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Brain RNA and Hypophysectomy; A Topographical Study

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Abstract

The present study was designed to localize the effect of hypophysectomy on brain RNA content at a topographical level. For this purpose, a brain dissection method, based on morphological differences between various brain areas, was developed. In these areas, total cell RNA was measured and expressed as the RNA/DNA ratio. The highest ratio was found in the cortex cerebri (ca. 2.15) and hippocampus (2.21), whereas cerebellum (0.42) and bulbus olfactorius (0.95) showed the lowest values. Hypophysectomy reduced the RNA content mainly of brain-stem structures (ca.–24%), i.e., the thalamus, hypothalamus, mesencephalon, and medulla oblongata. Nevertheless, a small but significant reduction was found in the rostral cortex. In view of previous studies, it is suggested that this disturbed RNA metabolism in brain-stem structures could have a bearing on the impaired avoidance performance of hypophysectomized rats.

Key words
Hypophysectomy
Brain dissection
RNA

Our laboratory has been interested in the role of pituitary-adrenal system hormones in active avoidance conditioning; part of these studies concerned the performance of hypophysectomized rats [de Wied, 1964, 1969; de Wied *et al.*, 1972]. It has been reported that hypophysectomy markedly impairs the acquisition of a conditioned avoidance response in shuttle box conditioning [Applezweig and Baudry, 1955; de Wied, 1964]. Recent studies have also shown that removal of the pituitary causes a marked alteration in RNA metabolism in the brain [Gispen *et al.*, 1970]. This alteration is probably related to the poor performance of rats so treated [Gispen *et al.*, 1971] and it is tempting to speculate that hypophysectomy affects central nervous function, leading to impaired avoidance behavior [de Wied

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et al., 1972]. Our previous studies were concerned with the biochemical effects of hypophysectomy at a subcellular level in the brain. The present report deals with an attempt to localize the effect of hypophysectomy on RNA metabolism by measuring RNA content in various parts of the brain.

Materials and Methods

Animals and Surgery

Male albino Wistar rats of an inbred strain, weighing 110–120 g, were used. Hypophysectomy was performed via the transauricular route under light ether anesthesia. Macroscopic examination of the sella turcica, loss of body weight, and adrenal atrophy (both adrenals weighing less than 10 mg) served to confirm that the surgery had been performed correctly.

Time Schedule of the Experiment

Hypophysectomy and sham operations were performed on day 1. Animals remained in their home cages for 21 days and were subsequently sacrificed by decapitation. A group of intact rats weighing 110–120 g were decapitated on the day of the biochemical analysis and used as controls.

Brain Dissection

Immediately after decapitation, the skull was opened and the brain was rapidly removed and placed on an ice-cooled PVC plate. Following removal of the blood vessels and membranes from the surface of the brain, dissection was performed within 5 min, using two pairs of sharp forceps. Figures 1–4 represent various aspects of the brain, schematized in such a way that relevant borders of the dissected tissues are shown. The various tissues are numbered and the numbers correspond to those used in the figures. The dissection method is based on morphological differences between the brain regions and takes into account natural borders, such as ventricles, nerve fibres, etc.

Fig. 1a, b. Ventral aspect and medium sagittal section of the brain. For details on dissection see Materials and Methods. Key to numbers:

1 Amygdala with overlying cortex pyriformis
2 Commissura anterior
4 Brachium pontis
5 Bulbus olfactorius with tractus olfactorius
6 Cerebellum
7 Crus cerebri
8 Corpus callosum
9 Cortex cerebri
13 Hippocampus
15 Hypophysis
16 Hypothalamic fissure
17 Hypothalamus
18 Corpora mammillare
19 Medulla oblongata
20 Medulla spinalis
21 Mesencephalon
23 Chiasma opticum
24 Paraflocculus
25 Pons with pyramidal tract
26 Area preoptica
27 Fissura rhinalis
28 Septum
29 Thalamus
A Transverse section A
B Transverse section B

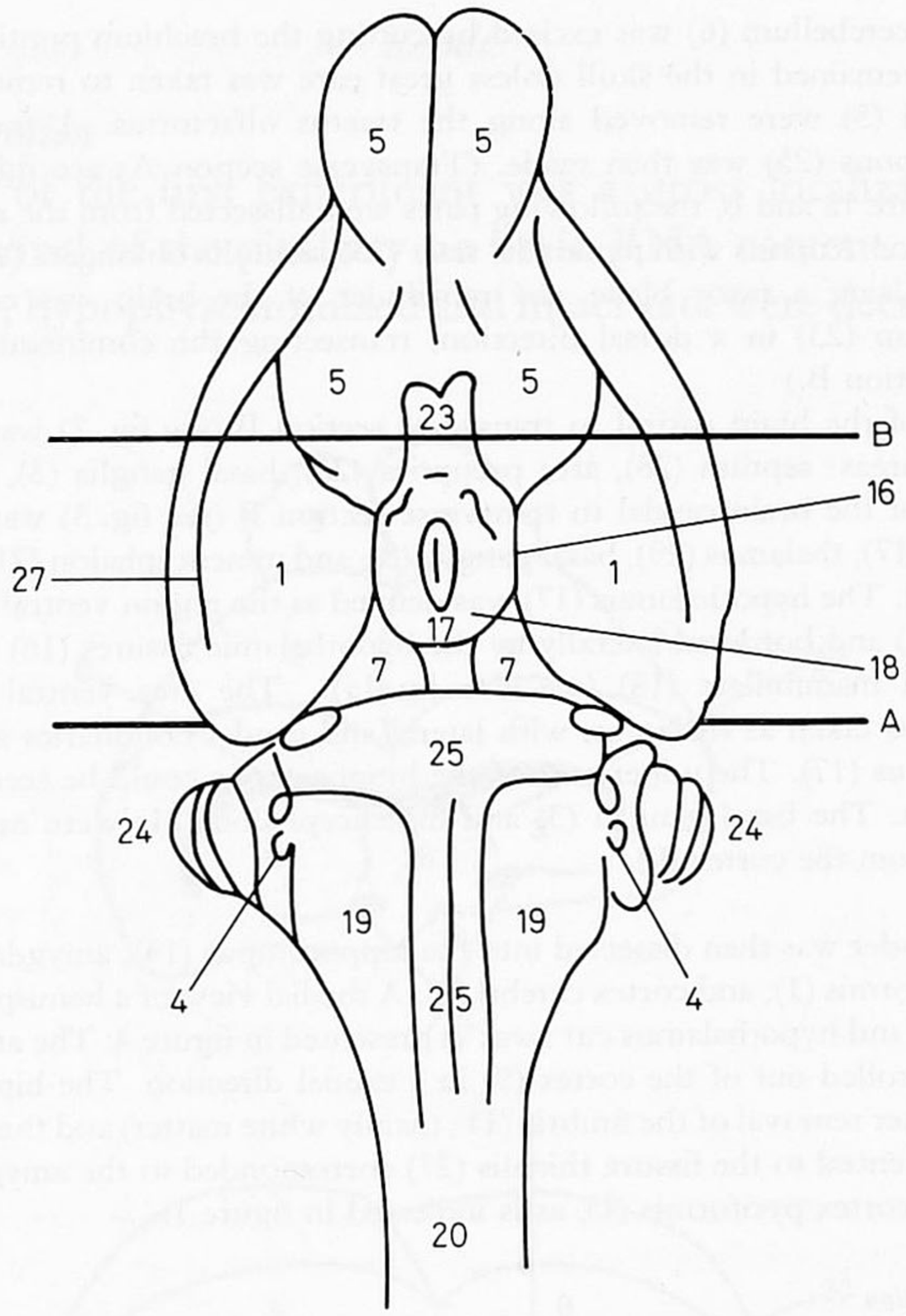
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Fig. 1a

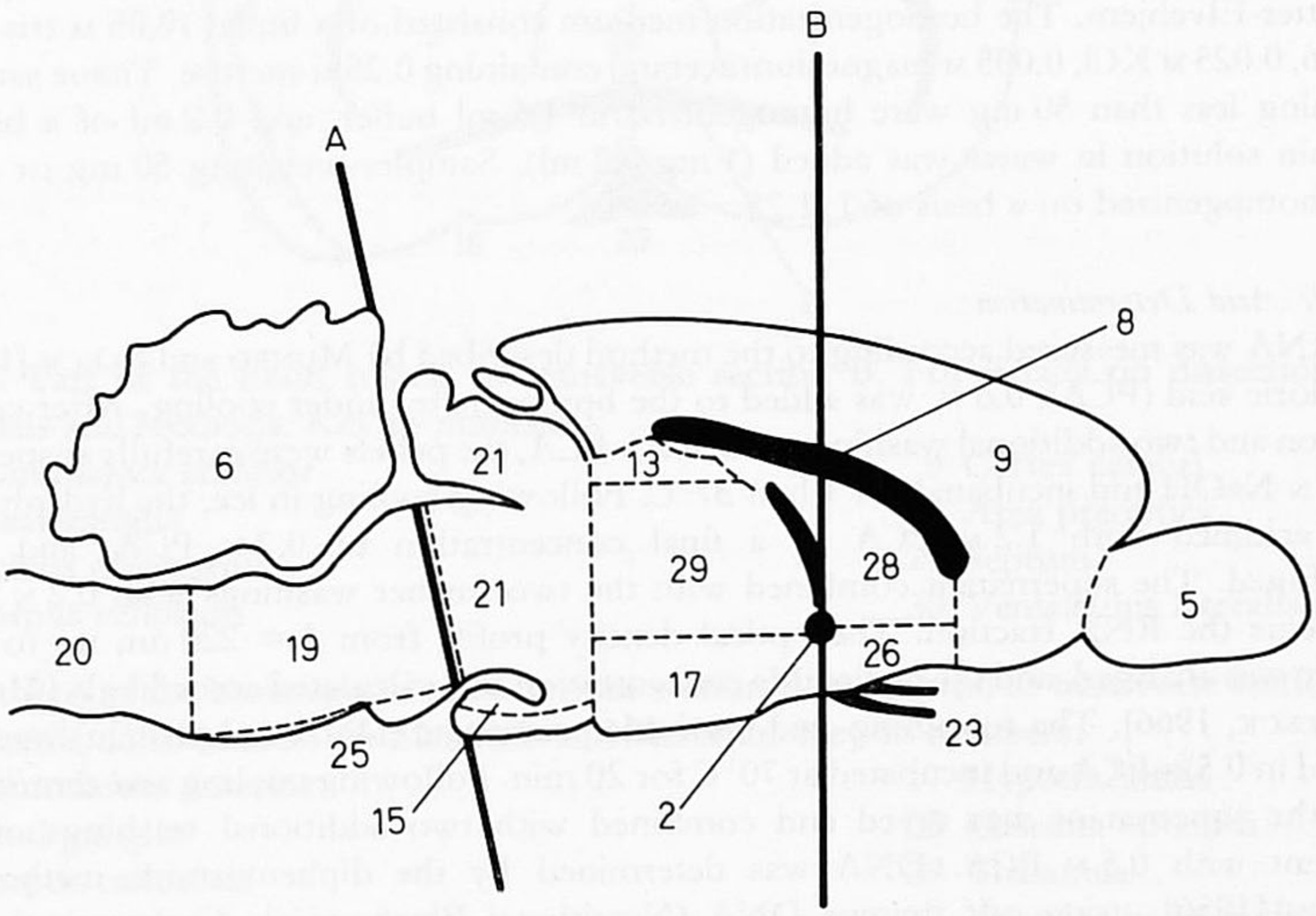
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Fig. 1b

First, the cerebellum (6) was excised by cutting the brachium pontis (4). The paraflocculus (24) remained in the skull unless great care was taken to remove it also. The bulbi olfactorii (5) were removed along the tractus olfactorius. A transverse section rostral to the pons (25) was then made. (Transverse section A; see fig. 1a and b.) As depicted in figure 1a and b, the following parts were dissected from the region caudal to transverse section A: pons with pyramidal tract (25), medulla oblongata (19), and medulla spinalis (20). Using a razor blade, the remainder of the brain was cut through the chiasma opticum (23) in a dorsal direction, transecting the commissura anterior (2). (Transverse section B.)

The part of the brain rostral to transverse section B (see fig. 2) was dissected into the following areas: septum (28), area preoptica (26), basal ganglia (3), and cortex (9).

The part of the brain caudal to transverse section B (see fig. 3) was dissected into hypothalamus (17), thalamus (29), basal ganglia (3), and mesencephalon (21). The pituitary was not studied. The hypothalamus (17) was defined as the region ventral to the commissura anterior (2) and bordered laterally by the hypothalamic fissures (16) and posteriorly by the corpora mammillare (18) (see also fig. 1a). The area ventral to the corpus callosum (8) was taken as thalamus, with lateral and caudal boundaries as described for the hypothalamus (17). The upper part of the hippocampus could be seen after removal of the thalamus. The basal ganglia (3) and mesencephalon (21) were easily recognized and removed from the cortex (9).

The remainder was then dissected into the hippocampus (13), amygdala with overlying cortex pyriformis (1), and cortex cerebri (9). A medial view of a hemisphere with most of the thalamus and hypothalamus cut away is presented in figure 4. The area indicated by 12 and 13 was rolled out of the cortex (9) in a caudal direction. The hippocampus (13) was obtained after removal of the fimbria (11; mainly white matter) and the gyrus dentatus (12). The area ventral to the fissura rhinalis (27) corresponded to the amygdalar complex with overlying cortex pyriformis (1), as is indicated in figure 1a.

Tissue Fractionation

After weighing, the various parts of the brain were homogenized manually, at 4 °C, using 5 up-and-down strokes of a loose-fitting teflon pestle in a homogenizer, according to Potter-Elvehjem. The homogenization medium consisted of a buffer (0.05 M tris-HCl, pH 7.6, 0.025 M KCl, 0.005 M magnesium acetate) containing 0.25 M sucrose. Tissue samples weighing less than 50 mg were homogenized in 1.5 ml buffer, and 0.2 ml of a bovine albumin solution in water was added (1 mg/0.2 ml). Samples weighing 50 mg or more were homogenized on a basis of 1:1.25.

Nucleic Acid Determination

RNA was measured according to the method described by MUNRO and FLECK [1966]. Perchloric acid (PCA), 0.6 N, was added to the homogenate, under cooling. After centrifugation and two additional washings with 0.2 N PCA, the pellets were carefully suspended in 0.3 N NaOH and incubated for 1 h at 37 °C. Following cooling in ice, the hydrolysates were acidified with 1.2 N PCA to a final concentration of 0.2 N PCA, and then centrifuged. The supernatant combined with the two further washings with 0.2 N PCA represents the RNA fraction. The optical density profile from $\lambda = 220$ nm up to $\lambda = 320$ nm was analyzed, and the nucleotide concentration was calculated accordingly [MUNRO and FLECK, 1966]. The remaining acid-insoluble precipitate (DNA and protein) was suspended in 0.5 N PCA and incubated at 70 °C for 20 min. Following cooling and centrifugation, the supernatant was saved and combined with two additional washings of the sediment with 0.5 N PCA. DNA was determined by the diphenylamine method of BURTON [1956], using calf thymus DNA (Nutritional Biochemicals Corporation) as a standard.

Results

Gross Localization

The aim of the first experiment was a gross localization of the effect of removal of the pituitary on brain RNA content. At 3 weeks after surgery, hypophysectomized and intact rats were decapitated and

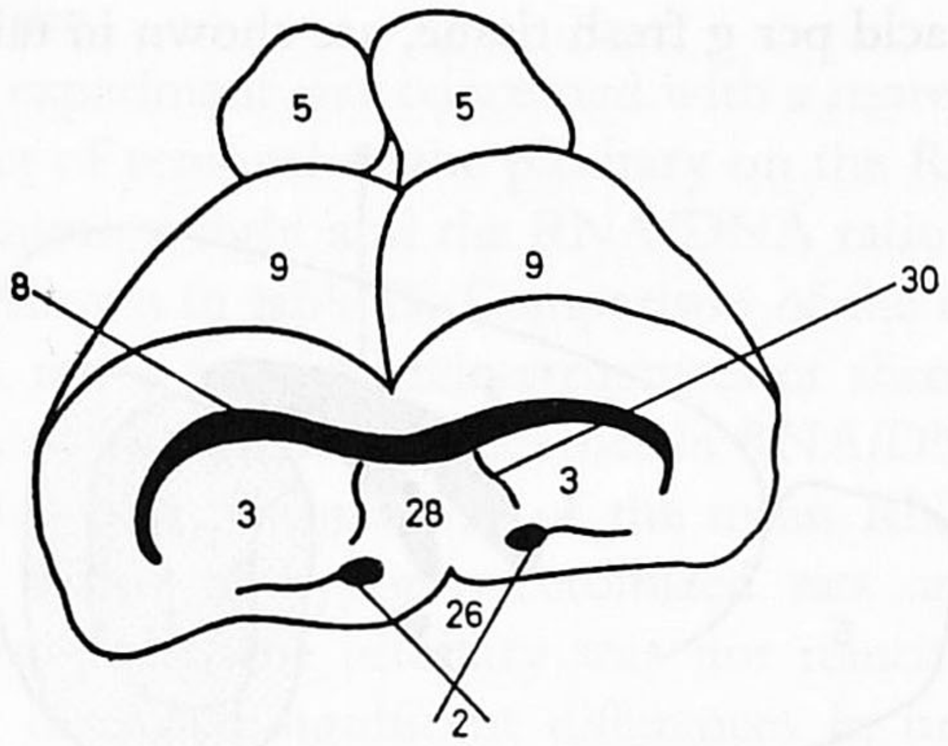

Fig. 2

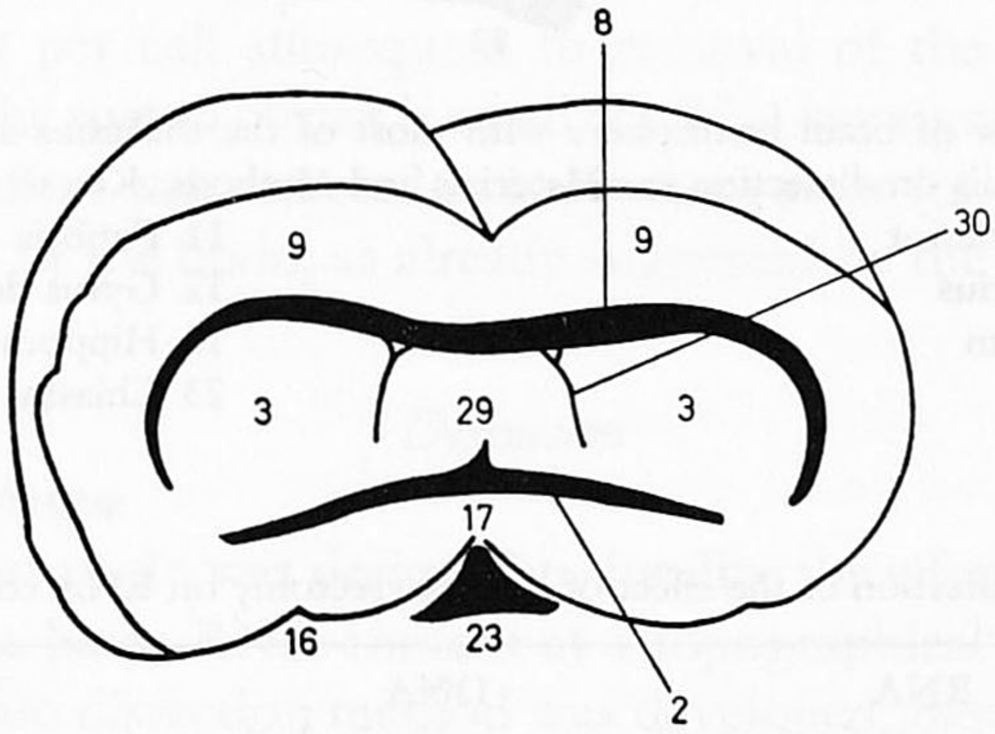

Fig. 3

Fig. 2. Part of the brain rostral to transverse section B. For details on dissection see Materials and Methods. Key to numbers:

2 Commissura anterior
3 Basal ganglia
5 Bulbus olfactorius
8 Corpus callosum
9 Cortex cerebri
26 Area preoptica
28 Septum
30 Ventriculus lateralis

Fig. 3. Part of the brain caudal to transverse section B and rostral to transverse section A. For details on dissection see Materials and Methods. Key to numbers:

2 Commissura anterior
3 Basal ganglia
8 Corpus callosum
9 Cortex cerebri
16 Hypothalamic fissure
17 Hypothalamus
23 Chiasma opticum
29 Thalamus
30 Ventriculus lateralis

their brains were removed and dissected into 3 major parts: cerebellum, cortex, and brain stem. The brain stem was defined as the subcortical part of the brain caudal to the transverse section B (fig. 1b). The cortex consisted of the cortex cerebri plus the subcortical structure rostral to the transverse section B, excluding the bulbi olfactorii. The brain parts were weighed and homogenized and the content of RNA and of DNA was measured. The data, calculated as mg nucleic acid per g fresh tissue, are shown in table I.

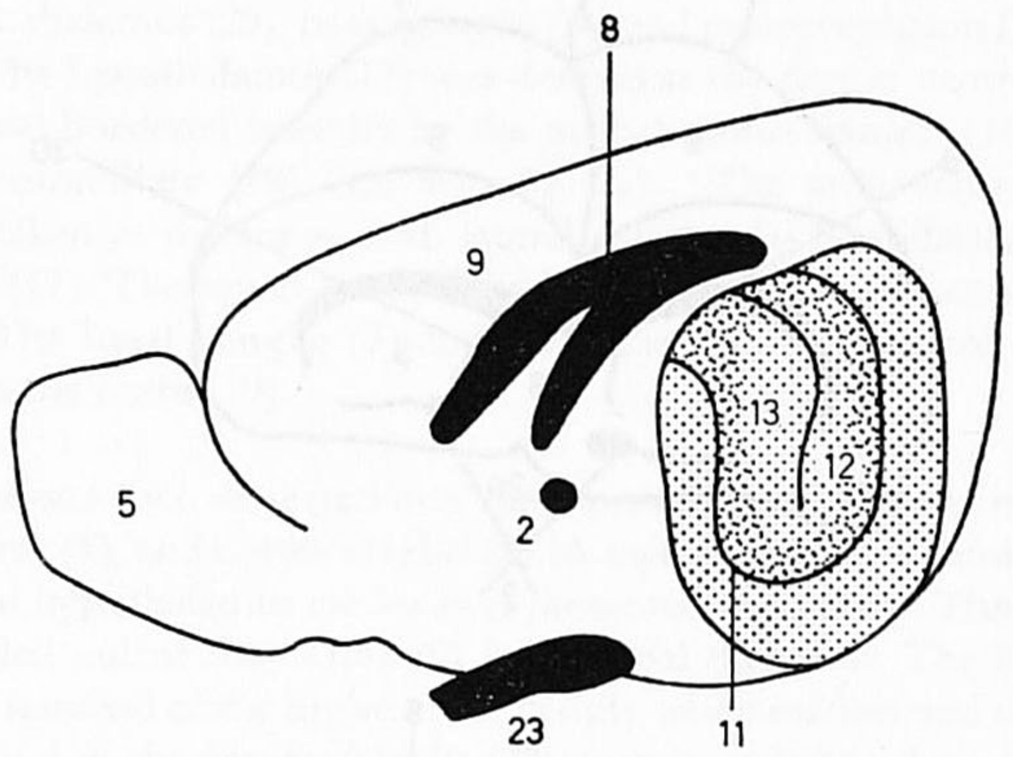

Fig. 4. Medial view of brain hemisphere with most of the thalamus and hypothalamus cut away. For details on dissection see Materials and Methods. Key to numbers:

2 Commissura anterior
5 Bulbus olfactorius
8 Corpus callosum
9 Cortex cerebri
11 Fimbria
12 Gyrus dentatus
13 Hippocampus
23 Chiasma opticum

Table I. Gross localization of the effect of hypophysectomy on RNA content in rat brain

	RNA mg/g fresh tissue	DNA mg/g fresh tissue	Ratio RNA/DNA	
Intact				
Cortex	1.77 ± 0.04[a]	1.03 ± 0.06	1.74 ± 0.07	(5)
Cerebellum	2.07 ± 0.08	4.91 ± 0.29	0.43 ± 0.01	(4)
Brain stem	1.46 ± 0.05	0.99 ± 0.04	1.47 ± 0.04[c]	(4)
Hypox[b]				
Cortex	1.55 ± 0.02	1.04 ± 0.04	1.51 ± 0.07	(6)
Cerebellum	1.90 ± 0.04	4.83 ± 0.23	0.40 ± 0.01	(5)
Brain stem	1.35 ± 0.03	1.13 ± 0.03	1.19 ± 0.04[c]	(5)

[a] Mean ± SEM.
[b] Hypophysectomized.
[c] $p < 0.025$ (t-test).
( ) = Number of rats.

As a measure of total RNA content per cell, the RNA/DNA ratio was introduced. It can be seen that there is no significant difference in the RNA/DNA ratio between control and hypophysectomized rats for cerebellum and cortex. However, the RNA/DNA ratio of brain-stem tissue from hypophysectomized rats was significantly lower than that of controls.

Defined Localization

The second experiment was concerned with a more exact localization of the effect of removal of the pituitary on the RNA content in the brain. The mean weight and the RNA/DNA ratio of the various brain parts are shown in table II. Comparison of the mean values of the RNA/DNA ratios for the brain structures of sham-operated and intact rats revealed no significant difference in RNA/DNA ratio in any of the areas. However, comparison of the mean RNA/DNA ratios obtained from brains of hypophysectomized rats and those from brains of rats in which the pituitary was not removed (intact and sham-operated) disclosed significant differences in brain-stem areas and in the rostral cortex cerebri. In these areas, there is a reduced RNA content per cell subsequent to removal of the pituitary. The reduction in the rostral cortex is small (11.2 %) but significant. A more pronounced effect was detected in brain-stem areas (ca. 15–22 %) than in other areas of the brain, as already suggested by the data in table I.

Discussion

Dissection Technique

The present study was designed to localize the effect of hypophysectomy on the brain RNA content at a topographical level. For this purpose, a brain dissection method was developed, based on morphological differences between the various brain regions. The areas used could be distinguished visually and were separated along natural borders, such as ventricles, nerve fibers, and myelinated and unmyelinated tissue. Only the division of the hypothalamus and thalamus was arbitrary. The hypothalamus was defined as ventral to the anterior commissure and the reproducibility of the dissection of these areas was based on the constant weight of the tissue samples in different experiments.

Topographical RNA Distribution

The RNA/DNA ratios of the various brain regions of intact rats determined in the present study are in good agreement with, but not

identical to, those reported by others [JACOB and MANDEL, 1966; SCHULZECK *et al.*, 1970; RAPPOPORT *et al.*, 1969]. The discrepancy in absolute values is probably the result of differences in methods of analysis and in the different extinction coefficients used for calculation. Nevertheless, the regional distribution for RNA in the brain reported here is similar to that described by other investigators. Differences in the RNA/DNA ratio between the various regions are related to the morphological characteristics of these regions. Since the DNA content per amount of tissue is proportional to the number of nuclei, one may conclude from the relatively high DNA content and low RNA/DNA ratio of the cerebellum and bulbus olfactorius that numerous small neurons are present in these areas, an assumption that is consistent with morphological data [RAPPOPORT *et al.*, 1969].

Effect of Hypophysectomy

It is obvious that hypophysectomy exerts its effect mainly on brain-stem areas. This observation corroborates the data of DE VELLIS and INGLISH [1968], who suggested that removal of the pituitary would affect brain-stem RNA metabolism in particular. Indeed, our previous work showed profound effects of hypophysectomy on uridine incorporation into RNA and on the polysome content in the brain stem [GISPEN *et al.*, 1970; GISPEN and SCHOTMAN, 1970]. However, in the experiment on gross localization, hypophysectomized rats were compared with intact rats that weighed 110–120 g and were, therefore, approximately 3 weeks younger than the former animals. It is known that the RNA and polysome content in the brain decreases during the life span of the rat [ADAMS, 1966; MURTHY, 1966; YAMAGAMI and MORI, 1970; DELLWEG *et al.*, 1968]. The observed difference between hypophysectomized and intact rats may, then, have been caused by the difference in age between these groups. It was partly for this reason that a sham-operated control group was introduced in the second experiment.

Although the RNA content of some brain areas tended to be lower in the sham-operated animals, no significant differences in the RNA/DNA ratio between the intact and sham-operated groups could be demonstrated. Comparison of the data obtained from hypophysectomized rats with those from rats that had a functioning pituitary (intact and sham-operated rats) revealed a clear-cut regional effect of hypophysectomy on brain RNA content. As expected from the first experiment, mainly brain-stem areas but also cortex, rostral to transverse

Table II. The influence of hypophysectomy and sham operation on RNA content of various areas of rat brain

	Mean weight, mg	Ratio RNA/DNA[a] intact	sham-operated	hypox[b]
Medulla spinalis	49.1± 7.1[c] (8)	2.06±0.06 (8)	2.07±0.12 (10)	1.70±0.10 (10)**
Pons and pyramidal tract	18.6± 1.5 (8)	1.72±0.11 (8)	1.65±0.09 (9)	1.54±0.08 (9)
Medulla oblongata	124 ±10 (8)	2.04±0.06 (8)	1.99±0.07 (7)	1.71±0.05 (10)**
Mesencephalon	143 ± 3 (8)	1.82±0.07 (8)	1.67±0.07 (10)	1.57±0.03 (10)*
Hypothalamus	33.1± 1.4 (8)	1.98±0.06 (8)	1.90±0.12 (10)	1.58±0.05 (10)**
Thalamus	44.2± 1.9 (8)	1.93±0.09 (8)	1.83±0.05 (9)	1.62±0.04 (10)**
Basal ganglia (caudal)	61.1± 3.9 (8)	1.74±0.08 (8)	1.72±0.08 (8)	1.59±0.08 (8)
Basal ganglia (rostral)	41.2± 3.0 (8)	1.82±0.08 (8)	1.82±0.14 (9)	1.67±0.08 (10)
Septum	12.6± 2.1 (8)	1.75±0.08 (8)	1.62±0.10 (8)	1.53±0.11 (10)
Area preoptica	25.8± 2.3 (7)	1.67±0.08 (7)	1.55±0.09 (8)	1.54±0.06 (9)
Hippocampus	81.4± 6.7 (8)	2.21±0.06 (8)	2.29±0.10 (10)	2.03±0.06 (10)
Amygdala with overlying cortex pyriformis	85.5± 4.5 (6)	2.34±0.04 (6)	—	2.21±0.03 (7)
Cortex cerebri (caudal)	386 ±11 (8)	2.08±0.05 (7)	2.01±0.07 (10)	1.94±0.04 (10)
Cortex cerebri (rostral)	302 ±15 (8)	2.25±0.04 (8)	2.18±0.11 (10)	2.01±0.04 (9)*
Bulbus olfactorius	84.5± 7.8 (8)	0.95±0.04 (8)	0.88±0.08 (8)	0.78±0.04 (8)
Cerebellum	198 ± 4 (8)	0.42±0.01 (8)	0.41±0.01 (11)	0.39±0.04 (10)

[a] mg RNA/g fresh tissue/mg DNA/g fresh tissue.
[b] Hypophysectomized.
[c] Mean ± SEM.
() Number of rats.
* $p<0.05$; ** $p<0.025$. Hypophysectomized versus intact plus sham-operated (*t*-test).

section B (fig. 1a and b), were involved. The small but significant difference in the rostral cortex could have been masked in the first experiment by the presence of other tissue in which there was no effect.

Removal of the pituitary has been reported to influence brain macromolecule metabolism in general [see review by GEEL and TIMIRAS, 1970]. Although there are reports on metabolic changes taking place in the cortex cerebri [DE VELLIS and INGLISH, 1968; GEEL and TIMIRAS, 1970; CHEEK and GRAYSTONE, 1969], other data suggest that it is in the brain stem that the metabolism of proteins and monoamines is particularly affected by hypophysectomy [DE MAIO, 1959; DE VELLIS and INGLISH, 1968; HYYPPA and VALAVAARA, 1970; SHCHEDRINA, 1970]. The hormones secreted by the pituitary and its target organs are known to exert an action on brain metabolism, especially in the developing brain [GEEL and TIMIRAS, 1970]. Not only growth hormone, thyroid hormone, and steroids, but, as was recently reported, ACTH itself might affect protein metabolism in central nervous structures [GEEL and TIMIRAS, 1970; JAKOUBEK *et al.*, 1970, 1971]. For the moment, one cannot ascribe the effect of hypophysectomy to the lack of one single hormone. It is more likely that the disturbed balance of a number of hormones would cause the observed phenomenon.

The fact that the effect is located mainly in the thalamus, hypothalamus, mesencephalon, and medulla oblongata might imply that the activity of an integrated system such as the reticular formation might be influenced by hypophysectomy. Furthermore, evidence is accumulating that the structure and function of thalamic and mesencephalic areas in particular are important in the regulation of conditioned avoidance behavior [VAN WIMERSMA GREIDANUS and DE WIED, 1971; DE WIED *et al.*, 1972; GLASSMAN, 1969; GISPEN *et al.*, 1971]. As was already pointed out in the introduction, hypophysectomized rats show a marked decrease in avoidance learning. It may, therefore, be hypothesized that the metabolism of macromolecules in this part of the brain in particular might be important for behavioral adaptation.

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