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Brain Cell RNA: Sexual Differences in the Rat

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Summary

Sexual differences in the total content of ribosomal RNA, established by cytophotometric measurements in neurones from selected brain regions, were studied in rats of the Wistar strain. In females of reproductive age, cyclic changes of RNA were synchronized with their oestrous cycle, the values being higher in the oestrous phase than in dioestrus. These changes were observed in pyramidal cells of the hippocampus and of the frontal cortex, in cells of anterior thalamus, of ventromedial and lateral hypothalamus and of tuberculi olfactorii. However, cycling cells were not disclosed in septum and thalamus posterior. A dependence upon the actual level of ovarial hormones was found in ventromedial hypothalamic cells only. In general, the RNA values in males of the same age corresponded to values of dioestrous females. The differences between newborn and 7-day-old pups were not marked enough and did not allow to define the critical period responsible for initiation of this sexual difference. In senescent rats, this difference persisted. During the stable phase of long-lasting dioestrus, the total RNA content in cells of the frontal cortex, hippocampus and hypothalamus was higher in females than in males of the same age which may suggest a faster reduction of this substance in aged males. The prolonged influence of oestrogens in the oestrous phase of the climacteric period (preceding the permanent dioestrus) decreased the RNA values in hippocampal and hypothalamic neurones even below the level established during the permanent dioestrus (and thus reached male values). On the contrary, in frontal cortical neurones, the female values remained higher in the permanent dioestrus as well as during long-lasting oestrus. A discussion concerns the possible participation of genetic determination and of the actual state of ovarial hormones in the manifestation of sexual differences in brain cells of the rat.

Key words

Laboratory rats – Sexual differences – Brain cells – Ribosomal RNA – Ontogeny – Hormonal influence

Introduction

The brain is generally considered to be one of the organs with sexual dimorphism characteristics. Differences between females and males were found in brain regions involved in the control of reproduction and sexual behaviour, as well as in those regions which have no relation to these functions. In the first case, it concerns the hypothalamus, implicitly the area preoptica, commonly considered as the region essential for the control of reproductive processes and hypophyseal secretion (for review see Naftolin *et al.* 1990, Gorski 1991, Leedom *et al.* 1994, Davis *et al.* 1996, López *et al.* 1996). Sexually different characteristics were also described in regions which do not represent the site of these functions, such as corpus callosum (Berrebi *et al.* 1988), brain cortex (Lustig *et al.* 1993, Witelson *et al.* 1995), mesencephalon (Raab *et al.* 1995), hippocampus (McEwen and Woolley 1994, for review see Reisert *et al.* 1996) and amygdala (Malsbury and McKay 1994).

The interpretation of the origin of structural and functional dimorphism in the brain, of its individual neurones and even of subcellular structures, were based on the concept of critical periods and on the assumption that gonadal steroids possess two functions: their first function is transient and can be considered as an activational one, the second function, on the contrary, is permanent and exerts organizational effects. In young mammals, the critical period (called by some authors the sensitive period or "time window") occurs at different stages of embryonic or postnatal development according to their species specificity. From the original basic brain of the female type (according to some authors directly from the female brain), the male type brain would be differentiated under the influence of testosterone metabolites. In rats, the critical period should be situated around the perinatal and neonatal age (for review see Gorski 1991) and it should have the properties of imprinting. On the other hand, recent papers report that this rigorous opinion should be limited to the hypothalamus and the pituitary gland (McCarthy *et al.* 1993, Hutchison *et al.* 1995, 1996, López *et al.* 1996, Mong *et al.* 1996). In other brain regions, the age limitation of the critical period to the perinatal and neonatal periods only does not appear to have general validity (Davis *et al.* 1995). It could be postponed, accordingly to the structural and functional maturity of different brain elements, up to postnatal day 10 (Jacobson *et al.* 1980) or even up to the period of sexual maturation (Reisert and Pilgrim 1991, Davis *et al.* 1996).

The absolute validity of the hypothesis of the critical period for sexual brain differentiation through the organizing influence of gonadal steroids was made somewhat doubtful by experiments carried on tissue cultures which concluded that some sexual dimorphism in the nervous system may be initiated independently of the action of gonadal steroids under genetic control (Kolbinger *et al.* 1991, Beyer *et al.* 1992, Delemarre-van de Waal *et al.* 1994, Lieb *et al.* 1994, Reisert *et al.* 1996), and may then be subsequently modified by differences in the hormonal environment (Beyer *et al.* 1991, Pilgrim 1994; for review see Reisert and Pilgrim 1991).

Recently there has been accumulating evidence that gonadal steroids regulate different morphogenic brain characteristics up to the cellular and subcellular level such as the number of neurones, axonal and dendritic growth and synapse formation in the neuronal system containing steroid receptors.

Pioneer work on this topic was performed by Pfeiffer (1936). He discovered the most important characteristics by which the cells of the anterior pituitary lobe qualitatively differ in females and males. The female cells displayed a phasic function (i.e. they are cyclic in their action), while the male ones have a tonic function (i.e. they are then acyclic). At present, this phenomenon has not drawn general attention of investigators. Consequently, we raised the question in the present paper, if there exist cells in the brain that might change their activity in a cyclic way in females, and remain functionally uniform in males. Furthermore, we wondered if this female cyclicity might be causally related to the actual level of ovarial hormones. We therefore decided to study the functional brain cell activity indirectly by estimating changes of the total content of ribosomal RNA in individual neurones, since a direct relation between the amount of this substance and the level of cell activity was first reported by Hydén (1964) and later by Kogan (1969) and Pevzner (1972).

Methods

Experimental groups

All animals were provided by our own breeding station of albinotic Wistar rats with outbred cross; adult individuals were fed a standard DOS-2b diet (analogous to the Purina Chow) and housed in plastic cages. The experiments included the following groups of laboratory rats of both sexes and of precisely known age:

A. Adult 90-day-old rats:

A1. This set comprised 18 intact animals of both sexes, i.e. 12 females (six of them being in oestrous and the other six in the dioestrous phase at the moment of sacrifice) and six males; the females and males were always selected out of siblings from the same litter.

A2. A set of 10 castrated females was operated during their dioestrous phase; RNA was assessed 40 days later.

A3. A set of 9 hypophysectomized females was also operated during their dioestrus and RNA was measured 40 days later. (One animal from the original group of 10 rats was discarded following histological verification that the pituitary excision was incomplete).

B. Young rats:

B1. Neonatal animals of both sexes, examined on the day of delivery, i.e. on day zero, were younger than 12 hours; 10 male and 10 female rat pups were selected from numbers of nest siblings (the hypothalamic male set only had 11 members).

B2. Similarly, two sets consisting of either 10 male or 10 female 7-day-old animals were selected in the same way from nest siblings.

C. Old rats:

These animals, originating from 8 litters and kept until weaning in cages where the number of young was restricted to 8 (4 females and 4 males), were observed since birth. From the age of 15 months, the oestrous cycle was examined (every time on 10 consecutive days once a month). It was observed that the cycle lasted 3–4 days as it is usual. At the age

of 23 months, approximately 50 % of the starting number of the females survived and their cycles became irregular: following one or two dioestrous records a pro-oestrus sometimes appeared. Those females that displayed a pro-oestrous or oestrous pattern in the vaginal cytogram during 21 subsequent days, were considered as individuals with a long-lasting oestrous phase; then, 8 females from this set were sacrificed and histological samples prepared. In another group of 8 females daily vaginal irrigations were further carried out. Afterwards, it was observed that the oestrous pattern was replaced by a permanent dioestrus in all females. When the dioestrus reached a duration of 21 consecutive days, the females were also sacrificed and their brain was subjected to histological and cytochemical examination. The age of males at the day of sacrifice was the same as that of females with permanent dioestrus. This procedure gave thus rise to the following three sets:

C1. Eight old females in long-lasting oestrus,
C2. Eight old females in permanent dioestrus,
C3. Eight old males.

Determination of the oestrous cycle

The phases of the oestrous cycle were established in females by the usual procedure according to their vaginal cytogram acquired by a single irrigation with 0.5 ml of saline once a day in the morning and by a following histological classification of stained elements in the fluid.

Histological tissue samples

From the brain of each animal decapitated without anaesthesia, tissue samples were dissected from 200–400 μm thick frontal slices with respect to drawings and stereotaxic coordinates in rat brain atlases (Krieg 1946, Paxinos and Watson 1986); from tuberculi olfactorii and from the frontal cortex only small tissue samples were cut out by free hand dissection. Afterwards, all samples were treated by the method of tissue crushing (Sandritter *et al.* 1966). Cells of the following structures were examined:

In the set A1: dorsal hippocampus (area CA1), septum, anterior and posterior thalamus, ventromedial and lateral hypothalamus, tuberculi olfactorii and the frontal pole of cerebral cortex.

In sets A2 and A3: ventromedial hypothalamus, tuberculi olfactorii and the frontal pole of the cerebral cortex.

In group B: ventromedial hypothalamus and the frontal pole of the cerebral cortex.

In group C: dorsal hippocampus (area CA1), ventromedial hypothalamus and the frontal pole of the cerebral cortex.

All types of the enumerated brain structures were excised from each animal in the corresponding group. The histological samples were fixed in Carnoy's solution for 12 h and stained by gallocyanine-chromalum (Kiefer *et al.* 1966).

Cytophotometry

In all brain regions the cells of the same type and size were measured. The total content of RNA insoluble in water (i.e. mainly ribosomal RNA) was detected in whole intact cells. In animals of groups A and C, the measurements were carried out on an integrating microdensitometer (Barr and Stroud, Ltd. UK) constructed according to Deeley (1955). The technical parameters were as follows: objective 100x, condensor nA 0.3, measuring point 0.7 μm, wavelength 560 nm.

In animals of the set A1 (i.e. in subsets of males and of oestrous as well as dioestrous females), 60 cells from each of the eight brain structures were always measured. Thus, the calculated mean RNA value represented one cell from a given structure of animals well-defined in advance as far as the hormonal state is concerned (e.g. displaying a regular and normal oestrous cycle). In neonatal and climacteric females of groups B and C having no oestrous cycle, it was necessary to take into consideration individual animals as representative samples.

In each of 24 animals (i.e. in 3 sets of 8 rats each) from group C, 50 cells were always measured in three brain structures; the average value referred then to one animal and structure, respectively.

Analogously, in those groups where animals were evaluated individually, i.e. following castration (set A2) or hypophysectomy (set A3), 60 cells were measured in three brain structures and their mean value was also related to one animal and a given structure.

The RNA measurements in animals of group B were carried out on a LEITZ TAS plus image analysis system combined with an automated LEITZ Orthoplan microscope (Auffermann *et al.* 1984, Böcking *et al.* 1988, Sánchez *et al.* 1990). In each animal of this group, 100 cells were measured in both brain structures and their mean value referred again to one animal and one structure.

The results are given in arbitrary units of the light absorption which correspond, however, to different standards of both cytophotometric equipments employed for our measurements. Moreover, the light absorption in brain cells can be influenced by the staining processes which, despite their standardization, do not occur under precisely identical conditions or simultaneously in all experimental groups. Therefore, quantitative data obtained in this way, having an important relative value, should be compared with the parallel animal groups only, as seen in the tables and figures presented in this paper, but not *vice versa* across incompatible groups.

Statistics

For any group or set comparison, the data were treated mainly by one-way analysis of variance (ANOVA) followed by the Tukey's multiple comparison test with calculation of the confidence interval (±D) at 5 % and 1 % significance levels (Snedecor and Cochran 1967). For the numeric backward classification of data obtained for every single animal in the corresponding treatment group, Student's t-test was used. This procedure classifying individual animals on the background of the whole group distribution was employed in those cases where an assortment of females according to the phases of their regular oestrous cycles was impossible to be predicted in advance, or in cases where their hormonal state was unequivocally defined by an operative intervention, e.g. castration or hypophysectomy. Consequently, this method was also applied in young rats (in both newborn and 7-day-old pups) and also in old rats. In this manner, three classes of individuals were formed according to their relation to the mean value of all other members of the group. Animals were assorted into lots having 1) a higher, 2) an equal, or 3) a lower total RNA content in corresponding brain cells than would correspond to the group or set mean.

Table 1. Values of the total RNA content in cells of eight brain regions in adult 90-day-old female and male Wistar rats (Set A1)

Brain structure	Dioestrous females (D)	Oestrous females (O)	Males (M)	F	±D(0.05) ±D(0.01)	*Contrasts* DxO p<	DxM p<
Hippocampus (pyramidal cells)	237.6±66.1	266.4±85.3	218.5±35.8	8.1	28.3 35.4	0.05	n.s.
Septum	115.9±17.8	117.9±19.7	119.8±23.2	0.6	8.8 11.0	n.s.	n.s.
Thalamus anterior	115.4±17.8	134.8±28.3	115.0±23.1	17.4	10.1 12.7	0.01	n.s.
Hypothalamus ventromedialis	136.7±22.8	167.7±37.9	145.7±43.9	11.8	15.5 19.4	0.01	n.s.
Hypothalamus lateralis	154.7±17.4	181.6±20.4	137.2±13.9	98.7	7.5 9.4	0.01	0.01
Tuberculi olfactorii	149.6±42.0	186.9±50.8	138.2±39.6	19.8	19.1 24.0	0.01	n.s.
Thalamus posterior	217.4±53.8	209.6±45.1	219.7±48.6	0.7	21.3 26.6	n.s.	n.s.
Frontal cortex	119.5±24.2	160.0±42.6	110.1±26.3	40.9	13.8 17.3	0.01	n.s.

Data of RNA (mean±S.D.) are given in arbitrary units of the light absorption as measured on an integrating microdensitometer (Barr and Stroud, UK). F (variance ratio), and ±D(0.05)/±D(0.01) (confidence intervals for mean differences at significance levels p<0.05 or p<0.01) were calculated for all three rat subsets (i.e. D, O and M), df/df was always 2/177. N = 60 for any of the three animal subsets and any brain structure under examination. The columns DxO and DxM reflect the validity of differences between dioestrous and oestrous females, as well as between dioestrous females and males, at p<0.05 or p<0.01 levels, respectively, according to Tukey's HSD procedure; n.s. – non-significant difference.

Results

A1. Adult 90-day-old rats

Differences in the total RNA content in individual cells from 8 selected brain structures were found between females in the oestrous phase (pro-oestrus and oestrus) and those in the dioestrous phase, the values being higher in oestrus. The values established in dioestrous females did not differ from those of the males with the exception of cells from the lateral hypothalamus, where males displayed still lower RNA values. The described typical relations prevailed in the pyramidal cells of the hippocampus (F = 8.1), in cells of anterior thalamic nuclei (F = 17.4), in the ventromedial (F = 11.8) and lateral hypothalamus (F = 98.7), in tuberculi olfactorii (F = 19.8), and in the

frontal pole of the cerebral cortex (F = 40.9). No differences were revealed in cells of the septum (F = 0.6, n.s.) and of posterior thalamus (F = 0.7, n.s.). In all ANOVA calculations within this set (A1), the explained and residual degrees of freedom (df/df) were equal to 2/177 (Table 1).

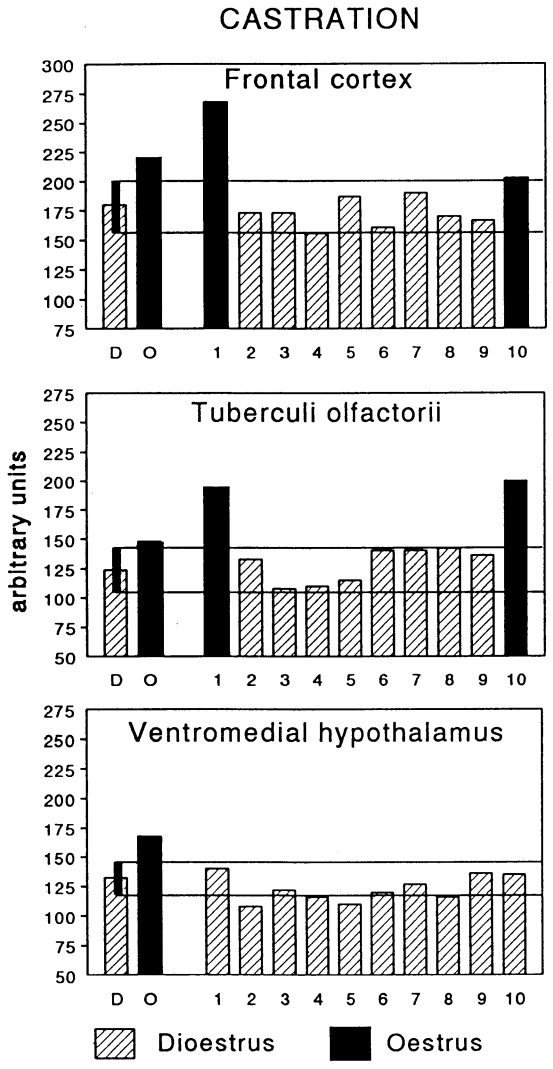

Fig 1. *Effect of castration on the total RNA content in pyramidal cells of the frontal cortex, in cells of tuberculi olfactorii and in cells of ventromedial hypothalamus in adult female rats. Columns represent mean RNA values as measured in individual castrated rats (No. 1 to 10) as well as in one control dioestrous (D) and oestrous rat (O). Same letters and numbers below the columns indicate that individual tissue samples of the three brain regions were always obtained from identical animals. The scale expresses relative RNA values in arbitrary units of light absorption. The two horizontal lines starting from column D delimit the borders of the minimal significant difference from the dioestrous level as given by the calculated confidence interval ±D (drawn by the small black bar at column D).*

A2. Castrated females

When comparing RNA values in cortical cells of individual castrated females with values obtained in one control dioestrous and another oestrous female, it can be concluded that this rat sample is heterogeneous (F = 38.9). Two of ten castrated females had a higher RNA content than that in the control dioestrous female. A similar pattern was observed in tuberculi olfactorii, where cell samples from the same two animals differed significantly when compared with control dioestrous values (F = 52.4). On the contrary, the RNA content in ventromedial hypothalamic cells of all castrated females were non-significantly scattered around control dioestrous values or attained even lower levels (F = 25.4). The df/df were always 11/708 (Fig. 1).

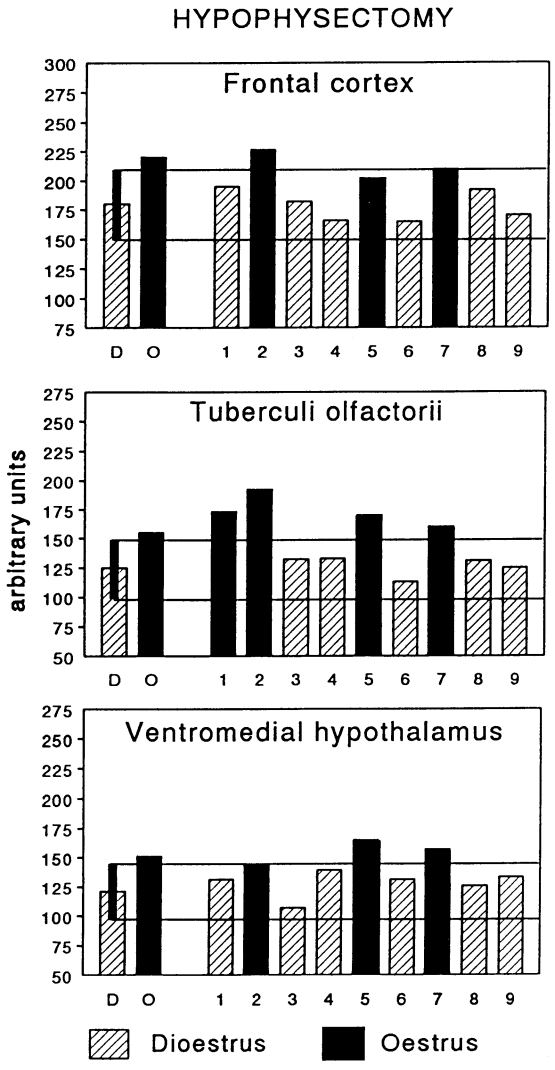

Fig 2. *Effect of hypophysectomy on the total RNA content in cells of the same three brain regions as in Figure 1. In this series, nine hypophysectomized rats only were confirmed by histological verification and thus statistically evaluated. For further explanation see the legend to Fig. 1.*

Table 2. Values of the total RNA content in cells of two brain regions in young (0-day-old and 7-day-old) female and male Wistar rats (Group B)

Brain structure	*0-day-old rats* Females	Males	*7-day-old rats* Females	Males	F (df/df)	±D(0.05) ±D(0.01)
Frontal cortex	7.8±0.9	6.7±0.5	12.4±1.3	13.5±1.4	99.6 (3/36)	1.28 1.59
Hypothalamus ventromedialis	9.8±0.6	7.4±0.8	11.6±1.6	13.1±1.7	38.6 (3/37)	1.55 1.88

Contrasts			
between females and males	*0-day-old rats*	*7-day-old rats*	
	no (diff.=1.1<1.28; n.s.)	**no** (diff.=1.1<1.28; n.s.)	frontal cortex
	yes (diff.=2.4>1.88; p<0.01)	**no** (diff.=1.5<1.55; n.s.)	hypothalamus
Contrasts between 0-day-old and 7-day-old rats	*Females*	*Males*	
	yes (diff.=4.6>1.59; p<0.01)	**yes** (diff.=6.8>1.59; p<0.01)	frontal cortex
	yes (diff.=1.8>1.55; p<0.05)	**yes** (diff.=5.7>1.88; p<0.01)	hypothalamus

Data of RNA (mean ±S.D.) are given in arbitrary units of the light absorption as measured by the LEITZ TAS plus image analysis system (Ernst Leitz, Wetzlar, Germany). F (variance ratio) and ±D(0.05)/±D(0.01) (confidence intervals for mean differences at significance levels p<0.05 or p<0.01) were calculated for all four sets (i.e. 0-day-old and 7-day-old animals, both females and males) and the respective brain structure (N = 10 for any animal set and any brain structure, with the exception of the hypothalamus in 0-day-old males where N = 11). The comparisons reflect the validity of differences between individual groups according to Tukey's HSD procedure.

A3. Hypophysectomized females

Similarly, this set of operated animals was found to be heterogeneous. In three out of nine hypophysectomized females, the values of their brain cell RNA content ranged within the confidence interval of RNA values measured in the control oestrous female, while data from the other rats remained at the control dioestrous level. This result was confirmed for the regions of frontal cortex (F = 10.0), tuberculi olfactorii (F = 18.0), as well as for cells of ventromedial hypothalamus (F = 10.7); df/df were always 10/649 (Fig. 2).

B. Young rats

If four sets of young rats (i.e. 0-day-old and 7-day-old animals, both female and male) were statistically evaluated together, the ANOVA calculation did not confirm any sex difference at the cortical level in rats of the same age, as far as the cell RNA content was concerned. However, considerable differences became prominent between the younger and older animals, because the RNA values were higher in the latter group, thus stressing the age factor (F = 45.9; df/df = 3/37) (Table 2).

Table 3. Distribution of individual young rats (0-day-old and 7-day-old) into classes according to their mean total RNA content in brain cells of ventromedial hypothalamus and frontal cortex

Set	B1 (0-day-old)	B2 (7-day-old)
Class	*VENTROMEDIAL HYPOTHALAMUS*	
Higher	FFFFFFFFFF	F MMMMM
Equal	MM	FFFF MMMM
Lower	MMMMMMMMM	FFFFF M
	FRONTAL CORTEX	
Higher	FFFFF M	FFF MMMMMM
Equal	F MMM	F MM
Lower	FFFF MMMMMM	FFFFFF MM

Individual rats were allocated into particular classes (higher, equal, lower) according to the comparison of their RNA values in the respective brain structure with the mean value of all other 19–20 set members. F – individual female, M – individual male.

Table 4. Differences between male and dioestrous female rats in the total RNA content of brain cells expressed in % (values of dioestrous females being taken as 100 %)

Brain structure	Adult rats 90-day-old rats	Old rats aged over 2 years
Frontal cortex	−7.9*	−13.6**
Hippocampus (pyramidal cells)	−8.0*	−21.2**
Hypothalamus ventromedialis	+6.6*	−12.4

*Calculated according to the formula 100(M−F)/F, where M and F are the measured mean RNA values in males and females, respectively. * non-significant difference (for details see Table 1). ** significant difference (for the probability level see Table 5).*

The numeric backward classification of individual newborn animals based on hypothalamic cell values showed that the distribution of values in females and males with regard to the mean of all other newborn rats had a mutually polar pattern: the females belonged in 100 % to the class marked as "higher"; on the contrary, 82 % of males were placed in the "lower" class. At the age of 7 days, despite a non-significant sex difference of the mean values, the polar distribution of females on the one hand, and males on the other, remained preserved, but in a somewhat reversed sense: 50 % of males belonged to the "higher" and 40 % to the "equal" class, 50 % of females to the "lower" and 40 % to the "equal" class (Table 3).

As for the frontal cortex, newborn female values displayed a trend to form two separate subsets: 50 % of the values were higher and 40 % lower than the mean value of the set. The newborn males formed a relatively homogeneous group, 60 % of them belonging to the "lower" and 30 % to the "equal" class. At the age of 7 days, these distributional trends were preserved with small deviations only: the females were classified in 30 % into the "higher" and in 60 % into the "lower" class, males in 60 % into the "higher" and in 20 % into the "equal" class (Table 3).

This may suggest a slower rise of brain cell RNA in females (by 38 % in the cortex and 16 % in the hypothalamus) and a more rapid rise in males (by 49 % and 44 %, respectively).

C. Old rats

In aged animals, sexual differences persisted in all the brain structures examined, if the females had reached a stable dioestrous state. Their total RNA content in brain cells was still higher than in the males. Nevertheless, this situation represents another quantitative relation between the sexes, when compared to young adult rats; in most brain regions, except the lateral hypothalamus, the RNA values of 90-day-old dioestrous females agreed fully with the values of males, small deviations not surpassing the limits of the confidence interval (mean±D). However, in old rats, the percentage of the male/female difference was 2–3 times greater (Table 4). This may suggest a more rapid decline of brain cell RNA in aged males than in females.

Table 5. Values of the total RNA content in cells of three brain regions in old female and male Wistar rats, aged over two years (Group C)

Brain structure	Dioestrous females (D)	Oestrous females (O)	Males (M)	F p<	±D(.05) ±D(.01)	*Contrasts* DxO p<	DxM p<	OxM p<
Frontal cortex	207.6±25.0	209.4±19.9	179.4±19.4	4.88 0.025	27.15 35.16	n.s.	0.016	0.011
Hippocampus (pyramidal cells)	256.3±45.3	214.5±22.8	201.9±13.2	7.08 0.005	38.09 49.33	0.041	0.011	n.s.
Hypothalamus ventromedialis	168.9±16.1	149.5±19.8	148.0±7.2	4.59 0.025	19.28 24.97	0.049	0.008	n.s.

Data of RNA (mean±S.D.) are given in arbitrary units of light absorption as measured on an integrating microdensitometer (Barr and Stroud, UK). F (variance ratio) and ±D(0.05)/±D(0.01) (confidence intervals for mean differences at significance levels p<0.05 or p<0.01) were calculated for all three groups (i.e. D, O and M), df/df were always 2/21. N = 8 for any animal set and any brain structure under examination. The columns DxO, DxM, and OxM reflect the validity of differences between dioestrous and oestrous females, between dioestrous females and males, as well as between oestrous females and males, at indicated significance levels obtained by t-tests; n.s. − non-significant difference.

The long-lasting influence of oestrogens in the oestrous phase of the climacteric period caused different effects in brain structures. It lowered the RNA values in hippocampal and hypothalamic neurones, whereas it exerted smaller changes in cortical neurones. Consequently, at the hippocampal and hypothalamic levels, the RNA values were diminished in oestrous females down to values of old males ($F = 7.08$ for hippocampus; $F = 4.59$ for hypothalamus). Furthermore, at the cortical level, the RNA values in both dioestrous and oestrous females were equal, but always higher than those in the males ($F = 4.88$); for all three brain regions, df/df = 2/21 (Table 5).

The numeric backward classification of individual animals gave the following results: as for hypothalamic cells, the females in long-lasting oestrus were accumulated in the two extreme subsets, different from the mean; 50 % of them belonged into the "lower", 37.5 % into the "higher" class. As for hippocampal and cortical neurones, their cluster had a more homogeneous form: 50 % were classified into one of the remote classes and the remaining 37.5 % into the neighbouring "equal" class.

Table 6. Distribution of individual old rats into classes according to their mean total RNA content in brain cells of the hippocampus, ventromedial hypothalamus and frontal cortex

Class			
	HIPPOCAMPUS		
Higher	DDDDD	O	
Equal	DD	OOO	MMM
Lower	D	OOOO	MMMMM
	VENTROMEDIAL HYPOTHALAMUS		
Higher	DDDDD	OOO	
Equal	DD	O	MMMMM
Lower	D	OOOO	MMM
	FRONTAL CORTEX		
Higher	DD	OOOO	
Equal	DDDDD	OOO	MMM
Lower	D	O	MMMMM

Individual rats were allocated into particular classes (higher, equal, lower) according to the comparison of their RNA values in the respective brain structure with the mean value of all other 23 set members. D – individual dioestrous female; O – individual oestrous female; M – individual male.

As far as all brain structures studied are concerned, the females in permanent dioestrus and males were concentrated in homogeneous groupings, where the majority (62.5 %) occupied one class and the remainder usually belonged to the neighbouring one, but never to the other extreme class. The sexual difference was still present: dioestrous females were placed, in general, in the "higher" class (the hippocampus and hypothalamus) or in the "equal" one (cortex); males were then usually ranged into the "lower" class (cortex, hippocampus) or in the "equal" one (hypothalamus) (Table 6).

Discussion

It has been demonstrated in this study that brain neurones undergoing cyclic changes of their ribosomal RNA content really exist during the reproductive period of adult female rats; the measured RNA values were higher in the oestrous phase and lower in the dioestrous phase of the cycle. However, such cycling cells were not found in all investigated brain regions, but their location could not be deduced from the adherence to certain specialized brain functions, systems or circuits; this fact was clearly demonstrated in the septum – an important formation of the limbic system – where such cycling neurones were not found; on the other hand, they were observed in the hippocampus and in anterior thalamic nuclei which also constitute the same system. Furthermore, they were not present in the posterior thalamus, but were repeatedly discovered in the frontal cortex, in the rhinencephalon and in hypothalamus. Such a pattern cannot be explained on the basis of contemporary knowledge, concerning brain evolution or the functional organization of the central nervous system. With the exception of the hypothalamus, cyclic RNA changes obviously do not depend on the levels of gonadal steroids; they simply run synchronously with the oestrous cycle.

The results are in agreement with earlier studies. Hutchison and Beyer (1994) reported sex differences in aromatase activity of hypothalamic cells in tissue cultures, but this effect was not confirmed in cultured cortical cells. Garcia-Segura *et al.* (1993) described the cellular distribution of histone in the arcuate area of the hypothalamus as being dependent upon the oestrous cycle, namely upon the levels of plasma oestradiol. Ovariectomy was also effective, since it increased the number of immunoreactive results in the opposite direction. In females, the percentage of immunoreactive neurones was lower. It is regrettable that these authors did not compare yet another brain structure with the hypothalamus.

Experiments yielding results on procedures contrary to castration, i.e. administration of gonadal hormones, did not provide unambiguous results.

Neonatal administration of testosterone propionate to 5-day-old females, and oestrogen to males of the same age (during the critical period) resulted in effects seen later in adulthood, and were manifested in males by a permanent increase in total cell RNA content and by a transformation of the cyclic type of cells of the nucleus paraventricularis of females into an acyclic type. These changes were limited to the hypothalamus only, the hippocampal cells remained unaffected (Nováková *et al.* 1971).

On the contrary, McEwen and Woolley (1994) reported that the administration of progesterone and antiprogesterone to adult females proved that changes in the density of synapses both in the hypothalamus and in the hippocampus (area CA1) depends upon the plasma hormone level. Moreover, the number of dendritic spines changed in dependence on the spontaneous oestrous cycle. The disagreement between the findings of these authors and our previous results is probably due to the age of the experimental animals, i.e. to an increased sensitivity to the high doses of these drugs in young animals.

Evaluation of the effects of experimental treatments such as ovariectomy, hypophysectomy, administration of high doses of gonadal steroids, or results achieved in tissue cultures (i.e. in a non-integrated and non-regulated mass of individual cells) may be ambiguous by the artificial character of such interventions. We therefore believed that a better and more adequate control could be found under physiological conditions of the organism, when ovaries are not yet (or no more) fulfilling their specific regulatory function. Such a situation occurs twice in the life of females: in the juvenile period before sex maturation, and in the old age. Leedom *et al.* (1994) analysed the developmental changes in the number of synapses in the nucleus arcuatus. During the reproductive life of adult female rats, circulating oestrogen modulates the synaptology of this structure. 30–50 % of axosomatic synapses disappeared following the preovulatory oestrogen surge from dioestrus to pro-oestrus; a repeated long-lasting pro-oestrus in old females finally caused a reorganization of the synaptology regulated by the oestrogen-induced gonadotrophin surge. During the senescent constant oestrus in aged females, the membrane organization appears indistinguishable from the males. Naftolin *et al.* (1990) elucidated the processes of creation of sex-differentiated elements during youth, and their loss in old age, by the effects of oestrogens on synaptogenesis in the newbornas well as by a synaptic remodelling in adulthood. Under a long-lasting influence of oestrogens in senescent females, this remodelling resulted in a reduction of synapses. Oestrogen imparts sex differences in the rat brain during sexual maturation by shaping synaptology, postsynaptic membranes and glial cells. In our experiments, no sexual differences were observed among cells of the frontal cortex in newborn rats; in the hypothalamus, the RNA values of females were higher than those of the males. On the contrary, Lustig *et al.* (1993) did not find any sexual differences in three neural specific messenger RNAs either in the cortex or in the hypothalamus of newborn rats. This is not in agreement with our present study. It may be due to the fact that Lustig *et al.* (1993) used another rat strain (Sprague-Dawley) in their experiments than we did (the albinotic Wistar strain). It is well established that Wistar rats are less stress-resistant than non-albinotic strains. Since females are more sensitive to stress than males at the age under study (Jans *et al.* 1985), perinatal alterations of hormones in the mother and pups may therefore act selectively, i.e. with a stronger effect upon females; then, the observed increase of cell RNA content would be non-specific and transient by nature. Incidentally, our studies have confirmed this presumption by the fact that sexual differences were observed neither in the cortex, nor in the hypothalamus of 7-day-old animals.

The results of our studies on intracellular structures may support the concept of neuropil reorganization (regression) in old females caused by a long-lasting effect of oestrogen: in fact, the period of long-lasting oestrus decreased the neuronal quantity of ribosomes in aged females to male values. However, this conclusion should be restricted to brain cells of those regions that take part in the control of ovaries (i.e. the hypothalamus), or in the modulation (inhibition) of the hypothalamus itself (i.e. hippocampus). On the contrary, in the frontal cortex which is considered to be indifferent from this point of view, female cells retain their higher RNA content in comparison with the males and contributed in this way to preservation of the sexual difference in old age and in a given hormonal situation.

Authors investigating the influences of ovarial hormones on the senescent brain did not respect the fact that the phase of long-lasting oestrus is replaced by the permanent dioestrus, where the production of oestrogens is minimal. We therefore consider this life period to be the most appropriate to search for the mechanisms how sexual differences in the brain appear and develop since they are spontaneous and fully physiological. Hence, the results could help in differentiating between the contribution of genetic and hormonal factors in the manifestation of sexual differences at the level of brain cells.

In conclusion, it follows from our present study that the sexual differences of certain brain structures are genetically determined for two reasons:

1) In general, the cycling alterations of the total RNA content in brain cells of young females in the course of their reproductive life period were found synchronous with, but not dependent upon, the oestrous cycle; on the contrary, hormonal dependence

was manifested in hypothalamic cells only, this representing the single exception not following the rule,

2) During the period of permanent dioestrus of aged females, the RNA values in all brain structures were higher than in males of the same age, including the hypothalamus.

It may be expected that this basic genetic program is extended in course of the reproductive life period as well as in the climacteric oestrous phase of females by the ability of neurones from specific control regions of the brain to produce a response reflecting the actual hormonal situation. However, we did not observe that the expression of this genetic program was already acting at early postnatal stages in rats.

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