reticulum and lamellar complex. The failure of previous investigators to find adenylate cyclase activity in the intracellular structures of the cell, in our opinion, may be due to the effect of the prolonged fixation, the use of ATP as substrate, and also, perhaps, to other causes. Evidence of the intracellular localization of adenylate cyclase also was given by the results of other studies [1, 2, 8] in which activity of hormone-sensitive adenylate cyclase was found in the fraction of membranes of the endoplasmic reticulum and lamellar complex from hepatocytes and in isolated thymocytes.

The results of the present investigation thus indicate that adenylate cyclase is present not only on the plasma membrane, but also in the intracellular structures of brain neurons and glial cells.

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MORPHOMETRIC STUDY OF PURKINJE CELLS IN THE DOG CEREBELLAR CORTEX

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The cerebellum, which plays an important role in reflex responses of the brain stem and higher levels of the CNS, not only performs the function of "movement modulator" [3], but also, as experimental and clinical investigations [15] have shown, it participates in sensory integration, in learning skilled movements, in visual and auditory discrimination, regulation of emotions and the level of wakefulness, and pain perception. The Purkinje cells (PC) are the only neurons in the cerebellum along whose axons information leaves the cerebellum. That is why information on their number and structural state is essential in order to understand the functions of this part of the brain. It must be recalled that although close together, or even neighboring, PC have different specific functions [3], i.e., the PC population is heterogeneous. Investigations by various morphological techniques [12, 14] have revealed pale, dark, and intermediate PC. However, their arrangement in the cerebellar cortex is not yet known.

The object of this investigation was to study the distribution of different types of PC in three sagittal zones of the cerebellum, differing in their physiological characteristics [6].

EXPERIMENTAL METHOD

Experiments were carried out on seven mature mongrel dogs of both sexes. The brain was removed from the skull immediately after the animals had been killed under electrical anesthesia. The right half of the cerebellum was divided in the sagittal plane into three parts, corresponding to medial (I), intermediate (II), and lateral (III) zones [9]. After

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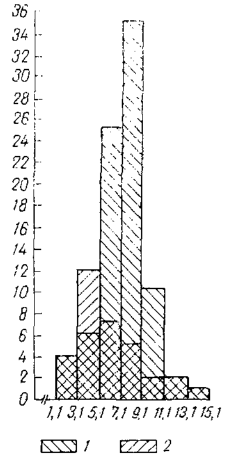

Fig. 1

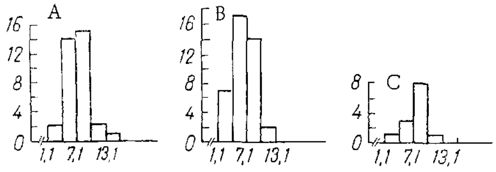

Fig. 2

Fig. 1. Histograms of distribution of PC by number per millimeter length of layer in upper and lower parts of cerebellar gyri. Abscissa, number of PC/mm length of layer; ordinate, number of situations corresponding to intervals plotted along abscissa. 1) Upper part of gyri, 2) lower part of gyri.

Fig. 2. Histograms of distribution of PC by number per millimeter length of layer in anterior, middle, and posterior lobes of medial zone of cerebellum. A) Anterior, B) middle, C) posterior lobes of cerebellum. Remainder of legend as to Fig. 1.

TABLE 1. Number of Normal and Modified PC in Different Functional Zones of Dog Cerebellar Cortex (M ± m)

Zone	Normal PC		PC with changes		Total number of PC/mm length of layer
	per mm length of layer	% of total number of PC in zone	per mm length of layer	% of total number of PC in zone	
I	5,9±0,2	89	0,7±0,1	11	6,6±0,3
II	6,8±0,3	92	0,6±0,1	8	7,4±0,3
III	7,4±0,7	94	0,5±0,2	6	8,0±0,6

fixation for 2 h in Carnoy's fluid followed by standard processing, the material was embedded in paraffin wax. Sections 5-7 μ thick were cut from the paraffin blocks and stained with cresyl violet by Nissl's method. The layer of PC was drawn from sections with the aid of a photographic enlarger (×10).

The length of the PC layer on the drawing was measured by means of a curvimeter. The PC were counted and their morphology studied under the microscope with a magnification of 400 times. For each zone of the cerebellum studied the total number of PC, the number of normal PC (pale, dark, intermediate), and the number of PC with morphological changes were counted per millimeter length of the PC layer. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The results of analysis of the number of PC in the functional zones of the cerebellum are given in Tables 1 and 2. As Table 1 shows, the total number of PC in zone I was significantly smaller than in zones II and III ($P_{I-III} < 0.05$; $0.05 < P_{I-III} < 0.1$). The number of PC with morphological changes in zones I, II, and III did not differ significantly. The main changes discovered were those of "heavy disease" of the neurons, with swelling and lysis of the basophilic substance. PC with pericellular edema, hydropic changes, and vacuolation of the cytoplasm were seen. However, the percentage of PC with morphological changes did not exceed 11% of the total number of PC.

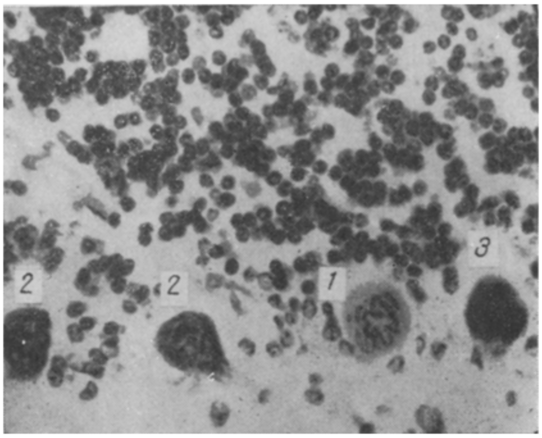

Fig. 3. Cerebellar cortex of intact dog: 1) pale PC, 2) intermediate PC, 3) dark PC. Cresyl violet by Nissl's method, 400 ×.

TABLE 2. Number of Different Types of PC in Medial, Intermediate, and Lateral Zones of Dog Cerebellum (M ± m)

Zone	Pale PC		Dark PC		Intermediate PC	
	per mm length of layer	% of total number of PC in zone	per mm length of layer	% of total number of PC in zone	per mm length of layer	% of total number of PC in zone
I	3,6±0,2	61,0	0,11±0,05	1,7	2,2±0,3	37,3
II	4,3±0,4	63,0	0,12±0,04	1,8	2,4±0,2	35,2
III	4,6±0,4	62,6	0,05±0,02	0,7	2,7±0,4	36,7

Differential analysis of the different types of normal PC showed (Table 2) that the different zones differed in the number of pale PC: They were fewer in number in zone I than in zones II and III ($P_{I-III} < 0.05$; $0.05 < P_{I-II} < 0.1$). The number of dark and intermediate PC was practically the same in the different zones. A heterogeneous distribution of PC also was found in the upper and lower parts of the gyri of the cerebellum. Histograms of distribution of PC by number per millimeter length of the PC layer in the corresponding regions of gyri are illustrated in Fig. 1. In the upper part of the gyri the number of PC was significantly greater than in the lower part (7.5 ± 0.2 and 5.3 ± 0.4 respectively; $P < 0.001$).

By distinguishing regions in the drawings of the sections of zone I corresponding to the anterior, middle, and posterior lobes of the cerebellum, the number of PC in them could be counted. Histograms of distribution of PC by their number per millimeter length of the PC layer in the anterior, middle, and posterior lobes of the cerebellum are given in Fig. 2. The difference between the total number of PC in these regions was not significant.

The morphometric investigation yielded quantitative and qualitative characteristics of the PC population in the medial, intermediate, and lateral zones of the cerebellum. These zones differ in their connections with different regions of the cerebral cortex, in their relative proportion of "peripheral inputs," and they participate in the control of different types of movements [6]. The medial zone of the cerebellum, corresponding to the vermis, and the intermediate and lateral zones, corresponding to the hemisphere, differ in their phylogenetic origin and in their times of ontogeny, as well as in their vulnerability to various pathological processes [13]. It has also been suggested that they even have different mediators [10]. Morphological differences in the total number of PC and in the number of pale PC between the medial, intermediate, and lateral zones revealed by their experiments thus reflect the functional heterogeneity of these regions. The longitudinal zones in the cerebellum are not only functional but also structural units. That is evidently why, during transverse division of the cerebellum into anterior, middle, and posterior lobes, no differences in the number of PC are formed.

The significant difference between the number of PC in the upper and lower parts of the cerebellar gyri shown by these experiments confirms the results obtained previously by qualitative evaluation of PC density [9].

Regions in which the number of PC per millimeter length of layer is greater than in other regions were thus discovered. These are zones II and III, corresponding to the hemisphere, and within the zones, the upper part of the gyri. It was shown previously that in these regions of the cerebellum the PC are smaller and have a narrower distribution of their dendritic tree [11]. Consequently, correlation exists between the number of PC per millimeter length of layer and the dimensions of the cells and arborization of their dendrites.

Differential analysis of the different types of PC (pale, dark, intermediate) (Fig. 3) showed that differences in the number of PC in the medial, intermediate, and lateral zones are connected with the number of pale PC. Pale and dark PC have different concentrations of RNA, DNA, and phospholipids [14] and they differ in their acid phosphatase activity [12]. The dark PC have a more highly developed lamellar complex [8] and they contain more free ribosomes and cisterns of the endoplasmic reticulum. The cytoplasm of the dark cells is much more osmiophilic than that of the pale cells. The dimorphism of PC is linked with the different functional states of the dark and pale cells [12]. The dark cells have a higher level of metabolism, and after a period of active work they become pale, when they are in a state of relative rest, but through intracellular physiological regeneration the pale cells change once more into dark [1].

Consequently, the number of dark and pale PC is a fundamentally important parameter reflecting the ratio between active and "resting" cells, and it is this ratio which determines the stability of function of an organ [5]. The fact that although the number of pale PC and the total number of PC in the zones studied in these experiments were different, the relative percentages of the different types of PC in these regions were similar is particularly interesting from this point of view.

As was pointed out above, besides normal PC, cells with morphological changes also were found. Some of these changes were transient in character, such as lysis of the basophilic substance [4]. Other changes, especially swelling, reflect the state of intensive activity of the neurons [2]. However, PC with "heavy disease," leading to irreversible changes in the neurons, and also with vacuolation of the cytoplasm, evidence of aging of the cell according to Bessis [7], also were found. The presence of a relatively small number of PC with changes of this type is in accordance with views regarding age changes in neurons [4], the result of which is a decrease in the number of PC with age [7].

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