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Monoaminergic Dysregulation on Diestrus-2 and Estrus Through High Emotional Reactivity

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SFIKAKIS, A., Z. PAPADOPOULOU-DAIFOTIS, M. SFIKAKI AND J. MESSARI. *Monoaminergic dysregulation on diestrus-2 and estrus through high emotional reactivity.* PHARMACOL BIOCHEM BEHAV **60**(1) 285–291, 1998.—Rats with great differences in emotional reactivity, during weighing and handling for vaginal smear screening were examined on diestrus-2 (DE-2), proestrus (PE), and estrus (E). Rats with high emotional reactivity (HR), interpreted as trait anxiety, had different serotonergic and dopaminergic profile in hypothalamus–preoptic area (HY-PA) and striatum (Str) and thymus weight lower than that found in rats with low emotional reactivity (LR). In HY-PA of rats with HR when compared to rats with LR, increased 5-hydroxyindoleacetic acid (5-HIAA), 5-HIAA/serotonin (5-HT) ratio, and 3,4-dihydroxyphenylacetic acid (DOPAC) and in Str increased DOPAC and DOPAC/dopamine (DA) ratio were found only on DE-2, paralleled by increased adrenal weight and decreased thymus weight. In Str, a significant effect of HR on 5-HIAA was found only on E, in parallel with increased 5-HT and decreased DOPAC and DOPAC/DA ratio when compared to rats with LR. The results suggest that activation of 5-HT and DA in HY-PA and DA in Str through HR is apparent only on DE-2 while, conversely, on E suppression of striatal DA it is apparent with 5-HT dysregulation. These findings might have some relevance to the predisposition of women with trait anxiety to premenstrual syndrome. © 1998 Elsevier Science Inc.

Emotional reactivity Serotonin Dopamine Diestrus Proestrus Estrus
Relevance to premenstrual syndrome

FEMALE rats with great differences in emotional reactivity to vaginal smear screening (VSS) but regular estrous cyclicity (37) might prove useful to gain information relevant to disorders in humans, related to reproductive cycle such as premenstrual dysphoria, commonly called premenstrual syndrome (PMS). This syndrome, with most prominent symptoms such as irritability, tension, and depression, only at the late luteal phase, has an incidence of 3–8% in women during their reproductive years (18,32). From a review of the literature it may be concluded that there are no known abnormalities of gonadal steroid levels in PMS (34). Increasing evidence has recently shown that serotonergic dysregulation may be involved in the pathogenesis of PMS (21,31,33,44,51). On the other hand trait anxiety is considered as a predisposing factor to PMS (16,17,49).

Experience from previous works (37,38) and unpublished observations prompted us to consider the possibility that female rats, showing emotional reactivity to simple handling for weighing and repeated resistance to handling for VSS, might represent individuals with trait anxiety, when compared to rats with no or rare resistance to VSS, provided that handling is always conducted by the same person, under similar conditions.

The present study was designed to investigate whether increased emotional reactivity, as expressed by behavioral resistance, under similar duration of handling for VSS, may affect serotonin (5-HT) and dopamine (DA) activity in hypothalamo–preoptic area (HY-PA) and striatum (Str) on diestrus-2 (DE-2) and also on two other stages of the estrous cycle proestrus (PE) and estrus (E).

Our interest in HY-PA was occasioned by previous findings (37,38) showing that prolonged duration of handling for vaginal screening, combined with high emotional reactivity, augments serotonergic activity in HY-PA and activates the hypothalamic–pituitary–adrenal (HPA) axis. This interest was further stimulated by a study in humans (30) reporting HPA axis abnormalities in PMS patients compared to normal women. Striatum was examined because it includes the nucleus accumbens, the brain area linked to the reward system (11,15).

The three consecutive stages DE-2, PE, and E of 4-day estrous cycles were examined for the following reasons: the day

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of DE-2, which precedes the ovulatory surge, taking place on the afternoon of PE (48) under our lighting schedule, might correspond to the follicular phase of the human cycle, while PE might correspond to the periovulatory phase. The stage of E during the a.m. hours that follow ovulation, taking place after midnight (36), might correspond hormonally to the late luteal phase of the human cycle.

A possible correspondence of E to the late luteal phase is derived from findings showing that in rats, as opposed to humans, a true luteal phase with high progesterone and estradiol levels is missing, because the corpora lutea formed after ovulation are not functional (52). For this reason the level of estradiol and progesterone are low at a.m. on E, being preceeded by the high levels of these two hormones on PE (10,52). This decline has an analogy to the decline in levels of estradiol and progesterone at the late luteal phase in humans, from the preceeding high levels of these hormones that characterize the true luteal phase (42,52). One might argue that metestrus (diestrus-1), which follows E and precedes DE-2, is closer to the late luteal phase than E. Against this argument is the presence of relatively high levels of progesterone and low levels of estradiol on diestrus-1 (DE-1) and the fact that DE-1 is not preceeded by high levels of progesterone and estradiol (10,52).

To assess the effect of repeated emotional reactivity to handling on the HPA axis, the weight of the thymus was used as an approximate index of the amount of experienced chronic stress (12,20,37). The weight of the adrenals was also measured in all the studied rats being considered as a useful index of the degree of chronic activation of the HPA axis (2,3,5,7,37,43).

METHOD

Animals–General Procedure

Virgin female rats of the Wistar strain, born and raised in our laboratory, living under automatically controlled light cycle (lights on from 0600 to 2000 h, 21–23°C), were studied at 3–3.5 months of age. The stage of the estrous cycle was determined by vaginal smears, which were obtained between 0900 and 1100 h. Rat chow and drinking fluid were available ad lib. The handling for VSS was preceeded by simple handling for weighing on 6–7 days. Handling for weighing was continued during the whole experiment preceeding each time handling for VSS. All handled rats were autopsied after decapitation at the end of each experiment on DE-2, PE, or E between 0900 and 1200 h.

At autopsy, the two adrenals, uterus, and thymus after being removed, trimmed from surrounding fat and connective tissue on a filter paper saturated with saline, were weighed to the nearest 0.01 mg on an electric balance. On PE, the uterus was weighed both with intralumenal fluid and after the extrusion of fluid. For neurochemical estimation of monoamines the brain was rapidly dissected on ice and subsequently the HY-PA and the Str consisting of caudate putamen and nucleus accumbens were removed. The tissue was weighed just after the removal, then sonicated in $HClO_4$ and the supernatant after centrifugation was stored at −80°C until the analytical estimations.

Experimental Design

The individual behavior of the rats was recorded at each handling including the first 6–7 days of handling for simple weighing, without VSS.

Because the purpose of the present study was to compare rats with great differences in resistance after similar duration of handling to increase the chances to reveal rats hypersensitive to environmental stimuli, a 2-day interval free of handling was always included between each two sessions of 4 or 5 consecutive days of handling for VSS.

The expected two stages of the cycle, according to preceeded vaginal smears, were recorded for these 2 days. The regularity of the 4-day cycle, DE-1, DE-2, PE, and E, was verified by vaginal smears taken on the following 5 or 4 consecutive days. Rats with a 5-day or 6-day estrous cycle were discarded.

Care was taken that half of the rats autopsied on each particular day of cycle DE-2, PE, or E, were sacrificed after short handling for VSS, seven to nine handlings, and half after prolonged handling 12–15 handlings. Rats with short handling increased the chances to reveal those with resistance, because long duration of handling per se is paralleled by increased resistance (37). For this reason the sum of reactions in rats with low resistance ranged from 0–5 and in rats with high resistance from 6–22, because five reactions corresponding to 14 handlings for VSS were considered as low resistance while six reactions corresponding to eight handlings for VSS were considered as high resistance. Care was taken that the six studied groups, three with low resistance and three with high resistance, did not differ in age, body weight, and duration of handling.

The sum of the reactions was calculated for each rat and represented its resistance score. The following reactions were recorded during handling for weighing [1–6] and during handling for VSS [7–12]: 1) attempts to hide during removal from the cage of roommates being the last or next to last to be picked out. 2) Attempts to escape from the cage or from the box during waiting time. 3) Resistance during removal from the cage, which imposed grasping from the tail. 4) Vocal reaction before starting handling for VSS. 5) Defecation during waiting time before grasping for VSS. 6) Urination during waiting time. 7) Uneasiness and restlessness during grasping the rat gently by the skin of the neck and back, to be immobilized in the upright position for VSS, which imposed repeated grasping for immobilization. 8) Vocal reaction during handling for VSS. 9) Urination during handling for VSS. 10) Defecation during handling for VSS. 11) Resistance during touching of the vagina. 12) Agressiveness during the procedure for VSS with an attempt to bite.

Methods for Neurochemical Estimations

The analytical estimations were done by High-Performance Liquid chromatography (HPLC) using electrochemical detection. A reverse-phase ion pair chromatography was used in all analysis of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), serotonin (5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) (27,41). The mobile phase consisted of a phosphate buffer at pH = 3.9, containing methanol and 5-octylsulfate (Merck, Germany) as the ion-pair reagent. The sensitivity of the assays were always tested using external standards 5-HT, 5-HIAA, DA, and DOPAC (Sigma, St. Louis, MO). The HPLC system used was BASLC4B with an amperometric detector. The electrode used was glassy carbon, the columns were chrompack HPLC column glass 100 × 3 mm Sperisorb 50DS and an integrator LKB 2221 was also connected with the above-mentioned HPLC system. Additionally, the turnover rate of DA (DOPAC/DA) and 5-HT (5-HIAA/5-HT) was also calculated, to have a better evaluation regarding the dopaminergic and serotonergic function.

Statistical Analysis

A two-way ANOVA was used to examine the effect of the two factors: the phase of cycle and the high resistance vs. low

resistance (HR vs. LR) and the interaction between these two factors on the concentration of 5-HT, 5-HIAA, DA, and DOPAC, and on the 5-HIAA/5-HT ratio and DOPAC/DA ratios in HY-PA and Str. This was followed by a one-way ANOVA to examine the effect of phase of cycle on the above parameters in rats with similar resistance and the effect of (HR vs. LR) on the same day of the cycle.

Each ANOVA was followed by Sheffe's multiple range test to evaluate whether the differences among or between the groups were statistically significant. For individual comparison the unpaired Student's *t*-test was used preceeded by the logarithmic conversion of values when needed.

RESULTS

Table 1 shows the duration of handling, age, and body weight in the six studied groups of rats and their emotional reactivity as depicted by the resistance score. It also shows that uterine weight on DE-2 was lower compared to PE and E both in rats with LR and rats with HR, while on E from HR rats the weight was higher when compared to that of LR rats. Adrenal weight in rats with LR was higher on PE and E when compared to DE-2 (Table 1).

Comparison between rats with LR and HR on DE-2 revealed a difference in adrenal weight (Table 1). On the other hand, the thymus weight in these rats was inversely related to adrenal weight being decreased in HR rats (172.3 ± 14.82 mg/100 g b.wt.) vs. LR rats (222.4 ± 18.97, $p < 0.05$).

Evaluation of thymus weight, measured in 10 out of 13 rats sacrificed on PE and 10 out of 12 rats sacrificed on E, revealed that a thymus weight of 210.8 ± 8.87 mg/100 g b.wt. in 10 rats with LR (five PE and five E) with resistance score 2.9 ± 0.52, was significantly higher ($p < 0.01$) than the thymus weight of 177.2 ± 8.71 found in 10 rats with HR (five PE and five E) with resistance score 13.7 ± 1.77.

The two-way ANOVA for 5-HT (cycle phase × HR vs. LR) revealed a significant cycle effect, $F(1, 2) = 5.366$, $p < 0.00980$ without an effect of HR vs. LR. Further analysis by one-way ANOVA revealed a cycle effect only in rats with HR, $F(2, 15) = 3.759$, $p < 0.0475$, showing a rise of 5-HT on PE compared to DE-2 and E (Fig. 1). The Student's *t*-test, less strict than Sheffe, indicated that 5-HT on PE was also increased in rats with LR, but only vs. DE-2 ($p < 0.05$). After two-way ANOVA there were no cycle and no HR vs. LR effects on 5-HIAA, but there was a significant cycle × HR vs. LR interaction, $F(2, 2) = 3.521$, $p < 0.0415$. The one-way ANOVA revealed cycle effect on 5-HIAA, only in rats with LR, $F(2, 17) = 4.20$, $p < 0.0305$, with an increase of 5-HIAA

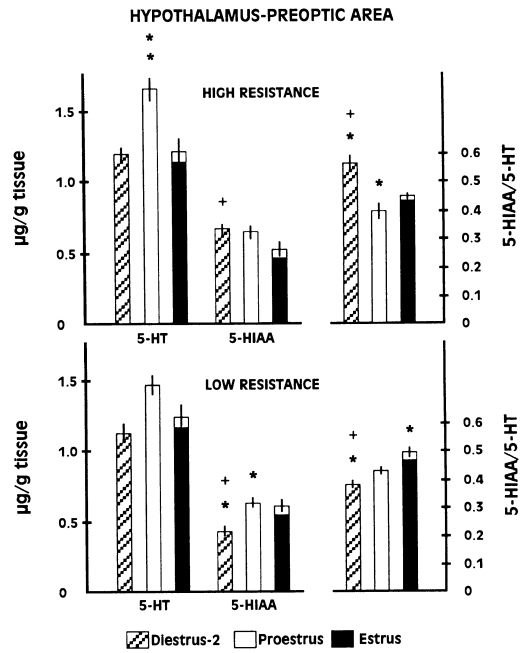

FIG. 1. The serotonergic profile in hypothalamus–preoptic area examined at 0900–1200 h, on the 3 consecutive days of 4-day estrous cycles in rats with low resistance to handling for vaginal screening (n = 6–7/group) and rats with high resistance (n = 6/group) as evaluated after recording behavior during handling of similar duration. 5-HT = serotonin, 5-HIAA = serotonin metabolite 5-hydroxyindoleacetic acid, 5-HIAA/5-HT = serotonin turnover. Mean ± SE. See text for significance levels by two-way ANOVA and one-way ANOVA followed by Sheffe. *$p < 0.05$ between days of cycle bearing asterisk in rats with similar resistance. **$p < 0.05$ vs. the other 2 days of cycle. †$p < 0.05$ between rats with different resistance bearing this sign on the same day of cycle.

TABLE 1

RESISTANCE SCORE UTERINE AND ADRENAL WEIGHT ON THREE DIFFERENT STAGES OF THE ESTROUS CYCLE, IN RATS WITH DIFFERENT RESISTANCE DURING HANDLING

	Low Resistance			High Resistance		
	Diestrus-2	Proestrus	Estrus	Diestrus-2	Proestrus	Estrus
	(7)	(7)	(6)	(6)	(6)	(6)
Resistance score*	1.71 ± 0.60	2.4 ± 0.64	2.8 ± 0.65	10.16 ± 1.44	11.16 ± 1.90	14.5 ± 2.52
Duration for VSS (days)	11.14 ± 1.50	11.7 ± 2.36	10.33 ± 1.08	12.0 ± 1.78	11.0 ± 1.86	11.6 ± 1.66
Duration for weighing (days)	17.8 ± 1.4	17.8 ± 1.18	17.5 ± 1.17	17.6 ± 1.38	17.1 ± 1.30	17.1 ± 1.07
Age (days)	96.8 ± 3.37	97.3 ± 1.52	96.5 ± 1.31	101.3 ± 4.07	99.1 ± 3.17	101.6 ± 2.74
Body weight (g)	173.4 ± 9.63	183.8 ± 8.33	172.8 ± 15.54	169.0 ± 10.68	175.0 ± 7.85	165.8 ± 3.28
Uterine weight (mg/100 g b.wt.)	156.0 ± 11.95†	257.0 ± 14.8	218.2 ± 10.52	164.0 ± 17.28†	269.9 ± 9.60	257.3 ± 11.13‡
Adrenal weight (mg/100 g b.wt.)	25.5 ± 1.26	28.7 ± 1.28§	29.7 ± 1.67§	29.5 ± 1.42§	28.7 ± 2.6	30.9 ± 2.24

*Sum of reactions for each rat during handling for weighing and handling for vaginal smear screening (VSS). Data are expressed as means ± SEM of number of rats in parentheses.

†$p < 0.005$ vs. proestrus and estrus.

‡$p < 0.05$ vs. estrus low resistance.

§$p < 0.05$ vs. diestrus-2 low resistance.

on PE compared to DE-2 (Fig. 1). On the other hand, HR vs. LR affected 5-HIAA only on DE-2, $F(1, 11) = 10.922$, $p < 0.007$, with an increase of 5-HIAA in rats with HR compared to rats with LR (Fig. 1).

A significant cycle × HR vs. LR interaction was also revealed for the 5-HT turnover (5-HIAA/5-HT ratio), $F(2, 2) = 7.603$, $p < 0.0020$. Further analysis by one-way ANOVA showed that 5-HT turnover was affected by the phase of cycle, $F(2, 17) = 4.507$, $p < 0.0269$, in rats with LR, with an increase on E compared to DE-2 (Fig. 1). The cycle effect on the 5-HIAA/5-HT ratio was different in rats with HR, $F(2, 15) = 4.61$, $p < 0.0275$, showing an increase on DE-2 vs. PE (Fig. 1). A HR vs. LR effect was found only on DE-2, $F(1, 11) = 12.016$, $p < 0.0053$, showing an increase in 5-HIAA/5-HT ratio on DE-2 of rats with HR vs. rats with LR (Fig. 1).

For DOPAC in HY-PA there was a significant two-factor interaction, $F(2, 2) = 4.556$, $p < 0.0184$. By further analysis, a cycle effect was found only in rats with HR, $F(2, 15) = 4.307$, $p < 0.03$, but the increase on DE-2 did not reach significance after Sheffe. Only on DE-2 was an HR vs. LR effect found on DOPAC, $F(1, 11) = 4.966$, $p < 0.048$, indicating an increase on DE-2 in rats with HR compared to rats with LR (Fig. 2).

For striatal 5-HT two-way ANOVA revealed significant effects of the day of cycle, $F(1, 2) = 8.493$, $p < 0.0011$, and of HR vs. LR, $F(1, 2) = 4.459$, $p < 0.0426$, and a significant interaction between these two factors, $F(2, 2) = 3.436$, $p < 0.0445$. In rats with LR a significant cycle effect was found, $F(2, 17) = 5.257$, $p < 0.0167$, with an increase of 5-HT on PE vs. DE-2 and E (Fig. 3). In rats with HR the cycle effect, $F(2, 17) = 5.982$, $p < 0.0123$, was different, expressed by a significant increase of 5-HT on PE and E vs. DE-2 (Fig. 3). Only on E a significant HR vs. LR effect for 5-HT was found, $F(1, 10) = 11.537$, $p < 0.0068$ with a significant increase of 5-HT on E of rats with HR (Fig. 3). Two-way ANOVA for 5-HIAA in Str, revealed a significant effect only of HR vs. LR, $F(1, 2) = 5.371$, $p < 0.027$. After one-way ANOVA, this effect of resistance was found significant only on E, $F(1, 10) = 4.784$, $p < 0.05$, but the increase of 5-HIAA on E of rats with HR did not reach significance after Sheffe (Fig. 3). This increase in 5-HIAA on E of rats with HR compared to LR was found significant after the Student's t-test ($t = 2.18$, $p < 0.05$).

Two-way ANOVA for DOPAC in Str revealed a highly significant interaction between cycle day and high vs. low resistance, $F(2, 2) = 6.658$, $p < 0.0038$. A significant effect of the day of cycle was found only in rats with HR, $F(2, 15) = 11.2$, $p < 0.0011$, with a significant increase of DOPAC on DE-2 vs. PE and E (Fig. 4). DOPAC was affected by HR compared to LR on DE-2, $F(1, 11) = 6.763$, $p < 0.0247$, and on E, $F(1, 10) = 7.748$, $p < 0.0193$, however in the opposite way. On DE-2 DOPAC was increased, while on E, DOPAC was decreased in rats with HR compared to rats with LR (Fig. 4). The results for DA turnover (DOPAC/DA ratio) in Str were similar to those for DOPAC, as revealed by a highly significant interaction between the two factors, $F(2, 2) = 8.564$, $p < 0.0010$. A highly significant effect of the day of cycle on DOPAC/DA ratio was found only in rats with HR, $F(2, 15) = 12.615$, $p < 0.0006$, with an increase on DE-2 vs. PE and E (Fig. 4). A significant resistance effect on DOPAC/DA ratio was also found on DE-2, $F(1, 11) = 14.721$, $p < 0.0028$, with a significant in-

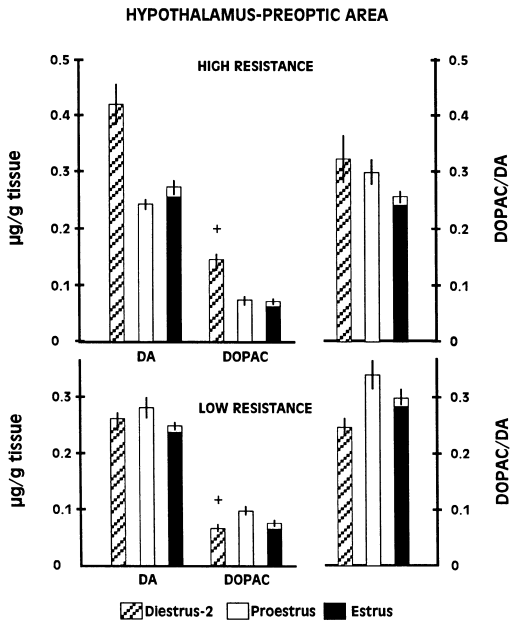

FIG. 2. The dopaminergic profile in hypothalamus–preoptic area, on the 3 consecutive stages of 4-day estrous cycles examined at 0900–1200 h. DA = dopamine, DOPAC = dopamine metabolite 3,4-dihydroxyphenylacetic acid, DA/DOPAC = dopamine turnover. Mean ± SE. See text for significance levels determined by two-way ANOVA and one-way ANOVA followed by Sheffe in low resistance rats (n = 5–7/group) and high resistance rats (n = 6/group). †$p < 0.05$ between rats with different resistance on the same day of cycle.

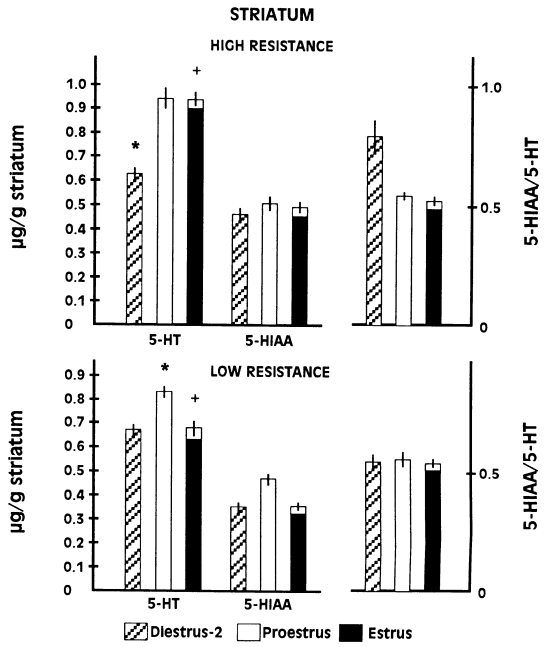

FIG. 3. The serotonergic profile in striatum on the 3 consecutive days of 4-day estrous cycles examined at 0900–1200 h in rats (n = 6–7/group) with low resistance to handling for vaginal screening and rats (n = 6/group) with high resistance, as evaluated after recording behavior during handling of similar duration. 5-HT = serotonin, 5-HIAA = serotonin metabolite 5-hydroxyindoleacetic acid, 5-HIAA/5-HT = serotonin turnover. Mean ± SE. See text for significance levels by two-way ANOVA and one-way ANOVA followed by Sheffe. *$p < 0.05$ vs. the other two stages in rats with similar resistance. †$p < 0.05$ between rats with different resistance bearing this sign on the same day of cycle.

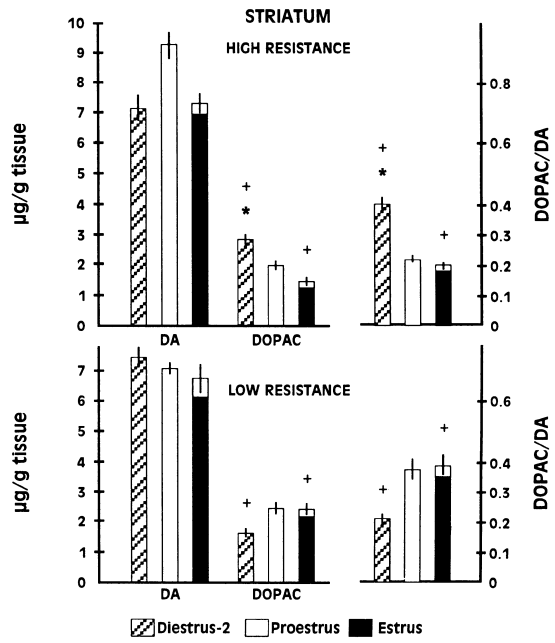

FIG. 4. The dopaminergic profile in striatum on the 3 consecutive days of 4-day estrous cycles examined at 0900–1200 h in rats (n = 6–7/group) with low resistance and in rats with high resistance (n = 6/group) as evaluated after recording behavior during handling for vaginal screening of similar duration. DA = dopamine, DOPAC = dopamine metabolite 3,4-dihydroxyphenylacetic acid, DOPAC/DA = dopamine turnover. Mean ± SE. See text for significance levels determined by two-way ANOVA and one-way ANOVA followed by Sheffe. *$p < 0.05$ vs. the other two stages of rats with similar resistance. †$p < 0.05$ between rats with different resistance bearing this sign on the same day of cycle.

crease in rats with HR (Fig. 4). On the other hand, on E the effect of resistance, $F(1, 10) = 6.846$, $p < 0.0258$, was of the opposite order, because, in rats with HR, the DOPAC/DA ratio was significantly lower than in rats with LR.

DISCUSSION

The results of the present study indicate a different serotonergic and dopaminergic profile in HP-PA and Str of rats with HR from that found in rats with LR.

On the other hand, the findings of decreased thymus weight in 16 rats with HR when compared to 17 rats with LR suggest higher activation of the HPA axis in rats with HR. These findings are interesting because abnormality of the HPA axis in women with PMS suggested increased activity of HPA axis both during their follicular and luteal phases (30). The higher activation of the HPA axis in rats with HR should be related to the emotional response and reactivity, during the procedure for simple handling and during the procedure for VSS. Behaviors such as attempts to hide or escape, defecation, urination, or vocal reaction preceeding handling for VSS might be interpreted as trait anxiety. Behaviors during handling for VSS might also suggest trait anxiety because they expressed reactions due to hyperresponsiveness to the sensory stimuli of VSS, an event that elicited none or rare reactions in rats with LR.

The serotonergic profile in HY-PA of rats with LR demonstrates an increase of 5-HIAA on PE compared to DE-2 and an increase in serotonin turnover on E compared to DE-2, suggesting serotonergic activation on PE and E of rats without trait anxiety, because according to previous studies (1,24,25,40) increase in 5-HIAA and/or 5-HT turnover provide evidence for increased 5-HT activity.

In view of the inhibitory role of 5-HT_Y in feeding (8), the finding of increased 5-HT activity in HP-PA on PE and E compared to DE-2 is in line with decreased food intake on these two stages, demonstrated in previous studies (39,45,46). In a recent study a 5-HT_{IA} agonist-induced hyperphagia was less evident on PE and E than on DE (47). This difference was interpreted as an estrous cycle modulation of somatodendritic autoreceptors of the raphe nucleus. Downregulation of these autoreceptors on PE and E would facilitate 5-HT neuronal release, which is compatible with our findings of serotonergic activation on PE and E.

On the other hand, because reduced ^{3}H-5-HT binding in HY-PA has been demonstrated on PE and E vs. DE (6), the increase in 5-HT activity on PE and E might also be related to a decrease of 5-HT_{IA} somatodendritic autoreceptors in HY-PA. In view of the presence of 5-HT cells in the hypothalamic dorsomedial nucleus and the ventrolateral hypothalamus (26), this possibility should be further investigated.

In rats with HR instead of increased 5-HT turnover on E vs. DE-2, an increase in 5-HT turnover was found on DE-$_Y$2 vs. PE.

The activation of the serotonergic system in HP-PA on DE-2 of rats with HR, as shown by the increase in 5-HIAA and 5-HT turnover, compared to rats with LR, was paralleled in these rats by an increase in adrenal weight and decrease in thymus weight, suggesting that activation of the HPA axis by high emotional reactivity goes in parallel with serotonergic activation in HY-PA on DE-2.

These findings on DE-2 confirm previous results (37,38), but they add further information namely that HR per se, which might be interpreted as trait anxiety, goes in parallel with increased serotonergic activity in HY-PA and is followed by activation of the HPA axis.

The cycle effect on DOPAC on DE-2 vs. PE and E in HY-PA of rats with HR was not found significant, but the HR vs. LR effect on DE-2 was found significant indicating a rise of DOPAC in rats with HR. Because DE-2 is hormonally closer to male rats than PE and E, the increase in DOPAC might be related to an activated CRH hypothalamic system as shown in male rats and mice (9,14,29).

The serotonergic and dopaminergic activation found in rats with HR on DE-2 was not apparent in HY-PA on PE and E. This might be attributed to the presence of estrogen-dependent nuclei, where the changing hormonal milieu might modify, through estradiol and progesterone, the synthesis, metabolism, concentration, and turnover of 5-HT and DA (23).

On the other hand, the increase in adrenal weight of rats with LR on PE and E vs. DE-2 might be related to hyperhaemia of the pelvic cavity, very pronounced on PE and E compared to DE-2 (unpublished observation). This temporary increase in wet adrenal weight on PE and E, apart from individual variations, might mask differences in adrenal weight due to mild chronic stress.

A common finding in rats with LR and HR was the increase of 5-HT on PE, apparent both in HY-PA and in Str (Figs. 1 and 3). This increase should not be considered as an index of increased neuronal activity, because it was not paralleled by increased 5-HIAA and/or increased 5-HT turnover. Because of increased estradiol on PE (10,19,39) this rise in 5-HT might be attributed to decreased metabolism of 5-HT through an estrogen-dependent decrease of type A monoamine oxidase (22).

In Str in rats with HR, a significant rise of 5-HT was found on PE but also on E vs. DE-2. Furthermore, only on E, out of the three phases, was 5-HT significantly increased in rats with HR compared to LR. These results suggest that the rise in 5-HT on E should be attributed to a cycle effect associated with increased emotional reactivity. The 5-HT increase could be attributed to stress-dependent decrease of monoamine oxidase (22) and to increased 5-HT synthesis through a stress-induced increase of tryptophan hydroxylase but also to higher neuronal release of 5-HT in rats with HR compared to rats with LR (4). The rise of 5-HT on E in rats with HR and the finding that the main effect of HR vs. LR for striatal 5-HIAA, was revealed as significant only on E, suggest a dysregulation of the serotonergic system in Str on the day of E related to trait anxiety. Considering increasing evidence that serotonergic dysregulation may be involved in the pathogenesis of PMS (21,32,33,44,51) and considering the greater hormonal similarity of E to the premenstrual phase in humans from DE-2 and PE, the finding of serotonergic dysregulation in Str of rats with trait anxiety only on E is very interesting if we also consider the prevalence of PMS in women with trait anxiety (16,17,32).

The major finding of the present study was the differential effect of repeated emotional reactivity on DOPAC and DA turnover in Str on DE-2 and E. The results provide evidence for dopaminergic activation on DE-2, no effect on PE and dopaminergic suppression on E. Because DE-2 is hormonally much closer to male rats than PE and E, our findings of increased DOPAC and DA turnover on DE-2 of rats that experienced repeated stress agree with the results in male rats showing increased nigrostriatal and mesolimbic DA release after repeated daily stress (13).

The dopaminergic suppression on E of rats with trait anxiety might be linked to dysregulation of the serotonergic system, because it was paralleled by an increase in 5-HT and 5-HIAA when compared to E of rats without trait anxiety.

The mechanism is unknown at present, and we also ignore in what area of striatum this dopaminergic suppression takes place: the caudate putamen or nucleus accumbens? Further investigation for clarification of the brain area involved and for the elucidation of the mechanism responsible for this dopaminergic suppression on E is necessary. Dopaminergic dysfunction has been associated with several experimental models for depression in male animals (50). In the chronic mild-stress model in male rats, reduced consumption of sucrose was restored by the chronic administration of antidepressants but was reversed by the acute administration of DA antagonists (35), while reduced consumption of sucrose was restored by selective D_2, D_3 agonists and reversed by a DA receptor antagonist (28). The overall results in Str of rats with trait anxiety, showing dopaminergic suppression in parallel with serotonergic dysregulation on E, might have some relevance to the predisposition of women with trait anxiety to PMS.

On the other hand, dopaminergic activation in striatum on DE-2 compared to PE and E in rats with trait anxiety, raises the question as to the effect of this activation on the respective postsynaptic receptors on the days following DE-2. In case of a downregulation of these receptors in rats with trait anxiety, a functional deficit of the respective monoaminergic system might be expected on the following days, especially if coupled with decreased neuronal release (53). Further research using cycling female rats as an experimental model, with great differences in emotional reactivity, might prove useful to gain information for disorders in women, associated with trait anxiety like PMS and vulnerability to addiction (11). Further investigation should also include DE-1, distinct hypothalamic nuclei, other brain areas, measurements of steroid hormones, and other behavioral tests related to trait anxiety and depression.

In conclusion, the overall results of the present study suggest that repeated emotional reactivity to VSS results in activation of the HPA axis and is associated with serotonergic and dopaminergic dysregulation differing in HP-PA and Str. This dysregulation is differentially affected on DE-2 PE and E. The differentiation is mostly pronounced between DE-2 and E in Str, expressed by dopaminergic activation on DE-2, and dopaminergic suppression on E paralleled by serotonergic dysregulation.

We cannot answer at present at what extent the differences in serotonergic and dopaminergic activity between rats differing in emotional reactivity may be relevant to their emotional state and at what extent these differences may be related to different activation of the HPA axis.

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