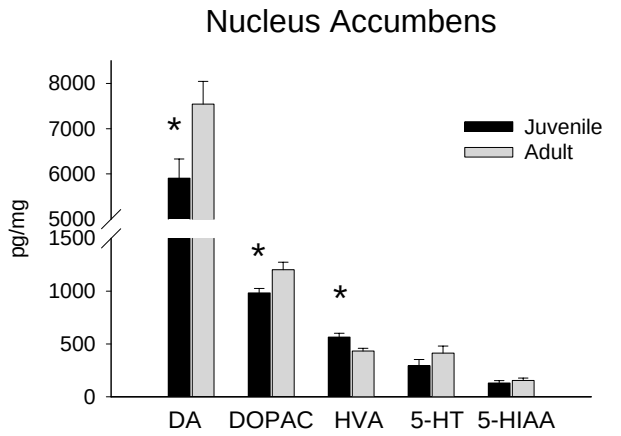


Fig. 2. DA, DOPAC, HVA, 5-HT, and 5-HIAA content in the juvenile and adult NAC. There was higher DA and DOPAC and lower HVA content in adult compared with juveniles ($^{*}P < 0.03$). Data expressed as pg/mg (mean ± S.E.).

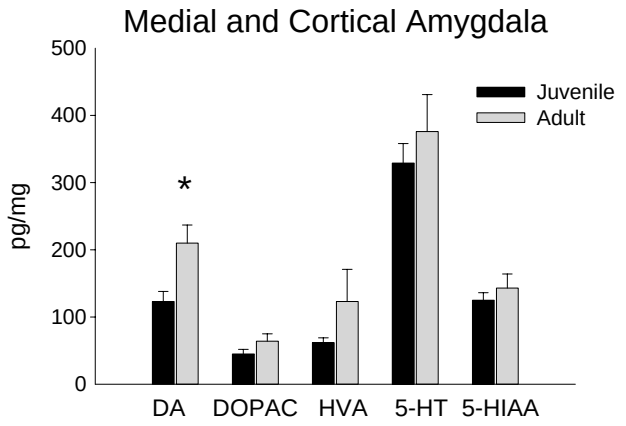


Fig. 3. DA, DOPAC, HVA, 5-HT, and 5-HIAA content in the juvenile and adult MCA. There was higher content of DA in adults compared with juveniles ($^{*}P < 0.01$). Data expressed as pg/mg of tissue (mean ± S.E.).

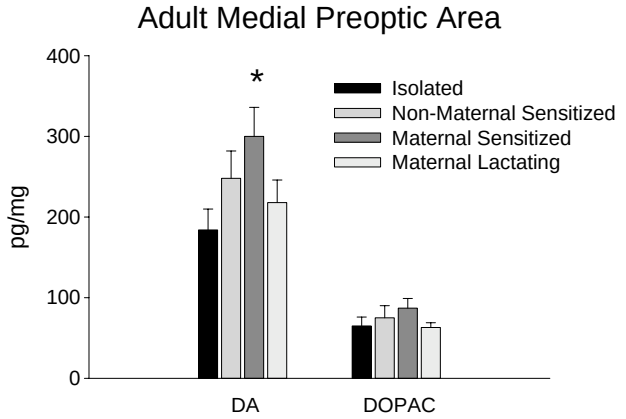


Fig. 4. Concentration of DA and DOPAC measured by HPLC in the adult MPOA, across the behavioral groups. There was higher content of DA in maternal sensitized when compared with isolated females ($^{*}P < 0.03$). Data expressed as pg/mg of tissue (mean ± S.E.).

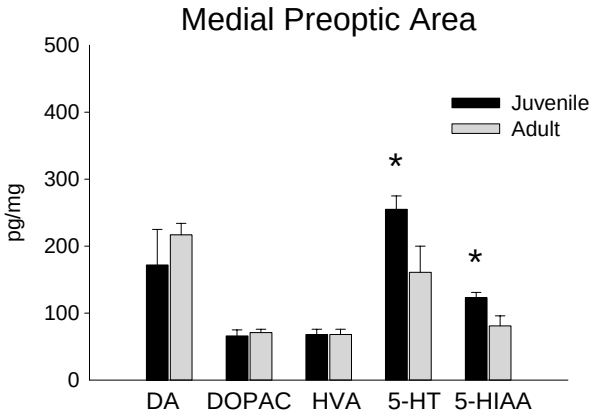


Fig. 5. DA, DOPAC, HVA, 5-HT, and 5-HIAA content in the juvenile and adult MPOA. There was higher content of 5-HT and 5-HIAA in juveniles compared with adults ($^{*}P < 0.05$). Data expressed as pg/mg of tissue (mean ± S.E.).

PLSD, $P < 0.026$). There were no other statistically significant differences in the amount of DA, 5-HT or their metabolites in the MPOA across the behavioral groups adult or juvenile (Fig. 4; Tables 1 and 2).

Adult females exhibiting pup-induced maternal behavior had statistically significantly higher levels of DA than did juveniles exhibiting pup-induced maternal behavior (300 ± 36 versus 146±21 pg/mg wet weight, respectively) (Tables 1 and 2; *t*-test, $P < 0.005$). There were no other statistically significant differences across other comparable juvenile or adult groups.

Regardless of their behavioral group, the juveniles had statistically significantly higher than 5-HT and 5-HIAA in their MPOA than did adults (Fig. 5, *t*-test, $P < 0.05$ and $P < 0.02$, respectively).

## 4. Discussion

We found that the levels of dopamine and serotonin in the NAC, MPOA, and MCA undergo postnatal developmental changes as juveniles become adults. The amount of DA and DOPAC in the NAC and MCA was higher in adults than in juveniles, while the 5-HT and 5-HIAA in the MPOA were lower in adults than in juveniles. These results suggest that higher basal DA activity in the NAC and MCA and lower basal activity of 5-HT in the MPOA are found in adults compared with juveniles.

The correlation of these neurochemical changes with our behavioral findings showing a rapid induction of pup-induce maternal behavior in juveniles compared to a slow induction in adults suggests that these changes may support these behavioral differences. An increased 5-HT activity in the MPOA and the reduced DA activity in the NAC and MCA could facilitate interaction with pups in juveniles and the subsequent fast induction of maternal behavior. It seems, however, unlikely that such neurochemical changes are only

related to differences in maternal responsiveness, rather we propose that they may underlie more broadly functional differences in the processing of information or salience from novel stimuli resulting in the general differences in sociability, and response to novelty found in juveniles compared with adults. Our current behavioral work suggests that juveniles are generally less neophobic to both novel social and object stimuli [63]. These interpretations are further supported by the fact that DA has been implicated in the mediation of the response to novelty, principally acting in the NAC [1,3,8,33,56,57,59], and adult response to novelty including the salience of pup stimuli, has been characterized as an inhibition or aversive response [18–20,43,45].

The data additionally show increased DA content in the MPOA of adults induced into maternal behavior compared with both isolated adults and juveniles induced into maternal behavior. This suggests that dopamine has a role more complex than simple inhibition of these behavioral components once the system has matured, and that the function may vary across different structures in this behaviorally important neural circuit.

### 4.1. Concentration of dopamine and serotonin and metabolites

Our neurotransmitter and metabolite levels in the adult NAC are in good accord with levels reported in prior work [15,34]. Less is known about transmitter concentrations in the MPOA and MCA and since some of these data were gathered using specialized microdialysis it is difficult to compare data directly [29,32,46,54,76], however, our data are in agreement with that of others for the amygdala [60]. Our dopamine related levels in the MPOA were slightly lower than those of Du et al. [14] probably since our samples were slight rostral to theirs, and thus rostral to the somas of dopaminergic neurons in the caudal MPOA and the rostral anterior hypothalamus [11,62]. Lonstein, et al. [40], also using HPLC on postmortem samples, found that dopamine levels in the MPOA change across the peripartum and postpartum period in correlation with behavioral and parturtional events during a period we did not examine. However, our data do accord well with several of their other data points, virgins and lactating females. Since their virgin females were at diestrous, and our virgin control isolates were taken at random cycle points this suggests that the levels of these transmitters in the MPOA do not vary substantially across the cycle. Possibly the additional temporal precision of microdialysis is critical to examine this type of regulation since others have found that in the female rat, extracellular DA is responsive to hormonal state [42].

We considered two methodological issues, the wet weight basis of our calculations and developmental differences in myelination, and believe these issues do not limit the use of our data to support our conclusions. In the present study, all neurotransmitter and metabolite amounts were calculated and expressed as pg/mg wet weight of tissue, and since we found that data points from similar conditions were in agreement with Hohn and Wuttke [31] who used pg/mg protein, both calculations are valid. The myelin component of the brain is rapidly developing in juveniles during the second to fourth postnatal weeks, reaching adult levels much later [9]. By logic, less myelin would result in higher neurotransmitter concentration per mg of tissue in the juvenile. We, however, found that either the concentration of serotonin was higher in juveniles while dopamine was lower, or there were no differences across the ages depending upon the brain region. Therefore, differences in myelin cannot be the basis for the differences we have documented in juveniles and adults.

### 4.2. Dopamine increases with maturation

We found that dopamine and serotonin levels had different patterns in juveniles compared to adults, and so we will discuss each neurotransmitter separately. Adults had higher concentrations of DA and metabolites than did juveniles in the accumbens and amygdala. Our results accord with lower dopamine concentration and turnover rates in the NAC of 20-day-old rats, compared to older rats reported by Hohn and Wuttke [31]. Other components of the dopaminergic system also differ since adults have a higher density of $D_1$ receptors and DA transporter in the NAC and a lower density of $D_2$ autoreceptors receptors than juveniles [10,67–69,70].

Since stimulation of autoreceptors in dopaminergic nerve terminals inhibits DA synthesis and release in those terminals [64,71,75], it is possible that the reduced DA-related activity in these brain regions of the juvenile may be mechanistically related to differences in the levels of DA receptors and transporters. Indeed mouse models lacking the dopamine transporter have a reduction in DA and an increase in HVA in the striatum [35].

Possibly a lower density of presynaptic $D_2$ autoreceptors in adults could disinhibit DA synthesis, and result in higher dopamine levels in adults. A higher density of DA transporter in adults would increase the re-uptake of DA reducing the extracellular degradation of DA. This may contribute to a lower concentration of HVA in the adult NAC compared with juveniles that we found. Other mechanisms such as adult–juvenile differences in the activity of enzymatic degradation in the extracellular space by catechol-*O*-methyltransferase (COMT) and intracellular monoamine oxidase (MAO) may also be involved.

### 4.3. Serotonin decreases with maturation in the MPOA

In contrast to higher dopamine levels in two regions in adults, we found decreased serotonin levels in adults in a single region, the MPOA. These data accord with previous reports of higher levels of the 5-HT metabolite 5-HIAA in the cerebrospinal fluid in both prepubertal humans and rats compared with adults [30,61]. Decreased 5-HT and 5-HIAA content in the adult MPOA suggests decreased 5-HT based neural activity.

Key differences between prepubertal juveniles and reproductively mature adults are likely to involve changes that occur during puberty, and that are hormonally driven particularly in this brain region. Pubertal changes are, however, complex and beyond the scope of our focus. However, since 5-HT has been implicated in reproductive behavior and function [2,16,23,72], this juvenile to adult decrease suggests there may be a precise hormonally driven set point for these transmitters in adult reproductive function and such hormonal events may not yet be occurring in the juvenile. Whether 5-HT changes in the MPOA found in the present experiment were dependent on ovarian hormones is not known because all rats had intact reproductive organs; however, the MPOA is well known to play a critical role in the hormonally dependent maturation of reproductive neuroendocrine and behavioral functions [4,38,47,73].

### 4.4. Comparisons across behavioral groups

We found no changes in DA or 5-HT associated with pup exposure or the expression of maternal behavior in the continuous presence of the pups in the NAC or MCA. Changes in DA release in the NAC are associated with the maternal response to pups upon being reunited with pups [25,26,28]. Perhaps only specific behavioral components of responses to pups are under the mediation of dopamine in these regions, e.g. the motivational components of the behavior or the initial pup directed actions which occur at different time points in the behavioral sequence than we used.

We, like Lonstein et al. [40] who was using a different paradigm, found no changes in 5-HT in the MPOA associated with pup exposure or expression of maternal behavior. We, like Lonstein et al. [40], found no simple correlation between dopamine levels in the MPOA and maternal behavior in the parturient female at day 4 or 7 postpartum. We did find an increase in DA in the adults that were induced into maternal behavior by pup exposure compared to isolated adults. The maternal juveniles induced by pup stimuli did not show an increase in DA content. This change may not be needed in juveniles that may be less inhibited, or alternatively, increased DA in adults might be related not to maternal state but to responses to pup stimuli. Perhaps juveniles exposed to pups for the same time would also have resulted in these higher dopamine levels. For example, non-maternal adults, and lactating females exposed to pups for shorter period of time ($7 \pm 1$ days and 4 days, respectively) than maternal adults ($10 \pm 1$ day), showed intermediate values for DA in the MPOA, suggesting that longer pup exposure may contribute to higher DA levels.

While this one data point suggests that DA in the adult MPOA may be implicated in the expression of maternal behavior or responsiveness to pups as stimuli, the general pattern of results across the behavioral groups suggests an additional possibility. Perhaps these neurotransmitters do mediate maternal behavior or social responsiveness by acting within several of these areas, but the changes in neurotransmitter content correlated with this naturally occurring spontaneous behavior or exposure to the natural pup stimulus is either quantitatively smaller or has a much shorter temporal course than we could measure. Perhaps it is not mechanistically realistic to expect the large changes in these neurotransmitter levels found after pharmacological agents are used in the central nervous system. Instead, modest and tightly temporally regulated levels of neurotransmitters within behaviorally critical regions might mediate such a behavioral sequence.

The model of DA and 5-HT action in the MPOA in the mediation of male sexual behavior may inform future studies on maternal behavior [12,13,32,41,53]. Particularly interesting is the work in the male showing that neural activity in the medial amygdala enhances levels of extracellular dopamine in the male MPOA and that an intact medial amygdala is necessary for both copulatory ability and DA response in the MPOA [12,13]. Our data showing that there are differences in the juvenile versus adult in the MPOA and amygdala suggest that there may also be similar neurotransmitter based co-ordination across these two regions for the regulation of maternal behavior. Such multi-region data is informative as to how these circuits might work as a unit to yield their respective complex behaviors.

## 5. Conclusions

The present study demonstrated that developmental changes in the DA and 5-HT systems occur between 20 and 60 days of age in brain regions that participate in the neural circuit that supports maternal behavior in rats. Increased basal DA activity in the NAC and the MCA, and decreased basal activity of 5-HT in the MPOA may affect the initial response to pups, delaying the induction of maternal behavior in adults. In addition, these findings suggest that developmental changes in the level of DA and 5-HT receptors may also occur in the MCA and MPOA, respectively, as previously found in the NAC. Future studies must examine the causality of these changes by using region-specific administration of neurotransmitter analogues to determine whether the juvenile state can be induced prematurely to change into the adult behavioral state, or whether the adult state can be altered to return to the state in which maternal behavior is more easily induced perhaps by greater positive responsiveness to salient novel stimuli.

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