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Research report

Sexual dimorphism in number and proportion of neurons in the human median raphe nucleus

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Abstract

The number and proportion of neurons in the median raphe nuclei stained by the Golgy–Cox and Nissl methods was compared in males and females infants. When subjects are matched by age and cause of death the number and proportion of fusiform, ovoid and multipolar cells differs significantly between sexes at different ages.

Theme: Development and regeneration

Topic: Developmental genetics

Keywords: Neural sex dimorphism; Raphe nucleus; Serotonergic system; Golgy–Cox study

1. Introduction

Morphological sexual differences have been found in several areas of the human brain, i.e. preoptic area [17], Onuf nucleus [13], amygdala [8], corpus callosum and anterior commisure [1,3], planum temporale [7] and posterior temporal cortex [19]. Men's brain seems to be functionally and neuroanatomically more lateralized than women's brain [2]. The median raphe nucleus (mnr) is the largest of all the raphe nuclei in human brainstem and is part of the serotonergic system [10]. The serotonergic nuclei are involved in neuronal firing, human emotions control, reproduction and sexual behavior [15]. This wide structural sexual dimorphism of the nervous system makes it premature to assign the dimorphic sexual behavior to only one nucleus of the brain [11].

In a preliminary study we found sexual dimorphism in the number and proportion of the different types of neurons in the mnr [5]. Now we extend this study to older infants confirming that finding and studying its ontogeny. Since the Golgy–Cox method (GCm) stains between 1 and 20% of neurons we added the Nissl method (Nm) to assess most of stainable cells.

2. Material and methods

The autopsies of four males and four females were performed at the Roberto del Río Hospital (Santiago, Chile), according to the ethical norms accepted at that time in Chile. Infant data are summarized in Table 1. They were born after a normal pregnancy. Brains were obtained and fixed in 10% neutral formalin within 12 h postmortem [4]. Blocks 3 mm thick cut nearby plane 19 were processed [12] by the GCm [16]. Blocks were fixed, placed in collodion and cut 120 μm thick. Five histological sections were reduced, mounted under synthetic resin and paired by the neuroanatomical patterns. Additionally, a transversal block 3 mm thick at plane 19 [12] from brainstems of two infants were cut in slices 10 μm thick and stained by the Nm. For this method, in both mnr, 3 rostral, 3 middle and 4 caudal sections were used. Random numbers were assigned to slices to ensure blind assessment. All the properly stained neurons were drawn. Fig. 1 shows the type of neurons with the GCm and Nm.

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Table 1
Clinical and anatomopathological features of the eight infants

	Female A-1[b]	Female A-2	Female A-3	Female A-4	Male B-1[b]	Male B-2	Male B-3	Male B-4
Age at death	9 days	23 days	150 days	2 days	3 days	22 days	210 days	2 days
Gestational age (wks)	37	38	38	32	39	38	40	32
Delivery	Normal	Cesarean	Normal	Normal	Normal	Normal	Normal	Normal
Birth weight (g)	2.680	2.920	3.160	1.860	3.200	3.600	2.900	1.535
Birth height (cm)	49	48	47.5	43	50	50	46.5	44
Cause of death	Asphictic syndrome	Pneumonia	Pneumonia	Asphictic syndrome	Asphictic syndrome	Pneumonia	Pneumonia	LVH[a]
Brain weight (g)	285	349	743.4	226	325	347.5	774.5	220
Delay before autopsy	6 h	5 h	4.5 h	4 h	10 h	2 h	6.5 h	3.5 h
Stain method	Golgi–Cox	Golgi–Cox	Golgi–Cox	Nissl	Golgi–Cox	Golgi–Cox	Golgi–Cox	Nissl

[a] Lateral ventricular hemorrhage.
[b] A-1 and B-1 were studied previously [5].

GCm: Five drawings of each case were made by the aid of a camera lucida and an eye-piece in the ocular at ×100. Each drawing corresponded to a volume of 2400 μm dorsoventral, 800 μm medio-lateral and 120 μm rostral caudal. (Fig. 2). All distinguishable neurons were counted. The dorsal boundary of the drawings coincided with the upper third of the medial longitudinal fasciculus (mlf) (Fig. 2). Perikaryon and dendritic branches of ovoid (O), multipolar (M) and fusiform (F) cells were drawn.

Nm: Neurons whose nucleus and nucleulus (one or more) were sharp on one focus plane per area (500 μm medio lateral and 750 μm dorsoventral) were drawn (×400) and counted. Large (12.5 μm ore more) and small (less than 12.5 μm) neurons can be recognized on the drawings (Fig. 1).

Neuron counting was based on the drawings. These drawings corresponded to the above mentioned volume of the mnr for the GCm and the transversal section for the Nm. We tested four hypotheses: (1) The mean number of neurons per counting unit is equal for both sexes (both

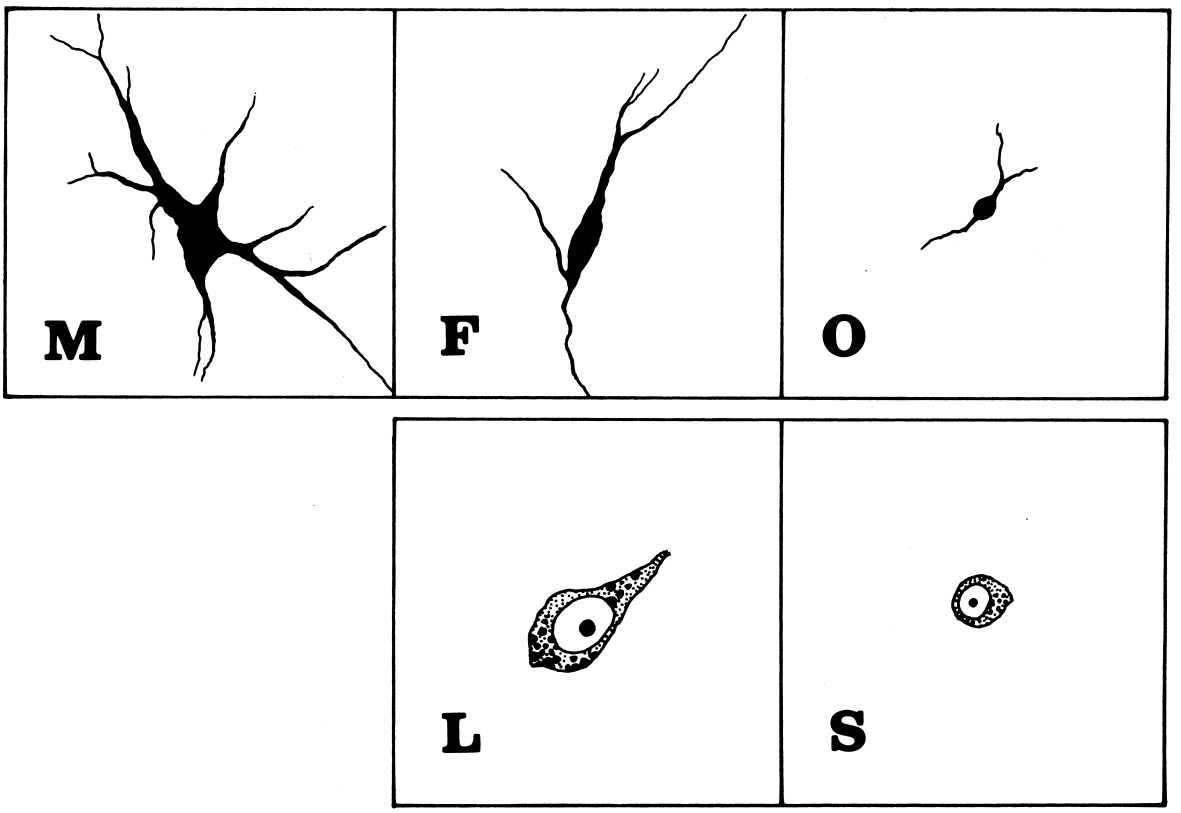

Fig. 1. Camera lucida drawings of the neuron types from the mnr: Multipolar (M), fusiform (F) and ovoid (O) cells (GCm, ×100) and two neuron types: large (L) and small (S) (Nissl method, ×400).

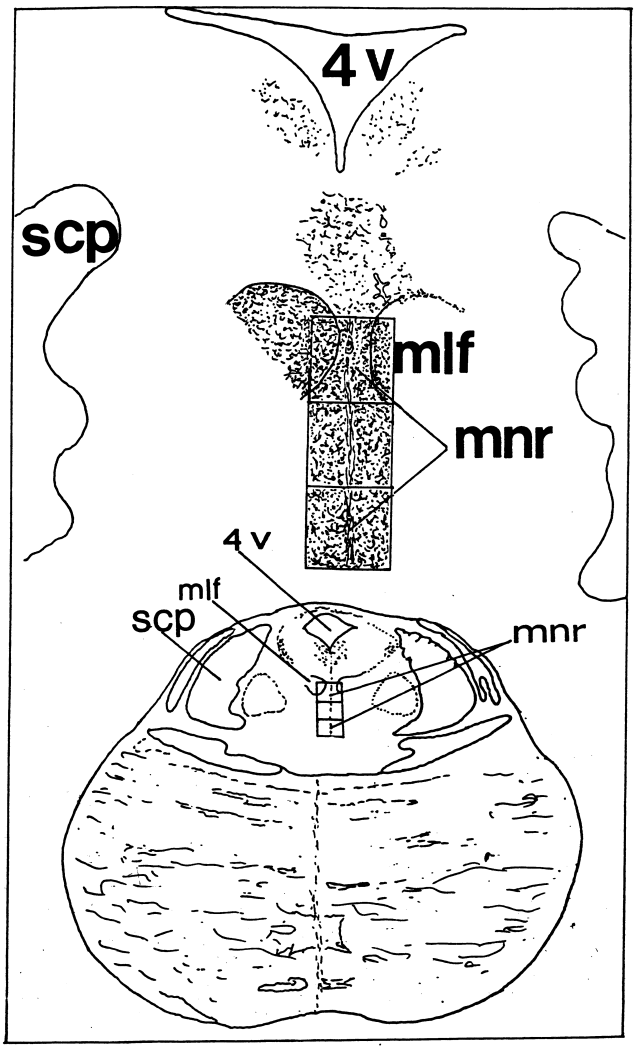

Fig. 2. Magnification of the region from where drawings were made and a transversal section of the rostral pons. Four V fourth ventricle; scp superior cerebellar peduncle.

stain methods). (2) The mean number of neurons per counting unit is the same across the different ages of our sample (GCm). (3) The proportion of the three types of neurons for the GCm and the two types of neurons for the Nm is homogeneous for sexes. (4) The distribution of the types of neurons is homogeneous for ages (GCm).

Since results showed significant heteroscedasticity, we did not perform ANOVAS. Means were compared by two '*t*'-tests. One of them (*t'*) allows for unequal and unknown variances by correcting degrees of freedom (Welch–Satterhway solution) and the other is the standard *t*-test [9]. The non parametric Wilcoxon's rank-sum test was added [9]. Since the Wilcoxon's test does not inform of the amount of the differences (among means and variances) we conserved the *t*-test with the least significance. Hypotheses on distribution of neuron types were tested by a chi-square for homogeneity. Degrees of freedom are indicated as subscripts.

3. Results

Figs. 3–8 show the photomicrographs and their drawings of the 9/3, 23/22 and 150/210 days female/male pairs respectively. Greater cellularity of the female mnr at the three ages is evident, mainly for multipolar (M) and ovoid (O) neurons. The dendritic network was denser in females. M neurons and glial cells seemed larger in males. Drawings with the Nm are shown on Fig. 9. A remarkable finding is the greater cellularity found in the female sample.

Table 2 shows comparisons of the number and types of neurons from GCm drawings. At the three ages females had more neurons than males excepting for fusiform (F) neurons at 9/3 days. Among the nine possible comparisons between sexes six were clearly significant ($P<0.02$). Non significant comparisons were found for F neurons at 9/3 days, M cells at 23/22 days and for O neurons at 150/210 days. Highly significant differences ($P<0.01$) were found in M cells at 9/3 and at 150/210 days and in F and O cells at 23/22 days. The number of neurons was larger in females across the three ages. The Wilcoxon's test yielded

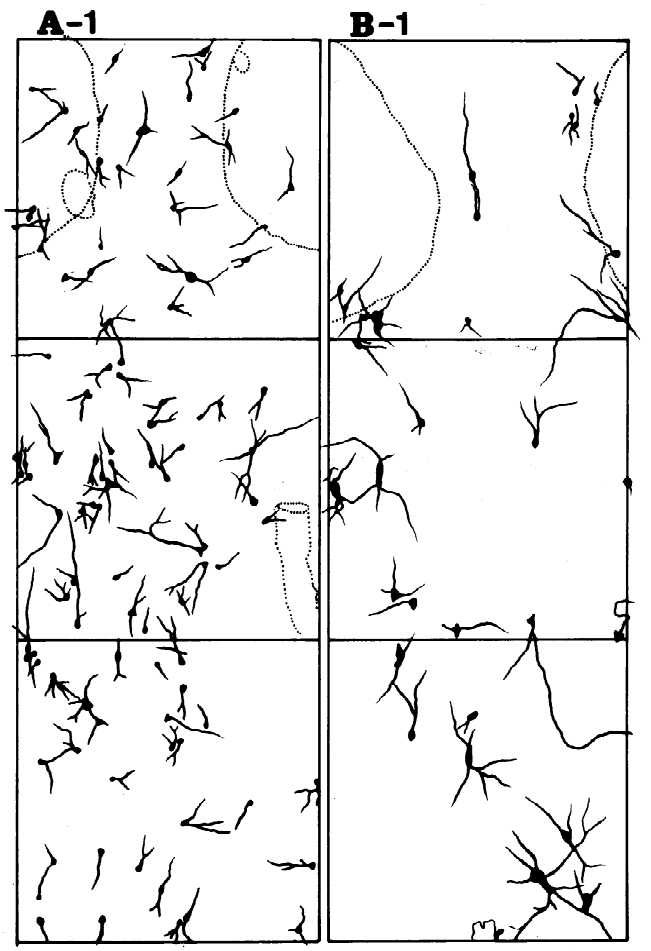

Fig. 3. Drawings of neurons from the mnr with the GCm. Left side A-1: 9-day-old female; right side B-1: 3-day-old male. Dotted lines represent the border of the mlf or blood vessels.

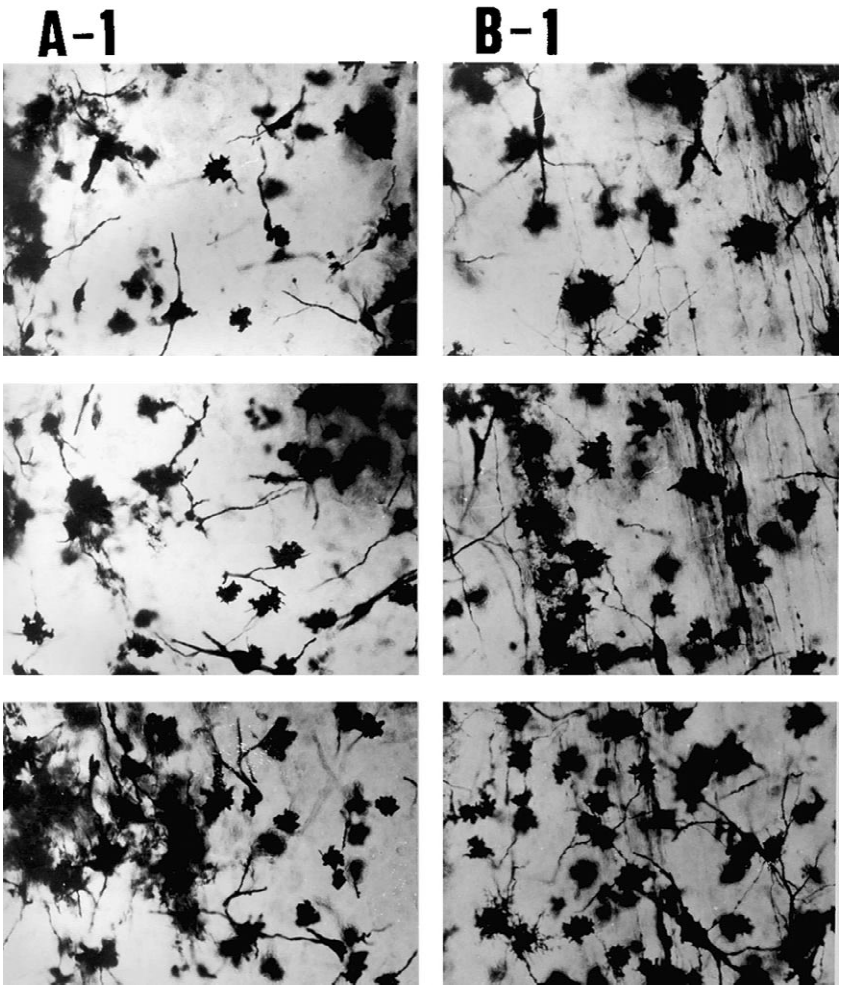

Fig. 4. Photomicrographs of the mnr of the same groups as in Fig. 3. (GCm, ×100).

Table 2
Female to male comparisons of the mean number of neurons per unit and significance at the three ages with the Golgi–Cox method[a]

Neuronal types	Females			Significance		Males		
	N	Mean	S.D.	P_t	P_w	*N*	Mean	S.D.
Age:		9 days					3 days	
Fusiform	5	5.40	1.95	0.5960	>0.5	5	6.20	2.588
Ovoid	5	35.00	15.00	0.0185	0.00794	5	9.00	2.121
Multipolar	5	19.00	3.53	0.0009	0.00794	5	8.20	3.114
Age:		23 days					22 days	
Fusiform	5	16.40	4.16	0.0090	0.01587	5	7.80	3.768
Ovoid	5	46.80	10.55	0.0006	0.00794	5	14.40	8.019
Multipolar	5	27.00	6.12	0.0890	0.22222	5	19.80	5.630
Age:		150 days					210 days	
Fusiform	5	10.40	3.58	0.0168	0.01587	5	4.60	1.673
Ovoid	5	25.20	13.42	0.2277	0.30952	5	16.40	5.857
Multipolar	5	27.40	10.11	0.0082	0.00794	5	6.80	3.962
	Total cells							
9–3 days	5	59.40	13.37	0.0015	0.00794	5	23.4	5.814
23–22 days	5	90.20	12.93	0.0005	0.00794	5	42.0	13.982
150–210 days	5	63.00	22.00	0.0206	0.00794	5	27.8	8.497

[a] *N* = number of drawings, S.D. = standard deviation, P_t = probability for the least significant *t*-test, P_w = Wilcoxon's test probability.

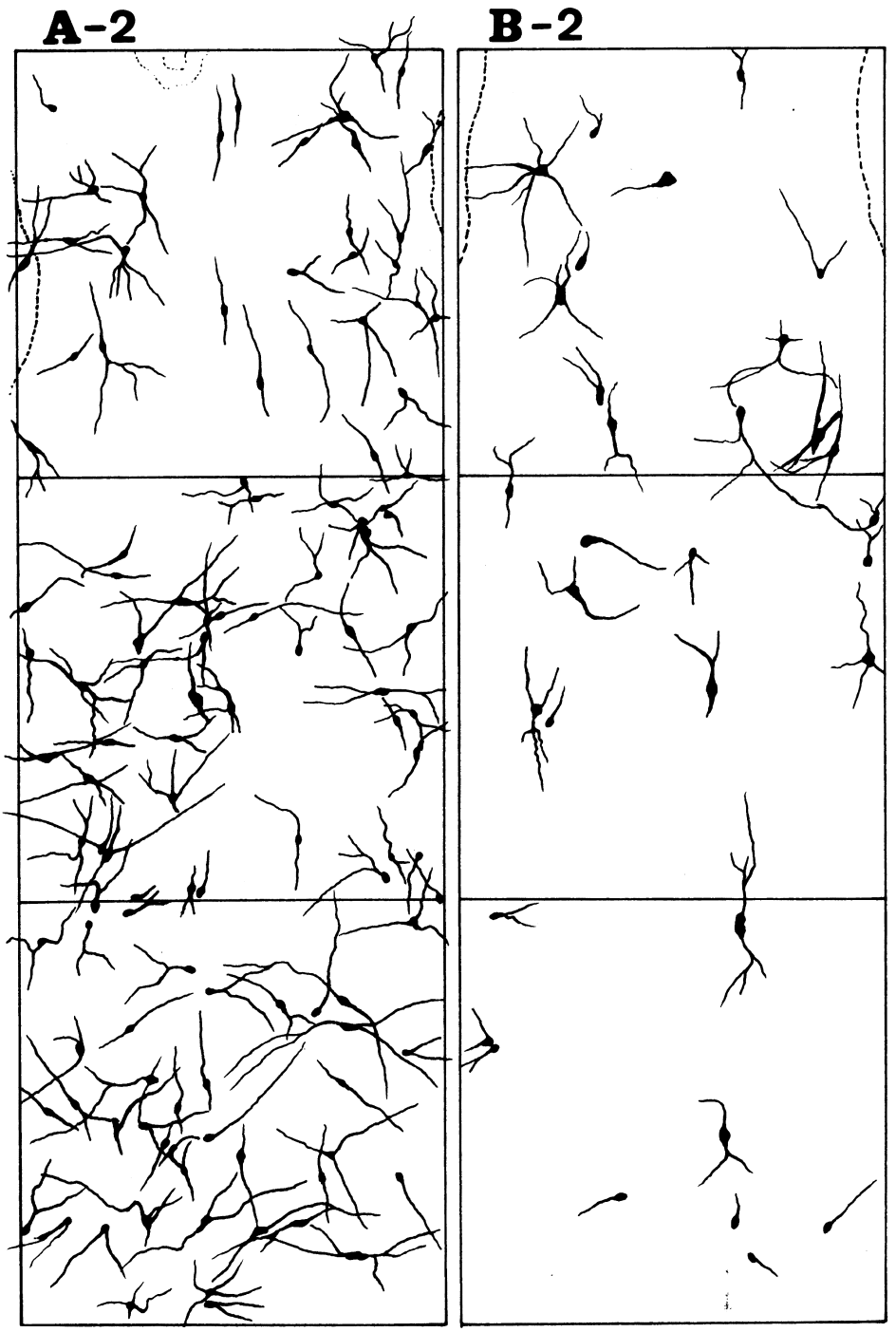

Fig. 5. Drawings of neurons from the mnr with the GCm. Left side A-2: 23-day-old female; right side B-2: 22-day-old male infant. Note the greater cellularity and dendritic network in A-2.

similar significant findings, but this test has some limitations to evaluate large differences.

There are nine possible comparisons with three types of neurons among three ages. In females five of them resulted significant. The nine days female had less F neurons than the 23 days one ($P=0.002$) and the 150 days one ($P=0.029$), and less M neurons than the 23 days one ($P=0.039$). The 23 days female had more F and O neurons than the 150 days one ($P=0.04$ and $P=0.022$ respectively). Thus, only the small number of F neurons in the 9 days infant was clearly significant. In males only three significant comparisons were found. The 3 days male had less M cells than the 22 days one ($P=0.05$) and less O neurons than the 210 days one ($P=0.045$). The only clearly significant comparison was that found in M neurons between the 22 and 210 days males ($P=0.003$).

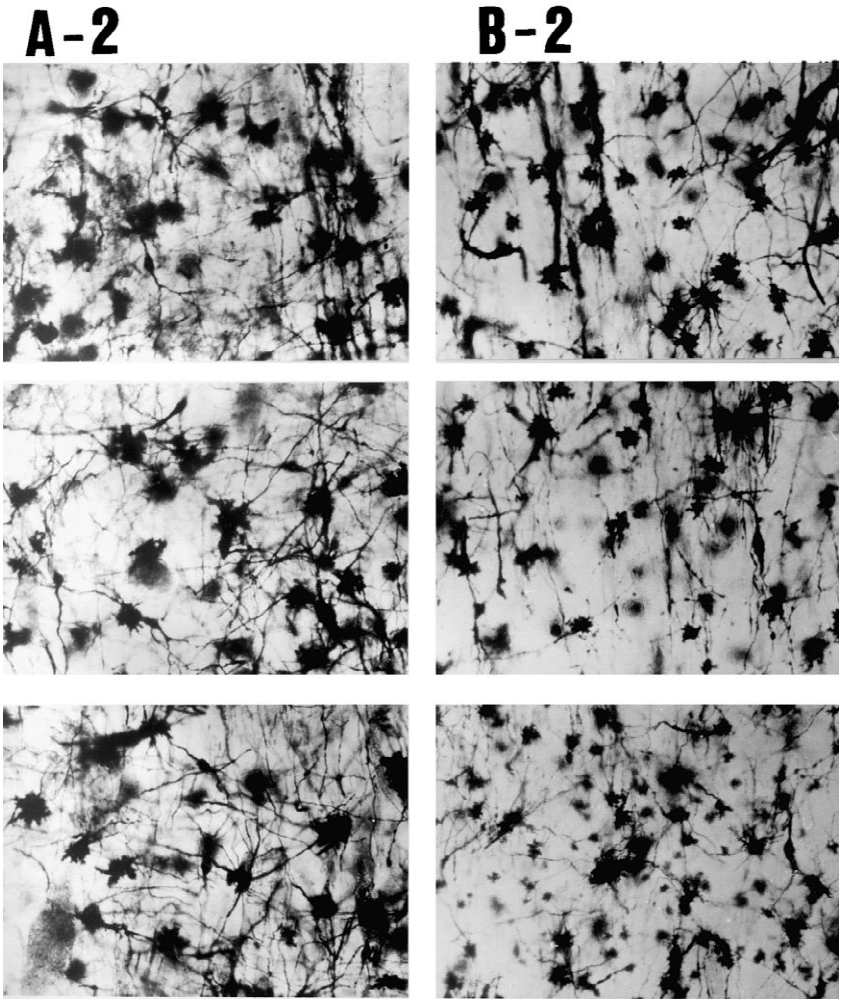

Fig. 6. Photomicrographs of the mnr as in Fig. 5. (GCm, ×100).

The total number and proportion of different types of neurons were calculated from Table 2 multiplying means by 5 (number of drawings). To these numbers we applied a χ^2_2 test for heterogeneity to estimate differences in the proportion of neurons. At the three ages we found a highly significant sexual dimorphism on the proportion of the neuron types. Infants 9/3 days presented a remarkable sex dimorphism in the neurons distribution ($P=3\times10^{-6}$) due mostly to a low proportion of F cells in the female and O cells in the male. At 23/22 days the female had significantly more O and less M cells than the male ($P=21\times10^{-6}$). At 150/210 days the female had significantly lower frequency of O and higher frequency of M cells ($P=0.00022$). The distribution of cells among females at the three ages was significantly heterogeneous ($P=2\times10^{-6}$) due to a very low proportion of F cells in the 9 days infant. In males there was a significant heterogeneity ($P=13\times10^{-6}$) in the neuron distribution mostly due to a high frequency of M cells at 22 days and of O cells at 210 days. The proportion of the total count of neural types, including both sexes, was homogeneus among the three ages ($P=0.117$).

Table 3 shows analyzes for the Nm. The female infant had more small and large cells than the male infant ($P=0.0831$ and $P=0.009$ respectively). The proportion of both types of neurons was homogeneus between sexes ($P=0.3098$).

4. Discussion

Of our four hypotheses the clearest refutations were those related to the equality of number and proportion of neurons between males and females in the mnr. Females had more neurons than males. To our knowledge there are no other studies on sexual dimorphism of the reticular formation performed by the GCm and Nm. The differences in the number and proportion of neurons among ages within sexes were significant, but not consistently so due to a few extreme values. A systematic higher number of

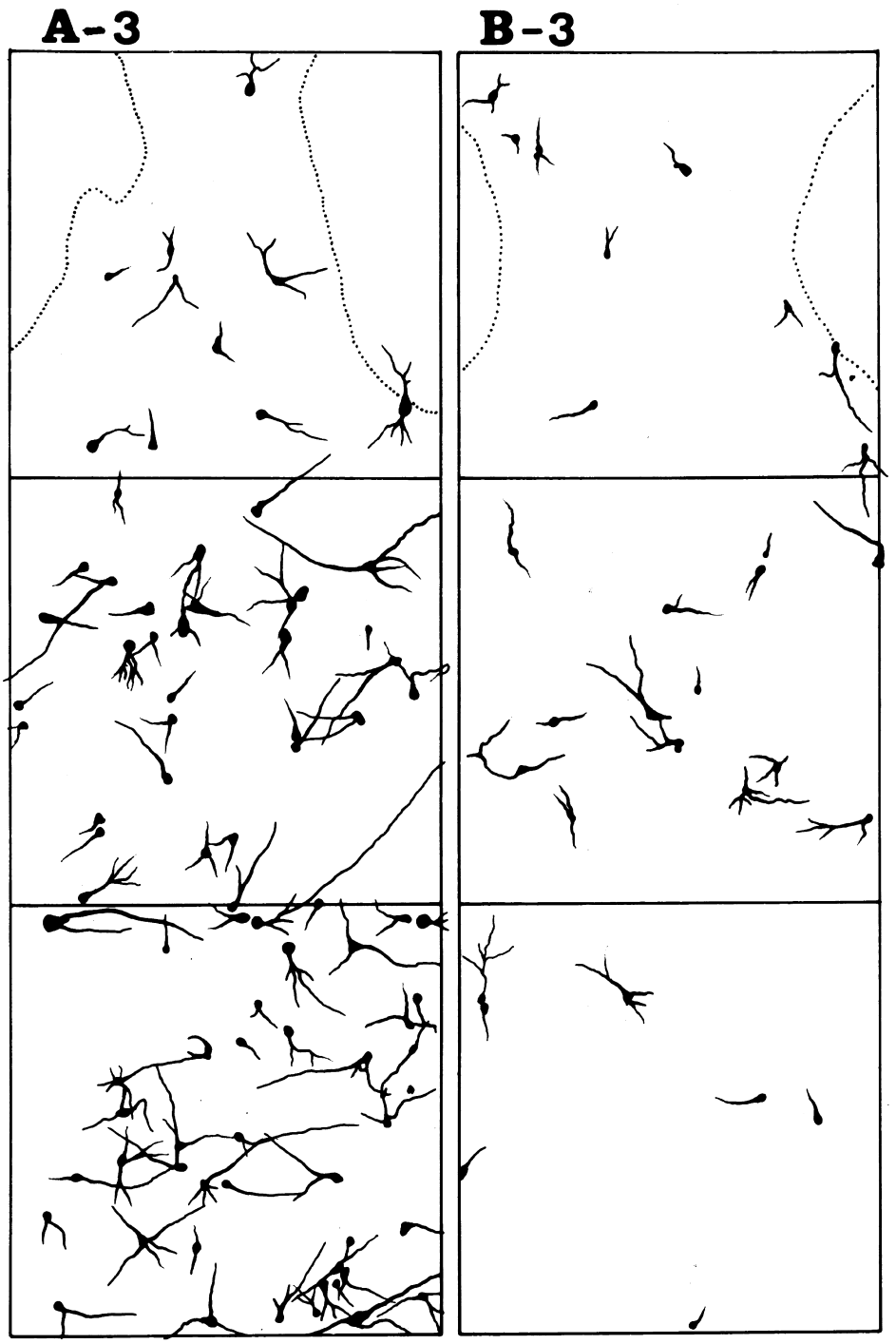

Fig. 7. Drawings of neurons from the mnr with the GCm. Left side A-3: 150-day-old female; right side B-3: 210-day-old male infant. Note scarce number of neurons in B-3.

neurons was found in the 23/22 female/male couple. This could mean that a real increase in the number of neurons took place after birth, as was previously reported in the human sexually dimorphic nucleus of the hypothalamus [17] and in the vasopressin and oxytocin containing neurons of the pig hypothalamus at puberty [18]. Other studies have reported higher neuronal densities and neuronal numbers in different males human cortical loci, a greater density of neurons in women posterior temporal cortex and an increase in the neuropil/neuronal processes in female cerebral cortex [6,14,19].

This study is limited to a total of eight infants only. The results are based on the staining methods that we used, the GCm stains around 1–20% of the neurons. However, the

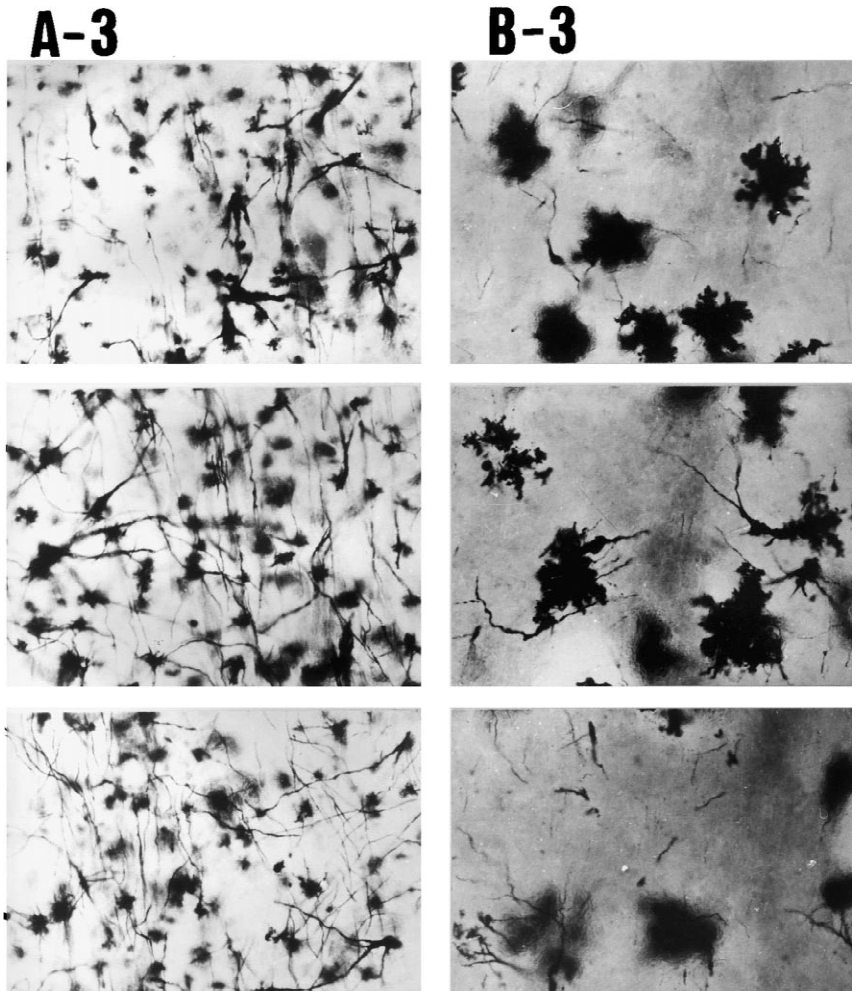

Fig. 8. Photomicrographs of mnr as in Fig. 7. Note the large size of astrocytes in 8/B-3. (GCm, ×100).

Table 3
Comparison of the number and proportion of neurons between a female (aged 2 days) and a male (aged 2 days) according to the Nissl method[a]

	Female			Significance		Male		
	N	Mean	S.D.	P_t	P_w	*N*	Mean	S.D.
	Number							
Small neurons	10	31.1	11.16	0.0083	0.00684	10	18.2	8.05
Large neurons	10	32.6	9.72	0.009	0.01150	10	21.9	5.76
Total	10	63.7	20.13	0.005	0.00389	10	40.1	11.05
	Proportion							
		Small	Large	Total	Small	Large	Total	
Neurons		311	326	637	182	219	401	
%		48.82	51.18		45.39	54.61		
			$\chi_1^2 = 1.031$		$P = 0.3098$			

[a] N = number of drawings, P_t = probability with t test. P_w = Wilcoxon's test probability.

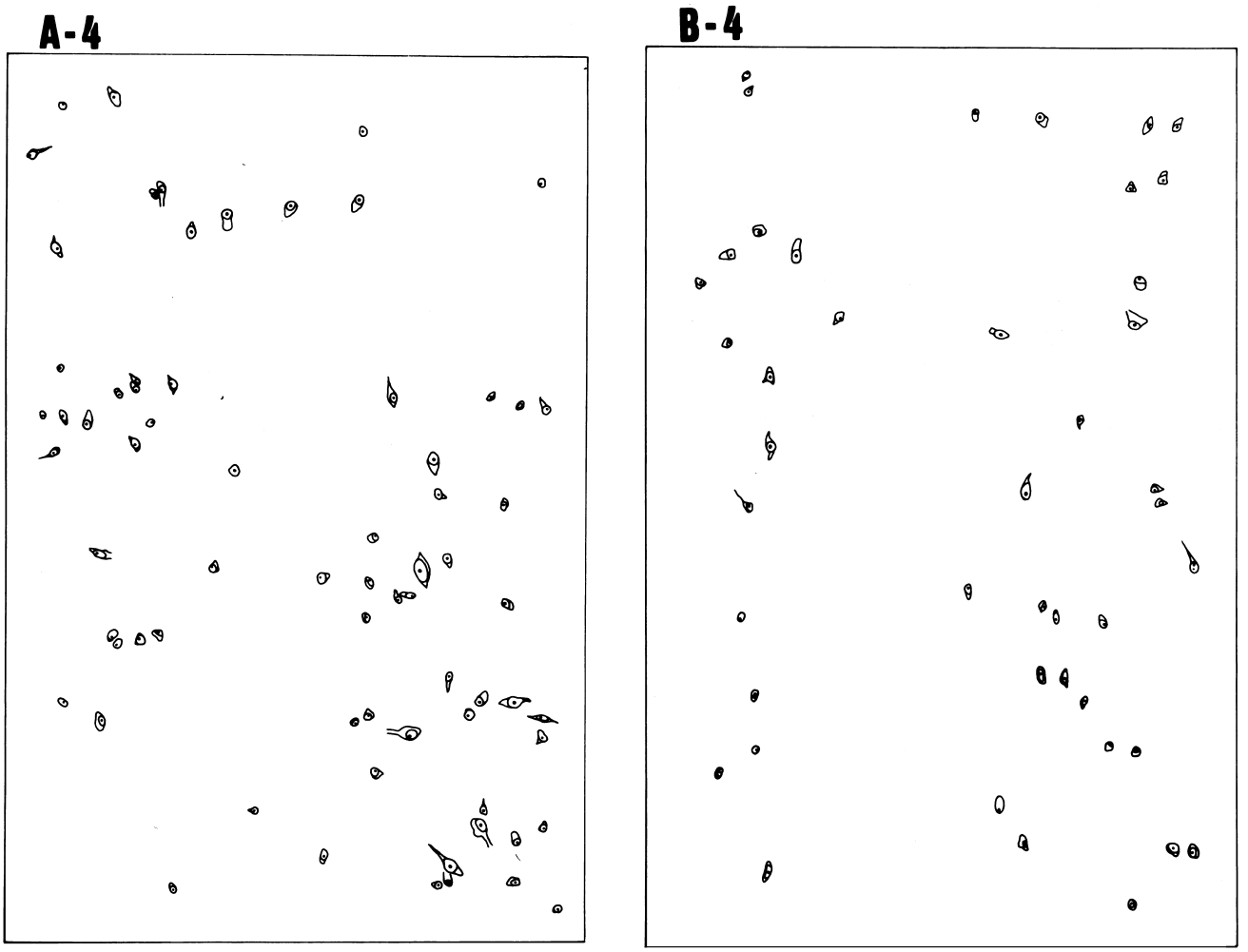

Fig. 9. Drawings of neurons from the mnr with the Nissl method. Large and Small neurons in a 2-day-old female (A-4) and in a 2-day-old male (B-4). In A-4 both neuron types are more numerous. (magnification ×400).

Nm stains most of the neurons and is in agreement with the findings of the GCm. This study has to be replicated and extended, using larger samples and focusing in other brain regions as well. These neurohistological differences between sexes could have implications in sex dimorphic behavior and neuropsychiatric disorders.

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