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# Monoamine Neurotransmitters and Metabolites During the Estrous Cycle, Pregnancy, and The Postpartum Period

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DESAN, P. H., W. W. WOODMANSEE, S. M. RYAN, T. K. SMOCK AND S. F. MAIER. *Monoamine neurotransmitters and metabolites during the estrous cycle, pregnancy, and the postpartum period.* PHARMACOL BIOCHEM BEHAV **30**(3) 563–568, 1988.—A wide variety of behavioral changes in the female rat have been associated with the estrous cycle, pregnancy, and the postpartum period and their accompanying hormonal fluctuations. Since monoamine systems have been implicated in the control of these behaviors, the present study examined the tissue concentrations of norepinephrine (NE), dopamine (DA), and serotonin (5HT) and their primary metabolites dihydroxyphenylglycol (DHPG), 3-methoxy-4-hydroxyphenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC), and 5-hydroxyindoleacetic acid (5HIAA) in the anterior cerebral cortex, hippocampus, and cerebellum during the estrous cycle, late pregnancy, and the early postpartum period. Results show no significant differences in neurotransmitter or metabolite levels during the estrous cycle in any of the three brain regions examined. However, profound differences were observed between samples from late pregnancy, early postpartum, and the estrous cycle. In general, NE and 5HT levels in all three brain regions fell from normal values during late pregnancy and rose in the early postpartum period; levels of their metabolites rose in postpartum samples. Conversely, DA levels were elevated in late pregnancy and depressed in the early postpartum period in anterior cortex, while DOPAC levels were depressed in both late pregnancy and the early postpartum period. The finding of changes in monoamine metabolism associated with pregnancy and its termination could be important in understanding the increased susceptibility to affective illness in women during the postpartum period.

Estrous cycle Pregnancy Monoamines HPLC/ECD

MANY aspects of behavior and neural function are influenced by the hormonal changes which occur during the estrous cycle, pregnancy, and postpartum period in rodents. In addition to changes in sexual and maternal behavior, general activity [23], pain sensitivity [5], feeding behavior [19], aggressiveness [6], seizure susceptibility [31], intracranial self-stimulation [21], body temperature [15], and conditioned avoidance learning [29] have been found to vary with stage of estrus and pregnancy. Behavioral changes which are produced by exposure to environmental stressors seem to be particularly sensitive to stage of estrus. For example, the magnitude and nature of "stress-induced analgesia" strongly depends on the stage of estrus and estrogen levels [25].

Some of these changes may be mediated by monoaminergic systems in the brain. For example, monoaminergic systems in hypothalamic and limbic regions appear to control the appropriate occurrence of sexual behavior and the release of pituitary hormones during the estrous cycle. Monoamine levels [2, 12, 22, 28], monoamine turnover [33], and the activity of synthetic and degradative monoaminergic enzymes [1, 12–14] in various limbic regions vary during the estrous cycle and in ovariectomized rats treated with estrogen and/or progesterone [13, 14, 22, 32, 33]. Monoamine and metabolite levels have also been shown to fluctuate throughout the course of pregnancy in the fore-, mid-, and hindbrain of mice [8,9]. Some of these changes may reflect direct effects of gonadal hormones [13,14]. For example, some catecholaminergic neurons of the brain stem are known to concentrate estrogen [26].

These studies on alteration of monoamine metabolism have focused only on subcortical regions, which receive a variety of noradrenergic, dopaminergic, and serotonergic inputs. The locus coeruleus and the dorsal raphe provide diffuse, poorly topographic, noradrenergic and serotonergic inputs, respectively, to widespread regions of the brain [17,18]. In particular, the cerebral cortex, hippocampus and

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cerebellum receive all or most of their noradrenergic and serotonergic input from the locus coeruleus and the dorsal raphe. In addition, the cortex receives a dopaminergic input from the mesocortical cells of the substantia nigra and ventral tegmental area [16]. Little information is available about the modulation of these major monoamine systems during the estrous cycle. This study measured tissue levels of the principal monoamine neurotransmitters and, more importantly, their metabolites in samples of anterior cerebral cortex, hippocampus, and cerebellum collected on each of the four stages of the estrous cycle, day 19 of pregnancy and day 6 of the postpartum period.

## METHOD

### *Chemicals*

All compounds, with the exception of ascorbic acid oxidase, were purchased from Sigma Chemical Co., St. Louis, MO. The enzyme ascorbic acid oxidase was obtained from Boehringer Mannheim GnbH, West Germany.

### *Subjects*

Subjects were Holzman-Sprague-Dawley female albino rats (250 g) bred at the University of Colorado, Boulder and housed individually. The rats were maintained on a 12:12 light/dark cycle with the light condition beginning at 0700. Food and water were available at all times. Samples were obtained for each of the four days of the estrous cycle (n=8/group), the 19th day of pregnancy (n=5), and 6th postpartum day (n=5). Maintenance of the four day cycle was assessed by the daily examination of vaginal smears and the onset of pregnancy was determined by the first sperm-positive day following the introduction of males. Only rats displaying stable consecutive estrous cycles were used and the estral stage was confirmed at the time of sacrifice.

### *Procedure*

The animals were killed between 1300 and 1600 on the selected day of the estrous cycle, the 19th day of pregnancy, and postpartum day 6. The time of sacrifice corresponded to a point in the light-dark cycle in which behavioral differences during the days of the estrous cycle have been studied by this laboratory [25]. The cerebellum, hippocampus and anterior cortex were dissected from each animal, frozen on dry ice, and stored at −70 degrees celsius. The tissue was thawed in pH 5.0 0.1 M acetate buffer solution containing 1.0 $\mu$M 3,4-dihydroxybenzylamine HBr (DHBA) as an internal standard, sonicated and centrifuged at 17000×g for thirty minutes. The supernatants were divided into aliquots of 200 $\mu$l and refrozen at −70 degrees celsius. For analysis, samples were thawed and 20 $\mu$l of a solution of ascorbic acid oxidase (0.1 M acetate buffer solution, pH 5.0, containing 1.0 mg/ml) was added. Ascorbate oxidase was used to remove excess ascorbate from the sample preparation before analysis, a necessary step to resolve early eluting peaks.

### *Monoamine Measurements*

High performance liquid chromatography (HPLC) with electrochemical detection was used to determine the content of norepinephrine (NE), dopamine (DA), serotonin (5HT), the principal dopaminergic metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), and the principal serotonergic metabolite, 5-hydroxyindoleacetic acid (5HIAA) in the tissue samples. The monoamines and their metabolites were separated by ion pairing, reverse phase chromatography on a Varian 5000 chromatograph with a Beckman column (15 cm × 4.6 mm) packed with 5 $\mu$m $C_{18}$ODS silica beads. This analytical column was preceded by a guard column (3 cm × 4.6 mm) hand packed with reverse phase silica beads (Vydac, Varian, Sunnyvale, CA). The mobile phase (pH 4.00) consisted of 8% HPLC grade methanol, 0.135 M citric acid, 0.19 M sodium acetate, 0.3 mM ethylene diamine tetraacetic acid (EDTA), and 0.46 mM 1-octane sulfonic acid (OSA) in deionized water.

One set of sample aliquots was used for the measurement of NE, DA, 5HT, DOPAC, and 5HIAA, using conventional oxidative electrochemical detection (LC-3A, BAS, Inc., West Lafayette, IN). An oxidation potential of 0.75 volts was employed for all measurements using this method. A second set of sample aliquots from cortex and cerebellum were assayed for DHPG and MHPG using a serial oxidative/reductive detection system. This method employs a multi-electrode detector recently described [4]. In this method, column outflow is passed serially through three "coulometric" electrochemical cells (5020 Guard Cell followed by 5011 Dual Cell, connected to Model 5100A Coulometric controller, ESA Inc., Bedford, MA). These cells oxidize or reduce a high proportion of chromatographic species passed through them when operated at appropriate potentials. The first detector is operated at a potential of +0.40 volts and serves to oxidize species of interest and the second detector serves to screen out easily reduced interfering compounds at a potential of 0.0 volts. The third detector is used for peak quantification of compounds at −0.40 volts. NE metabolites can be measured accurately with this method because coeluting species either rereduce at the second electrode or fail to rereduce at the third.

The catecholamine, indoleamine, and metabolite contents of the tissue were determined by comparison with chemical standards and corrections for recovery were calculated using the DHBA standard. All concentrations were expressed as nanograms of compound per gram of wet tissue weight. The statistical significance of differences in monoamine and metabolite levels were assessed by a one-way analysis of variance (ANOVA) and preplanned comparisons, carried out for each compound studied. One-way ANOVAs were performed across the four days of the estrous cycle and across all six groups. Samples from pregnancy and the postpartum period were compared with one another and to samples from the pooled estrous cycle data by using preplanned contrasts. A rejection level of $p<0.05$ was chosen for all comparisons.

## RESULTS

### *Monoamine and Metabolite Content During the Estrous Cycle*

Results of this study showed only small changes in neurotransmitter or metabolite content during the estrous cycle in all of the three brain regions examined, none of which were statistically significant (one-way ANOVA over the four estrous cycle stages). NE levels in the hippocampus were slightly depressed in proestrus as compared to the three remaining days of the cycle (Fig. 1D). 5HT levels were constant across the cycle in all brain regions, whereas levels of its metabolite, 5HIAA, exhibited some variation (Figs. 1C, D, 2B). DA levels increased slightly on proestrus but DOPAC concentrations in the cerebral cortex remained constant during the four day cycle (Fig. 1B).

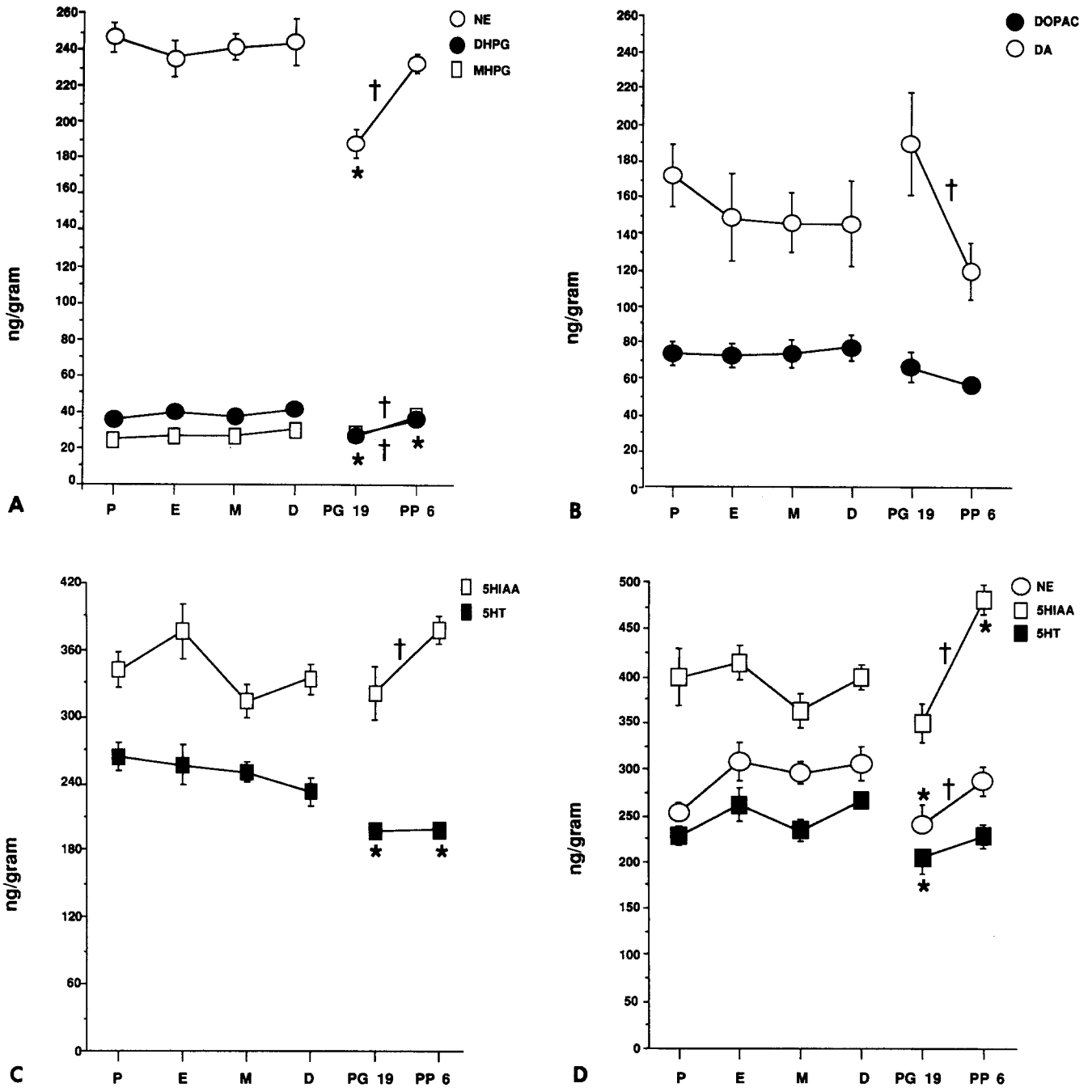


FIG. 1. Variation in levels of monoamine neurotransmitters and their metabolites in **cerebral cortex** and **hippocampus** during the estrous cycle, pregnancy and postpartum period. The experimental groups are abbreviated as follows: P, proestrus; E, estrus; M, metestrus; D, diestrus; PG 19, day 19 of pregnancy; PP 6, day 6 of postpartum. (A) Levels of NE and its metabolites, DHPG, and MHPG in cortex. (B) Levels of DA and its metabolite, DOPAC, in cortex. (C) Levels of 5HT and its metabolite, 5HIAA, in cortex. (D) Levels of NE, 5HT and 5HIAA in the hippocampus. Lack of error bars indicate error (SEM) is smaller than the symbol. Asterisks indicate significant differences between pregnancy day 19 or postpartum day 6 and the estrous cycle, $p<0.05$. Daggers show cases in which pregnancy and the postpartum period differ significantly from one another, $p<0.05$.

*Monoamine and Metabolite Content as a Function of Pregnancy*

The greatest changes noted in the study occurred between samples collected in late pregnancy and the early postpartum period. A rise in NE levels occurred in all three brain regions analyzed (Figs. 1A, D, 2A). This was accompanied by a rise in MHPG and DHPG levels in both regions analyzed for noradrenergic metabolites, cortex and cerebellum (Figs. 1A,2A). 5HT levels rose modestly between pregnancy and the postpartum period in hippocampus and cerebellum (Figs. 1D,2B) but did not change in cortex (Fig. 1C). 5HIAA levels rose dramatically in all three regions (Figs. 1C, D, 2B). DA de-

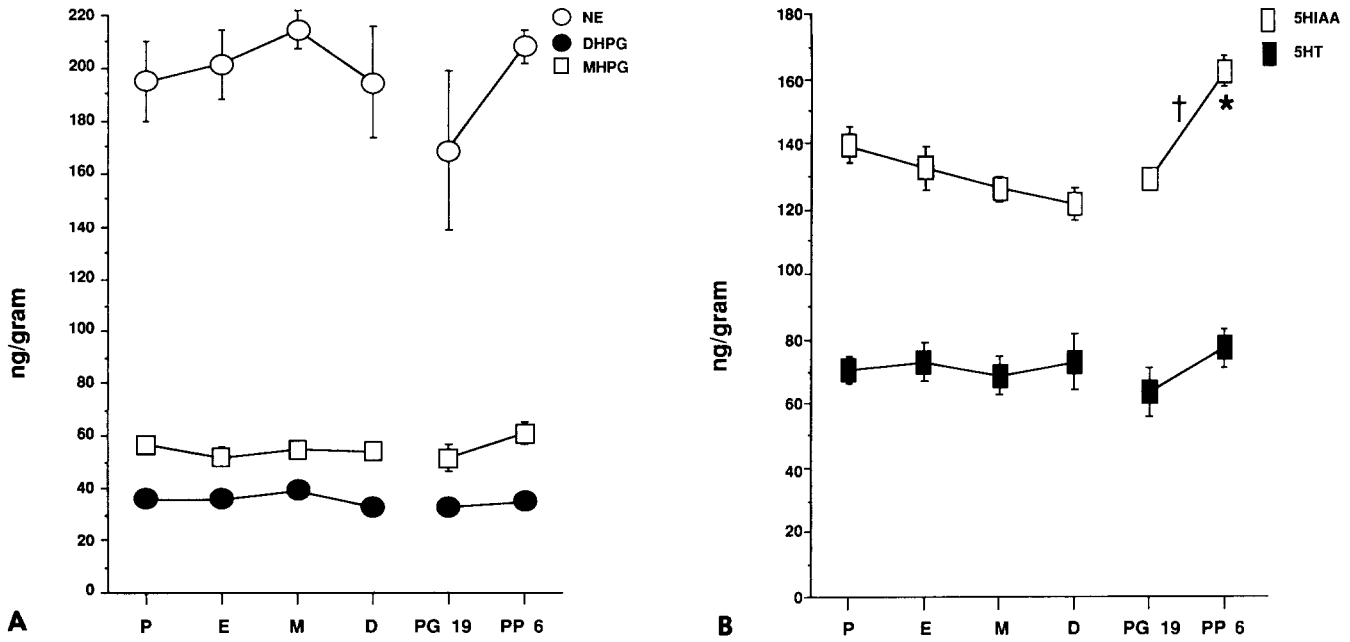


FIG. 2. Variation in levels of monoamine neurotransmitters and their metabolites in cerebellum during the estrous cycle, pregnancy, and postpartum period. Abbreviations are as in Fig. 1. (A) Levels of NE and its metabolites, DHPG and MHPG, in cerebellum (B) Levels of 5HT and its metabolite, 5HIAA, in cerebellum. Lack of error bars indicate error (SEM) is smaller than the symbol. Asterisks indicate significant differences between pregnancy day 19 or postpartum day 6 and the estrous cycle, $p<0.05$. Daggers show cases in which pregnancy and the postpartum period differ significantly from one another, $p<0.05$.

creased markedly in cortex while DOPAC remained unchanged (Fig. 1B).

Systematic differences were seen between values during the estrous cycle and values in pregnancy or the postpartum period. In each brain region, NE and 5HT concentrations were at their lowest values obtained across all groups during pregnancy. In contrast, their principal metabolites, MHPG. and 5HIAA, reached maximum values during the postpartum period. The neurotransmitter DA reached its highest value during pregnancy and its lowest value during the postpartum period. DOPAC content was lower during pregnancy and the postpartum period than at any point in the estrous cycle.

## DISCUSSION

This study examined steady-state levels of NE, DA, and 5HT and their respective primary metabolites in the rat anterior cerebral cortex, hippocampus, and cerebellum during the estrous cycle, late pregnancy, and early postpartum period. The results showed generally small and insignificant variations in monoamines and monoamine metabolites during the estrous cycle in each of the three regions for which measurements were obtained. NE and its metabolites, DHPG and MHPG, were largely constant during the cycle except for a modest decrease in NE content on proestrus in the hippocampus. By contrast, large changes in NE levels have been found in limbic system regions, usually a decreased concentration during proestrus and estrus [2,22]. Similarly, significant changes in DA and DOPAC concentrations were not observed at any point in the four day cycle in cortex; a variety of patterns of change in DA level and turnover have been noted in limbic regions and in the basal ganglia [2]. While 5HT levels remained constant, small cyclic changes were seen in the concentrations of serotonin's metabolite, 5HIAA. 5HIAA levels were highest on estrus in the cortex and hippocampus, while in the cerebellum they were elevated on proestrus. While 5HT levels vary in some subcortical structures during the estrous cycle, no significant changes were seen in the cerebral cortex [12]. It is noteworthy that 5HT receptor density increases during late proestrus and estrus [34]. Thus, an increase in cortical 5HIAA (perhaps reflecting neurotransmitter turnover) may be accompanied by an increase in postsynaptic serotonergic sensitivity in cortex. Our data indicate that the neurotransmitter and metabolite levels in cortical structures do not behave in the same manner as they do in subcortical regions during the estrous cycle. However, our results cannot be interpreted to exclude the possibility that cortical changes may have occurred at times other than the ones we sampled.

The most notable differences observed in this study occurred in pregnant and postpartum animals. The results of a previous study of monoamine neurotransmitter levels in fore-, mid-, and hindbrain of mice during each day of pregnancy agree with some of the present results on transmitter levels [8,9]. In that study, NE concentration in forebrain samples, which did not include basal ganglia, was found to be decreased relative to diestrus values during most of pregnancy with a trend to increased values toward term. 5HT levels were depressed in midpregnancy but increased above diestrus values toward term. DA levels were similar to diestrus levels through middle pregnancy but decreased at its end. Although these trends in terminal pregnancy resemble the changes noted between late pregnancy and the early postpartum period in this study, comparison is difficult

among results with different sample dissections in a species with a different length of pregnancy. An abstract from the same laboratory on monoamine levels in identical regions in the postpartum period, indicates some similarities and some differences with the present study [7].

Our results show little change in monoamine metabolism across the estrous cycle but large changes in pregnancy and the postpartum period. Whether such changes reflect changes in presynaptic neurotransmitter release in these systems in unclear because the inference of functional activity of a transmitter system from neurotransmitter and neurotransmitter metabolite level is not straightforward. While transmitter level may remain the same, decrease, or even increase during increased impulse activity, the levels of transmitter metabolites MHPG (both free and sulfated), DOPAC, and 5HIAA increase during increased firing in noradrenergic [11], dopaminergic [24], and serotonergic [10] systems, respectively, and in general correlate with other methods of assessing transmitter release. Less information is available for the noradrenergic metabolite DHPG, but it also appears to rise (both free and sulfated form) during increased activity [27]. Thus, the relatively constant levels of monoamine metabolites measured during the estrous cycle in this study presumably indicate a relatively constant level of impulse activity, transmitter release, and metabolism. The increase in MHPG and 5HIAA levels between late pregnancy and the postpartum period may indicate an increased level of activity in noradrenergic and serotonergic systems innervating the cortex and cerebellum. However, such inferences may be inaccurate in the presence of increased transmitter levels. A concurrent increase in transmitter level could reflect either a general facilitation of system function or an increased transmitter pool turning over at an unchanged rate. The hypothesis of decreased noradrenergic function during pregnancy is supported by a recent study of catecholamine levels and turnover, as measured by transmitter depletion after inhibition of synthesis, during pregnancy in the mouse [30]. These authors report an increase in NE content accompanied by an increase in NE turnover between late pregnancy and day of birth in both cerebral cortex and hippocampus. However, they did not find major changes in DA levels or turnover in the cerebral cortex. The decreased levels of DOPAC during both pregnancy and the postpartum period, compared with the estrous cycle levels, could indicate a less active dopaminergic innervation of the cortex. It is important to emphasize that changes in tissue levels of transmitter and transmitter metabolite could reflect changes in synthesis, intracellular turnover, and degradation in neurotransmitter pathways as well as changes in transmitter release during the estrous cycle and pregnancy.

Changes in monoamine metabolism during pregnancy and the postpartum period may be of importance because they might play a role in affective changes reported to occur during this period. In humans this represents a period of time during which there is an increased susceptibility to depressive disorders [3], and alterations in both noradrenergic and serotonergic function have been suggested as causal factors in affective change [20].

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