A QUANTITATIVE STUDY OF THE SUPRAOPTIC NEURONS OF THE ALBINO RAT AND TWO DESERT RODENTS, GERBIL AND KANGAROO RAT

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ABSTRACT

A QUANTITATIVE STUDY OF THE SUPRAOPTIC NEURONS OF THE ALBINO RAT AND TWO DESERT RODENTS, GERBIL AND KANGAROO RAT

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Desert-dwelling rodents are, of necessity, efficient water economizers. Much of the recent interest in these animals has been focused on the renal and hormonal factors associated with the water conservation process. The important role of the supraoptic nucleus in elaborating the hormones essential for appropriate renal function, is a well-documented fact in the laboratory rat. The present study addresses itself to a quantitative analysis of these neurons in two desert rodents, the gerbil and kangaroo rat.

After appropriate histological preparation, the brains of these animals and those of two normal albino rats and two water-deprived albino rats were examined for signs of neurosecretory activity. The cells of the supraoptic nucleus (both the anterior nucleus and the much-neglected posterior portion, or "tuberal supraoptic nucleus") which reacted positively to neurosecretory staining, were then examined to assess the extent of activity in these nuclei.

The quantitative measures applied included estimates of the number of cells per nucleus, cell size, and number of nucleoli per cell.

The kangaroo rat, one of nature's most competent water economizers, with its ability to gain weight on a diet free from exogenous water, seems to demonstrate a functionally more active supraoptic nucleus than that found in either the gerbil or laboratory rat. This desert rodent has relatively more supraoptic neurons per gram body weight and more double nucleoli per cell than either of the other two animals. There is also some indication that cell size (relative to body weight) is greater in this animal than in the gerbil or laboratory rat. Correspondingly, the gerbil has more supraoptic cells, relative to body weight, than the laboratory rat; the data suggests, in addition, that gerbil supraoptic cells are larger than those in the rat. The fact that the supraoptic cells of a normal laboratory rat deprived of water for five days demonstrate changes that approach the conditions found in desert rodents, indicates that increases in cell size and number of nucleoli may be adaptive mechanisms which desert rodents have capitalized upon.

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ABBREVIATIONS USED IN FIGURES 1-5

MAM.	.mammillary area
NSO-A.	.supraoptic nucleus, anterior division
NSO-T.	.supraoptic nucleus, tuberal division
OFT.	.olfactory tubercle
OT	.optic tract
P	.area of the pons

INTRODUCTION

A desert environment would be swiftly fatal to the albino rat. The physiological systems that this animal has evolved for survival in his native temperate environment would be inadequate to handle the stress imposed by an arid climate. Lack of water, or more precisely, lack of a system adequate to cope with a harsh restriction of water, would certainly be a major factor contributing to its demise. Desert rodents, on the other hand, display a remarkable capacity to conserve water, thus ensuring their existence. Animals such as Australian desert mice (MacMillen and Lee, 1967), the Peruvian desert mouse, Phyllotis gerbillus (Koford, 1968), the kangaroo rat, Dipodomys merriami (Schmidt-Nielsen and Schmidt-Nielsen, 1950), the Egyptian gerbil, Gerbillus gerbillus (Burns, 1956; Schmidt-Nielsen, 1962) and the Mongolian gerbil, Meriones unguiculatus (Winkelmann and Getz, 1962), can subsist on a diet of air-dried seeds and other dry plant substances without additional drinking water.

What is it, then, about the desert rodent's physiology, that allows him to actually thrive in an environment unsuitable for many other species?

For the desert animal existing on a diet of dry grain, water is available from the preformed water absorbed in the grain and from metabolic water. These animals concurrently minimize body water loss by the production of a very concentrated urine, dry feces, absence of sweating, and restriction of skin and respiratory losses (Lockwood, 1963).

Renal Factors

The production of a very concentrated urine, much higher than that of the laboratory rat (MacMillen and Lee, 1967) is probably one of the desert rodent's most valuable means of economizing water loss. The kidney of the desert rodent comes equipped with certain adaptive features which seem to account for its heightened ability to restrict water loss. Gollschalk and Mylle (1959) have found that there is a strong correlation between the lengths of the segments of the renal tubule and the ability to concentrate urine. Three species of desert rodents, with capacities for high urine concentration, G. gerbillus, J. jaculus (Khalil and Tawfic, 1963) and Dipodomys merriami (Vimtrup and Schmidt-Nielsen, 1952), have distal and collecting ducts which differ in length and structure from those of the albino rat; they have a distinctive morphology and are much longer.

Hormonal Factors

Tubular readsorption efficiency is, of course, also dependent on the level of circulating antidiuretic hormone

(ADH), a hormone whose storage site is in the posterior lobe of the pituitary. Neurosecretory stains which stain the material of the posterior pituitary also selectively stain certain regions of the hypothalamus. The currently accepted hypothesis is that neurosecretory cells of the hypothalamus elaborate ADH (or its precursor), which then diffuses down the axons of the hypothalamic cells to the terminals in the neurohypophysis (Scharrer and Scharrer, 1963).

While three distinct areas in the vertebrate brain have been identified which react to neurosecretory stains, viz., the paraventricular, supraoptic and mammillo-infundibular nuclei (Smith, 1951), the main ADH manufacturing plant seems to be the supraoptic nucleus (NSO), since continued antidiuretic function is dependent on the integrity of the NSO, the hypothalamico-hypophyseal tract, and posterior pituitary (Fisher, Ingram and Ranson, 1938).

In desert rodents, with their "superior" kidneys, one would also expect to find evidence of a highly developed ADH system. Some evidence to this effect has already been adduced. Enemar and Hanström, 1956 (as cited in Thorn, 1958), have noted that the neuro-hypophyseal lobe of desert rodents and hibernating rodents is relatively larger than in species living in more temperate regions. Ames and van Dyke (1950) have demonstrated that the pituitary of D. merriami contains more ADH (0.9 milliunits/μg.) than

does the pituitary of the albino rat (0.3 milliunits/μg.); they have also found that the concentration of ADH excreted in the urine of this desert mammal is considerably higher than that found in the urine from the laboratory rat or dog. Howe and Jewell (1959) found that the posterior lobe of the pituitary of the desert rat, Meriones meriones, occupies about 17 percent of the total volume of the hypophysis, while in the laboratory rat it only accounts for about 10.5 percent of the total gland. They also noted that in 10-day water-deprived animals of this species, there was only a scant amount of neurosecretory material in the hypothalamus, indicating a depletion in response to the deprivation stress. Khalil and Tawfic (1963), who have examined the hypothalamo-hypophyseal neurosecretory systems of two desert rodents, J. jaculus and G. gerbillus, assert that there is a higher amount of active ADH synthesis in the cells of the NSO of these animals under normal conditions than there is in the albino rat. Their evaluation was based on certain criteria--staining characteristics, the large size of the cells, size and position of the nuclei, the large size of the nucleoli, and the position of the Nissl substance. Castel and Abraham (1969) have investigated the hypothalamic neuro-hypophyseal system in two species of spiny mice. Their results show marked changes in the system with increasing days of water deprivation on a seed diet.

Other than the evidence cited above, little has been contributed to our knowledge of NSO influence on water conservation function in desert rodents. The supraoptic nucleus deserves specific attention.

Neuroanatomical Considerations

First of all, within the supraoptic nucleus, an anatomical distinction should be made. It consists of two spatially separated portions: the anterior nucleus, which lies slightly caudal to the most rostral point of the optic chiasma, and the tuberal nucleus, a region which commences immediately behind the optic chiasma with cells clustering on the medial side of the optic tract. The tuberal portion of the supraoptic nucleus is seldom expressly considered; when mention is made of the NSO, unless otherwise specified, the authors are presumably referring to the main portion--the anterior supraoptic nucleus.

In most mammals, the tuberal NSO would be considered part of the anterior NSO if not for the discontinuity between the two areas, the small number of cells in certain species (Auer, 1951), and its diffuse arrangement of cells in some species (Auer, 1951; Bodian and Maren, 1951). However, the cells themselves appear to be morphologically similar to those of the anterior nucleus; both portions of the nucleus feature large polygonal or bipolar cells (Westwood, 1962; Malone, 1916; Legait, 1955).

The small amount of data that presently exists indicates that the tuberal NSO is not only morphologically but also functionally similar to the anterior NSO. Peterson (1966) noted that this area in the albino rat stains much in the same manner as the cells of the anterior portion, indicating neurosecretory activity. (Only Cotte and Picard (1968) report that the tuberal portion of the nucleus fails to show signs of neurosecretion.) Cells in both areas show signs of cellular distortion and a general chromatolytic response when the animals have been deprived of water. Cells of both areas degenerate in much the same fashion when hypophysectomy is performed (Bodian and Maren, 1951), or when the hypothalamus is isolated (Bleier, Bard and Woods, 1966). For the above reasons, any study that involves itself with a description of the supraoptic hormonal system, must concern itself with both portions of the nucleus.

Statement of Purpose

The purpose of the present study was to provide a systematic comparison between the neurosecretory supraoptic cells of desert rodents, both anterior and tuberal portions, with those of the laboratory rat. The comparisons were based on certain quantitative measures, i.e., cell size, number of cells in a nucleus, and number of nucleoli per cell. Caspersson's extensive work with various cell types

(1950), has led him to the conclusion that conspicuous changes in protein metabolism within a cell's nucleus are correlated with nerve function. Edstrom, Eichner and Schör (1961) have demonstrated that the ribonucleic acid content (and therefore nuclear protein metabolism) in NSO cells increases during water deprivation. It was therefore anticipated that the NSO cells in the animals selected for study would show signs of nucleolar activity in correspondence to the ability of these animals to resist dehydration. It was also expected that the number and size of the NSO cells would reveal a correlation with the differing abilities of these animals to withstand water deprivation.

The cells were first tested for reaction with neurosecretory stains to establish that the regions under study in the desert rodents were the functional homologues, that is, neurosecretory centers, of those regions in the laboratory rat.

Reliance on these stains for demonstrating neurosecretion was based on its correlation with information derived from several sources. Bachrach (1964) has demonstrated a secretory cycle in the NSO cells of the laboratory rat. He placed his rats on a dehydration treatment, since this is a well-known method for inducing significant ADH mobilization. Single animal groups were sacrificed at eight

days in the dehydration period and after periods of 12 days of subsequent rehydration. From the changes of ribonucleic acid content and Nissl pattern of the cells and their change in size, a systematic pattern emerged which indicated a definite secretory cycle; changes in amount of Gomori-positive material reflected the same cycle. Dawson's work (1966) indicates a correlation between microscopic findings and bio-assay determinations of neurosecretory activity. He investigated the staining pattern of the neuro-hypophysis and hypothalamus of fetal and postnatal rats, and found that the onset of ADH production in fetal rats, as detected by staining, occurs on the eighteenth gestational day, which coincides with the findings by bio-assay technique. In addition, the two techniques demonstrate a parallel portrayal of progressive amounts of neurosecretion in maturing rats, increased production reflected by density of staining, or, in the case of bio-assay, by the number of units of extractable hormone.

In the present study, both neurosecretory staining characteristics, and three quantitative measures of NSO cells, were used to provide a composite picture of cellular activity in two desert rodents and the laboratory rat. The gerbil and kangaroo rat were the two desert animals chosen. The hypothesis was that desert animals compensate for restricted water availability by being able to conserve the

water that is available to them, through the use of the anti-diuretic hormone-producing system. While both the gerbil and kangaroo rat are able water economizers, the kangaroo rat is superior to the gerbil in its ability to concentrate urine and gain weight on a diet free from exogenous water (Winkelmann, 1962; Schmidt-Nielsen, 1951). It was therefore anticipated that the kangaroo rat would reflect this difference in the measures of neurosecretory cellular activity.

METHOD

Subjects

Subjects were two, adult, male kangaroo rats (*Dipodomys merriami*), two, adult, male gerbils (*Meriones unguiculatus*), and four male Holtzman albino rats, 100-110 days old on arrival to the laboratory.

Procedure

The eight animals were housed in individual wire cages and maintained on a diet similar to their normal regimen; for the desert animals, mixed seeds (approximately 12% preformed water by weight) were available ad libidum; the albino rats were allowed access to Wayne Mouse-Breeder Blox and water.

When their weights had stabilized, the gerbils, kangaroo rats and two of the albino rats were anesthetized with ether and perfused through the left ventricle with physiological saline and formalin; the two treatment animals were kept in their cages five additional days, without access to water, before being sacrificed. The brains were removed and embedded in celloidin. Serial sections (15 μ thick) through the hypothalamus were taken; one brain of each species was sectioned in a horizontal plane, the other

in a sagittal plane. Alternate sections of each brain were stained with the following: (1) Thionin was used as the standard cell stain, (2) Bargmann's modification of Gomori's Chrome-Alum-Hematoxylin for neurosecretion, and (3) Gomori's Aldehyde-Fuchsin for neurosecretion. Two neurosecretory stains were used for comparative purposes; however, the Chrome-Alum-Hematoxylin stain prepared for this study did not act selectively, and therefore could not be used for purposes of demonstrating neurosecretion. (For details on histological preparation of the tissue refer to Appendix B.) The stained sections were mounted on glass slides and covered with "1" size cover-slips (0.13-0.16 mm thick).

Estimates of cell populations of the NSO were obtained. A sample of supraoptic cells was examined to determine cell size and number of nucleoli. (Refer to Appendix B for a description of the sampling procedure and methods of obtaining measurements.)

RESULTS

Neurosecretory Staining

Figures 1-4 show the relationship between the anterior and tuberal portions of the NSO, and the surrounding hypothalamic tissue in the normal albino rat (NR), the five-day deprived albino rat (DR), the gerbil (G), and the kangaroo rat (KR). Figure 5 is an example of the same nuclei stained with Aldehyde-Fuchsin. The darkly stained supraoptic nuclei are readily distinguishable from the paler surrounding tissue which remained unreactive. Under high power the cells are usually not recognizable as discrete units but as irregularly-shaped packets or clumps of stained material. When individual cells can be identified, the darkest staining material appears massed at the periphery. The supraoptic nuclei in all animals reacted with the neurosecretory stain.

Number of Cells

Results of cell counts are presented in Figures 6a and 6b. Figure 6a indicates the absolute number of cells for each animal; Figure 6b shows number of cells per gram body weight. Because of the fact that some sections of NSO tissue in the normal rat were lost in histological preparation,

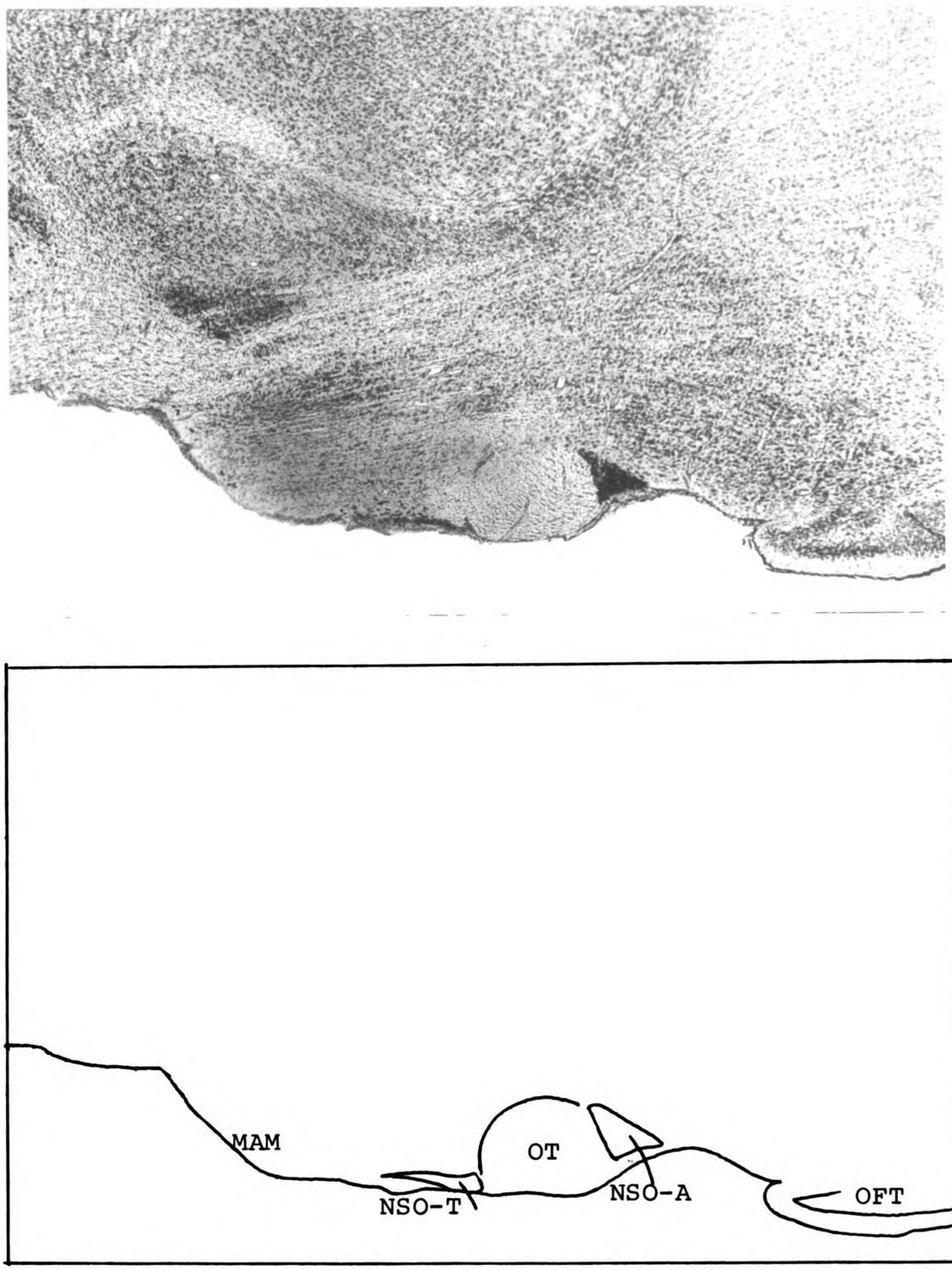
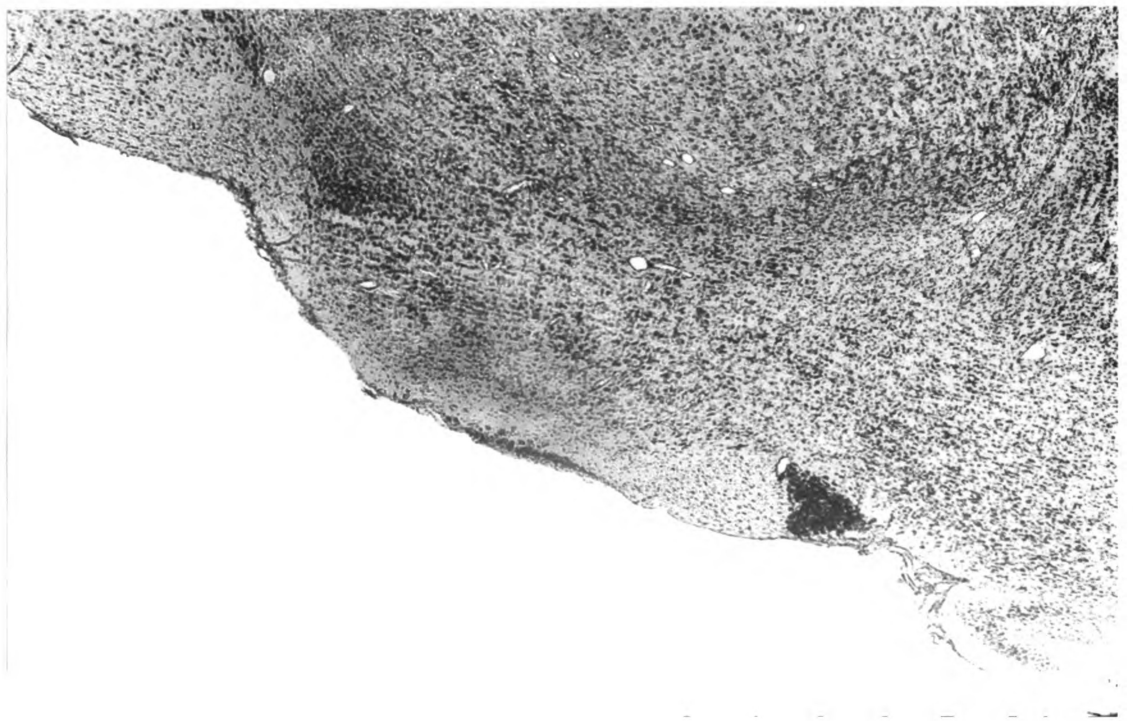


Figure 1. Photomicrograph of a sagittal Thionin-stained section through the hypothalamus of a normal albino rat at the level of the supraoptic nuclei. Magnification: x 30.



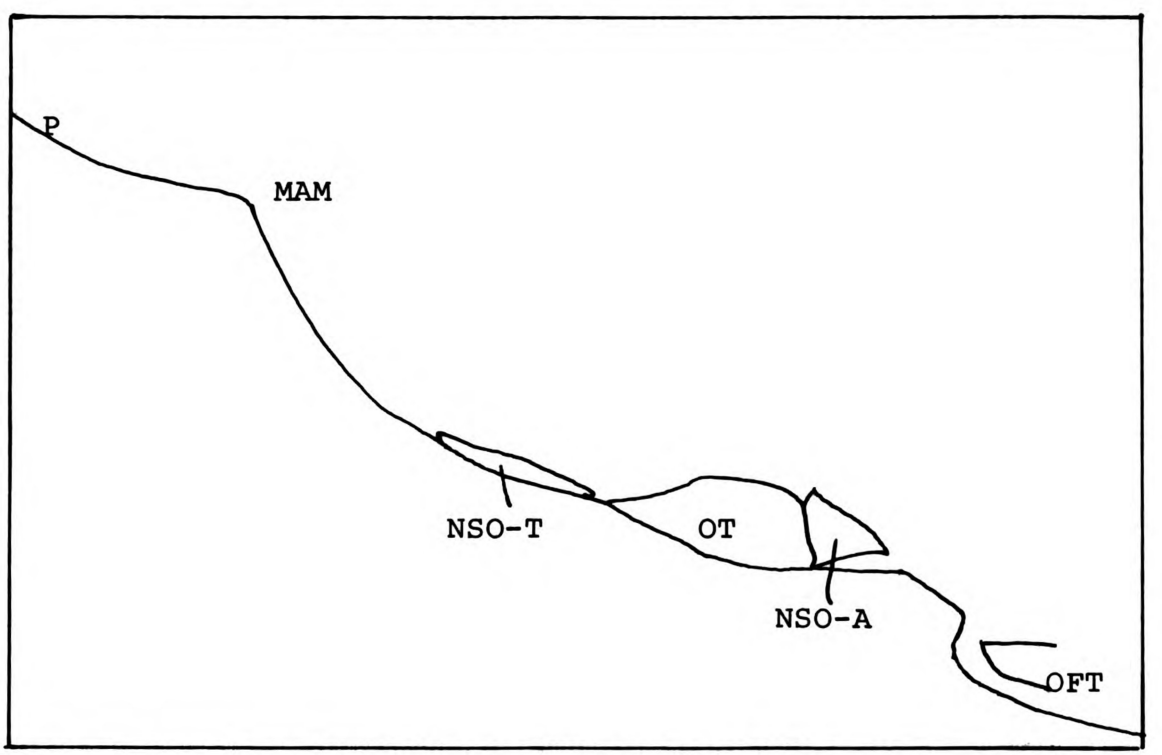


Figure 2. Photomicrograph of a sagittal Thionin-stained section through the hypothalamus of a five-day water-deprived albino rat at the level of the supraoptic nuclei. Magnification: x 30.

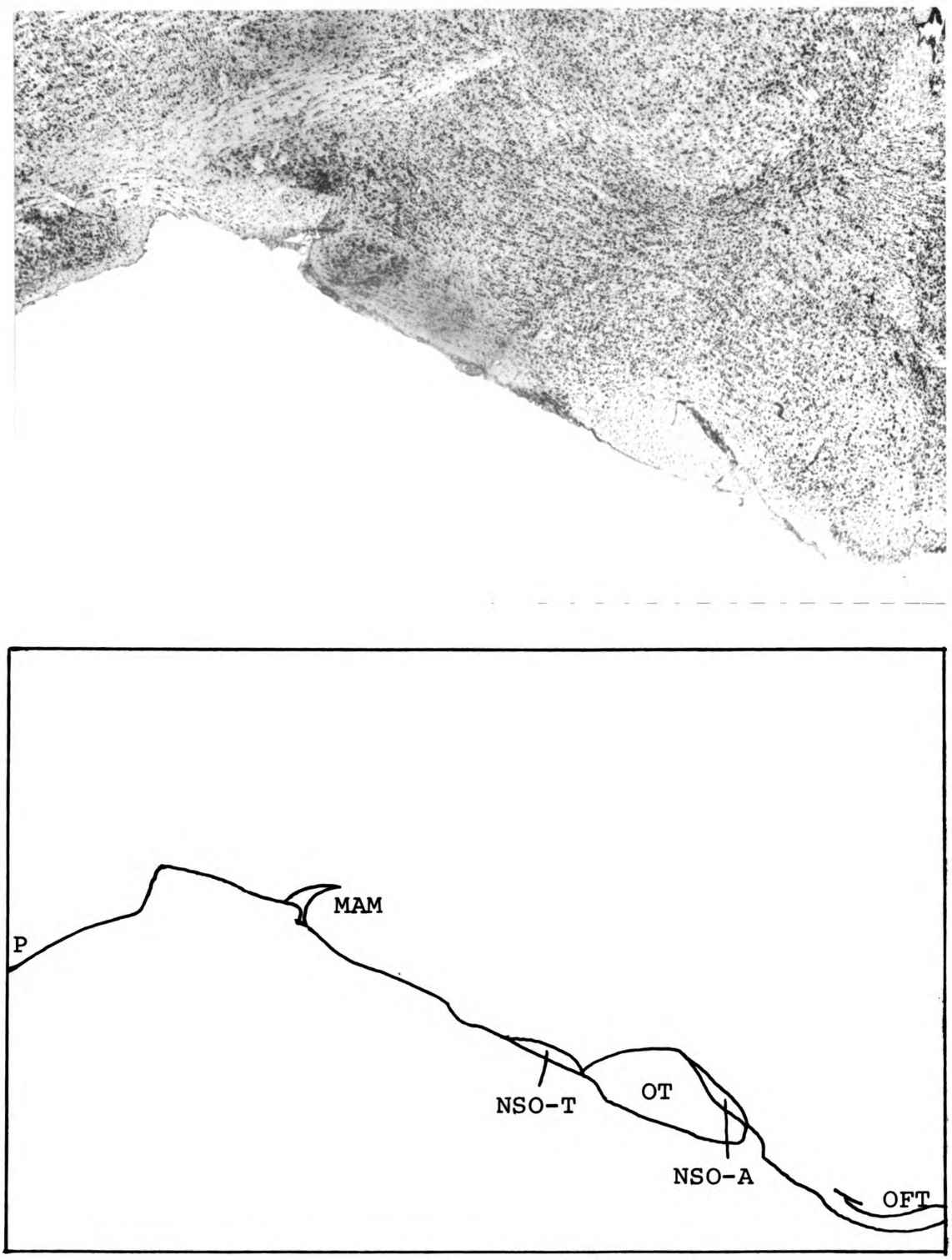


Figure 3. Photomicrograph of a sagittal Thionin-stained section through the hypothalamus of a gerbil at the level of the supraoptic nuclei. Magnification: x 30.

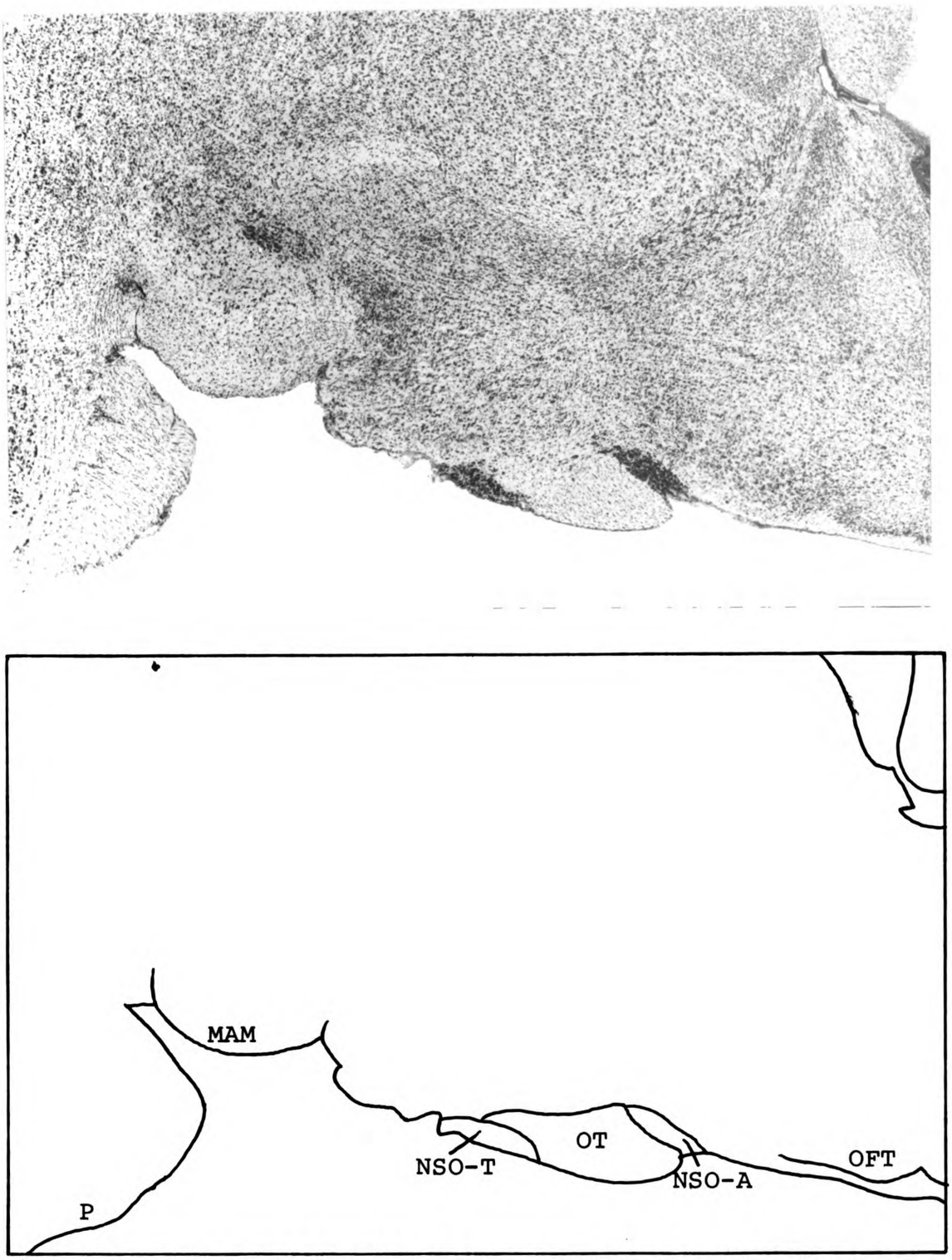


Figure 4. Photomicrograph of a sagittal Thionin-stained section through the hypothalamus of a kangaroo rat at the level of the supraoptic nuclei. Magnification: x 30.

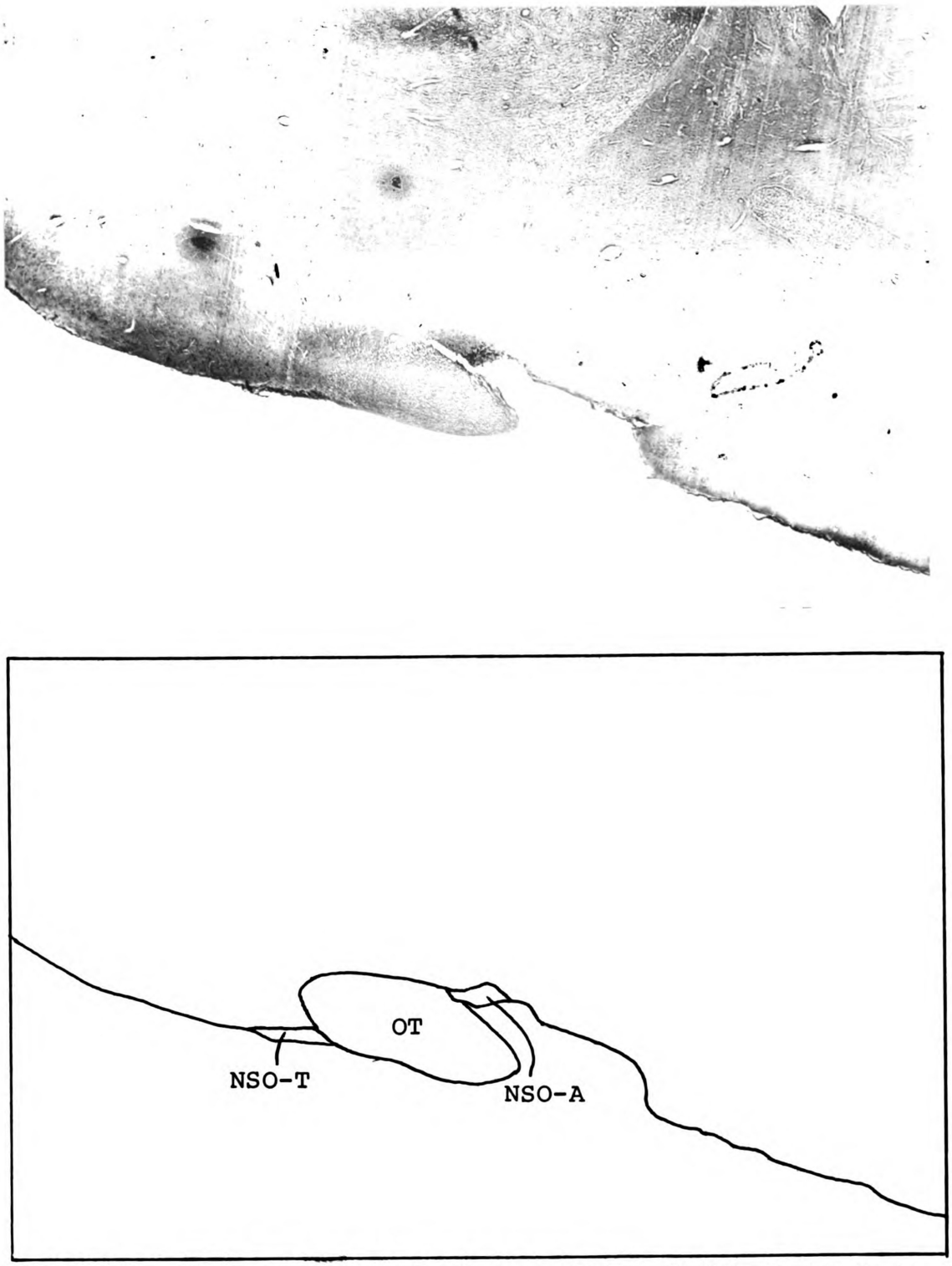


Figure 5. Photomicrograph of a sagittal Aldehyde-Fuchsin stained section through the hypothalamus of a normal albino rat at the level of the supraoptic nuclei. Magnification: x 30.

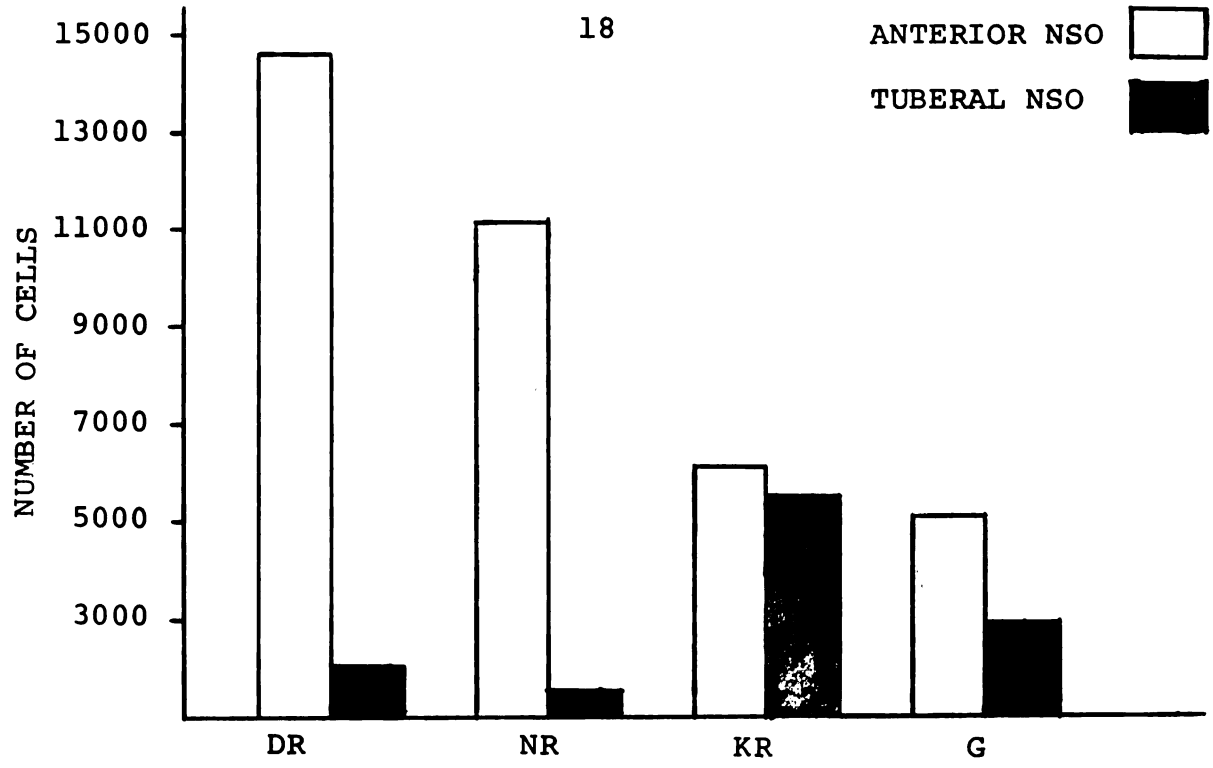


Figure 6a. Number of NSO cells by nuclear division in four animals.

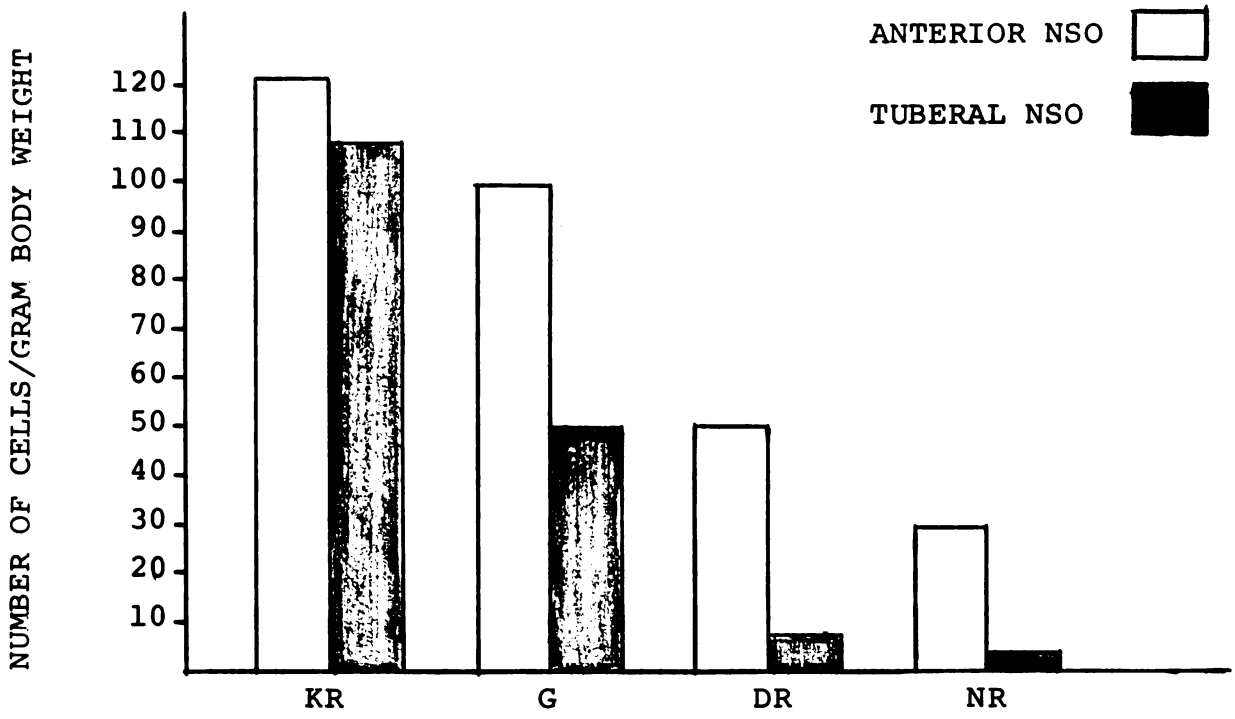


Figure 6b. Number of NSO cells, expressed as number of cells per gram body weight, by nuclear division, in four animals.

we relied on the data of Hatton and Johnson (1968). The fact that their estimate of the number of cells in the albino rat is smaller than ours cannot be attributed to the deprived state of our rats, but is probably due to section thickness differences; since their sections were 10 μ thicker, it is assumed that a percentage of their cells escaped observation. Bodian and Maren's estimates (1951) came closer to ours, but the weights of their rats could not be obtained. In any case, the absolute number of cells in the rat, normal or deprived, exceeds that of either the kangaroo rat or gerbil; KR has a greater number than G. In relation to body weight, KR has the largest number of cells, G next, followed by DR and NR. (The deprived rat does not actually have more cells; the apparent differences between DR and NR is only a result of a large weight loss in the deprived rat, causing, by way of numerical transformation of the data, the deprived rat to have more cells per unit weight.)

Cell Size

Mean cell areas in horizontal and sagittal planes for each animal are presented in Table 1. Differences in cell area between planes appeared to be due to differential shrinkage of the brains rather than to the orientation of the cells, since (1) ratio comparisons of tuberal to anterior NSO remained

Table 1. Mean cell areas of supraoptic neurons.

Animal	Anterior NSO	Tuberal NSO
Deprived Rat Sagittal	198.64 μ^2 (S=1.55)	207.11 (S=1.83)
Deprived Rat Horizontal	191.86 μ^2 (S=1.31)	230.87μ^2 (S=1.92)
Normal Rat Sagittal	131.49μ^2 (S=1.21)	131.69μ^2 (S=1.21)
Normal Rat Horizontal	151.28μ^2 (S=1.02)	156.12μ^2 (S=1.41)
Gerbil Sagittal	73.72μ^2 (S=.59)	73.76μ^2 (S=.59)
Gerbil Horizontal	136.53μ^2 (S=1.43)	140.29μ^2 (S=1.25)
Kangaroo Rat Sagittal	154.38μ^2 (S=1.40)	160.25μ^2 (S=1.24)
Kangaroo Rat Horizontal	132.81μ^2 (S=1.37)	148.80μ^2 (S=1.02)

essentially constant except for the deprived rat (Table 2), and (2) there was no consistent biasing toward larger cells in one particular plane. The basis for selecting planes, then, for purposes of inter-animal comparisons, was the plane with the least amount of shrinkage. For the normal rat this was the horizontal plane; for the gerbil, the horizontal; for the kangaroo rat, the sagittal. Selecting the plane for the deprived rat posed a problem, for while the sagittal DR anterior appeared to suffer less shrinkage than the horizontal, the situation in the tuberal portion was reversed. The sagittal plane was finally chosen over the horizontal on the basis of two observations: (1) The ranges of the horizontal anterior and tuberal cells were contained within the ranges of the sagittal anterior and tuberal cells (horizontal anterior: 142.56 - 256.20 μ^2, sagittal anterior: 136.36 - 287.19; horizontal tuberal: 152.89 - 305.79, sagittal tuberal: 140.50 - 367.77) and (2) a comparison of means between anterior and tuberal supraoptic cells revealed significant differences in only the deprived rat horizontal, and possibly deprived rat sagittal or kangaroo rat sagittal. (See Table 3, * items.)

Mean cell areas and standard errors of the four animals in the planes selected are displayed in Figures 7a and 7b. Figure 7a depicts the absolute values of cell area, while Figure 7b shows cell areas expressed as number of units per gram body weight.

Table 2. Cell area ratios computed as the ratio between tuberal and anterior portions of the NSO.

ANIMAL	HORIZONTAL	SAGITTAL
DEPRIVED RAT	1.20	1.04
NORMAL RAT	1.03	1.00
GERBIL	1.03	1.00
KANGAROO RAT	1.12	1.04

Table 3. A summary of Student's t values from a comparison of means conducted on cell area results.*

			Kangaroo-Rat		Gerbil		Normal Rat		Deprived Rat		Deprived Rat	
			Sagittal		Horizontal		Horizontal		Horizontal		Sagittal	
			A	T	A	T	A	T	A	T	A	T
Kangaroo Rat	Sagittal	A										
		T	1.07*									
Gerbil	Horizontal	A	3.06									
		T		3.89	.68*							
Normal Rat	Horizontal	A	.61		2.88							
		T		.75		16.29	.95*					
Deprived Rat	Horizontal	A	6.70		9.77		8.32					
		T		10.55		13.48		10.70	5.74*			
Deprived Rat	Sagittal	A	7.25		13.60		8.71		1.14			
		T		7.25		10.43		7.57		3.07	1.21*	

x: Values of t greater than 2.62 are significant at the .01 level of confidence (df=98). 1.98 .05
1.29 .20
1.04 .30

*: Designates anterior to tuberal comparisons within species and plane.

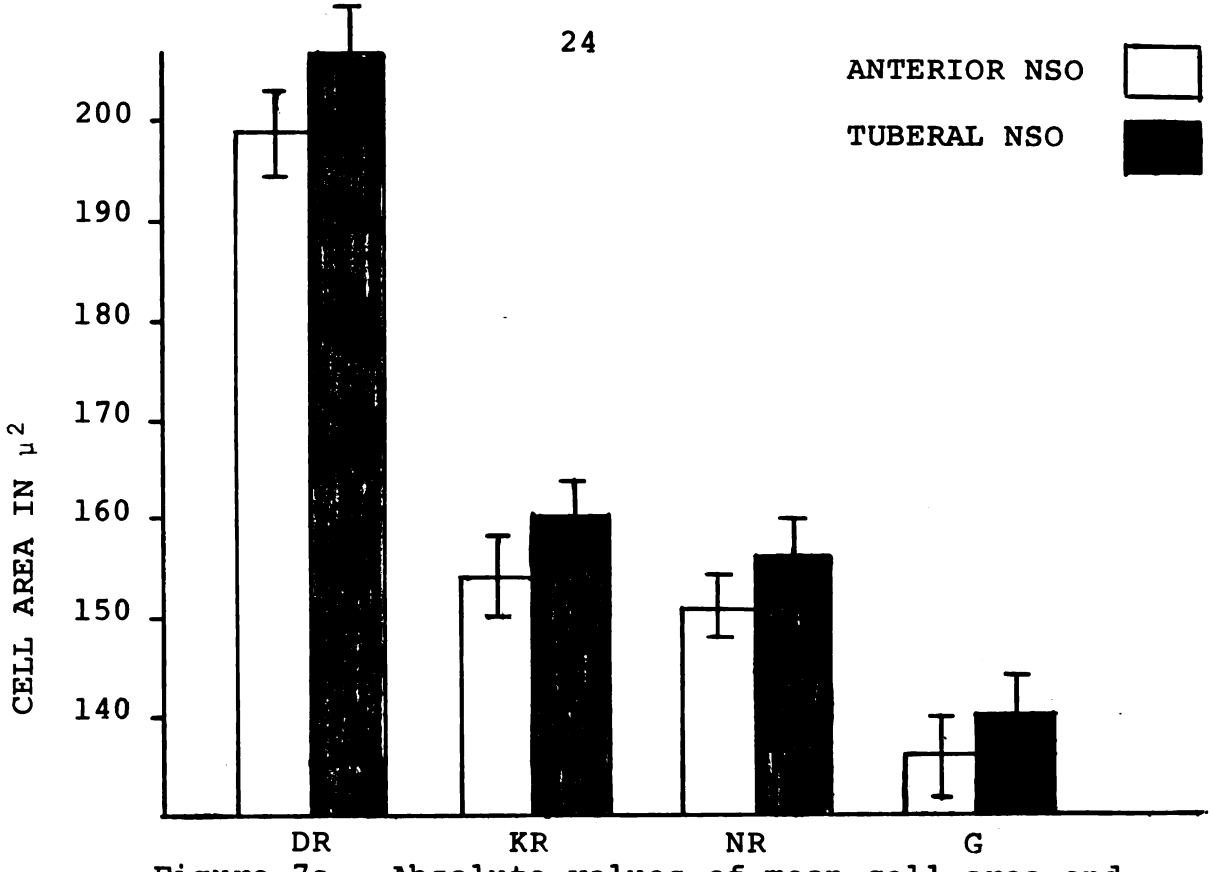


Figure 7a. Absolute values of mean cell area and standard error of supraoptic neurons in four animals.

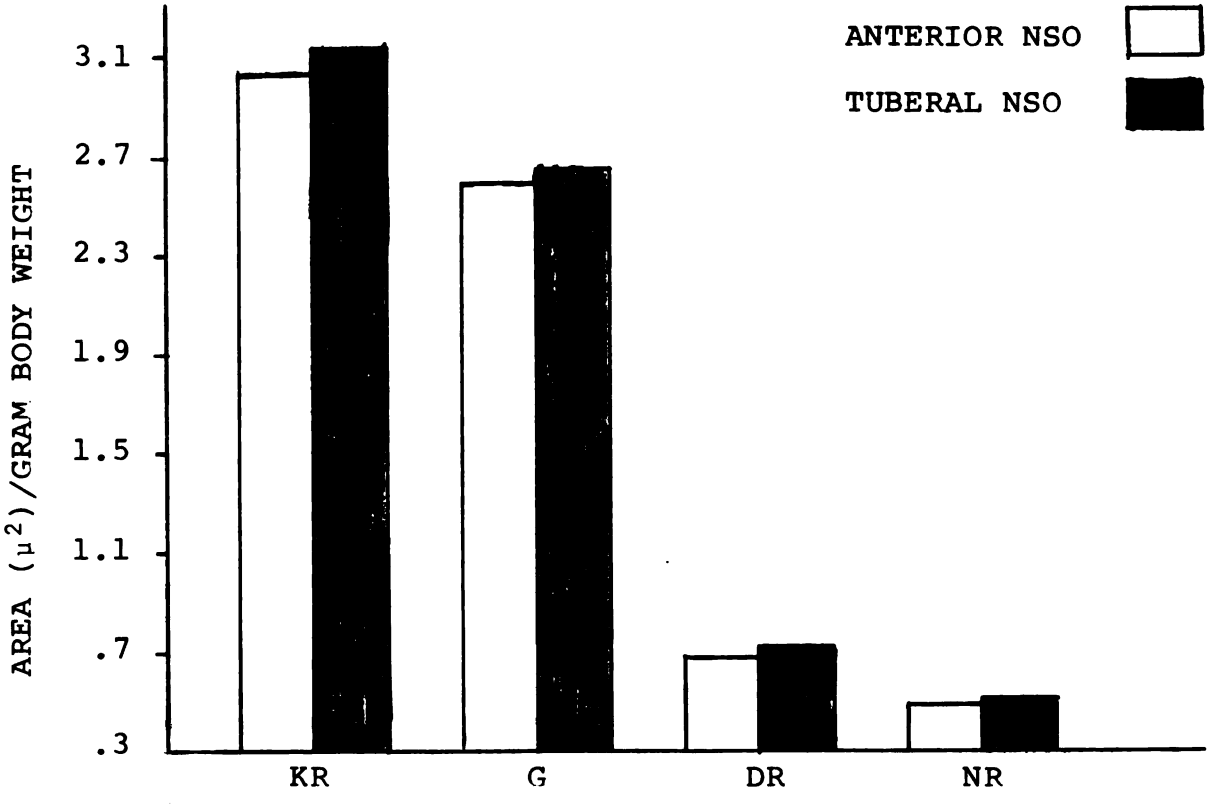


Figure 7b. Cell area of supraoptic neurons in four animals, expressed as number of units per gram body weight.

A comparison of cell size means between animals (Tables 1 and 3) revealed that KR=NR, G<NR, G<KR, G<DR, NR<DR, KR<DR.

Deprived rat and normal rat show a marked difference in their cell size. In order to determine whether the deprived rat's larger cells were due to the deprivation treatment, reflecting some kind of heightened metabolic activity, or to some mechanical factor such as shrinkage or an osmotic effect caused when perfusion was performed, NSO cell areas were compared to a control group of hippocampal cells. If the enlarged cells in the deprived rat were solely a nuclear effect, i.e., an ADH mobilizing response to the deprivation stress, the difference should not be reflected in hippocampal cells which have no known specific neurosecretory function.

Areas of 20 hippocampal cells in each animal, in both planes, were obtained. The means and standard errors of these cellular areas are presented in Figure 8, along with those of the anterior supraoptic cells. Anterior cells were chosen arbitrarily over the tuberal cells; most anterior and tuberal cells were not different in cell area (Table 3). Figure 8 indicates that hippocampal cells and anterior NSO cells are of approximately the same size, with the exception of the deprived rat and horizontal plane gerbil. The disparity between hippocampal cell size and NSO cell size in

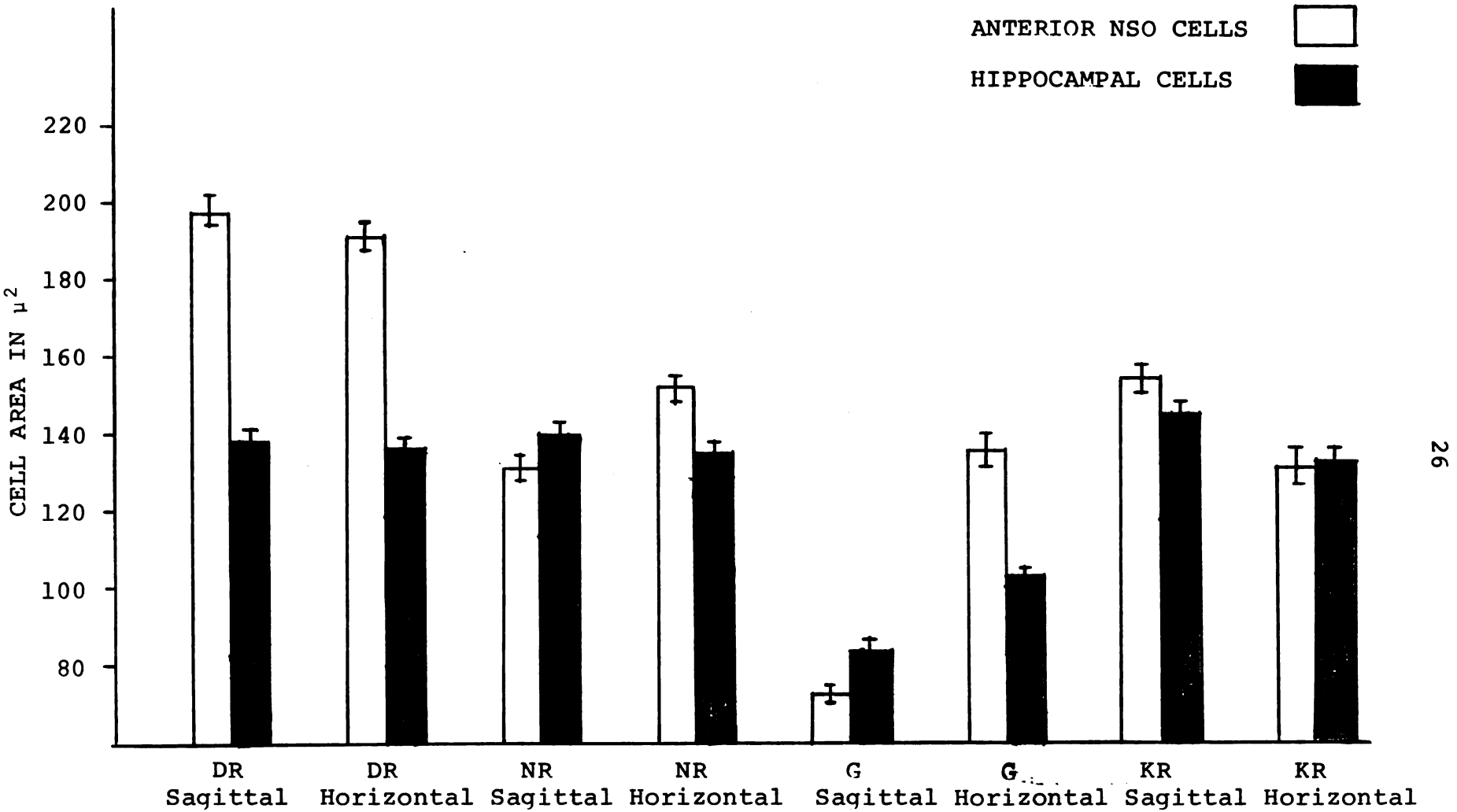


Figure 8. Mean cell area and standard error of anterior NSO and hippocampal neurons.

the deprived rat may be attributed to a NSO nuclear effect in response to deprivation: Hippocampal cells remain essentially the same size from normal to deprivation condition, while NSO cells do not. The reasons for the gerbil differences were not immediately apparent.

For purposes of cell size comparisons among the other animals, NSO cell areas were converted to hippocampal cell units (HCU), that is, expressed as the ratio of anterior NSO cell area to hippocampal cell area. It was anticipated that this transformation would subtract inter-animal shrinkage factors and that ratio comparisons of cell size means within animals, across planes, would remain constant; however, as Figure 9 indicates, they did not--the gerbil had the most discrepant HCU values across planes; values for NR and KR also varied. The apparent ratio differences between the two planes, within animal groups, makes cell size comparisons among animals, based on this particular transformation, impossible. These results indicated that the shrinkage factor may not even have been consistent within a particular brain.

Number of Nucleoli

Figure 10 shows the proportion of cells possessing single, double, and triple nucleoli in each of the four animals. The sample size of 100 cells is a result of

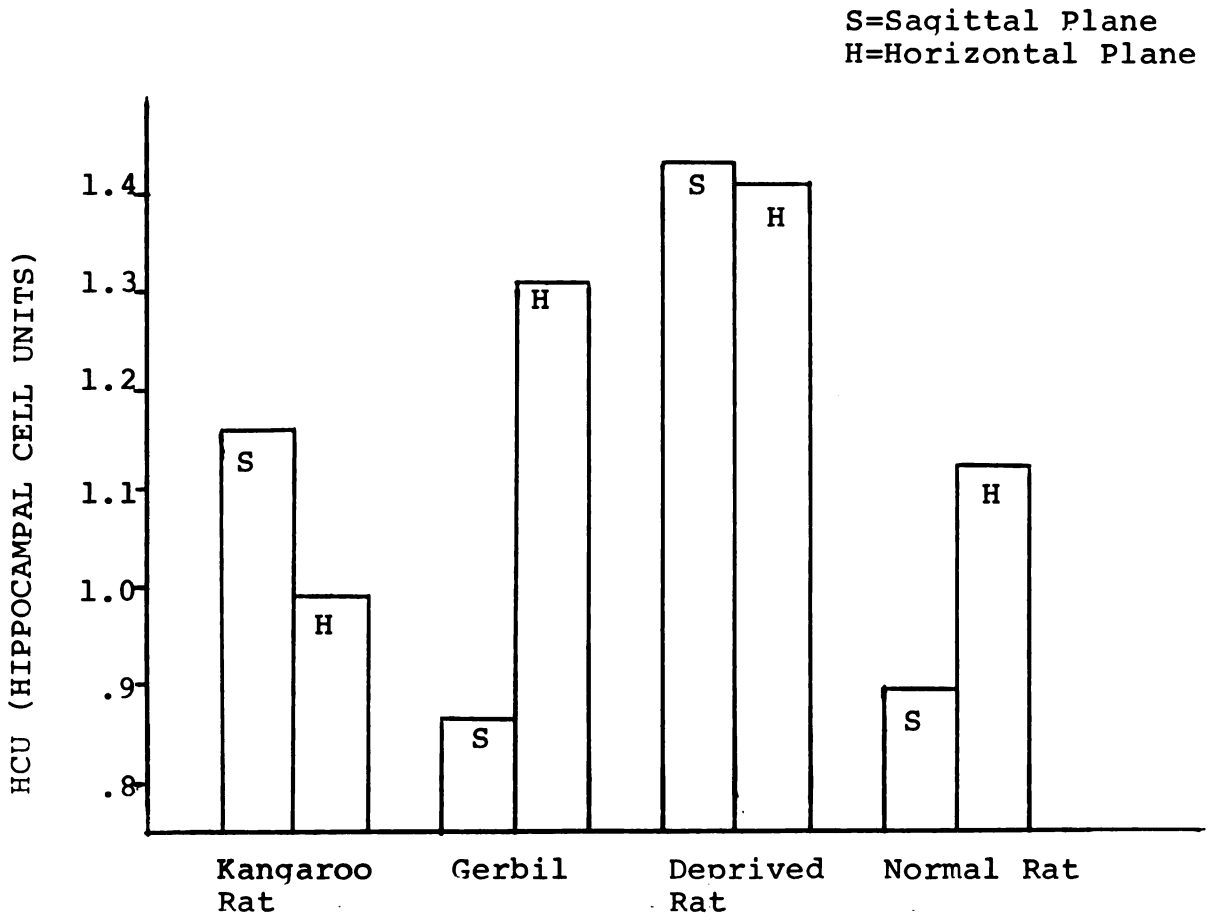


Figure 9. Results of cell area ratios between anterior NSO and hippocampal neurons.

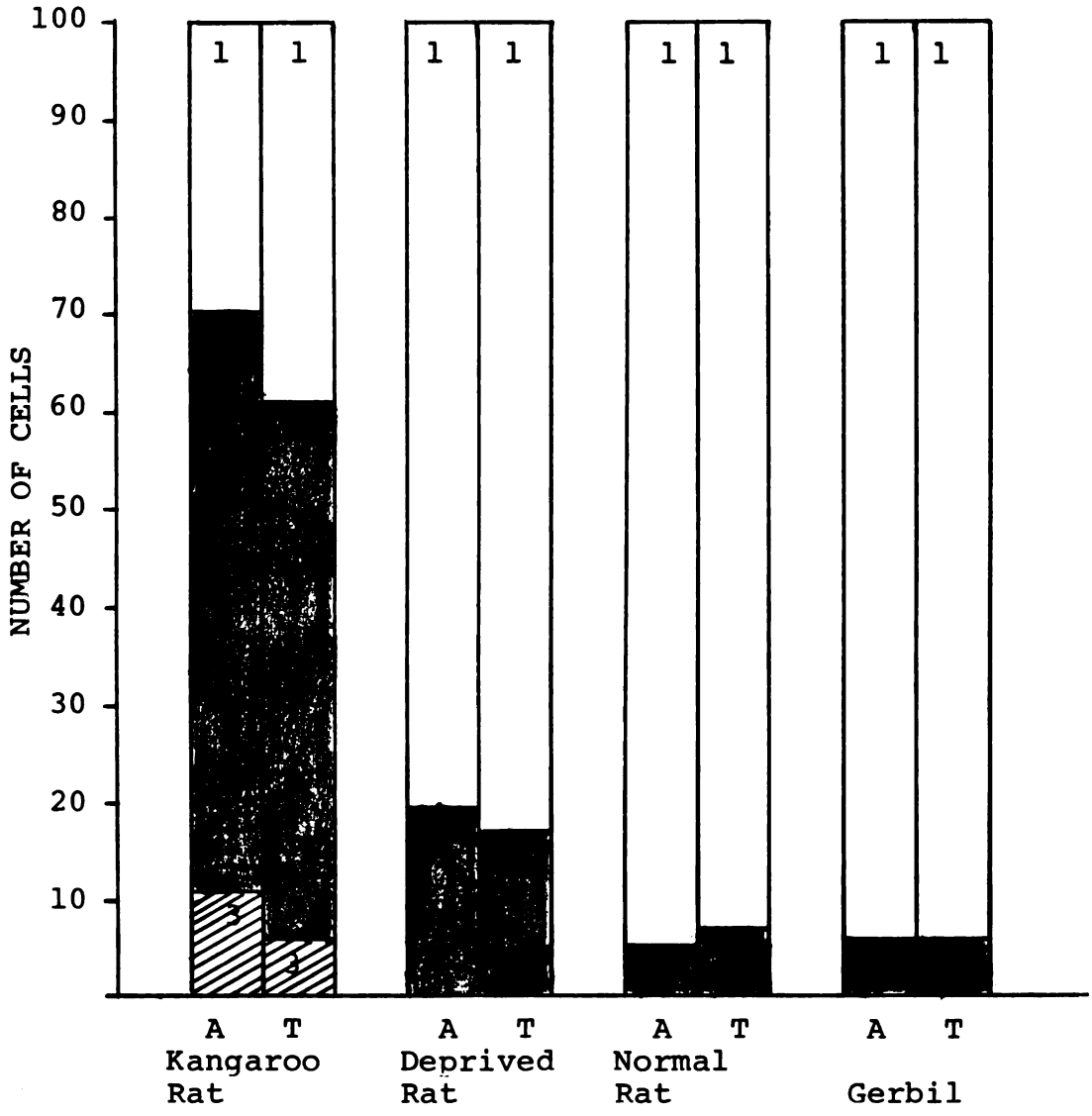


Figure 10. Number of nucleoli per cell, for both anterior (A) and tuberal (T) divisions of NSO, in four animals.*

*Number of nucleoli indicated on upper portion of bar segments.

combining the 50 cells examined in both sagittal and horizontal planes. The majority of cells in the KR have double nucleoli. In G, DR, and NR the cells have mainly single nucleoli, but the DR has a greater number of cells with double nucleoli than either G or NR. Figures 11-14 demonstrate the appearance of the nucleoli.

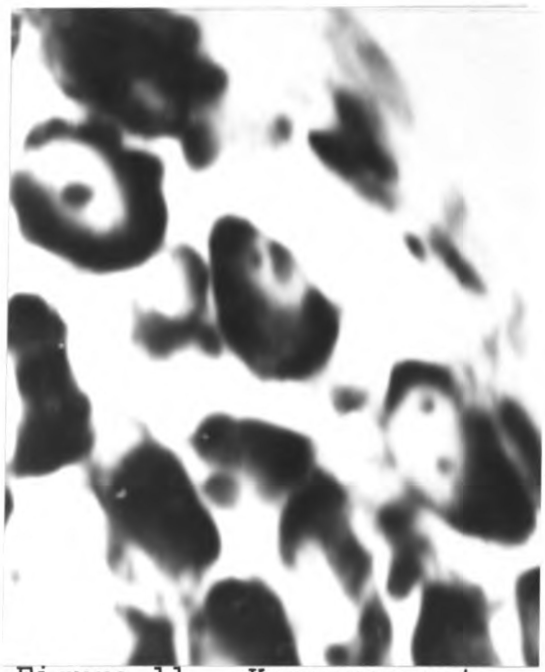


Figure 11. Kangaroo rat

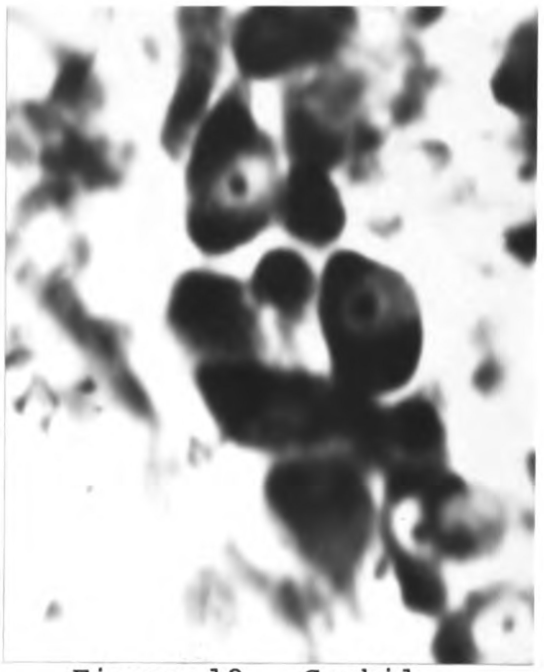


Figure 12. Gerbil

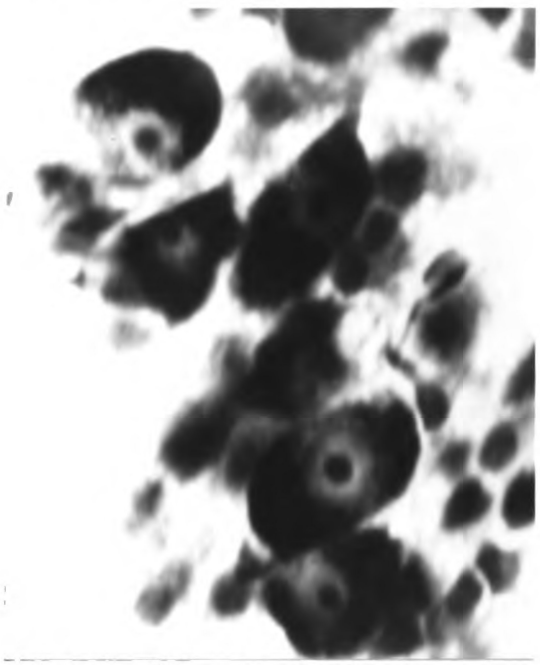


Figure 13. Normal rat

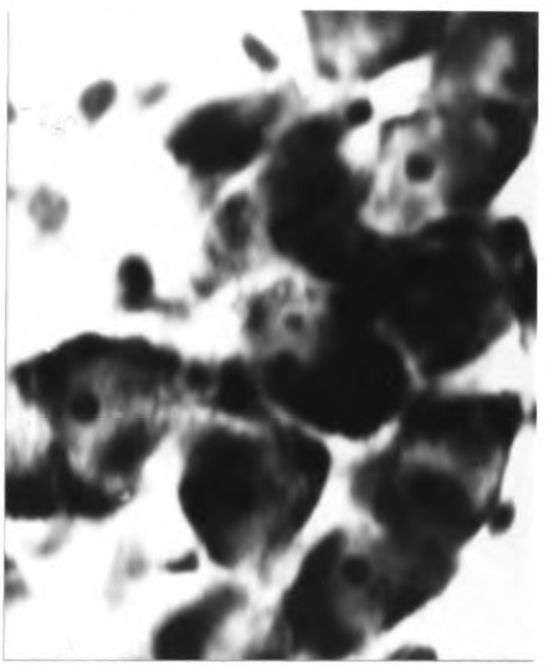


Figure 14. Deprived rat

Figures 11-14. Photomicrographs of Thionin-stained tuberal supraoptic neurons. Note the presence of double nucleoli in kangaroo rat NSO cell (lower right field) and deprived rat NSO cell (center field). Magnification: x 2,500.

DISCUSSION

Demonstration of Neurosecretion

Treatment of the hypothalamic tissue of these animals with Aldehyde-Fuchsin stain was intended to demonstrate the presence of neurosecretion in the anterior and tuberal cells, and thus the function of these cells as neurosecretory agents.

Peterson (1966) reported that both tuberal and anterior portions of the NSO in normal rats show signs of neurosecretion. Cotte and Picard (1968), on the other hand, reported that there were no morphological signs of neurosecretion in the posterior part of the NSO. Our results confirm those of Peterson. Both anterior and tuberal portions of the NSO in the three rodent species so treated did selectively react with the neurosecretory stain--surrounding tissue failed to react. It was, however, not possible to infer any degree of differential neurosecretory activity among the animals merely on the basis of the staining results. Differences in staining intensity may have been due to concentration of the stain at its time of use. Quantitative assessment of numbers of neurosecretory units was not possible due to the limited resolution powers of the light microscope.

Number of Cells

Estimates of the number of cells contained within the laboratory rat supraoptic nuclei vary somewhat according to different reports. Bodian and Maren (1951) cite an anterior NSO size of 14,740 cells and a tuberal size of 2,015. Hatton and Johnson (1968) report figures of 11,246 for the anterior, and 1,575 for the tuberal. Our estimates more closely coincide with those of Bodian and Maren, with an averaged count (both planes) of 14,616 for the rat anterior and 2,066 for the tuberal portion. Differences in the estimates may be attributed to counting criteria, ease in distinguishing cells from one another, and individual differences between rats.

Hatton and Johnson (1968) have also obtained estimates of gerbil cell populations: 4,068 cells in the anterior portion and 3,108 in the tuberal. These correspond to our figures of 5,262 and 2,910. It is not surprising that the two different estimates of the tuberal population coincide more closely than those of the anterior portion; the lower packing density of the tuberal provides for much more ease and certainty in tabulating individual cells.

Our estimation of kangaroo rat cell populations are 6,234 and 5,541 for the anterior and tuberal, respectively. There are no other published estimates available for

comparison. However, in the present experiment, a sample of these cells was counted by another observer, and the inter-scorer reliability was within 10 percent.

Figure 6a shows the absolute number of NSO cells for each animal. Since this data seemed to bear little relationship to the information concerning the differential kidney efficiencies and differential abilities of these animals to withstand water deprivation, the data were covaried with body weight. The new results showed a relationship more in accord with the other behavioral and physiological data. As Figure 6b indicates, relative to body weight, the kangaroo rat has the largest number of supraoptic cells; the number of NSO cells per gram body weight in the gerbil exceeds that of the laboratory rat.

However, there is the possibility that the difference in cell numbers between the laboratory rat and the two desert rodents may be due to factors of body size partially independent of the need to economize water; number of cells varies with body weight, but a minimum number of cells is necessary to maintain anti-diuretic function. Thus, the relationship between body weight and number of cells will not be a valid index in extreme cases, i.e. in animals of a very small size. However, when two animals of similar ecologies and of the same body size (gerbil and kangaroo rat) are compared, results differ in the predicted direction,

with the most competent water economizer, the kangaroo rat, showing the greater number of NSO cells.

Differences in number of cells may also actually represent family differences. Since each of the three rodent species examined were members of different families (kangaroo rat--Heteromyidae; gerbil--Cricetidae; albino rat--Muridae), additional species will have to be investigated in order to furnish a more complete answer.

In the three species examined in the current study, the anterior portion of the nucleus is always larger than the tuberal, but the ratio of tuberal to anterior varies. If we allow an evolutionary trend in the desert rodents toward acquiring larger numbers of supraoptic cells for neurosecretory control in economizing water loss, it becomes apparent that the tuberal plays a large role in this adaptive mechanism. In the normal laboratory rat the tuberal portion accounts for 12.1% of the total supraoptic nucleus, while in the gerbil it occupies 33.1%, and in the kangaroo rat, 46.6% (see Appendix C). Again, these figures may also represent family differences.

Cell Size

Albino Rat: Various researchers have commented on the large size of supraoptic cells but there are only a few references as to its actual dimensions. Enestrom (1967) cites a mean cell area of 119.34 μ^2 in the rat. Our own

results show a cell area of 131.49 μ^2 (Table 1). However, since the kind of fixatives and methods of embedding brain material employed by different experimenters vary, figures such as these cannot be validly compared. The only meaningful comparisons are those of relationships found within an individual experiment. Enestrom (1967) reports that cell volume of laboratory rat NSO cells changes with successive days of water deprivation; in his rats, cell size had increased by the fourth day, had further increased by the seventh day, but receded by the twelfth day. Peterson (1966) reports nucleolar enlargements with a five-day deprivation period. Bachrach (1964) also reports cell component enlargements (cytoplasm, nucleus, and nucleolus) with deprivation. Our results of cell size enlargement coincide with the above findings, and moreover, specify that these cell enlargements take place equally in both the anterior and tuberal portions of the nucleus.

Desert rodents: Relatively little data is available for inter-species comparisons. Khalil and Tawfic (1963) describe "large" cells in the two desert rodents J. jaculus and G. gerbillus, but no quantitative comparisons are available. On the basis of absolute size, our results show that KR cells are the same size as NR cells, both being larger than those of G and smaller than DR cells. When cell size

is expressed in relation to body weight, KR has the largest cells, followed by G, DR, and NR, in that order. This structural relationship parallels the data concerning the ability of these animals to tolerate restriction of water, with the KR as most competent, followed by G, and laboratory rat finishing as a poor third. The cell size value for DR moves in the direction of the value for the desert rodents; in other words, G and KR under natural environmental conditions function in the way that a laboratory rat would when placed under similar, but for him, unnatural and stressful conditions. The fact that a laboratory rat will soon expire when maintained without exogenous water, while the kangaroo rat thrives and the gerbil manages to get by, suggests that increasing cell size (and presumably metabolic and neurosecretory function) is both a species and an individual survival mechanism.

It should also be noted that family differences may play a part in cell size differences among the animals. This possibility will have to be investigated in other rodent species. The fact that the cells of the laboratory rat increase in size from the normal to the deprivation condition, lends support to the habitat-adaptation hypothesis.

When anterior NSO cells were covaried with hippocampal cells, the results failed to corroborate the cell size relationships among animals previously indicated--ratio

comparisons of cell size means within animals across planes did not remain constant. This would seem cause for questioning the validity of the cell size distinctions previously made, until other factors are taken into account. Figure 9 represents the results of a transformation of NSO cell size data based on an arbitrarily chosen nuclear group, the hippocampal cells. The actual meaning of these results is not totally apprehensible. First of all, it is not apparent which, if any, of the inter-plane, intra-animal cell size values are statistically different; the sample size (number of animals) is not large enough to allow such distinctions. One must also question the suitability of using the hippocampal cells as the covariate. Although we assumed no specific neurosecretory function on the part of hippocampal cells and, indeed, these cells did not react with Aldehyde-Fuchsin stain in the manner that supraoptic neurons do, we must consider the possibility of differential effect on these cells from other sources. Furthermore, the possibility that there may be cell size differences among different portions of the hippocampus itself, must certainly be considered.

Since the validity of this measure itself must be called into question, any conclusions as to "real" cell size distinctions, based on this measurement and subsequent transformation, would be equivocal. Cell size comparisons based on selection of the plane with least apparent shrinkage and normalized for the size of the animal by ratios of

cell area to body weight (see Figure 7b), do not, then, appear to have been invalidated by the conflicting hippocampal results. Furthermore, the fact that the cell size distinctions originally made are in harmony with other structural-functional considerations (relative number of NSO cells, ability of the animals to withstand water deprivation), should enhance the credibility of such distinctions. However, since the hippocampal results did deviate from expectation, and do not substantiate the earlier measure, the definitive statement on cell size distinctions must be suspended until larger groups of animals have been tested.

Number of Nucleoli

The nucleolus of a cell plays a central role in cytoplasmic protein synthesis. Caspersson's work (1950) with various types of nerve cells, and the results cited by other researchers, have lead him to the conclusion that conspicuous changes in protein metabolism are correlated with nerve function. During intense activity, a cell's protein-forming system must be able to replace expended proteins at a rapid rate. Cells such as the supraoptic neurons, which, in addition to normal cellular functions, must produce protein-rich secretions, can be expected to place a great demand on their protein-synthesizing centers.

Edstrom, Eichner and Schör (1961), determined the concentration of ribonucleic acid (RNA) in isolated hypothalamic neurons. (RNA is an important element in the biosynthesis of protein.) When rats were deprived of water for seven days, a treatment known to stimulate increased production of ADH, RNA content of supraoptic neurons increased to over double the original amount. Bachrach (1964) has also demonstrated increased RNA production in the supraoptic neurons of deprived rats; he reported a corresponding increase in the size of the nucleoli of these cells.

In the present study no quantitative measure was made of the size of the nucleoli; however, a rather striking difference between animals, in the number of nucleoli per cell, became apparent. The normal laboratory rat supraoptic neurons are predominently uni-nucleolar. Only 5 percent (anterior NSO) and 7 percent (tuberal NSO) of the cells had double nucleoli. After five days of water deprivation, the figures increased to 18 percent in the anterior and 14 percent in the tuberal. Fifty-eight and 57 percent of KR neurons had double nucleoli; there was also a small percent of cells possessing triple nucleoli. It would appear that the large number of cells with double nucleoli in KR, and increased numbers in DR, were results of cellular hyperfunction, i.e., responses to physiological demands for a high level of hormonal production. An examination of the

cells with double nucleoli revealed that each nucleolus was approximately one half the size of the nucleoli of single nucleolar cells in the same animal. Why these particular cells developed two nucleoli instead of merely increasing the size of the existing one is cause only for speculation: Is it a case of a degenerative phenomenon? The KR would seem to indicate that this is not so; these animals can live indefinitely on a diet of dried seeds with continual ADH production. Our animals, which had been maintained for approximately twenty days on a seed diet, were uniformly high in cells with double nucleoli. Perhaps two small nucleoli are more efficient than one large one for intensive protein synthesis. (Recall that under deprivation conditions a small percentage of rat supraoptic neurons develop double nucleoli.) This is all, of course, only speculation. It seems a likely possibility until the gerbil is considered.

The number of cells with double nucleoli in the gerbils we examined, is just as small as in the normal laboratory rat. Here is an animal who is able to get along almost as well as the kangaroo rat in a desert environment; however, the majority of the gerbils' cells were uni-nucleolar.

Several things must be taken into consideration before basing any assumptions on these data. Bachrach (1964) has demonstrated that supraoptic neurons go through a

number of phasic responses during deprivation and rehydration. The size of the cytoplasm, nucleus, and nucleolus changes during these treatments. Enestrom (1967) has shown that as days of deprivation increase, the cell expands to a certain size, and then decreases somewhat. Perhaps individual cells are able to increase the nucleolar size up to a certain point without impairing function; after that point has been reached, division of the nucleolus takes place. (Recall that double-nucleolar cells have nucleoli which are about one half the size of those in single-nucleolar cells.) Both nucleoli may start to develop in size, competing for materials in the nucleus, such as the nucleolus-associated chromatin; this competition may eventually cause one of the nucleoli to degenerate and allow the remaining one to assume full function and to develop to the size of the original nucleolus, until it too undergoes division and the cycle is repeated. (Cases of cells with triple nucleoli may be due to a failure of one nucleolus to degenerate.) If this is indeed the case, the disparate appearance of the G and KR cells can be accounted for: The KR and G may have been sacrificed at what were actually different periods in a phasic cycle of the cells. Even though both species were maintained on the same diet, and were not sacrificed until their weights had stabilized, the conditions for the two animals were

not necessarily equivalent in terms of physiological demands imposed on the cells for ADH production. Castel and Abraham (1969) have shown that the spiny mouse, Acomys russatus, displays a marked increase in NSO cells with multiple nucleoli as days of deprivation are increased.

In order to clarify the meaning of the nucleolar results in the gerbil, it would be desirable to investigate NSO functioning at different intervals along a deprivation continuum. Such data may reveal that the picture furnished by the specimens used in this study is an incomplete one, the complete picture revealing a phasic response to deprivation.

Summary

The kangaroo rat, one of nature's most competent water economizers, with its ability to gain weight on a diet free from exogenous water, seems to demonstrate a functionally more active supraoptic nucleus than that found in either the gerbil or laboratory rat. This desert rodent has relatively more supraoptic neurons per gram body weight and more double nucleoli per cell than either of the other two animals. There is also some indication that cell size (relative to body weight) is greater in this animal than in the gerbil or laboratory rat. Correspondingly, the gerbil has more NSO cells, relative to body weight, than the laboratory rat; the data suggests, in addition, that

gerbil NSO cells are larger than those in the rat. The fact that the supraoptic cells of a normal laboratory rat deprived of water for five days demonstrate changes that approach the conditions found in desert rodents, indicates that increases in cell size and number of nucleoli may be adaptive mechanisms which desert rodents have capitalized upon.

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APPENDIX A

Materials

APPENDIX A

Materials

Animals

1. Kangaroo rats obtained from The Pet Farm, Miami, Florida.
2. Gerbils ordered from Chickline, Vineland, New Jersey.
3. Albino rats, Holtzman strain, from Madison, Wisconsin.

Miscrosopic Equipment

1. Zeiss microscope
2. Photochanger
3. Extension tube
4. Whipple-Hauser disc

Photographic Equipment

1. Cameras: Zeiss Icon microscope camera and a 5" x 7" plate camera and optical bench arrangement.
2. Film: (1) Kodak High Contrast Copy, (2) Kodak Metallographic Plates.
3. Printing paper: (1) Kodak Kodabromide - F-5, (2) Kodak Photographic Paper - AZO F-5.
4. Contact Printer
5. Photo Enlarger

APPENDIX B

Procedure

APPENDIX B

Procedure

Celloidin Embedding

1. Perfuse brain as soon as animal is anesthetized, or as soon as possible after death. Perfuse first with saline (.87% Na Cl), followed by a formalin mixture (10% formalin, .87% saline).
2. Remove brain from skull, immerse in 10% formalin in .9% saline for five days.
3. Place brain in running tap water overnight.
4. Dehydrate through graded alcohols:

80% alcohol	1 day
fresh 80%	1
95% alcohol	2
fresh 95%	3
used absolute alc.	1
fresh absolute	1
ether-alcohol (50/50)	1/2
thin celloidin	7
medium celloidin	7
thick celloidin	14

Wide mouth specimen jars are satisfactory for the dehydration process and for the first three celloidins. To make thin celloidin use 5 gms Nitrocellulose to 100 cc ether alcohol; medium celloidin is 15 gms Nitrocellulose to 100 cc ether alcohol; thick is 25 gms Nitrocellulose to 100 cc ether alcohol. The ether alcohol is made with 2 parts ether to 1 part absolute alcohol.

5. On the last day in thick celloidin place brain in a paper box filled slightly with thick celloidin. Cover with thick celloidin and position brain with a probe.
6. When the celloidin becomes thick enough not to adhere to the finger when pressed, place the block in an airtight container and this within another airtight container. After bubbles in the celloidin have disappeared, place the block in a desiccator along with several small vials of chloroform. Place the lid on the desiccator and seal tightly. After the celloidin has become firm cover the block with 70% alcohol and let stand until the block can be handled easily. Store the block in fresh 70% alcohol until ready to mount and section.

Staining Neurosecretory Material
(for use with Celloidin embedded tissue)

The following procedure works satisfactorily with sections of tissue 15 μ thick; if the sections are much thinner or thicker, experimental modifications will be necessary to determine the procedure best suited for the material.

A. Removing the celloidin

1. Select sections and place them in a petri dish filled with 95% alcohol. Agitate the paper under solution and float the sections off.
2. Place the sections serially in a second petri dish filled with clove oil. The celloidin will melt away from the edges of the tissue almost immediately, but the celloidin in the tissue takes longer to come out. Fifteen u sections take about 2-2 1/2 hours using fresh clove oil.
3. Transfer the sections to zylene for 15 minutes. Repeat this with three more changes of zylene.

B. Rehydrating the tissue

1. Move the sections at 20 minute intervals through a series of graded alcohols, i.e., from 100% to 90, to 70, to 50, to 30.
2. Place the sections in a dish filled with distilled water for 20 minutes and then transfer them to a fresh change of water for another 20 minutes.

C. Mounting the sections

Arrange sections on gelatinized slides. Keep the water on the slide down to a minimum in order to keep the gelatin from becoming too dilute to hold the sections on when they dry; after placing each individual section on the slide, blot brush on paper toweling at intervals while orienting the sections--when it is in the desired position blot the section with brush until it adheres without moving when the slide is tilted. By the time most of the sections are on the slide, some will have begun to dry out and if allowed to get too dry, the edges will begin to curl up. Do not allow this to happen or sections will stain unevenly. When the sections have all dried at least once, place the slide in a tray and submerge in distilled water. Take slides through the staining baths.

D. Gomori's Aldehyde-Fuchsin
(Adapted after Conn, H. J., Darrow, M. A., and Emmel, V. M. Staining Procedures. Biological Stain Commission, 1962, Williams and Wilkins Co., Baltimore.)

1. Oxidize in potassium permanganate one minute.
   (.3 g $KMnO_4$, 100 ml distilled water, .3 ml concentrated H_2SO_4.
2. Rinse in distilled water.
3. Bleach in 2% sodium bisulphite two minutes.

4. Wash in running tap water five minutes.

5. Stain in aldehyde fuchsin mixture overnight. (Add 1 g basic fuchsin to 200 ml boiling water. Boil 1 minute; cool and filter. Add 2 ml concentrated HCl and 2 ml paraldehyde. Leave stoppered at room temperature. When mixture has lost reddish fuchsin color and is deep purple (3-4 days), filter and discard filtrate. Dry precipitate on filter paper in oven. Remove and store. Dissolve 0.25 g in 50 ml of 70% alcohol. Stain may be used for several months, but filter before each new use.)

6. Leave in acid alcohol 15-20 minutes. (0.5% HCL in 70% alcohol.)

7. Leave in 95% alcohol from 6-12 hours until light enough so that stained neurosecretory areas will stand out from the background when placed under the microscope.

8. Take through 3 changes of xylene (10 minutes in each).

9. Cover slip.

E. Alternative Stain: Bargmann's Modification of Chrome-Alum-Haematoxylin Method of Gomori (Adapted after Pearse, A. G. E. Histochemistry: theoretical and applied. 1961, J. and A. Churchill, Ltd., London)

1. Leave sections 12-24 hours in 1 part Bouin's with 1 part of 3-5% chrome-alum at 37° (Bouins: 50 ml

picric acid, 10 ml commercial formaline, 5 ml glacial acetic acid, 35 ml distilled water.)

2. Wash in tap water until colorless.
3. 1 minute in potassium permanganate - sulphuric acid solution: 1.3% $KMnO_4$ in 0.3% H_2SO_4.
4. Rinse in distilled water.
5. Decolorize in 1% oxalic acid, 1 1/2 min.
6. Wash in tap water, 1 minute.
7. Stain 12 minutes in haematoxylin solution:

   haematoxylin 0.5 g

   distilled water 50.0 ml

   when dissolved add

   potassium dichromate (5%) 2 ml

   2.5% sulphuric acid 2 ml

   ripen 48 hours--can be used as long as a metallic film is present. Store in refrigerator. Filter before use.
8. Differentiate for 1 1/2 minutes in 0.5% HCL in 70% alcohol.
9. Wash in tap water, 2-3 minutes.
10. Dehydrate rapidly, 2 minutes in each solution (30, 50, 70, 80, 90, 100% alcohol).
11. Take through 3 changes xylene (10 minutes in each).
12. Cover slip.

Thionin Stain (Nissl Method)

1. Float sections off paper into distilled water.
2. Place sections in a steaming bath of 1% aqueous thionin solution (buffered to pH 4.0) and leave in 54° oven 15 minutes.
3. Transfer the sections through two changes of distilled water.
4. Place sections in 80% alcohol and agitate for 1-2 minutes then to fresh analine alcohol (50cc aniline, 450 cc 95% alcohol). Allow the sections to remain here to complete differentiation.
6. When sections have reached the desired color transfer them to 95% alcohol. Transfer to fresh 95% for three additional times to fully remove the aniline.
7. Put through 1 change of 100% alcohol.
8. Take through oil of cajeput for clearing.
9. Take through 4 changes of xylol to remove cajeput oil.
10. Mount on slides and cover slip.

Measurements

(1) Cell Area

Supraoptic nucleus: Cells of the NSO were magnified 2200 times through a Zeiss microscope with a photo-changer attachment (objective: x 40; eyepiece: x 12.5; distance from floor to lens: 37.5 cm.). The sample consisted of 50 randomly selected cells (five cells from each of five

sections through the left and right portions of the nucleus) for each nucleus, anterior and tuberal, in each animal, in both horizontal and sagittal planes. A cell's outline was traced when its nucleolus was in focus. The areas of the cells were obtained by measurement with a Keuffel and Esser compensating polar planimeter.

Hippocampal cells: Cell area for hippocampal cells was obtained in the same manner described for the NSO cells, the only difference being the number of cells sampled. Five cells from each of two sections through the left and right portions of the hippocampus of each animal were measured.

(2) Number of Nucleoli

The sample consisted of 50 cells from each nucleus in each animal and plane. The number of nucleoli was recorded for each individual cell in the sample.

(3) Number of Cells

By placing a Whipple-Hauser disc in the eyepiece of the microscope, a grid could be superimposed on a section of the NSO. Number of cells contained within successive squares were tabulated to yield a total number of cells per section. Only cells with nucleoli visible were counted. In instances where there were two or more nucleoli per cell, only one was tabulated. All thionin stained sections through the nucleus were examined and counted in this manner.

Since the thionin set of sections represented only every third section through the nucleus, the number of cells per section was multiplied by three in order to take into account the cells in the intervening sections.

APPENDIX C

Raw Data

NSO cell areas in square centimeters as determined by planimetry, for each animal, in each plane, both anterior and tuberal portions of NSO. N=50.

Kangaroo Rat								Gerbil							
Sagittal Plane				Horizontal Plane				Sagittal Plane				Horizontal Plane			
Anterior		Tuberal		Anterior		Tuberal		Anterior		Tuberal		Anterior		Tuberal	
11.1	6.9	9.5	8.4	7.2	8.0	7.2	5.0	3.6	3.6	3.5	4.6	7.5	6.5	8.1	5.9
7.8	9.0	6.6	9.7	5.3	3.7	4.6	4.7	3.3	4.8	3.1	3.8	5.2	6.1	7.3	5.6
8.0	7.2	7.3	6.9	7.0	5.9	7.4	5.7	3.3	3.0	3.7	3.7	4.5	5.1	7.7	5.9
7.8	9.0	6.2	10.7	7.4	6.3	6.8	6.2	3.4	2.5	3.8	2.6	8.2	7.5	9.1	6.3
6.1	7.2	6.8	8.1	6.0	5.3	6.0	8.0	3.6	3.3	3.6	2.9	9.8	5.8	7.8	7.0
7.7	6.3	8.5	7.5	6.4	7.9	6.6	7.2	3.8	4.2	4.1	4.6	7.6	7.9	6.1	7.7
8.7	7.2	6.6	8.2	8.7	4.4	8.3	6.9	3.1	3.4	3.6	3.3	8.1	4.9	7.1	6.3
8.8	8.4	7.5	6.0	5.6	7.5	7.9	5.5	3.2	4.8	4.3	3.8	8.6	6.2	9.8	6.4
7.0	8.0	7.1	7.8	3.8	6.0	9.9	5.1	2.7	3.0	4.4	4.0	5.6	6.8	10.5	6.3
7.0	6.8	9.0	9.5	8.6	7.7	6.6	7.3	3.4	4.3	4.4	3.8	8.0	7.0	5.8	5.9
7.9	5.3	6.4	7.1	8.0	4.9	9.0	6.9	3.5	3.2	5.3	3.3	5.1	5.9	7.1	6.4
10.0	6.0	6.4	7.4	5.4	6.4	8.1	6.2	3.3	3.6	4.5	3.8	3.2	8.6	6.1	6.1
3.9	7.4	8.0	7.3	6.7	6.6	8.3	6.9	4.3	2.7	3.6	4.2	5.8	6.7	6.4	7.7
8.2	6.2	9.5	9.7	9.1	6.3	9.4	6.2	3.3	4.6	3.8	2.8	5.7	7.6	8.2	5.9
6.1	6.2	10.7	7.6	5.7	7.1	11.4	6.7	3.4	2.7	4.3	3.1	5.1	6.9	6.5	7.1
6.3	8.5	9.2	5.0	4.3	5.5	3.6	8.2	3.6	3.8	4.8	2.2	7.8	5.8	6.5	6.0
7.6	7.9	8.7	7.1	4.6	7.9	6.4	8.3	4.0	3.7	2.3	2.6	5.7	6.5	7.3	4.5
7.0	7.7	8.7	7.7	4.6	4.3	10.9	6.2	5.1	4.5	2.6	2.8	7.9	5.8	7.9	7.1
7.0	8.4	8.9	6.5	7.0	7.9	6.7	7.9	3.7	2.8	4.2	3.0	6.5	5.8	6.0	4.8
5.8	8.8	9.0	6.1	7.9	6.8	7.4	5.2	3.6	3.8	3.4	3.3	7.3	6.8	8.0	8.4
7.1	5.5	6.7	7.4	6.0	6.6	5.6	7.6	3.4	3.6	2.9	3.3	9.6	5.2	7.0	4.8
7.4	5.5	7.8	7.9	8.6	5.9	5.5	8.4	3.2	3.6	3.5	3.5	7.8	4.0	7.9	6.1
7.4	10.4	7.3	6.6	4.9	6.3	7.8	7.0	3.8	3.9	2.9	3.6	5.6	5.3	5.5	6.5
9.0	6.8	6.4	7.9	6.0	8.7	7.9	8.0	3.7	2.4	3.7	3.3	9.0	5.5	6.8	4.3
10.1	6.2	7.6	7.3	6.7	6.0	6.5	8.0	3.2	4.1	3.4	2.9	8.0	7.0	8.0	6.0

NSO cell areas (cont'd.)

Normal Rat								5-Day Deprived Rat							
Sagittal Plane				Horizontal Plane				Sagittal Plane				Horizontal Plane			
Anterior		Tuberal		Anterior		Tuberal		Anterior		Tuberal		Anterior		Tuberal	
5.3	5.4	8.3	7.5	8.6	8.5	6.7	10.2	11.1	6.6	11.0	12.6	7.6	8.9	9.7	13.2
7.4	5.0	8.5	7.3	10.1	7.7	5.8	7.8	12.8	8.3	8.2	9.9	10.2	8.7	10.5	13.5
7.3	6.7	5.9	6.2	7.7	5.9	5.3	9.3	12.2	7.6	8.7	9.8	8.4	10.3	8.7	10.3
6.2	8.1	5.8	6.3	7.0	6.0	5.0	8.7	11.6	8.9	9.1	13.6	7.6	9.8	12.1	11.5
6.3	8.8	6.9	6.0	11.1	6.0	7.7	7.5	11.5	11.2	9.1	9.1	6.9	9.0	10.8	11.3
4.8	7.5	4.6	7.5	10.5	4.5	6.0	9.4	9.7	11.4	8.7	10.9	8.3	7.4	11.4	11.9
5.3	6.9	5.7	5.6	9.6	6.9	9.0	9.6	8.4	8.6	13.5	8.2	9.2	8.3	10.1	14.0
5.3	5.7	8.2	7.2	7.5	5.5	7.2	8.8	7.7	10.1	9.2	9.9	9.7	7.7	13.7	10.3
6.3	6.7	4.7	7.3	7.7	6.0	7.2	6.3	7.9	9.5	8.7	11.1	8.6	10.3	11.8	8.0
4.8	5.3	7.0	6.2	8.9	7.7	8.5	6.7	9.5	10.6	9.0	10.0	10.0	9.4	12.3	7.4
6.0	4.3	7.1	6.4	6.7	6.1	5.6	8.2	10.5	8.0	9.6	10.0	7.8	12.4	12.5	8.9
4.7	6.7	5.6	6.5	8.9	8.5	6.7	6.5	7.6	8.9	10.1	10.3	12.1	9.9	10.8	9.9
6.0	5.0	6.9	5.4	8.0	6.0	9.5	6.1	8.8	12.8	9.4	10.9	9.7	11.8	12.1	11.6
7.9	7.1	5.7	5.9	8.1	6.5	8.1	9.7	9.0	11.8	8.4	9.9	7.6	9.4	8.7	14.8
8.9	6.6	7.0	5.9	6.6	7.3	7.7	7.2	9.0	8.8	9.8	17.8	9.5	10.1	9.4	10.5
10.0	4.5	6.8	4.8	6.0	6.6	8.2	7.3	8.1	8.8	9.2	7.9	8.0	9.9	9.8	11.4
7.2	6.1	4.9	6.7	6.9	4.6	8.0	6.7	8.3	8.5	10.9	12.2	9.9	8.2	10.2	10.3
7.1	5.7	6.1	6.1	7.4	8.9	6.6	7.4	13.9	8.7	9.2	8.4	8.3	9.3	11.9	16.6
5.0	5.3	6.3	5.9	6.3	5.8	6.3	5.9	10.2	8.9	9.2	9.5	11.3	8.4	9.7	13.5
6.8	6.9	6.7	5.7	7.3	7.5	7.1	6.8	8.4	9.4	10.9	8.9	9.6	8.0	9.4	8.5
6.7	6.2	6.4	7.0	7.2	8.6	6.7	7.4	10.3	10.4	10.1	7.3	8.8	10.5	9.2	10.5
6.4	8.0	5.6	5.2	5.6	5.6	7.6	7.2	8.3	10.0	9.4	9.3	10.9	8.5	14.3	12.8
7.2	5.7	6.6	7.7	7.4	6.3	6.7	8.3	9.5	10.0	9.3	11.6	10.3	8.9	9.8	9.8
6.8	5.0	8.7	5.0	8.7	8.3	10.8	9.2	8.2	10.6	10.3	11.8	7.9	12.2	10.7	13.0
6.7	6.6	6.3	6.1	8.0	7.0	10.4	5.2	9.0	10.8	12.5	6.8	8.7	10.1	14.3	11.3

Hippocampal cell areas in square centimeters as determined by planimetry, for each animal, in both planes. N=20.

Kangaroo Rat		Gerbil		Normal Rat		Deprived Rat	
Sagittal	Horizontal	Sagittal	Horizontal	Sagittal	Horizontal	Sagittal	Horizontal
6.3	7.2	3.6	5.0	7.6	5.3	6.7	6.6
6.7	6.0	4.3	4.5	6.5	7.1	6.6	6.8
7.6	6.4	4.6	5.0	8.4	6.3	6.5	6.1
6.4	7.3	4.4	4.4	6.9	6.7	7.6	7.3
5.5	5.8	4.0	5.3	6.7	7.2	6.0	7.8
6.8	5.6	4.0	5.1	7.0	6.9	6.3	5.8
6.8	5.7	5.0	5.1	8.3	6.3	6.9	6.0
6.9	6.6	4.4	5.6	7.1	6.3	7.5	7.5
7.0	6.3	3.5	6.0	6.0	7.1	5.9	6.6
7.9	5.5	3.9	5.2	6.3	6.2	5.7	6.8
6.2	6.7	4.0	5.6	6.3	6.9	6.1	7.1
7.0	5.7	3.6	5.1	6.8	6.3	7.4	6.1
7.8	5.1	4.2	5.9	6.1	6.1	7.1	6.4
7.0	7.3	3.9	4.7	5.5	6.5	7.6	6.2
7.5	6.4	3.9	5.3	6.5	7.4	7.5	6.2
8.1	7.1	3.6	4.0	6.8	5.7	7.5	5.3
6.9	7.5	3.6	4.2	6.8	6.8	6.7	5.6
7.9	7.1	4.1	4.5	6.3	6.7	5.8	8.1
8.1	7.1	5.1	4.4	6.5	6.7	6.1	6.8
6.3	6.6	4.1	5.4	7.5	6.3	6.8	6.3

Number of cells for each 15μ thick section through the anterior and tuberal nuclei, in each animal and plane.

Kangaroo Rat (Sagittal Plane)
Anterior NSO

Section Number	Cell Count	Section Number	Cell Count
27	5	225	42
30	54	228	38
33	82	231	35
36	75	234	39
39	80	237	36
42	65	240	39
45	54	243	33
48	41	246	35
51	49	249	29
54	70	252	30
57	72	255	19
60	82	258	8
63	74	261	6
66	74		
69	74		
72	50		
75	48		
78	35		
81	30		
84	20		
87	10		
90	8		
93	2		
174	1		
177	2		
180	4		
183	10		
186	12		
189	21		
192	30		
195	41		
198	40		
201	47		
204	59		
207	56		
210	52		
213	42		
216	43		
219	41		
222	35		

Kangaroo Rat (Sagittal Plane)
Tuberal NSO

Section Number	Cell Count	Section Number	Cell Count
45	1	171	18
48	0	174	16
51	3	177	16
54	9	180	31
57	23	183	33
60	33	186	29
63	48	189	34
66	83	192	48
69	83	195	42
72	84	198	36
75	79	201	50
78	62	204	54
81	57	207	42
84	74	210	45
87	57	213	24
90	59	216	31
93	54	219	29
96	44	222	32
99	39	225	34
102	21	228	26
105	17	231	15
108	18	234	9
111	14	237	4
114	13	240	1
117	13		
120	4		
123	15		
126	12		
129	6		
132	1		
135	13		
138	1		
147	2		
150	5		
153	11		
156	22		
159	18		
162	15		
165	19		
168	16		

Kangaroo Rat (Horizontal Plane)

Anterior NSO		Tuberal NSO	
Section Number	Cell Count	Section Number	Cell Count
108	46	117	5
111	109	120	27
114	148	123	11
117	134	126	17
120	80	129	3
123	36	132	33
126	57	135	13
129	55	141	81
132	43	114	6
135	4	117	3
141	74	120	42
144	112	123	17
102	60	126	20
105	102	129	5
108	126	132	41
111	92	135	84
114	70	138	81
117	59	141	38
120	61		
123	66		
126	75		
129	67		
132	72		
135	52		
141	97		
144	148		

Gerbil (Sagittal Plane)

Anterior NSO		Tuberal NSO	
Section Number	Cell Count	Section Number	Cell Count
3	22	27	3
6	50	30	31
9	44	33	42
12	58	36	59
15	53	39	65
18	36	42	68
21	42	45	71
24	50	48	36
27	70	51	28
30	58	54	17
33	48	57	21
36	65	60	26
39	50	63	9
42	33	66	2
45	43	69	0
48	47	123	4
51	39	126	13
54	38	129	11
57	36	132	20
60	29	135	16
63	22	138	30
66	13	141	39
69	4	144	35
141	4	147	39
144	17	150	44
147	21	153	50
150	43	156	64
153	38	159	67
156	29	162	62
159	65	165	17
162	68	168	9
165	50	171	0
168	42	174	9
171	57		
174	54		
177	58		
180	46		
183	49		
186	75		
189	54		
192	28		
195	24		
198	17		
201	6		
204	6		

Gerbil (Horizontal Plane)

Anterior NSO		Tuberal NSO	
Section Number	Cell Count	Section Number	Cell Count
147	16	177	10
150	20	180	17
153	19	183	43
156	19	186	31
159	18	189	71
162	12	192	80
165	20	195	63
168	29	198	50
171	59	201	97
174	147	204	19
177	238	180	8
180	173	183	42
183	56	186	26
186	51	189	56
156	12	192	75
159	36	195	55
162	30	198	51
165	20	201	93
168	28	204	46
171	12		
174	42		
177	75		
180	99		
183	132		
186	152		
189	60		
192	39		

Deprived Albino Rat (Sagittal Plane)
Anterior NSO

Section Number	Cell Count	Section Number	Cell Count
15	2	189	68
18	65	192	103
21	17	195	122
24	99	198	90
27	62	201	112
30	91	204	117
33	135	207	121
36	171	210	109
39	112	213	129
42	129	216	127
45	134	219	100
48	143	222	113
51	125	225	113
54	139	228	118
57	136	231	113
60	129	234	120
63	108	237	88
66	108	240	96
69	100	243	99
72	90	246	74
75	82	249	6
78	69	252	16
81	45	255	47
84	44		
87	26		
90	11		
93	13		
96	9		
99	5		
156	7		
159	7		
162	5		
165	11		
168	27		
171	29		
174	43		
177	39		
180	49		
183	73		
186	70		

Deprived Albino Rat (Sagittal Plane)
Tuberal NSO

Section Number	Cell Count	Section Number	Cell Count
36	2	228	23
39	3	231	9
42	9	234	2
45	16	237	2
48	32		
51	36		
54	35		
57	37		
60	55		
63	47		
66	36		
69	22		
72	19		
75	18		
78	11		
81	10		
84	6		
87	4		
90	5		
93	1		
168	1		
171	6		
174	5		
177	4		
180	5		
183	6		
186	6		
189	10		
192	15		
195	21		
198	25		
201	30		
204	42		
207	24		
210	26		
213	38		
216	30		
219	21		
222	27		
225	27		

Deprived Albino Rat (Horizontal Plane)

Anterior NSO		Tuberal NSO	
Section Number	Cell Count	Section Number	Cell Count
189	6	204	2
192	5	213	8
195	1	216	31
201	28	219	46
204	217	222	91
207	606	225	58
210	775	228	76
213	424	210	20
216	192	213	45
219	15	216	69
192	5	219	36
195	17	222	42
198	59	225	38
201	242	228	12
204	624		
207	805		
210	535		
213	195		
216	42		

Weight of animals before perfusion

Animal	Weight in grams
Kangaroo rat (#1)	49
Kangaroo rat (#2)	53
Gerbil (#1)	56
Gerbil (#2)	50
Normal Rat (#1)	371
Normal rat (#2)	405
Deprived rat (#1)	298
Deprived rat (#2)	286