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Asymmetric calmodulin distribution in the hypothalamus Role of sexual differentiation in the rat

Marco A. Rodríguez-Medina[a,1], Alejandro Reyes[a], María Eugenia Chavarría[a], Marcela Vergara-Onofre[b], Enrique Canchola[b,*], Adolfo Rosado[b]

[a]*División de Investigación Biomédica, Unidad de Investigación Médica en Medicina Reproductiva, Hospital de Gineco-Obstetricia "Luis Castelazo Ayala", IMSS, México, D.F., Mexico*
[b]*División de Ciencias Biológicas y de la Salud, Universidad Autónoma Metropolitana, México, D.F., Mexico*

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Abstract

The Ca^{2+}/calmodulin (CaM) system plays important roles both in hypothalamic sexual differentiation and in the progesterone-induced facilitation of lordosis behavior in the adult rat. We recently showed sex-dependent differences in rat hypothalamic CaM levels, both in newborn and in adult animals. Here, we evaluated the presence of left–right hypothalamic asymmetries in CaM concentration in male and female rats, as well as the changes induced on these parameters by neonatal (1 h after birth) subcutaneous administration of tamoxifen (200 μg/rat) or testosterone (30 μg/rat). CaM was measured by RIA in each half of the hypothalamus (at 2, 6, 12, and 24 h and at 90 days after birth) in both control and treated animals. In untreated young rats (2–24 h after birth), CaM concentration was significantly higher in the right half of the hypothalamus of males, whereas in females, it was higher in the hypothalamic left half. Treatment of females with testosterone or tamoxifen to males, consistently reversed these results. In the hypothalamus of treated animals, we found higher CaM levels in the left half of males, as well as in the right half of females. In control adult females, CaM concentration was also higher in the left half of the hypothalamus, as it was in the right half of adult males. However, this asymmetry was lost after neonatal hormone manipulation. These results reinforce the role of CaM in the development of sex-related hypothalamic functions.

Keywords: Bilateral asymmetry; Calmodulin; Hypothalamus; Testosterone; Tamoxifen; Sexual differentiation

1. Introduction

In adult rodents, and in some other mammalian species, the pattern of sexual behavior (for review, see Goy and McEwen, 1980) and the distinctive type of gonadotropin secretion become irreversibly organized during the late prenatal or early postnatal period (Clemens et al., 1978; Hoepfner and Ward, 1988). Steroid hormones are known to participate in this process because perinatal treatment of female rats with aromatizable androgens or with other estrogen precursors results in defeminization and masculinization of the typical patterns of sexual behavior and of gonadotropin hormones release (Goy and McEwen, 1980; Ward and Renz, 1972), particularly of the surge secretion of LH. In contrast, perinatal gonadectomy of male rats or the application of tamoxifen, a well-known antagonist of estrogen action on the hypothalamus, induces feminization of the gonadotropin hormone releasing patterns and sexual behavior (Goy and McEwen, 1980; Södersten, 1978). However, it may be mentioned that sex differences in gonadotropin secretion between males and females may have been exaggerated. Thus, there are evidences that no sex-related differences in basal pulsatile LH secretion (Norman and Spies, 1986; Södersten and Eneroth, 1983) were observed.

We have shown that the Ca^{2+}/calmodulin (CaM) system plays an important role in the biochemical processes that mediate hypothalamic sexual differentiation in the rat (Rodríguez-Medina et al., 1993) and that it participates in the regulation of the lordotic behavior in adult female rats (Canchola et al., 1996). More recently (Rodríguez-Medina

* Corresponding author. Departamento de Biología de la Reproducción, Universidad Autónoma Metropolitana-Iztapalapa, Ave. Michoacán y Purísima, México, D.F. 09340, Mexico. Tel.: +52-5-804-4710; fax: +52-5-804-4730.

E-mail address: cancho@xanum.uam.mx (E. Canchola).

[1] Present address: Facultad de Estudios Profesionales-Zaragoza, Universidad Nacional Autónoma de México, México, D.F., Mexico.

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et al., 1998), we showed that CaM levels in the hypothalamus of the developing rat increase with age. We have also indicated that CaM concentration is significantly lower in 1-day-old females than in 1-day-old males and that this relation is reversed in normal adults, in which CaM concentration is significantly higher in females. Application of testosterone to females or tamoxifen to males 1 h after birth modified these sex-related differences in CaM concentration (Rodríguez-Medina et al., 1998).

On the other hand, the presence of important functional and behavioral differences related to the existence of bilateral (left–right) neurochemical and structural asymmetries has recently been stressed (Bradshaw and Rogers, 1993; Bianki et al., 1989; Harris et al., 1996; LaMendola and Bever, 1997). Many forms of asymmetry in rats and other animals depend on early experience and on the hormonal condition of the subjects (Bradshaw and Rogers, 1993; Denenberg et al., 1991; Schwarz and Rogers, 1992). Of particular significance is the growing number of reports signaling the importance of diencephalic bilateral asymmetry in relation to gonadal hormone receptors (McCormick and Singh, 1996; MacLusky et al., 1997), sexual function and/or sexual behavior (Harris et al., 1996; López et al., 1997; Sánchez et al., 1994; Von-Ziegler and Lichtensteiger, 1992). Significant differences in gonadal hormone sensitivity between the left and right sides of the hypothalamus in rats have also been reported (Harris et al., 1996), and Yahr and Greene (1992) remarked that unilateral manipulations of the hypothalamus selectively modify sexual behavior in rats.

To contribute to the understanding of the physiological mechanisms that regulate the perinatal sexual differentiation of the rat hypothalamus, we explore the possibility that bilateral asymmetric distribution of hypothalamic CaM may have an important role in hypothalamic sexual differentiation and if there are modifications in CaM hypothalamic distribution induced by hormone manipulation of the neonates.

2. Methods

2.1. Animals

Time-mated Sprague–Dawley rats were individually housed in a temperature-controlled colony room and were maintained on a reverse 14:10-h light/dark cycle with lights off at 8:00 AM. The time of birth for each pup was registered, the pups were weighed and sexed, and kept with their mothers on the same light/dark cycle. For the study of sexual behavior, sexually vigorous, trained males (studs) and sexually receptive females (stimulus) of the same strain were kept on a reversed lighting schedule. Stimulus females were adult, ovariectomized rats brought into behavioral estrous with subcutaneous injections of estradiol benzoate and progesterone, 48 and 4 h, respectively, prior to behavioral testing (Canchola et al., 1996).

2.2. Animal handling

One hour after birth, litters (~70 litters were used in this study) were randomly divided into two groups of equal size: control and experimental. Female pups belonging to experimental litters were subcutaneously injected with 30 μg testosterone propionate dissolved in 50 μl sesame oil (Arai and Gorski, 1968), and those belonging to control litters were injected with the oil vehicle alone. Males were similarly injected with either tamoxifen, 200 μg per animal in 50 μl sesame oil, or with the oil vehicle (Döhler et al., 1986; Rodríguez-Medina et al., 1998). After treatment, pups were maintained with their mothers. At five different times after birth (2, 6, 12, and 24 h and at 90 days of age), subjects belonging to different litters were randomly selected to be studied. To avoid the possible production of functional asymmetries elicited by peripheral handling (Bianki et al., 1989; LaMendola and Bever, 1997), care was taken to prevent dissimilar handling of the animals in the male and female groups.

2.3. Sexual behavior

Adult rats were weighed and randomly divided into two groups. The first group was sacrificed and utilized for the determination of the hypothalamic biochemical parameters. In the case of adult female rats, the stage of the estral cycle was determined by daily vaginal cytology (Smith et al., 1975), and the animals were always sacrificed when clear evidences of estrous were detected. To check the efficiency of the applied treatment, the second group was submitted to controlled male and female sex behavioral tests following procedures previously described (Canchola et al., 1996; Rodríguez-Medina et al., 1993). In short, the test for masculine sexual behavior took place 5–6 h after the onset of the dark phase. Five tests for homotypic or heterotypic masculine behavior were conducted. After a 5-min adaptation period, each subject was presented with a receptive, stimulus female. The occurrence of mounts, intromissions and ejaculatory behavioral patterns was recorded. The study of homotypic or heterotypic feminine sexual behavior was done as previously described (Canchola et al., 1996; Rodríguez-Medina et al., 1993).

2.4. Experimental

All animals were sacrificed by decapitation. After the removal of the cranium, the optic nerves were cut and the brain was turned back; the pituitary stalk and the brainstem were cut delivering the intact brain. Contaminating blood was washed away with isotonic buffer (NaCl 0.14 M, Tris–HCl tris-hydroxy-methyl amino methane hydrochloride 0.01 M, magnesium acetate 0.0015 M, pH 7.4). The brain was sliced into two halves under a stereoscopic microscope following previously described procedures (Rodríguez-Medina et al., 1993). Hypothalamic right and left halves

were then obtained by the procedure of Vangala et al. (1973), using established parameters (Rodríguez-Medina et al., 1998). Each obtained half was placed in a preweighed Eppendorf tube containing 1 ml of isotonic buffer. In the case of newborn rats (2, 6, 12 and 24 h), it was necessary to work with pools. Wet weight of the samples was determined as previously described (Rodríguez-Medina et al., 1993). Adult individual samples were processed for each variable studied. In all cases, samples were frozen in liquid nitrogen until processed.

Quantification of CaM was done using a commercial double-antibody radioimmunoassay kit purchased from DuPont NEN Research Products (Boston, MA) following the modifications previously described (Rodríguez-Medina et al., 1998). Protein determinations in the homogenized tissue and in the cytosolic fractions were done according to the procedure of Lowry et al., 1951.

2.5. Statistics

Statistical analysis was performed using a compatible PC microcomputer and the Sigma Stat II Statistical Package (Jandel, USA). Data in all tables and figures are expressed as means ± S.D. Statistical analysis were performed in neonates and adults separately. In the adults, a Sex × Treatment × Hemisphere three-way ANOVA was performed followed by multiple pairwise comparison (Tukey test) to obtain simple effects. The neonates were collapsed across age and then studied by a Sex × Treatment × Hemisphere three-way ANOVA.

3. Results

Since the sexual behavior tests in the adult rat demonstrated behavioral modifications not significantly different from those previously described (Rodríguez-Medina et al., 1998), detailed results are not included here. However, these results insure that the dose of the tested drugs, as well as the time of their application, were sufficient to induce the expected changes in the sexual behavior of the adult rats (Döhler et al., 1986; Etgen, 1979).

In newborn rats, no significant differences were found in body or hemihypothalamic weights (right versus left) with respect to either time after birth (2, 6, 12 and 24 h) or to hormonal treatment (tamoxifen or testosterone). Adult male rats, both control (354 ± 16 g) and tamoxifen treated (329 ± 18 g), were significantly heavier than control (256 ± 9 g) or testosterone (243 ± 12 g) treated females, but no statistical significant differences were found between control or treated animals of the same sex. In control adults rats, a statistically significant difference (P=.045) was found between the right half (29.4 ± 2.7 and 26.2 ± 3.0 mg, males and females, respectively) and left half (25.5 ± 3.1 and 31.3 ± 2.9 mg) hypothalamic weights, which was not observable in treated subjects: 27.4 ± 2.3 mg against 29.5 ± 3.0 mg in tamoxifen-treated males (right- and left-hypothalamic weights, respectively) and 28.4 ± 3.2 against 29.4 ± 3.5 mg in testosterone-treated females (same order).

The developmental pattern of CaM concentration (μg/mg protein) in the hypothalamus of the studied rats is shown in Tables 1 and 2. The amount of CaM in each half of the hypothalamus showed a similar behavior in both control and treated animals, decreasing between 2 and 6 h after birth and then increasing with age. Looking at the neonate data in Tables 1 and 2, it can be observed that the pattern of Treatment × Hemisphere differences was essentially the same

Table 1
CaM concentration of the right and left halves of a normal female rat hypothalamus and after neonatal administration of testosterone

		CaM concentration (μg/mg protein)			
		Control		Treated	
Time after birth	n	Right half	Left half	Right half	Left half
2 h	5	0.68 ± 0.08^{1}	0.96 ± 0.19^{2}	0.98 ± 0.08^{2}	0.55 ± 0.04^{1}
6 h	6	0.52 ± 0.06^{1}	0.72 ± 0.08^{2}	0.54 ± 0.05^{1}	0.40 ± 0.05^{3}
12 h	6	0.50 ± 0.06^{1}	0.97 ± 0.08^{2}	0.64 ± 0.05^{3}	0.48 ± 0.05^{1}
24 h	8	0.46 ± 0.05^{1}	0.79 ± 0.12^{2}	0.75 ± 0.06^{2}	0.56 ± 0.07^{3}
90 days	8	1.07 ± 0.08^{1}	2.52 ± 0.23^{2}	0.75 ± 0.08^{3}	0.94 ± 0.14^{4}

Numbers in the table are means ± S.D. from n samples, as indicated. Different superscripts in the same row indicate statistical difference (P < .05). All data are statistically different from their contralateral.

Table 2
CaM concentration of the right and left halves of a normal male rat hypothalamus and after neonatal administration of tamoxifen

		CaM concentration (μg/mg protein)			
		Control		Treated	
Time after birth	n	Right half	Left half	Right half	Left half
2 h	5	0.88 ± 0.09^{1}	0.60 ± 0.09^{2}	0.68 ± 0.05^{2}	0.96 ± 0.20^{1}
6 h	6	0.72 ± 0.20^{1}	0.43 ± 0.03^{2}	0.58 ± 0.06^{2}	0.71 ± 0.10^{1}
12 h	6	1.3 ± 0.15^{1}	0.72 ± 0.15^{2}	0.79 ± 0.07^{2}	1.20 ± 0.14^{1}
24 h	8	1.2 ± 0.12^{1}	0.67 ± 0.14^{2}	0.69 ± 0.10^{2}	1.01 ± 0.10^{1}
90 days	10	1.7 ± 0.08^{1}	0.83 ± 0.08^{2}	1.10 ± 0.08^{3}	1.19 ± 0.08^{4}

Numbers in the table are means ± S.D. from n samples, as indicated. Different superscripts in the same row indicate statistical difference (P < .05). All data are statistically different from their contralateral.

Table 3
Results obtained from a three-way analysis of variance of the data obtained in newborn rats (collapsed across time)

Source of variation	df	SS	MS	F	P
Sex	1	1.546	1.546	49.60	<.001
Hemisphere	1	0.165	0.165	5.30	.020
Treatment	1	0.029	0.029	0.92	.34
Sex × Hemisphere	1	0.033	0.033	1.07	.30
Sex × Treatment	1	0.043	0.043	1.38	.24
Hemisphere × Treatment	1	0.283	0.283	9.07	.003
Sex × Hemisphere × Treatment	1	3.486	3.486	111.82	<.001
Residual	184	5.736	0.031		
Total	191	11.321	0.059		

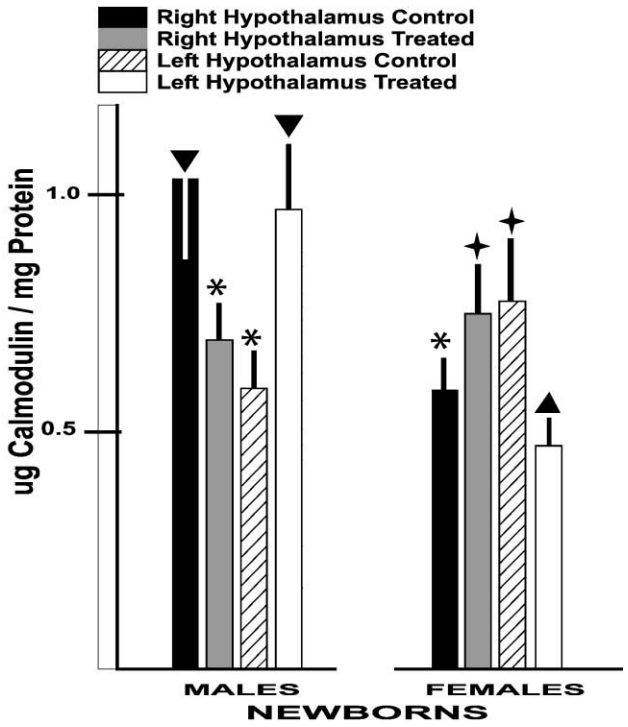

Fig. 1. Graphic representation of CaM concentration per milligram protein in the hemisectioned hypothalamus of normal and treated newborn rats. The results obtained at the four times studied have been collapsed across age (see text). Male rats were randomly divided into two groups. The control group received a subcutaneous (sc) injection of 50 μl of sesame oil. The treated group received a subcutaneous injection of tamoxifen (200 μg per animal in 50 μl of sesame oil). Female rats were randomly divided into two groups. The control group received a subcutaneous (sc) injection of 50 μl of sesame oil. The treated group received a subcutaneous injection of testosterone propionate (30 μg in 50 μl of sesame oil). The animals were sacrificed, and the brains were extracted and sliced into two halves (Vangala et al., 1973). The hypothalamic right and left halves were obtained (Rodríguez-Medina et al., 1993), washed and homogenized in Tris buffer, pH 7.4. CaM concentration was determined by a commercial double-antibody RIA kit. The bars indicate the means±standard deviations of six independent experiments. Different symbols above the bars indicate a significant difference ($P<.01$) in CaM concentration obtained by a three-way ANOVA and Tukey test. Significant differences in CaM concentration between the male and female groups under each one of the experimental conditions tested and that sex-related differences follow a reciprocal pattern between control and treated groups.

at all the neonate age points. Thus, there should be no significant effect of age or any interaction of age with the other variables. It was then considered appropriate to look at the neonate data after collapsing them across age and then performing a Sex × Hemisphere × Treatment three-way ANOVA (Table 3). This procedure showed that differences in the mean values among the different variables studied (sex, hypothalamic hemisphere and hormonal treatment, after allowing for the effects of differences in the other two variables) were only greater than would be expected by chance in the case of sex and hemisphere (Table 3), while the differences between the levels of treatment were not statistically different ($P=.34$), and that interaction between sex and hemispheric side in newborns depended on treatment, being significant both in the controls ($P<.001$) and in the treated animals ($P<.001$). Simple main effects obtained by an all pairwise multiple comparison procedure (Tukey test) are shown in Fig. 1.

Data obtained from adult animals (Tables 1 and 2) were independently analyzed by a Sex×Hemisphere × Treatment three-way ANOVA (Table 4). In this case, the differences observed in the mean values among the different variables studied, sex, hypothalamic side and hormonal treatment in the adult animals (also after allowing for the effects of differences in the other two variables) were all greater than would be expected by chance (Table 4). Interaction between sex and side in this group of animals depended on treatment, being significant on the controls ($P<.001$), but not in the treated animals ($P=.271$). Simple main effects obtained by an all pairwise multiple comparison procedure (Tukey test) are shown in Fig. 2.

In untreated control males, CaM was always significantly higher in the right half of the hypothalamus. This relation was reversed in untreated females, whose CaM concentration was significantly higher in the left half of the hypothalamus (Tables 1 and 2; Figs. 1 and 2). The highest concentrations of CaM were found in the right half of the hypothalamus of the adult control males and particularly in the left half of the hypothalamus of the adult control estrous females (Table 1 and Fig. 2).

Treatment, either testosterone in the females or tamoxifen in the males consistently inverted these relationships in newly born animals: CaM concentration was significantly higher in the left half of the hypothalamus in treated males, as well as in the right half of the hypothalamus of treated females (Fig. 1). No significant differences were found in CaM concentration between the left and right halves of the hypothalamus in treated male and female adult animals (Fig. 2). The effect of the endocrine treatment was particularly important in the adult estrous females, in which CaM concentration in the left half of the hypothalamus was almost three times lower in the testosterone-treated animals than in the control animals (Fig. 2). In general, CaM asymmetric distribution in the hypothalamus was modified according to the expected effect of the hormonal treatment; tamoxifen-treated males had CaM differential levels similar to those observed in control females, while in testosterone-

Table 4
Results obtained from a three-way analysis of variance of the data obtained in adult (90 days old) rats

Source of variation	*df*	SS	MS	*F*	*P*
Sex	1	0.173	0.173	7.77	.007
Hemisphere	1	0.630	0.630	28.24	<.001
Treatment	1	4.146	4.146	185.8	<.001
Sex×Hemisphere	1	5.587	5.587	250.4	<.001
Sex×Treatment	1	2.645	2.645	118.5	<.001
Hemisphere×Treatment	1	0.098	0.098	4.41	.04
Sex×Hemisphere×Treatment	1	4.532	4.532	203.1	<.001
Residual	56	1.249	0.022		
Total	63	19.06	0.303		

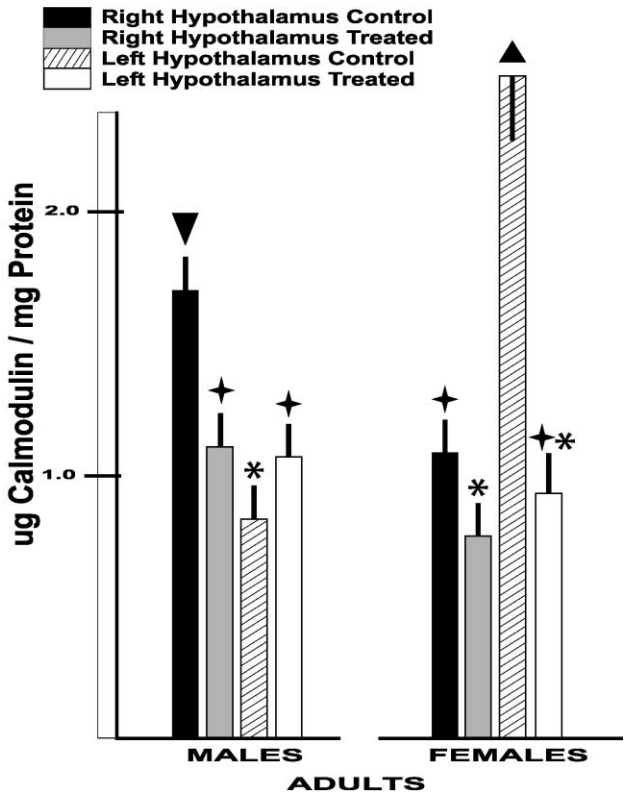

Fig. 2. Graphic representation of CaM concentration per milligram protein in the sectioned hypothalamus of normal and treated adult male and female rats (see Fig. 1). The bars indicate the means±standard deviations of the number of experiments indicated in Tables 1 and 2. Different symbols above the bars indicate a significant difference in CaM concentration ($P<.01$) obtained by pairwise multiple comparison analysis (Tukey test) from the results of a three-way ANOVA (Table 4). Significant differences were found between males and females of every group with the only exception of the left hypothalamus in control groups. As was observed in the case of newborns, the higher concentration were observed in the right hypothalamus of control males and in the left hypothalamus of control females.

treated females, CaM distributions were similar to those found in control males.

4. Discussion

There is good evidence that gonadal hormones in rats have asymmetrical effects on hypothalamic sexual differentiation and on the control of sexual behavior in the adults (Harris et al., 1996; Kimura, 1992). In addition, there is evidence that asymmetric hypothalamic sensitivity to hormonal influence is different in adult than in newborn animals (Nordeen and Yahr, 1983; Von-Ziegler and Lichtensteiger, 1992). In the newborn, the left side of the hypothalamus is particularly sensitive to the presence of estradiol, which reduces feminine sexual behavior in the adult without masculinization effects in the female rat (Von-Ziegler and Lichtensteiger, 1992). Estradiol implants in the left half of the hypothalamus of newborn female rats also suppress the cyclic pattern of LH secretion, inducing in the adult rat not only the suppression of the lordotic behavior but the presence of anovulatory cycles (Nordeen and Yahr, 1983). On the contrary, neonatal infusion or implantation of estradiol to the right hypothalamus strengthens the pseudomasculine behavior in adult female rats and has no effect on LH secretion in the adult rat (Nordeen and Yahr, 1983, Södersten, 1978).

Likewise, between Gestational Day 22 and Postnatal Days 6–15, there are changes in the asymmetric distribution of aromatase activity in the preoptic area, and in the posterior hypothalamic area (Swanson et al., 1990). At Postnatal Days 6 and 15, aromatase activity in females is higher on the left side of the hypothalamus than on the right side. However, in the preoptic area, a side difference is only detected in males (left side is higher). Since the proper function of the Ca^{2+}/CaM system is important for the hypothalamic sexual differentiation of the male rat (Norman and Spies, 1986; Rodríguez-Medina et al., 1993), the stage-dependent asymmetrical distribution of CaM during the first hours of extrauterine development would be expected to have significant influences during sex steroid-dependent hypothalamic differentiation. Twelve hours after birth seemed to be an important time in male hypothalamic differentiation, because in control males, CaM concentration rose abruptly in the right half of the hypothalamus and was kept high from this time until adulthood. In control females, however, almost no differences could be observed in CaM concentration in the initial hours of differentiation. CaM elevation in the left half of the hypothalamus of these animals must have occurred more than 24 h after birth.

In general, it may be said that hormonal manipulation of newborn rats—either tamoxifen in males or testosterone in females—tended to decrease the important differences observed in the CaM levels between the left and right halves of the hypothalamus in control animals. This was particularly true in the adults, in whom almost no differences were found in CaM levels between males and females, or between the right and left halves of the hypothalamus.

We have previously shown that administration of drugs interfering with the functioning of the Ca^{2+}/CaM system to adult female rats inhibits the progesterone-induced facilitation of lordosis behavior (Canchola et al., 1996). This important role of CaM in the hypothalamic regulation of sexual behavior in the adult is reinforced by our present data, indicating the presence of important contrasting asymmetries in CaM concentration between male and female rats, as well as the expected changes induced upon these parameters by neonatal treatment with tamoxifen or testosterone. CaM concentration was higher in the hypothalamic left half of the adult female and in the right half of the adult male. This pattern was reversed after neonatal hormonal manipulation. These results may be correlated with those of Nordeen and Yahr (1983), indicating that the left hypothalamus controls not only the presence of feminine sexual behavior, but also the cyclic release of gonadotropins. Similarly, Kimura (1992), proposed that the right hypothal-

amus controls the mount, intromission and ejaculatory male sexual behavior, while the left half of the hypothalamus regulates the female lordotic behavior. However, we must admit that some of our differences, particularly in adult females, might be related to variations in the hormonal milieu between androgenized and estrous control females.

The data of McCormick and Singh (1996) showed a left biased asymmetry between progesterone receptors in the frontal cortex and proceptive sexual behavior in the female rat. These authors found in the hypothalamus an association between the degree (but not direction) of progesterone receptors asymmetry and lordosis quotient (McCormick and Singh, 1996). However, Roy and Lynn (1987) have indicated that estradiol infusion into the right hypothalamus of adult females is more effective for producing lordosis than its infusion into the left half of the hypothalamus. In addition, LHRH levels in the adult female rat are significantly higher in the right half of the hypothalamus (Gerendai, 1987).

Diencephalic asymmetries are frequently sexually dimorphic and mediated by gonadal hormones. Exposure to hormones during the critical period of development may differentially affect the growth of each side of the diencephalon. The data of Corballis and Morgan (1978) suggest that some asymmetries produced during ontogenic development precede the effects that may be produced by exposure to gonadal hormones. Although our data refer only to newly born rats, asymmetric distribution of CaM seems to precede the postnatal sex-hormone effect on hypothalamic sexual differentiation and may support the suggestion of Corballis and Morgan (1978).

We have restricted our discussion about CaM asymmetric distribution to gonadal hormone effects on sexual behavior; however, the physiological participation of the Ca^{2+}/CaM system is crucial for cellular metabolism. Therefore, the significance of this asymmetry must surely surpass its involvement in sexual differentiation and sexual behavior. This will require further research to reach its total significance.

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References

Arai Y, Gorski RA. Protection against the neural organizing and activizing effect of exogenous androgen in the neonatal female and male rats. Endocrinology 1968;82:1009–13.

Bianki VL, Murik SE, Filippova EB. Interhemispheric asymmetry of positive emotional reactions in rats. Int J Neurosci 1989;47:193–201.

Bradshaw JL, Rogers LJ. Asymmetries in rats and mice. In: Bradshaw JL, Rogers LJ, editors. The evolution of lateral asymmetries, language, tool use, and intellect. San Diego: Academic Press, 1993. pp. 99–153.

Canchola E, Rodríguez-Medina MA, Dueñas-Tentori H, Mercado E, Rosado A. Ca^{2+}/calmodulin system: participation in the progesterone-induced facilitation of lordosis behavior in the ovariectomized estrogen-primed rat. Pharmacol, Biochem Behav 1996;54:403–9.

Clemens LG, Gladue BA, Coniglio LP. Prenatal endogenous androgenic influences on masculine sexual behavior and genital morphology in the male and female rats. Horm Behav 1978;10:40–53.

Corballis MC, Morgan JJ. On the biological basis of human laterality: I. Evidence for maturational left–right gradient. Behav Brain Sci 1978;2: 261–336.

Denenberg VH, Fitch RH, Schrott LM, Cowell PE, Waters NS. Corpus callosum: interactive effects of infantile handling and testosterone in the rat. Behav Neurosci 1991;4:562–6.

Döhler KD, Coquelin A, Davis F, Hines M, Shryne JE, Sickmöller PM, Jarzab B, Gorski RA. Pre- and postnatal influence of an estrogen antagonist and an androgen antagonist on differentiation of the sexually dimorphic nucleus of the preoptic area in male and female rats. Neuroendocrinology 1986;42:443–8.

Etgen AM. Antiestrogens: effects of tamoxifen, nafoxidine and CI-628 on sexual behavior, cytoplasmatic receptors, and nuclear binding of estrogen. Horm Behav 1979;13:97–112.

Gerendai I. Laterality in the neuroendocrine system. In: Ottoson D, editor. Duality and unity in the brain: unified functioning and specialization of the hemispheres. London: MacMillan, 1987. pp. 17–28.

Goy RW, McEwen BS. Sex differences in behavior: rodents, birds and primates. In: Goy RW, McEwen BS, editors. Sexual differentiation of the brain. Cambridge, MA: MIT Press, 1980. pp. 13–73.

Harris JA, Guglielmotti V, Bentivoglio M. Diencephalic asymmetries. Neurosci Biobehav Rev 1996;20:637–43.

Hoepfner BA, Ward IL. Prenatal and neonatal androgen exposure interact to affect sexual differentiation in female rats. Behav Neurosci 1988;102: 61–5.

Kimura D. Sex differences in the brain. Sci Am 1992;267:81–7.

LaMendola NP, Bever TG. Peripheral and cerebral asymmetries in the rat. Science 1997;278:483–6.

López E, Cruz ME, Domínguez R. Asymmetrical effects of the unilateral implant of pilocarpine on the preoptic anterior hypothalamic area on spontaneous ovulation of the adult rat. Arch Med Res 1997;28: 343–8.

Lowry OH, Rosebrough NJ, Farr A, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265–75.

MacLusky NJ, Bowlby DA, Brown TJ, Peterson RE, Hochberg RB. Sex and the developing brain: suppression or neuronal estrogen sensitivity by developmental androgen exposure. Neurochem Res 1997;22: 1395–414.

McCormick CM, Singh P. Asymmetry in progestin receptor levels and sexual behavior in female rats. Physiol Behav 1996;59:349–54.

Nordeen EJ, Yahr P. A regional analysis of estrogen binding to hypothalamic cell nuclei in relation to masculinization and defeminization. J Neurosci 1983;3:933–41.

Norman RL, Spies HG. Cyclic ovarian function in a male macaque: additional evidence for a lack of sexual differentiation in the physiological mechanisms that regulate the cyclic release of gonadotropins in primates. Endocrinology 1986;118:2608–10.

Rodríguez-Medina MA, Canchola E, Vergara M, Rosado A. Ca^{2+}/calmodulin system: participation in the rat sexual hypothalamic differentiation. Pharmacol, Biochem Behav 1993;46:697–702.

Rodríguez-Medina MA, Vergara M, Chavarría ME, Rosado A, Reyes A. Changes in hypothalamic calmodulin concentration induced by perinatal hormone manipulation in the rat. Pharmacol, Biochem Behav 1998; 61:445–50.

Roy EJ, Lynn DM. Asymmetry in responsiveness of the hypothalamus of the female rat to estradiol. Physiol Behav 1987;40:267–9.

Sánchez MA, López-García JC, Cruz ME, Tapia R, Domínguez R. Asym-

metrical changes in the choline acetyltransferase activity in the preoptic-anterior hypothalamic area during the oestrous cycle in the rat. Neuro-Report 1994;5:433–4.

Schwarz IM, Rogers LJ. Testosterone: a role in the development of brain asymmetry in the chick. Neurosci Lett 1992;146:167–70.

Smith MS, Freeman ME, Neill JD. The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology 1975;96:219–26.

Södersten P. Effects of antiestrogen treatment of neonatal male rats on lordosis behavior and mounting behavior in the adult. J Endocrinol 1978;76:241–9.

Södersten P, Eneroth P. Reproductive neuroendocrine rhythms. In: Balthazart J, Pröve E, Gilles R, editors. Hormones and behavior in higher vertebrates. Berlin: Springer, 1983. pp. 176–93.

Swanson HH, Houtsmuller EJ, Diaz R, Lowerse AL, Van der Poll NE. The preoptic area and sexual differentiation. In: Balthazart J, editor. Hormones, brain and behavior in vertebrates, vol. 1. Basel, Switzerland: Karger, 1990. pp. 30–40.

Vangala V, Naftolin R, Ryan K. Aromatization in the central nervous system of rabbits: effects of castration and hormone treatment. Endocrinology 1973;92:589–96.

Von-Ziegler NI, Lichtensteiger W. Asymmetry of brain aromatase activity: region and sex-specific developmental patterns. Neuroendocrinology 1992;55:512–8.

Ward IL, Renz FJ. Consequences of perinatal hormone manipulation on the sexual behavior of female rats. J Comp Physiol Psychol 1972;78:349–55.

Yahr P, Greene SB. Effects of unilateral hypothalamic manipulations on the sexual behavior of rats. Behav Neurosci 1992;106:698–709.