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ULTRASTRUCTURAL CHANGES IN RAT
PARAVENTRICULAR NUCLEUS FOLLOWING
DEHYDRATION AND REHYDRATION

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ULTRASTRUCTURAL CHANGES IN RAT PARAVENTRICULAR NUCLEUS FOLLOWING DEHYDRATION AND REHYDRATION

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ABSTRACT

ULTRASTRUCTURAL CHANGES IN RAT
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Previous quantitative ultrastructural analyses of the supraoptic and circularis nuclei in rats have revealed neuronal membrane appositions (somatic membranes separated by a 60 - 80 Å extracellular space with no intervening glial process). The proportion of somatic membrane in somato-somatic apposition varied during minimal dehydration and rehydration of the animal. This morphological plasticity could theoretically modulate ephaptic and/or trophic interneuronal interactions. A similar analysis was performed on the primarily vasopressin-containing lateral portion of the paraventricular nucleus. Rats (n = 4 per group) were non-deprived, deprived of water for 4, 12 or 24h, or rehydrated for 12 or 24h after an initial 24h of deprivation. Both the percentage of somatic membrane in somato-somatic apposition and the length of somatic membrane in somato-somatic apposition per unit of section area increased with dehydration. The number of small dense core vesicles (<1,600 Å) was decreased after 12h dehydration and again after 24h rehydration. Analysis of more experimental animals is needed to further characterize the responses of membrane appositions, dense core vesicles and, possibly, lysosomes to dehydration. Cilia were found emerging from magnocellular

somas and projecting into the neuropil, as previously reported in homologous nuclei of lower vertebrates where osmoreceptive function has been postulated.

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INTRODUCTION

Functional Organization of Magnocellular Nuclei

The paraventricular (PVN) and supraoptic (SON) nuclei are the most apparent neurosecretory nuclei of the mammalian hypothalamus (Bargmann and Scharrer, 1951). Both nuclei are highly vascularized (Ambach and Palkovits, 1974 a, b) and send strong projections to the posterior pituitary (Sherlock, Field, and Raisman, 1975 and others). The PVN was first implicated in the control of lactation. Early studies of the effects of lesions of the PVN or SON led to the hypothesis that neurons of the SON contained the hormone vasopressin (VP) for fluid regulation while the PVN contained oxytocin (OX) for a role in lactation, since SON lesions yielded diabetes insipidus and secondary polydipsia while the PVN lesions substantially reduced pituitary OX levels (Olivecrona, 1957). Furthermore, stimulation of the SON produced a marked antidiuresis, while stimulation of the PVN did not produce antidiuresis (Harris, 1947), but instead released OX (Cross, 1966). These results are now difficult to explain, since numerous immunocytochemical studies have reported OX and VP neurons in nearly equal numbers in the PVN and SON (see Defendini and Zimmerman, 1978 for review). In addition, a wealth of physiological and morphological data (see below) implicates both nuclei in fluid balance and in the regulation of lactation. Clearly, the early lesion and stimulation techniques were crude, and complicated by the substantial number of axons

emanating from the PVN and nucleus circularis (NC) and coursing in close proximity to the SON. However, despite the data which have now been obtained using more powerful techniques, little can yet be said of the reason for the existence of several distinct magnocellular groups (PVN, SON, NC and others) which contain some of the same hormones, while a morphologically, histochemically, and functionally similar assortment of cells is apparently confined to one nucleus, the magnocellular preoptic, in fishes and amphibians (for example, see Peter, 1977; Dixit, 1976; Watkins, 1975).

Functional differences between the PVN and SON are suggested by a karyometric analysis of female rats under various conditions of pregnancy, lactation and fluid balance (Bandaranayake, 1974). Increases in nucleolar and nuclear diameter were interpreted as indications of enhanced RNA and protein syntheses. Increases in these measures were found in both the SON and PVN following any of the treatments. However, the latency and magnitude of the responses suggested that the PVN was influenced more by pregnancy while the SON was more responsive to deviations in fluid balance. Furthermore, when nipples were unilaterally excised and allowed to heal prior to mating, a subsequent analysis of nuclear and nucleolar diameters revealed a laterality difference in the PVN but not in the SON or control (hippocampal) cells. Both before and during pregnancy, the neurons of the PVN exhibited greater signs of synthetic activation on the unoperated side. The projections by which sensory information reach OX-containing cells are not known. Since tritiated estrogens accumulate in the PVN and to a lesser extent in the SON (Stumpf and Sar, 1977), it may be that both afferent activity

and plasma hormone levels influence hormone synthesis, at least under conditions of pregnancy and lactation.

On morphological grounds, the magnocellular neurons of the rat PVN have been classified into two populations (Hatton, Hutton, Hoblitzell and Armstrong, 1976). Cells in the anteroventromedial portion of the nucleus ("medial," PV-M) were relatively fusiform. Posterior, dorsal and lateral to these cells, they found another group ("lateral," PV-L). Cells in the medial group exhibited a different orientation and were more fusiform than the lateral cells. Differences were also found in cell size and in the percentage of cells possessing multiple nucleoli ("multiples"). Immunohistochemical evidence suggests that cells containing OX and its associated protein neurophysin are preferentially located rostrally in the rat PVN (Swaab, Pool and Nijveldt, 1975), apparently within the medial group of Hatton, et al. VP-containing cells have been reported caudally (Choy and Watkins, 1977; Swaab, et al., 1975; Krisch, 1978), presumably within the lateral group. However, other reports place VP and its neurophysin centrally with OX peripherally within the nucleus (Sokol, Zimmerman, Sawyer and Robinson, 1976; Vandesande and Dierickx, 1975). Sokol, et al. (1976) have further suggested that some cells possess the capability of synthesizing the two hormones; the weight of present evidence still favors the one hormone one cell hypothesis for magnocellular neurons (Vandesande and Dierickx, 1975), although not definitively. These controversies may reflect immunohistochemical nonspecifity, strain differences or imprecise description and definition of locations and boundaries within the nucleus. Further complication comes from the recent observation of neurophysin within a group of large cells located posterior to the

other groups (Armstrong, Hatton, and McNeill, 1979). Since the cells of this group did not undergo chromatolysis following hypophysectomy (before extrahypothalamic projections had been documented, see below), they had been paradoxically named nucleus magnocellularis parvocellularis in order to distinguish them from the neurosecretory cells of the PVN (Bodian and Maren, 1951).

Other evidence tends to support the suggestion that most cells in the PV-M contain OX while those in the PV-L contain VP. Hutton (1975) found that the percentage of cells with multiple nucleoli, a possible indicator of synthetic activation, was elevated in PV-M during lactation and pregnancy; PV-L was not changed. Thus there were more multiples in PV-L than in PV-M in males but not in females. Medial cells were larger than laterals in females; the reverse was true for males (Hatton, et al., 1976). Following physiological levels of water deprivation, which result in the release of both OX and VP (Jones and Pickering, 1969 and others), similar cell size increases and nucleolar proliferation were found in the medial and lateral groups (Hoblitzell, Hatton and Armstrong, 1976). However, in animals which were maintained on a 12:12 light/dark cycle, substantial and parallel cell size changes were found in both the PV-L and PV-M (Armstrong and Hatton, 1978). No changes in nucleoli were detected in either division of the PVN, but the number of multiples did change in SON with the diurnal cycle. The implications of the circadian synthetic response, the similarity of response between the PV-M and PV-L, and of the disassociation of the nucleolar and cell size measures of synthetic activity are not clear. Some electrophysiological data also support the proposed medial/lateral distribution of hormones. According to one report, antidromically identified neurosecretory cells

in the PVN which were activated during lactation and estrus were typically ventral to other antidromically identified cells which did not appear to change (Freund-Mercier, Richard and Miro, 1975).

Numerous projections have been demonstrated recently from OX and VP-containing cells of the PVN to diverse areas in the basal forebrain, choroid plexus and ventricles, brain stem and spinal cord as well as to the external layer of the median eminence in addition to the neural lobe (for example, see Brownfield and Kozlowski, 1977; Buijs, Swaab, Dogterom and Van Leeuwen, 1978; Conrad and Pfaff, 1976; Saper, Loewy, Swanson and Cowan, 1976; Sofroniew and Weindl, 1978). Thus the PVN has the potential to influence much of the brain via projections as well as the body via the adenohypophysis and neurohypophysis. Little is known of the degree of collateralization, the precise location of cell bodies from which each projection originates, and the functional characteristics of these various magnocellular projections. The majority of extrahypothalamic projections appear to arise from the PVN rather than the SON. Likewise, the PVN also contains a population of parvocellular neurons apparently not found in SON (Gurdjian, 1927; Krieg, 1932). The functions and projections of these parvocellular neurons are not well understood.

A better understanding of the hormones, projections and functions of the cells within the magnocellular nuclei may provide a clue to the evolution of distinct supraoptic and paraventricular nuclei, found in reptiles, mamals and birds, from the presumably homologous neurosecretory cells of the magnocellular preoptic of fishes and amphibians (see Oksche, 1976; Vigh and Vigh-Teichmann, 1973; Vigh-Teichmann, Vigh and Aros, 1974). Likewise, consideration of comparative neuroendocrine

and neurocytological data may suggest further possibilities for mammalian investigations. For example, ciliated neurons have been found in the magnocellular preoptic and in the PVN of reptiles and have been proposed as osmoreceptors (see Vigh-Teichmann, et al., 1976), despite a lack of functional studies. The precise locations of osmo- or chemoreceptors which function in the regulation of drinking or excretion are not known in the mammal, although regulatory theory and physiological data strongly support their existence in some form (see Hatton and Armstrong, in press, for review).

In the short time since the diversity of efferents from the mammalian PVN has been recognized (and from magnocellular neurons of all vertebrate classes, see Hoheisel, Ruhle and Sterba, 1978 for references), several investigators have suggested roles for the PVN in addition to participation in the control of excretion and lactation. Electrical stimulation of the rat PVN causes ACTH to be released (Dornhorst, Carlson, Seif, Robinson, Zimmerman, and Gann, 1978). Brief immobilization produced an enhanced immunohistochemical staining for vasopressin within five minutes in parts of the PVN and in the peri-capillary layer of the median eminence, but not in the SON or neural lobe (Krisch, 1978). Previous work has suggested that VP may modulate the release of ACTH by CRF, rather than VP being the primary CRF (Yates, Russell, Dallman, Hedge, McCann and Dhariwal, 1971; see also Saffron and Schally, 1977 for review). Pharmacological, immunohisto-chemical and lesion studies also implicate the PVN in the corticoster-oid response to osmotic stress in the duck (Hoffman, Abel and McNeill, 1977). In ducks, a massive release of corticosteroids is triggered by osmotic stress, resulting in the excretion of salt via a nasal

gland. The PVN may be a site of hormonal feedback in this species, since it selectively accumulates corticosteroids (Abel, Takemoto, Hoffman, McNeill, Kozlowski, Masken and Sheridan, 1975). Projections from the PVN to the external layer of the median eminence (McNeill, Abel and Kozlowski, 1975) and brainstem (Schober, Trautmann, Naumann and Sterba, 1977) have been reported in birds. The intensity of neurosecretory staining in the external layer of the duck has been found to be related to plasma corticoid levels (McNeill, et al., 1975), which is consistent with the results of Krisch (1978) in the rat, which uses other steroid related mechanisms in the maintenance of electrolyte balance. Bilateral lesions of the PVN yield natriuresis (Keeler, 1959) and decreased aldosterone levels in rats (de Wied, Palkovits, Lee, van der Wal and de Jong, 1972). The mammalian PVN may thus be modulating plasma sodium levels via its projection to the external layer of the median eminence (Lobo-Antunes, Carmel and Zimmerman, 1977). Despite substantial evidence suggesting that VP and ACTH may influence learning (see de Wied, 1977 for review), the locations of relevant cell bodies or the site or mechanism of the apparent effects have not yet been determined. The numerous projections of the PVN should be investigated in this regard. Electrical stimulation of the mammalian PVN may also influence cardiovascular reflexes (Cirrello and Calaresu, 1978). The infusion of norepinephrine by cannula (Leibowitz, 1978) and knife cuts in the vicinity of the PVN (Gold, Jones, Sawchenko and Katapos, 1977) produced strong effects on feeding. More importantly, when norepinephrine levels were compared between genetically obese and control mice, differences were found only in the PVN (Cruce, Thoa and Jacobowitz, 1978).

Electrophysiological investigations of antidromically identified neurosecretory cells in the PVN and SON (i.e., the subpopulation of cells which sends axons to the neural lobe) have tentatively established distinguishable differences in firing pattern between OX- and VP-containing cells during enhanced hormone release (Wakerly and Lincoln, 1973; Lincoln and Wakerly, 1974; Poulain, Wakerly and Dyball, 1977). Cells were found in the SON and PVN which were dramatically activated prior to each milk ejection (m.e.); such cells were assumed to be releasing OX. Other cells were not activated prior to m.e. and were assumed to contain vasopressin, since their firing patterns became phasic after hemorrhage and returned to a nonphasic pattern when blood was reinfused. Antidromically identified cells of both types were found in both nuclei. There was a suggestion of a functional distinction between the PVN and SON, since m.e. neurons demonstrated a higher peak firing rate in the PVN (Poulain, et al., 1977). The phasic firing pattern has been commonly found in the SON and PVN under conditions of enhanced VP release (see also Wakerly and Lincoln, 1971; Harris, Dreifuss and Legros, 1975). Recent reports of other hormones in the magnocellular neurons of PVN and SON (enkephalin: Hokfelt, Elde, Johansson, Terenius and Stein, 1977; Rossier, Battenberg, Bayon, Shibagaki, Guillemin, Miller and Bloom, 1978; Sar, Stumpf, Miller, Chang and Cuatrecasas, 1978; somatostatin: Dubois and Kolodziejczyk, 1975; Bugnon, Fellmann and Bloch, 1977; and angiotensin II: Hokfelt, Elde, Fuxe, Johansson, Ljungdahl, Goldstein, Luft, Efendic, Nilsson, Terenius, Ganten, Jeffcoate, Rehfeld, Said, Perez de la Mora, Possani, Tapia, Teran and Palacios, 1978; but see also Changaris, Keil and Severs, 1978), if confirmed, could seriously complicate the

electrophysiological characterization of OX- and VP-containing cells. Likewise, there is a population of antidromically identifiable silent cells; neither the hormone contained by these cells nor their functions are known. Further information regarding the physiology of vertebrate neurosecretory systems can be found in recent reviews (Richard, Freund-Mercier and Moos, 1978; Hayward, 1977).

Ultrastructure of Magnocellular Nuclei

Ultrastructural analyses of magnocellular neurons have yielded fairly consistent results, despite differences in species, nuclei, method and duration of enhancement of synthesis, a frequent lack of quantification in experimental studies and advances in fixation techniques (see Morris, Nordmann and Dyball, 1978 for review). Zambrano and de Robertis (1966) examined the SON of the rat. As expected, the SON was extremely vascularized. Neurons were found abutted against capillary basement membranes; others were separated from them by single fine glial processes. The neurons appeared to be well developed for protein synthesis, each having a large nucleus and nucleolus, extensive endoplasmic reticulum and Golgi apparatus, and many ribosomes. Varying numbers of dense core vesicles (DCVs), ranging in diameter from 800-2,200 Å were found in the cytoplasm. In water replete rats, cells were found to vary in appearance; in particular, the rough endoplasmic reticulum (rER) varied from a collapsed form in some cells to a dilated form in others. When dilated, the rER often contained a flocculent material of low electron density, and the cytoplasm appeared to contain more ribosomes than were found in cells not having dilated rER. Fewer DCVs, which contain hormone or precursor (see Mason and Bern, 1977 for

review), were found in the cytoplasm of the cells with dilated rER, thus suggesting differences among the neurons of the SON of unstimulated animals in the degree of activation of the processes of synthesis, storage, transport, degradation and/or release of neurohormone. The proportion of cells with dilated rER was seen to increase after 48 hours of water deprivation. It was concluded that the SON of the rat in stable water balance contained cells in various stages of a synthetic activity cycle. Water deprivation increased the proportion of cells engaged in enhanced synthesis, thus synchronizing the metabolic activity of the supraopticohypophyseal system.

This hypothesis has been further advanced by Yukitake, Taniguchi and Kurosumi (1977), who classified neurons of the PVN of the unstimulated rat according to degree of dilation of rER and counts of DCVs within the cytoplasm. In addition, they observed the morphology of terminal boutons on the somas and the number of synaptic vesicles within those terminals (Kurosumi and Yukitake, 1977). Three forms of terminal were associated with the somas, but no differences were found in the number of each type of terminal found on the somas of each classification of cell. However, a correlation was found between the cell classification and the number of clear synaptic vesicles in the terminals on the cell body. Therefore, this correlation was suggested as evidence that synaptic activity in some way modulated synthetic activity. It should be noted that the direction of causation may be reversed, since via trophic factors, the metabolism of the PVN soma could conceivably influence the efficiency or metabolism of its afferent terminal and via retrograde axonal transport the distant soma, so that transmitters could be released preferentially onto cells which were in the most

appropriate stage for secretion. No attempt was made to test their cycle hypothesis by manipulating synthesis. Furthermore, immunohistochemical and tracing studies are needed to ascertain the degree to which the correlation between presynaptic and somatic morphology reflects the presence of cells which differ in hormone or efferent projection, with each cell type having separate sets of afferents.

Numerous reports indicate increased dilation of rER in rat magnocellular neurons following dehydration (Enestrom, 1967; Kalimo, 1975; Krisch, 1974, 1976, 1977; Morris and Dyball, 1974; Tweedle and Hatton, 1976, 1977). Apparently, dilation may occur with moderate dehydration (Morris and Dyball, 1974; Tweedle and Hatton, 1977; Zambrano and de Robertis, 1966), however much of the literature deals with the pathology of near lethal dehydration. With electron microscopic immunocytochemistry, Krisch (1977) demonstrated VP reaction product within the lumens of some of the profiles of dilated rER as well as in DCVs. This may represent prohormone in route to the Golgi apparatus for packaging, since synthesis in neurosecretory cells resembles that in other cells (see Mason and Bern, 1977 for review). In addition, it may also be an indication of extravesicular transport or storage of hormone (see Rougon-Papuzzi, Cau, Boudier and Cupo, 1978 and Krisch, 1977 for references). In either case, these findings may explain the poor correlation between immunohistochemical light microscopic and immunoassay results with the number of DCVs which can be found (Krisch, 1977). It may also explain increases in the intensity of VP immunohistochemical stain which may be seen with light microscopy in the PVN within five minutes after immobilization stress (Krisch, 1978). Recent evidence suggests that some hormone may be transported to the

neurosecretory endings in extravesicular form via the smooth endoplasmic reticulum (Rougon-Rapuzzi, et al., 1978).

After hypersecretion induced by severe dehydration, pronounced cytoplasmic vacuolization has been reported (for example, Krisch, 1974; Zambrano and de Robertis, 1966). A network of vacuoles 1-10 µm or more in diameter and filled with an electron lucent substance may predominate within the cytoplasm of grossly hypertrophied somas. Arguing for a cell synthetic cycle in the PVN of unstimulated rats, Yukitake et al. (1977, figure 2) depicted an enlarged neuron with dilated rER and with a single cytoplasmic vacuole measuring over 20 µm by 30 µm. Filled with a flocculent material, it was adjacent to smaller vacuoles as mentioned above which displaced the more typical cytoplasm and nucleus to one edge of the soma. Similar vacuoles are reportedly common in the SON but rare in the PVN of the unstimulated dog (Zambrano and de Robertis, 1967). Interestingly, the dog may store its neurohypophyseal hormones primarily within the somas and axons rather than in the pituitary (see Zambrano and de Robertis, 1967 for references). The role of fixation artefact and cellular pathology in the occasional occurrence of these curious forms is uncertain. Increased occurrence of vacuolized cells has been reported in the rat PVN during pregnancy (Kalimo, 1975) and in the human SON and PVN with pathological dehydration (Koep, Konigsmark and Sperba, 1970). These vacuolized cells are distinct from the enigmatic "dark" cells which are packed with ribosomes, DCVs and a fine electron dense material of unknown origin (for example, see Rechardt, 1969; Tweedle and Hatton, 1976).

The ultrastructure of the PVN has been the object of few systematic studies, although it has been mentioned as similar to the SON (Zambrano

and de Robertis, 1967, 1968a and others). In an abstract, Morris (1971) reported light and dark cells in the PVN which corresponded to those found in the SON by Rechardt (1969). The degree to which these dark cells are artefacts of fixation or disease, as suggested for dark neurons in other areas of the brain (Cammermayer, 1978), is yet unclear.

Flament-Durand (1971) briefly reported finding two cell types within the rat PVN. Her type 1 cell was typically magnocellular, with DCVs, lysosomes, and elaborate rER and Golgi apparatus. Without comment, she depicted two type 1 somas with plasma membranes in close apposition, without intervening glial process, for a length of over 1 μm. Similar membrane appositions have been noted in the SON and NC (Enestrom, 1967; Rechardt, 1969; Tweedle and Hatton, 1976, 1977). A second cell type was found with convoluted nucleus, few profiles of rER which wandered extensively through the cytoplasm and an occasional DCV of small diameter (75 μm). Type 2 cells were probably parvocellular cells of some sort, but she provided no clues to their precise location in or around the nucleus. Based on little credible evidence, she proposed that thyrotrophic hormone-releasing factor (TRF) might be secreted from these cells. It has since been reported that bilateral lesions "in the region of" the PVN substantially reduce hypothalamic and pituitary levels of TRF, leaving relatively high levels unchanged in nonhypothalamic brain (Jackson and Reichlin, 1977).

Kalimo (1971) provided the most extensive description to date of the fine structure of the PVN of the unstimulated rat. He reported "no striking differences" between the neurons of the SON and PVN. The nuclei and nucleoli were large and nearly spherical. Heterochromatin was often seen condensed along the nuclear membrane, and nucleoli often

contacted it. Nuclear pores were frequently observed. Rough endoplasmic reticulum was abundant, particularly in the peripheral cytoplasm. Cisternae of rER were occasionally seen in contact with the outer nuclear envelope. "Excessive" dilation of rER was not encountered. Extensive Golgi complexes were seen several microns from the nuclear membrane. Coated vesicles, DCVs, multivesicular bodies (MVBs) and lysosomes were found in proximity to the Golgi apparatus. Lysosomes ranged from 300-1,000 μm, and reacted positively for acid phosphatase, as did some parts of the Golgi bodies. DCVs were also common in the peripheral cytoplasm. The mean measured diameter of DCVs was 177 μm in the SON and PVN, however the distributions of diameters did differ in shape. Correcting for sectioned vesicles by using quantitative methods (and dubious assumptions), Kalimo (1971) computed that the actual mean diameter of DCVs following his histological techniques was 190 μm. The number of DCVs varied substantially among cells. Occasional dark cells were encountered, but were considered artefactual. Kalimo (1971, figure 2) also noted the existence of "close contacts" between the magnocellular neurons of the PVN. Such contacts have also been reported in a recent abstract (van den Pol, 1978).

As mentioned above, Yukitake et al. (1977; also Kurosumi and Yukitake, 1977) briefly examined the PVN for evidence of a cell activity cycle. Except for the findings of a few grossly enlarged neurons containing cytoplasmic vacuoles, their results were consistent with those of Kalimo (1971). Similar fixation procedures were followed by the two groups, thus the origin of these vacuoles is unknown.

Ultrastructural changes have been reported in the PVN following dehydration (Krisch, 1974; Kalimo, 1975; Morris and Dyball, 1974).

Furthermore, Kalimo and Rinne (1972) examined the SON and PVN of the Brattleboro rat, a recessive mutant which totally lacks the ability to synthesize VP and thus suffers from diabetes insipidus. No differences were found between the two nuclei of these chronically dehydrated animals. Neurons in both nuclei were hypertrophied, and there were few DCVs in somas or in the neural lobe, apparently due to hypersecretion of OX in the absence of VP. Similar results were obtained by Tasso and Rua (1978) who also observed membrane appositions, nematosomes, and more lysosomes than in the normal rat. Nematosomes have been reported in the SON of the normal rat (Tweedle and Hatton, 1976).

Krisch (1974) examined the distributions of mean diameters of DCVs per cell in the SON and PVN and in the neural lobe during pregnancy and water deprivation. Morphometric analysis of electron micrographs was used to investigate the responses of OX- and VP-containing cells, and of the PVN and SON, since immunohistochemical evidence that both hormones were found within both nuclei was not yet available (i.e., Burford, Dyball, Moss and Pickering, 1974; Swaab, et al., 1975; Vandesande and Dierickx, 1975). Mean DCV diameter was determined for each neuron or neural lobe terminal which was sampled. The distributions of these means were than analyzed. In normal rats, DCVs were larger and more frequent in the SON than in the PVN, and the difference was greater in females than in males. Mean DCV size per element (cell or neural lobe terminal) appeared bimodal in the neural lobe but not in the two nuclei. In pregnant females, cells in the SON and PVN were enlarged and had more DCVs and fewer lysosomes. Mean DCV size per cell increased in the PVN during pregnancy compared with female controls, possibly due to a depletion of the smaller (OX-containing?) vesicles.

Following 48 hours of thirst, both SON and PVN cells were altered. Dilation of rER was more commonly seen in the SON than PVN with dehydration. More extreme examples of dilation were seen with ten days of thirst. Two or ten days of water deprivation produced a decrease in the mean size of DCVs in the SON and a depletion of the larger (VP-containing?) vesicles from the neurohypophysis. Krisch's (1974) results are consistent with the finding of both hormones in both nuclei and with some functional differentiation between those nuclei. DCVs are also smaller in the PVN than the SON in the dog (Zambrano and de Robertis, 1967), and two populations of DCV sizes are reported in a toad (Zambrano and de Robertis, 1968b). Analysis of the sizes of vesicles as indicators of hormone have been superseded for the present by immunohistochemical methods. There are conflicting data regarding other factors such as fixation technique and age of vesicle and/or position along the hypothalamo-posthypophyseal tract which may influence size and staining characteristics, thus complicating attempts to morphologically distinguish hormones without highly specific immunohistochemical tools (for example, see Tasso, Rua and Picard, 1977). Precise analysis of small differences in vesicle size is further complicated by the need for consideration of section thickness. Sections through spherical structures yield underestimates of diameter, and the magnitude of the error is related to section thickness and to matters of sampling (Weibel, 1969).

Morris and Dyball (1974) substituted two percent NaCl for drinking water, a stimulus which increases the release of both OX and VP (Jones and Pickering, 1969; Dyball and Pountney, 1973). Rats were dehydrated for three days, and some were then rehydrated for a subsequent two days.

Similar effects were found in both the SON and PVN, although more cells initially had dilated rER in the SON. Cell size, the proportion of cells with dilated rER, and the number of MVBs increased with dehydration in both nuclei. DCVs were classified as "pale" (supposedly mature) or "dense" (immature) according to Cannata and Morris (1973). Small changes in pH or fixation duration appear to influence staining characteristics more than functional variables, thus complicating the analysis (Cannata and Morris, 1973; Morris and Cannata, 1973). Nonetheless, with precise control of fixation, the appearance of DCVs may be influenced by dehydration. Although the total number of DCVs was unaffected by dehydration, there was an increased proportion of dense (immature) vesicles in both nuclei with dehydration, and fewer lysosomes were found. The number of cells with dilated rER and the number of MVBs decreased with rehydration. In the PVN the rER remained somewhat dilated after rehydration. Rehydration also produced an increased number of lysosomes and an increased proportion of pale (mature) vesicles. The total number of vesicles increased during rehydration in the PVN. Morris and Dyball (1974) observed that the population of DCVs became more homogeneous following dehydration. A similar dehydration for a longer duration, 1.75 percent NaCl for 2 weeks, has been shown to increase the proliferation of astrocytes and endothelial cells in the SON and pituicytes (Patterson and Leblond, 1977). The PVN was not examined in that study.

Kalimo (1975) examined both the PVN and SON of rats under conditions of water deprivation and lactation. Neurons in both nuclei were hypertrophied with either stimulus, and the number of DCVs in the cytoplasm was reduced. Vacuolization of the rER (as described previously) was not seen with up to six days of water deprivation. Smaller changes

in the dilation of rER were not mentioned. However, vacuolization was seen in 15 percent of the neurons of the PVN and rarely in the SON after 2 - 2.5 weeks of lactation. This observation would seem to suggest that the formation of vacuoles is not entirely artefactual, although it may be pathological. Furthermore, this is additional evidence for different roles for the two nuclei during lactation.

Several studies have examined the ultrastructure of the PVN following more extreme manipulations. Hypothalamic deafferentation ("islands") caused a dramatic increase in the number of DCVs three days later in the PVN (Dyer, Dyball and Morris, 1973). These DCVs were assumed to contain primarily OX, since bioassay of tissue of the PVN showed a corresponding rise in OX activity. The physiological mechanisms underlying this synthetic response are unclear. Extracellular recordings from similar island preparations have revealed firing rates increased (Cross and Kitay, 1967) and decreased (Dyball and Dyer, 1971) compared with controls. Likewise, castration and ovariectomy produce a hypertrophy of both nuclei and dilation of rER in the rat (Zambrano and de Robertis, 1968a). Pituitary oxytocin levels also rise, and these effects were reversed by treatment with appropriate sex hormones. Norepinephrine levels have recently been shown to drop in the PVN following castration (Cruce, et al., 1978).

Possible direct or indirect endocrine influences on hypothalamic neurosecretion are further suggested by Murakami, Nakayama, Shimade and Hirata (1970), who found substantial hypertrophy, vacuolization and pathology in the PVN and SON following chronic administration of aldosterone. As mentioned previously, other evidence suggests that the PVN may participate in the control of steroid release and regulation

of plasma sodium. Similar ultrastructural changes were seen following chronic magnesium depletion (Murakami, Shimade, Inokuchi and Hirano, 1975). They cite evidence that this treatment substantially raises plasma aldosterone levels. Magnesium depletion could also conceivably disrupt any of the body's enzyme systems which utilize magnesium ions as a cofactor.

Swanson, Connelly and Hartman (1977, 1978) have analyzed the adrenergic innervation of the posterolateral PVN of ganglionectomized rats. Following 5-hydroxydopamine (5-OHDA) treatment, but not in control or reserpine pretreated animals, varicosities containing small granular vesicles (35 μm diameter) were commonly seen. Distinct synaptic membrane specializations, usually asymmetrical were seen with 19 percent of labeled profiles. The post-synaptic element was most often a dendrite, but occasional somas and a Herring body were seen with innervation. These terminals appear to correspond to terminals containing clear microvesicles in untreated animals. About five percent of these profiles were directly apposed to capillary basal laminas. Swanson et al. (1978) concluded that norepinephrine or epinephrine of central origin influences the PVN in several ways. First there is a traditional synaptic innervation of neurons. Second, the blood flow and permeability of this dense vascular network may be under direct neural control. Furthermore, because of the large proportion of terminals which lack post-synaptic specialization, the transmitter may also be released for diffusion to nearby neuronal, glial or vascular elements. This concept is consistent with the diffuse innervation of the brain by locus coeruleus (for example, see Jones and Moore, 1977). Changes in regional blood flow and permeability have been reported in

ganglionectomized monkeys following stimulation of the locus coeruleus (see Swanson, et al., 1978 for references). The role of capillary innervation in these changes has not been determined. However, changes in capillary function could be of particular importance in brain regions related to neurosecretion and fluid balance.

The hypothalamo-hypophyseal system has been the target of numerous cytotoxic investigations, primarily designed for the study of transport phenomena (for example, Boudier and Picard, 1976; Hindelang-Gertner, Stoeckel, Porte and Stutinsky, 1976; Vasquez and Amat, 1978). Colchicine appears to disrupt axoplasmic transport in this system without necessarily producing any morphologically detectable disruption of microtubules. Among the effects of colchicine treatment is an increased number of DCVs within the SON and PVN. Various ultrastructural anomalies have been reported in hypothalamic neurons following the injection of potent toxins (for example, see Hindeland-Gertner, et al., 1976).

Rationale for Experiment

Few of the ultrastructural studies of the mammalian hypothalamic magnocellular nuclei have included any quantification, despite no lack of reported changes following treatments. Recently, Tweedle and Hatton (1976, 1977) have measured changes in the SON and NC following minimal dehydration and rehydration. Furthermore, although the occurrence of somato-somatic membrane close appositions has been casually noted in the SON and PVN as well as elsewhere in nervous systems, Tweedle and Hatton have demonstrated substantial changes in the extent of these appositions following brief ecologically relevant degrees of stimulation of this system. Membrane appositions, which could theoretically permit

ephaptic interactions among neurons (Bennett and Auerbach, 1969), thus represent a potential mechanism for neural plasticity. Since the PVN clearly plays some role in fluid balance, and also contains neurons in close apposition (Flament-Durand, 1971; Kalimo, 1971), it seemed appropriate to examine this nucleus for comparison with the recent results for SON and NC (Tweedle and Hatton, 1976, 1977). Because it appears that VP-containing cells which project to the neurohypophysis may be located predominantly in the lateral portion of the nucleus, this investigation was of the PV-L.

METHOD

Subjects

Animals used in this study were male Sprague Dawley albino rats (Holtzman Co.) ranging in age from 90 - 120 days at sacrifice. The rats had been housed in group wire mesh cages under constant light in a room maintained at 21 - 23° C for at least 30 days with food and water constantly present. Rats were then moved into individual wire mesh cages with food and water available ad lib. One week later the experiment commenced.

Procedure

Deprivation. The methods for this experiment have been adapted from Tweedle and Hatton (1977). Twenty-four rats were used. Four rats were sacrificed without undergoing any water deprivation. Four animals were deprived of water (with food present) for four hours after they had been observed to terminate a voluntary drink. Other groups of 4 animals were deprived of water for either 12 or 24 hours prior to sacrifice. Following 24 hours of water deprivation, additional groups of 4 rats were then allowed to drink (rehydrate) for either 12 or 24 hours prior to sacrifice. Deprivations were scheduled so that all perfusions were performed between 10:00 A.M. and 2:00 P.M., and animals within each treatment group were perfused at times which were distributed within this interval. The schedule

of dehydration, rehydration, and perfusion was arranged so that one animal from each of the six treatment groups was perfused within a two day period. Each of these six animals was born on the same day, received similar pre-experimental treatment, and was histologically processed with solutions from the same mixtures. Since fixation was occasionally found to be inadequate, or tissue samples were lost or damaged in processing or sectioning, several additional animals were individually processed in accordance with similar procedures.

Histology. Rats were anesthetized with ether until breathing just ceased. The chest cavity was then opened and the descending aorta was clamped. Sodium heparin (0.2 ml) was injected into the left ventricle of the beating heart and allowed to circulate briefly through the brain. A perfusion cannula was introduced into the left ventricle and the right atrium was cut. Perfusion with 0.9% saline for 1 minute was followed with 250 - 300 ml. fixative (10 - 15 minutes). The fixative was 4.0% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3 - 7.4) containing 0.5% dimethyl sulfoxide. The perfusates and all subsequent solutions were used at room temperature. Following perfusion, the whole brain was quickly removed from the skull. Coronal slices were carefully made through the brain near the rostral extent of the third ventricles and through the cerebral aqueduct in order to expose the ventricular system, and the brain was placed overnight in fixative. The next day, the brain was placed into 0.15 M cacodylate buffer (pH 7.3 - 7.4) for 1 - 2 hours. Using a clean razor blade moistened in the same buffer, a block of tissue containing the hypothalamus was dissected from the brain. Under a dissecting microscope and using a moistened razor or stadie blade, coronal sections

(approximately 1 - 2 mm thick) were taken. Sections were placed on a translucent layer of paraffin and viewed under the same microscope using transmitted and/or incident light. Major visible landmarks included the anterior commissure, third ventricle, optic chiasm and optic tracts, fornix, SON, and occasionally the PVN. Cores of the PV-L were taken with an electrolytically etched 15, 17, or 18 gauge needle on each side of the section or sections where the nucleus was visible or expected to be found. Since the fornix was always visible in the hypothalamic slices, it was used as a target for cores that would contain its more medial fibers as well as the lateral parts of the PVN (see Figure 1). Notes were taken during coring on the estimated likelihood that a given section contained the PV-L. Because the angle of section often varied from true coronal, section thickness varied, and the PVN was often not visible, one could not be certain that a particular core contained an adequate sample of the PV-L. Therefore, cores were taken for more than one section, and cores from each section were placed in separate labeled vials.

The tissue samples were ejected from the coring needle into 0.15 M cacodylate buffer for 15 minutes, followed by another 15 minute rinse in buffer. Post fixation was performed by placing cores in 1.0% OsO_4 in 0.1 M cacodylate buffer (pH 7.4) for 2 - 3 hours. Following three 5-minute rinses in filtered water, cores were placed for 4 - 12 hours in 4.0% uranyl acetate in water and kept in the dark. After three 5-minute rinses in water, the cores were dehydrated sequentially with 50%, 70%, and 95% ethanol followed by 3 changes of 100% ethanol, each for 20 minutes. Cores were infiltrated with low viscosity resin (Spurr, 1969) by placing them

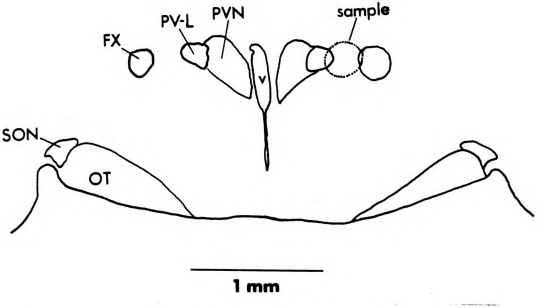

Figure 1. Drawing of a coronal section through rat hypothalamus showing position of sampled tissue. Fornix (FX) was a major landmark during coring and for determination of orientation during sectioning. Other visible landmarks in unstained tissue: OT-optic tract, SON-supraoptic nucleus, v-third ventricle. The PVN (including PV-L) was typically not visible during coring. Drawing from celloidin embedded section, thus the degree of shrinkage may differ from that in fresh, fixed or plastic embedded tissue.

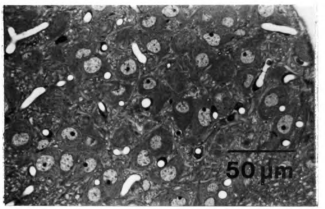

Figure 2. Micrograph of the PV-L of a 4 hour water deprived rat (1 µm thick plastic section stained with toluidine blue). Note vascularization, eccentric nuclei and prominent nucleoli.

sequentially, for 8 - 12 hours each and with occasional agitation, into 33%, 50%, and 66% plastic resin diluted with absolute ethanol, and then into 100% plastic resin. Polymerization was carried out for 12 - 16 hours in a laboratory oven which was maintained at 70° C.

Animals (cores in blocks) were sectioned, grids examined, photographed, printed and analyzed in a systematic order so that any change or improvement in the localization of the sample within specifically the lateral portion of the nucleus, or any technical change which would influence contrast, resolution, or measurement error could be expected to be distributed across the groups of animals. Blocks were thick and thin sectioned (approximately in the coronal plane) using glass knives and an ultramicrotome. The choice of blocks for sectioning was made on the basis of notes made during coring which suggested the cores that most likely contained the PV-L. Thick sections (1 - 2 μm) were taken of one or more cores in order to locate the PV-L. The large neurosecretory cells of the PV-L were found near the edge of the core and stained prominently with toluidine blue, especially in dehydrated and rehydrated animals (see Figure 2). Similar staining was seen with methylene blue. The number of capillaries per unit area was clearly higher in the PV-L than in surrounding hypothalamic regions. Parts of the fornix were seen across the section from the nucleus in the same or nearby sections. Silver ultrathin sections were taken of the PV-L, collected on uncoated 200 mesh copper grids, and post stained with lead citrate. After several grids had been taken, the knife was advanced 35 - 50 μm and more grids were taken. Thin sections were not taken within 50 μm from either end of a core, and additional cores were sectioned if necessary.

Sections were examined with an electron microscope, generally operated at 60 KV. Images were recorded on electron microscope sheet film. During the examination of grids on the electron microscope, a suitable section was chosen and grid squares containing the PV-L were noted. Photographs were systematically distributed among the suitable grid squares. For assessment of neuronal membrane apposition and dilation of rough endoplasmic reticulum, pictures were taken at 2,000x and enlarged 2.5x during printing. Care was taken so that an area was not sampled in more than one print per section or in near-adjacent sections. On rare occasions when prints of overlapping areas were found during analysis, the overlapping region of one print was randomly chosen for measurement. Pictures were taken from the same grid squares at 10,000x and enlarged 2.5x for use in the assessment of changes in vesicle populations of PV-L magnocellular neurons. A calibration test of the electron microscope (at 60 KV) during the course of this experiment revealed magnifications of 2,006x and 9,936x at the nominal values of 2,000x and 10,000x, respectively (J. Huntsberger, personal communication). No correction was made for these differences since they were small and would be expected to change during the operation of the scope. Magnification errors of a similar or greater magnitude, but still negligible, might be expected between uses of the enlarger. Approximately eight pictures were taken at each magnification from each of the two sets of grids. Suitable prints were analyzed, and additional sections were taken and photographed if necessary.

Morphometrics. During printing, the animal and negative numbers were written on the back of each print. To minimize the effects of experimenter bias, prints from several animals were shuffled together

prior to analysis. Low power prints (5,000x) were examined under an illuminated magnifying lens. The outlines of neuronal membranes were traced with felt markets. Different colors were used for apposed and non-apposed neuronal membranes. Somatic membranes and membranes of processes (longitudinally sectioned) that were visibly continuous with somas that were in the print were traced. Otherwise, cross sections of processes which did not exceed 6 μm in any direction were excluded. Cross sections of processes that exceeded this criterion were included if the cytoplasm within them was of a character similar to that seen in somas within the print. Locations where membranes which met these criteria were apposed, without intervening glial processes, to other membranes meeting the criteria, were defined as "somato-somatic appositions" (see Figures 3, 4). Numerous other appositions involving one or more neural elements not meeting these criteria were also observed. However, analysis of them would be complicated by the ultrastructural similarities between axons and dendrites of neurosecretory cells; therefore, these non-somato-somatic appositions were not measured in the present study. Somas which lacked neurosecretory vesicles and otherwise appeared not to be magnocellular were probably parvocellular and were very occasionally encountered among the magnocellular cells of the PV-L. These rare cells were included in the analysis of membrane apposition but disregarded for the assessment of the dilation of rough endoplasmic reticulum or vesicle populations.

For statistical purposes each print was considered a sample. Total length of somato-somatic membrane appositions (Lap, t) and total length of non-apposed membranes (Lmnap, t) were directly measured with a "plan measure." Percentage of neuronal membrane in

Figure 3. A. Arrows indicate extensive somato-somatic membrane apposition between magnocellular neurons of the PV-L in a 24 hour water deprived rat.

B. Enlargement of a somato-somatic membrane apposition (arrows) in a 24 hour deprived rat. Note the presence of astrocytic processes (asterisks) which often separate neuronal elements.

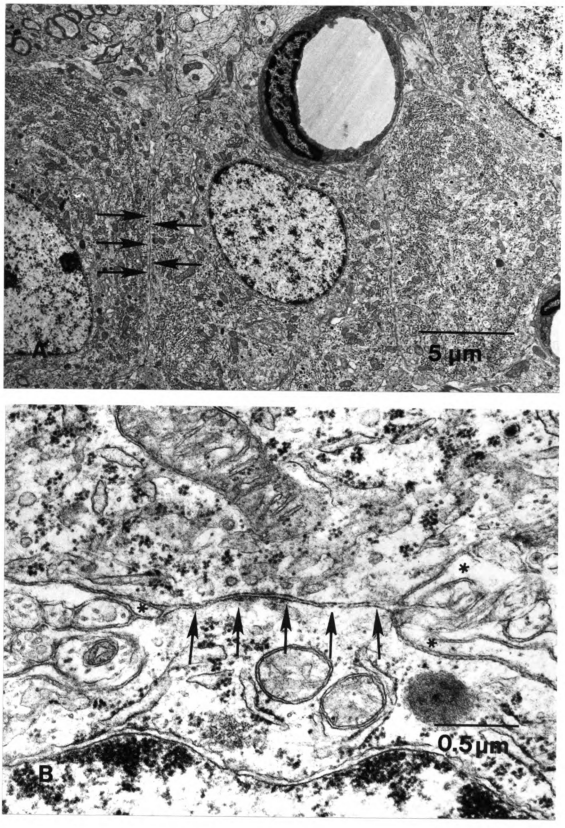

Figure 3.

Figure 4. Neuronal membrane close apposition between two magnocellular somas (arrows). Note the adjacent intervening glial process (asterisk).

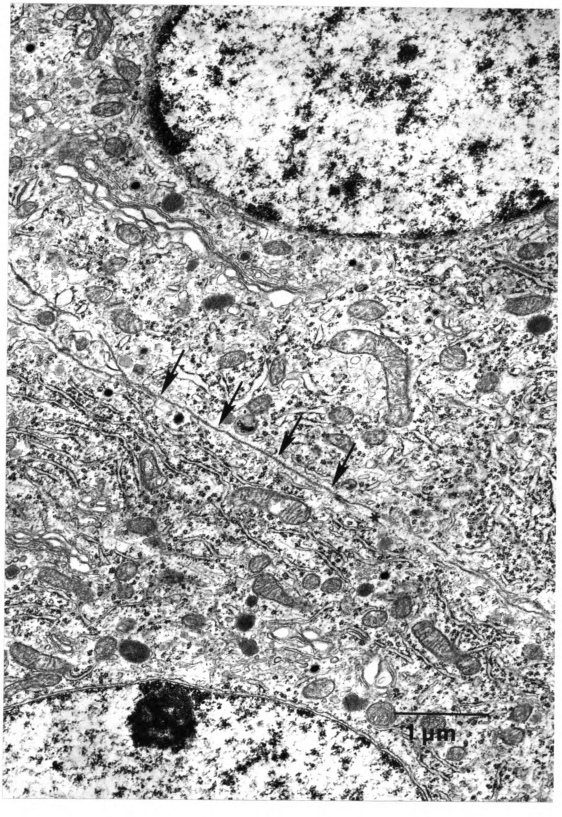

Figure 4.

apposition was defined by the following quotient[1]:

$$\frac{2 \times (\text{Lap, t}) \times (100)}{(\text{Lmnap, t}) + \{2 \times (\text{Lap, t})\}}.$$

The mean length of membrane appositions, number of appositions, number of appositions per cell exhibiting one or more appositions, and the number of cells having appositions which were visible within the print was determined for each print. Since functional print area varied, primarily due to occasional intervening grid bars, the number of appositions, the number of cells having appositions, and the total length of neuronal membranes in somato-somatic apposition was determined per 100 μm^2 of PV-L area. The proportion of cells having areas of dilated rough endoplasmic reticulum (see Figure 5) was determined for each print. Although this measure was fairly subjective, it was performed blindly and with frequent comparisons to a standard (Tweedle and Hatton, 1977, fig. 2) which depicted both dilated and nondilated rER. Membrane bound intranuclear vacuoles (see Figure 6) have been observed in the SON (Tweedle and Hatton, 1976). The proportion of magnocellular nuclei possessing these bodies was determined for each print. From these samples, means were determined for each animal.

Ultramicrographs of PV-L magnocellular cytoplasm taken at 10,000x and enlarged to 25,000x were scrutinized for changes in lysosomal and dense core vesicle populations. Electron density of cytoplasm appeared

[1]The term (Lap, t) is a measure of total length of somato-somatic appositions, whereas (Lmnap, t) is a measure of non-apposed membranes (m). The length of somatic membrane in somato-somatic apposition is {2 x (Lap, t)}. Therefore, {2 x (Lap, t)} appears in both the numerator and denominator, and the quotient yields the percentage of somatic membrane in somato-somatic apposition.

Figure 5. A. Note the dilation of rough endoplasmic reticulum (arrows) in cell at lower left of ultramicrograph of the PV-L of a 24 hour rehydrated rat. The rough ER of the adjacent cell is not dilated.

B. Enlargement of dilated rough ER of a 12 hour rehydrated rat. Notice the extensive presence of a flocculent substance within lumens of ER (arrows).

C. Extreme dilation of rough ER of a 12 hour rehydrated animal. This condition was rarely encountered but has been reported following severe dehydration and during lactation.

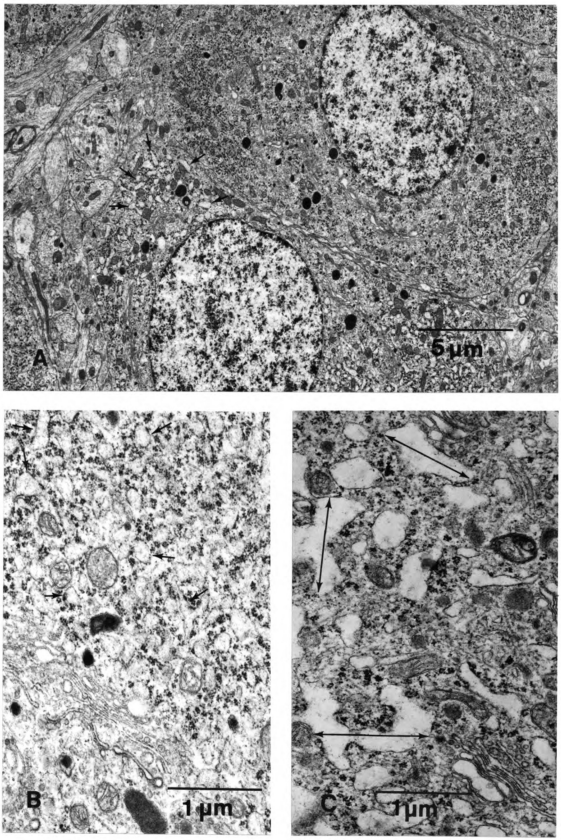

Figure 5.

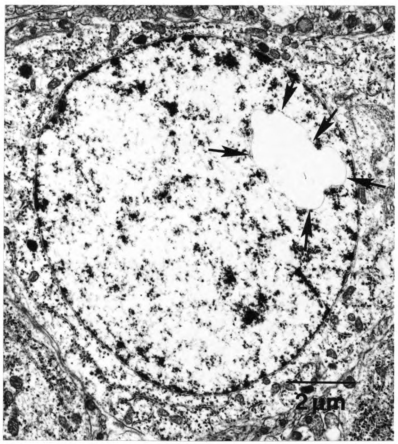

Figure 6. Membrane bound intranuclear vacuole (arrows) in the PV-L of a rat deprived of water for 4 hours.

to be variable within sections; however, it could not be reliably quantified. Extreme dark cells were not sampled, since they have been attributed to fixation artefact and cytopathology (for discussion, see Cammermeyer, 1978). Prints were analyzed blindly. Neuronal cytoplasmic area was determined with a planimeter and/or from measurements with a metric ruler. Counts were made of smaller dense core vesicles (DCVs, apparent diameter <1,600 Å), larger DCVs (>1,600 Å), and lysosomes (>4,000 Å, see Figure 7). A measuring magnifier was used to determine vesicle size, and measurements were taken from limiting membranes at orientations which maximized lengths. So-called "pale" neurosecretory vesicles (Morris and Cannata, 1973; Cannata and Morris, 1973) were excluded since they were indistinguishable from immature lysosomes (Tweedle and Hatton, 1977; see also Figure 8). Membrane bound vesicles of DCV size were occasionally seen with contents resembling nearby lysosomes in texture or electron density, or containing membranous inclusions. These structures were not counted as DCVs. Obvious lysosomes with diameters less than 4,000 Å were not included in counts of lysosomes because of the difficulties in distinguishing other small lysosomes from pale neurosecretory vesicles. Some obvious lysosomes were seen which were shaped so that they might appear to be DCVs in suitably oriented sections. There was judgement and error involved in the classification of structures as DCVs (counted) or pale vesicles/small lysosomes (not counted), and this judgement was probably influenced by print contrast and other histological, photographic and perceptual variables which may or may not have been distributed randomly across treatments. The potential confusion of certain DCVs and lysosomes has been mentioned previously (Boudier and Picard, 1976).

Figure 7. A. Note variation in sizes of DCVs (smaller arrows) in a 12 hour deprived rat. Electron dense lysosomes (larger arrows) are also prominent.

B. Extensive Golgi apparatus (arrows) and parallel profiles of rough ER are common in cytoplasm of neurosecretory cells (PV-L, 12 hour rehydrated rat).

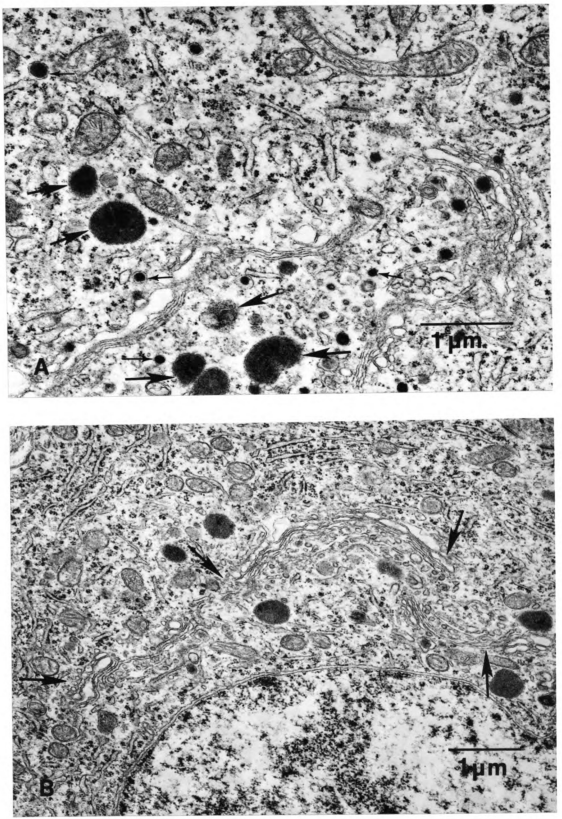

Figure 7.

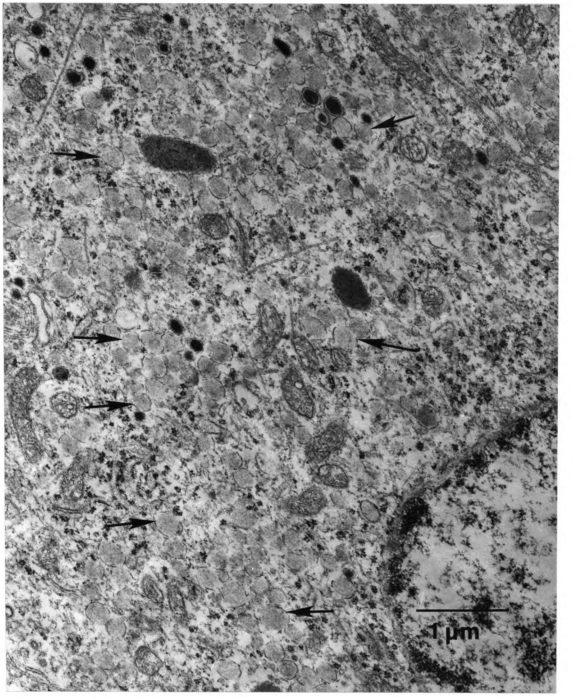

Figure 8. Magnocellular neuron in the PV-L of a 12 hour rehydrated rat containing numerous pale vesicles (arrows). Vesicles with clearly dense cores are also evident.

Counts made on material stained for neurosecretory material or acid phosphatase would be more valid. Multivesicular bodies with diameters greater than 4,000 Å were counted as lysosomes.

The data were analyzed using one way analyses of variance, fixed effects model. Pairwise and complex comparisons were made using Tukey's or Scheffé's post hoc tests, respectively. Trends were considered using orthogonal polynomials after discarding the four hour groups in order to obtain equal time interval spacing of treatments. Due to the extreme violation of parametric statistical assumptions by the intranuclear inclusion data (e.g., all animals in one group had the same score, 0 percent, while other groups had a large range of values), these data were tested with a Kruskal-Wallis nonparametric test. Since 14 of the 24 animals had no vacuoles, a Fisher's exact test was performed to determine whether the distribution of these animals was independent of treatment.

RESULTS

As expected, somato-somatic appositions were found in the PV-L. Data which suggest changes in neuronal somato-somatic appositions are depicted in Figure 9. Dehydration/rehydration influenced the percentage of total somatic membrane in somato-somatic apposition ($F = 2.838$; $df = 5,18$; $p < .05$), although there was much error variance (estimated proportion of variance accounted for by treatment $\hat{\omega}^2 = .277$). The percentage of somatic membrane in somato-somatic apposition appears to have increased with dehydration. However, no comparisons between individual groups were significant ($p > .05$). The total length of membranes in somato-somatic apposition per 100 μm^2 of PV-L was also affected by the treatment ($F = 2.951$, $df = 5,18$; $p < .05$; $\hat{\omega}^2 = .289$). On this measure, 12 hours deprivation produced an elevation over the 4 hour level ($p < .05$) and a cubic trend was found, suggesting a second increase during rehydration ($F = 5.184$; $df = 1,15$; $p < .05$)[1].

---

[1] A product-moment correlation indicated that these two measures of membrane apposition were highly correlated ($r = +.921$, $p < .00004$, Fisher's Z test). Since the same measurements of somato-somatic appositions were used as the numerators in the quotients which defined both measures (i.e., percentage of somatic membrane in somato-somatic apposition and total length of membrane in somato-somatic apposition per unit PV-L area), the denominators of both quotients must have been highly correlated. Therefore, there was a high linear correlation between total length of somatic membrane per sample (print) and PV-L sample area across treatments. Thus, the total length of sectioned somatic membrane per unit PV-L area was unaffected by neuronal hypertrophy or other effects of treatment.

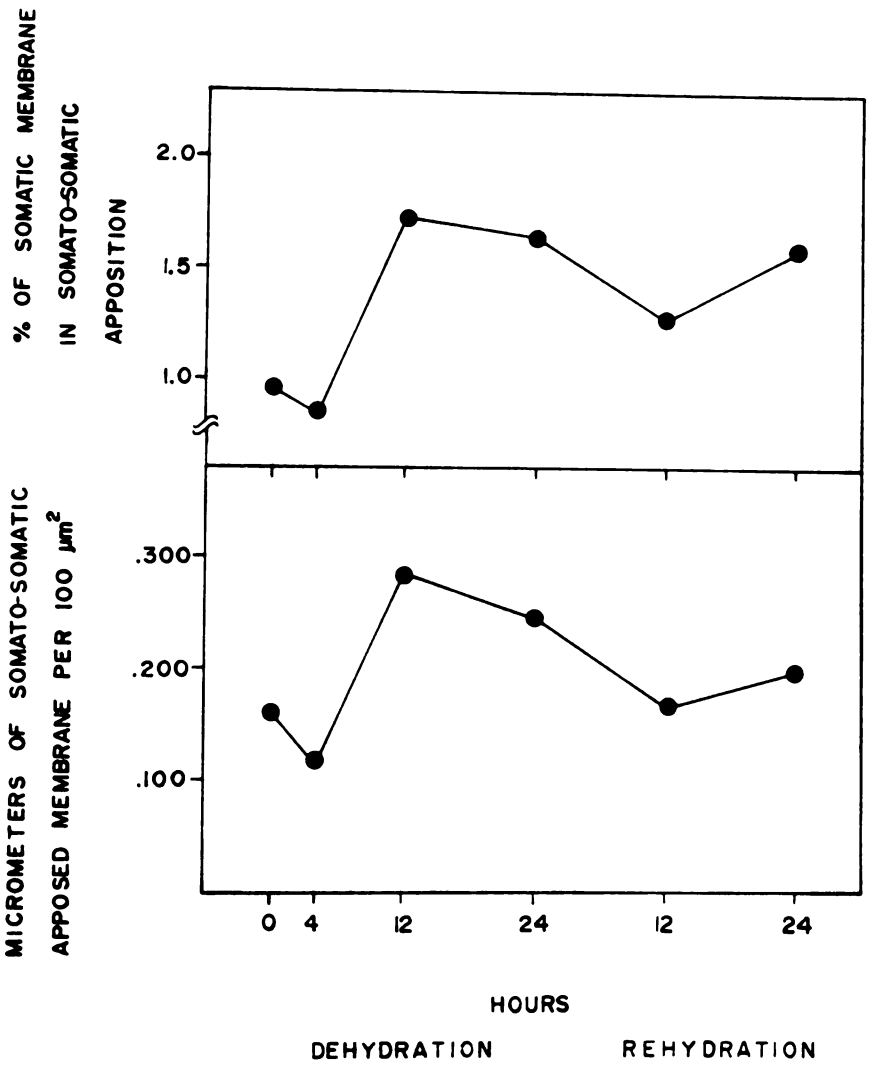

Figure 9. Effect of dehydration and rehydration on somato-somatic membrane appositions in the PV-L.

No changes were found in the number of somato-somatic appositions per 100 μm^2 of PV-L area ($F = 1.699$; $df = 5,18$; $p < .25$), the number of cells with somato-somatic appositions per 100 μm^2 of PV-L area ($F = 1.530$; $df = 5,18$; $p < .25$), or the mean length of somato-somatic apposition ($\bar{x} = 1.73 \pm .17$ μm; $F = .905$; $df = 5,18$; $p > .25$). Furthermore, an analysis of the number of appositions per cell showing at least one apposition revealed no change ($F = 1.052$; $df = 5,18$; $p > .25$; see Table 1).

No change was found in the percentage of magnocellular neurons with dilated rER ($\bar{x} = 21.80 \pm 1.92$; $F = .266$; $df = 5,18$; $p > .25$; see Table 2). Because of the extreme heterogeneity of variance found in the percentage of magnocellular nuclei containing vacuoles, non-parametric tests were considered appropriate. No vacuoles were found in 14 animals, including the 24 hour deprivation group and few were found after 4 or 12 hours. Ranges from 0 to over 30 percent were found among the control and rehydrated groups. The effect of treatment was not significant with this analysis ($H = 7.941$, $p < .20$; $\bar{x} = 5.294 \pm 2.030$; see Table 2). However, when animals were characterized as either possessing or lacking intranuclear vacuoles, the distribution of animals lacking vacuoles was not independent of treatment ($p = .017$, Fisher's exact test; see Table 3). The number of small (<1,600 Å) dense cored vesicles was influenced by treatment ($F = 3.163$, $df = 5,18$; $p < .05$; see Figure 10). The number of small DCVs per 100 μm^2 of magnocellular cytoplasm was below the baseline level after 12 hours of dehydration and again after 24 hours of rehydration (each $p < .05$). A trend analysis on small DCVs revealed linear ($F = 4.629$; $df = 1,15$; $p < .05$) and cubic ($F = 7.492$; $df = 1,15$; $p < .01$) trends. No change was found

Table 1. Cell contact characteristics*,**

	Group					
	0h	4h	12h	24h	24-12h	24-24h
Mean length of apposition (μm)	1.66	2.05	2.36	1.36	1.35	1.66
Number of appositions per 100 μm^2 of PV-L	.048	.032	.070	.090	.064	.065
Number of cells with appositions per 100 μm^2 of PV-L	.062	.041	.090	.104	.088	.094
Number of appositions per cell with at least 1 apposition	1.44	1.29	1.58	1.74	1.52	1.39

*Apparent differences in these data are not statistically significant.

**See also Figure 9.

Table 2. Magnocellular intraneuronal characteristics*

	0h	4h	12h	24h	24-12h	24-24h
% cells with dilated rER	15.97	19.33	24.90	20.84	20.12	29.65
% nuclei with intranuclear vacuoles**	9.66	.60	1.53	0	10.74	9.25
Number of large DCVs per 100 μm^2 of cytoplasm***	7.46	10.35	6.06	7.35	6.60	7.96
Number of lysosomes per 100 μm^2 of cytoplasm	9.82	10.96	8.22	10.33	9.12	9.72

*Apparent differences in these data are not statistically significant.

**See also Table 3.

***See also Figure 10.

Table 3. Presence of intranuclear vacuoles as a function of treatment*

	Control and rehydrated animals	Dehydrated animals
Vacuoles	8	2
No Vacuoles	4	10

*p = .017

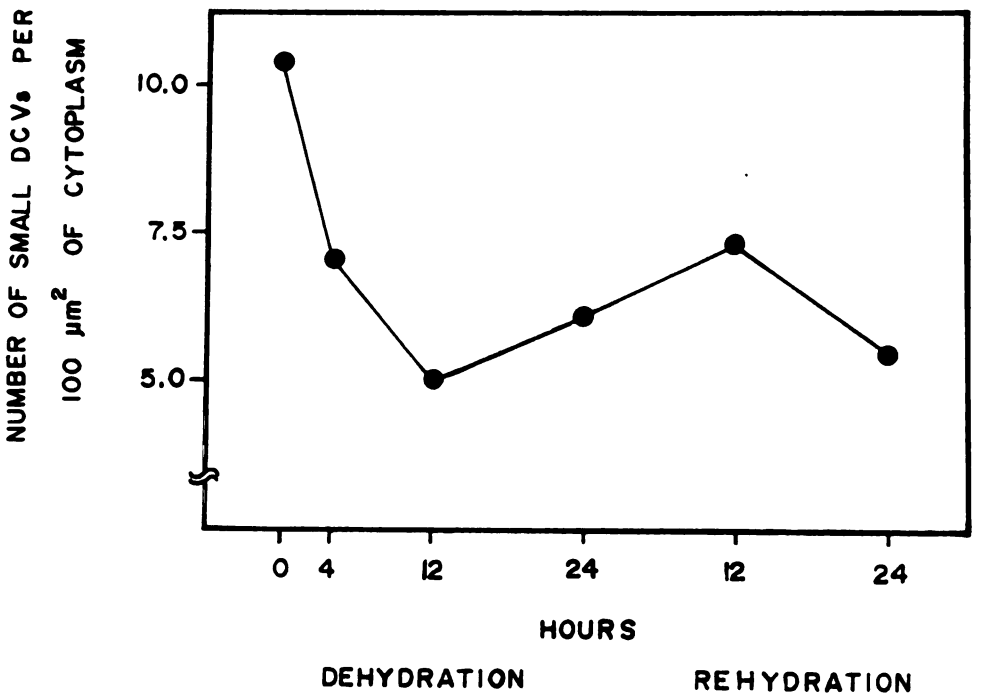

Figure 10. Number of small DCVs (<1,600 Å) per unit area of PV-L cytoplasm as a function of dehydration and rehydration.

in the number of large DCVs ($F = .706$; $df = 5,18$; $p > .25$) or lysosomes ($F = 1.078$; $df = 5,18$; $p > .25$; see Table 2).

The appearance of the PV-L was in substantial agreement with previous observations of the PVN (Kalimo, 1971, 1975; Morris and Dyball, 1974). The type 2 cells which Flament-Durand (1971) reported finding in the PVN, with extraordinarily long and wandering profiles of endoplasmic reticulum were only rarely encountered (see Figure 11). These cells contained relatively few ribosomes and a few possible DCVs (100 - 120 μm diameter). Several nematosomes were encountered within magnocellular neurons during this study (see Figure 12). Cilia were also seen in cross section in the neuropil of the PV-L. A 9+0 or 8+1 pattern of microtubules was evident, and in longitudinal section a cilium was seen emerging from a magnocellular soma (see Figure 13). An intranuclear rodlet was seen in one cell during this study (see Figure 14). The occurrence or morphology of the nematosomes, cilia or rodlet showed no obvious relation to treatment.

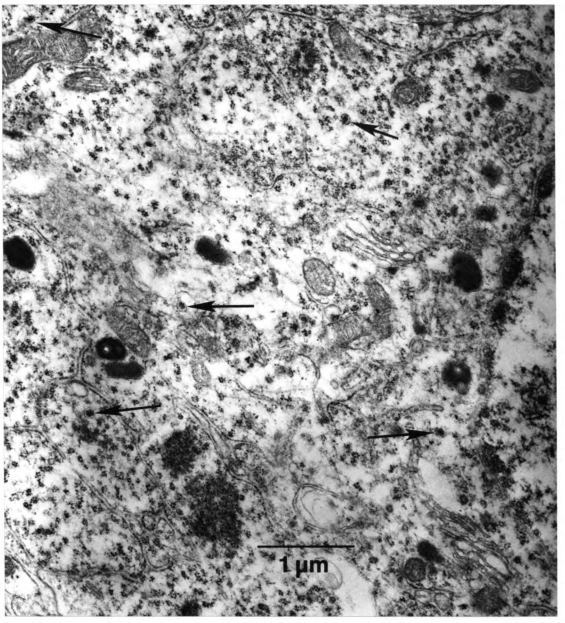

Figure 11. Cells resembling Flament-Durand's (1971) type 2 cell were infrequently encountered in the PV-L. Sparse distribution of long and branching profiles of endoplasmic reticulum is characteristic of these cells. Small DCVs (arrows) were occasionally seen in this 24 hour rehydrated rat.

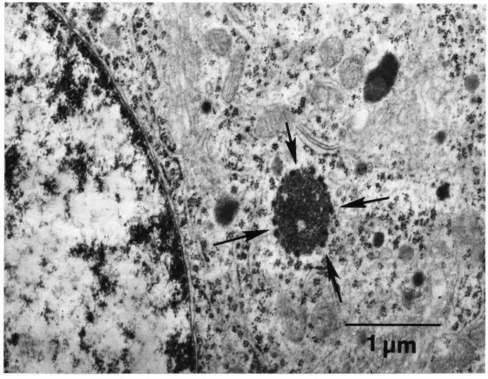

Figure 12. Nematosome in magnocellular PV-L cytoplasm of rat deprived of water for 12 hours.

Figure 13. Neuronal cilia. A. PV-L magnocellular neuron of a 4 hour water deprived rat. Arrow indicates cilium emerging from soma.

B. Enlargement of cilium in Figure 14A. Note presence of basal body at arrow.

C. Enlargement of cytoplasm of a "dark" magnocellular PV-L neuron of a 12 hour water deprived rat. Notice the presence of a dark cilium within an indentation in the somatic membrane (arrows) from which the cilium may have emerged. Close inspection reveals an apparent 9 + 0 pattern of microtubules.

D. Cytoplasm and neuropil in the PV-L of a non-deprived rat. Arrows indicate the probable presence of 2 cilia in near proximity.

E. Magnocellular PV-L neuron of a 24 hour deprived rat. Arrow indicates presence of a cilium adjacent to somatic membrane.

F. Enlargement of a cilium similar to that seen in Figure 14E from a 12 hour rehydrated rat. Eight pairs of microtubules encircle an eccentrically located pair.

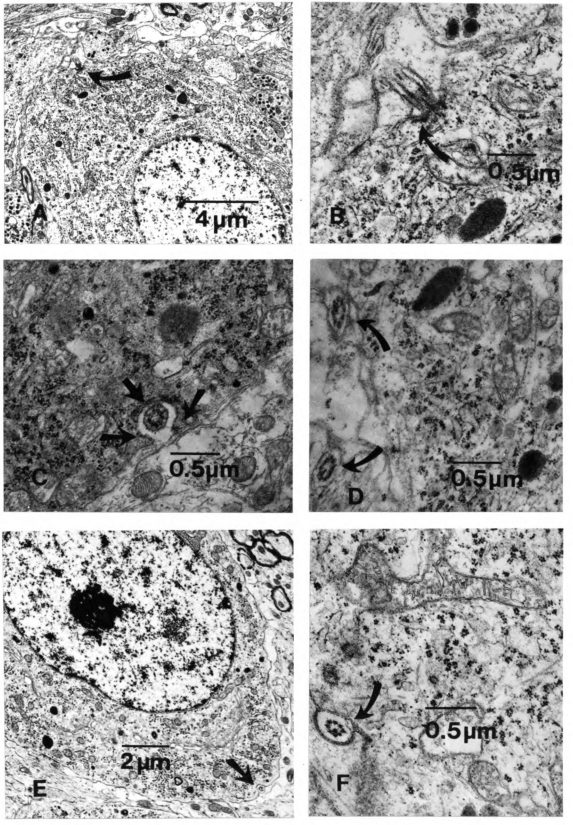

Figure 13.

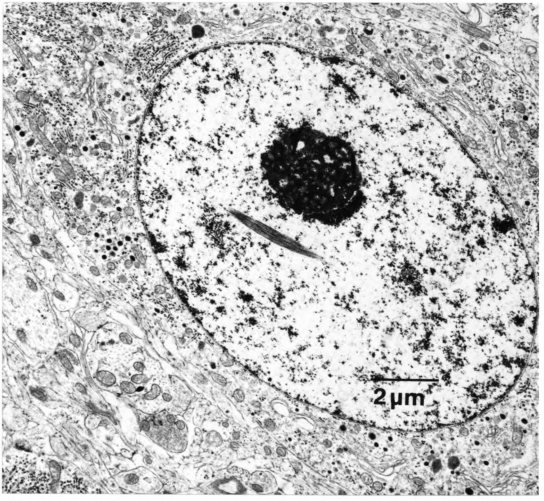

Figure 14. One intranuclear rod, composed of bundles of fibers, was found during this study in a magnocellular neuron of the PV-L of a 24 hour water deprived rat.

DISCUSSION

The procedures of the present study were similar to those of Tweedle and Hatton (1977). Nevertheless, caution should obviously be exercised in comparing the results of this study with others, or in the interpretation of negative results. Particularly, there may be differences in the variances of measures of SON, NC and PV-L due to differences in the structure and function of these nuclei. These nuclei may differ in complexity and uniformity. Furthermore, Tweedle and Hatton (1977) utilized more animals per group which afforded considerably more statistical power. The values obtained on measures of dilated rER and numbers of DCVs were somewhat dependent on subjective criteria. Thus changes in these measures are more appropriately compared than absolute levels.

Somato-Somatic Membrane Appositions

Somato-somatic membrane appositions were found in the PV-L, confirming previous reports (Flament-Durand, 1971; Kalimo, 1971). The distance between membranes in apposition was approximately 60 - 80 $\mathring{A}$, as reported in the SON and NC following similar experimental treatment and histological procedures (Tweedle and Hatton, 1976, 1977). Moreover, these appositions appeared to change with minimal dehydration, since the percentage of total somatic membrane in apposition and the total length of somatic membrane in apposition per 100 μm^2 were affected

by treatment. However, there was much variation within groups. Moreover, since the number of appositions per unit area, number of cells with apparent appositions per unit area, number of appositions per cell having at least one apposition, and the mean length of apposition were not significantly altered, the interpretation of the changes in total membrane in apposition is unclear. The issue is further complicated by the hypertrophy of neurons following stimulation (Hoblitzell, et al., 1976; Morris and Dyball, 1974 and others), which increases somatic surface area and correspondingly increases the length of sectioned membranes. Assuming (for illustration only) that somas are spherical, then both surface area and cross sectional area are proportional to the square of the radius.[1] Since the percentage of somatic membrane in apposition increased with dehydration at the same time that cell size probably increased, the amount of membrane in apposition must have increased faster than the change in total surface area. Geometric constraints would seem to dictate that for total membrane apposed to change, either the average size of apposition or the number of appositions, or both would also have to change. Thus, there were necessarily either type I or type II statistical errors made in the analysis of the membrane apposition data. Perhaps the measures of total membrane in apposition reached significance because of their sensitivity to variation in size and number of appositions, while the separate measures of apposition size and number were less sensitive. If this is the case,

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[1]The approximate proportionality of neuronal cross sectional area (the major proportion of PV-L area) with neuronal somatic surface area, as well as the possible hypertrophy of other constituents of the neuropil probably contributed to the high correlation between the two indices of membrane apposition pictured in Figure 9 (see footnote, p.42).

more conclusive results would be obtained with more animals. The significant results obtained on the measures of membrane apposition may instead have been erroneous (type I errors); however, these results were also consistent with and similar to previous findings in the SON (Tweedle and Hatton, 1976, 1977).

In the SON and NC, Tweedle and Hatton (1977) found that the percentage of total somatic membrane in somato-somatic apposition increased during 24 hours of dehydration and declined to baseline during rehydration. After 24 hours of dehydration the amount of membrane in apposition was approximately 2% in the SON and 10% in NC, whereas it was about 1.5% in the PV-L in the present study. In the PV-L, there was no indication of a further increase in apposition between 12 and 24 hours of deprivation, while the percentage of membrane in apposition increased monotonically in the SON up to 24 hours and continued to increase in NC for up to 5 days of dehydration (Tweedle and Hatton, 1976, 1977). Tweedle and Hatton (1976, 1977) also constructed montages of these nuclei and noted changes in the proportion of cells with appositions visible within thin sections. Since the absence of appositions in thin sections is certainly no assurance that the cells are not in apposition in unsampled sections, this was only a relative index of apposition. This measure was not used in the present study. Instead of constructing additional montages, the number of cells (or substantial parts thereof) with somato-somatic appositions was determined per print, using the same prints that had been used for the determination of the percentage of membrane in apposition and the assessment of rER. This measure of the number of cells in apposition per unit area is expected to be another relative index of apposition. The percentage of cells

exhibiting somato-somatic appositions visible within thin sections reached a peak in the SON and NC after 12 hours of deprivation. In the PV-L, the number of cells with visible appositions per unit area was not significantly altered. It was not possible in the PV-L to demonstrate whether changes in total apposition resulted from changes in the number or mean size of appositions. Likewise, over the course of 24 hours of dehydration and rehydration, Tweedle and Hatton (1977) could not answer the same question for SON or NC. Changes in their measure of the percentage of cells with appositions visible in thin sections do not necessarily reflect actual changes in the number of cells with appositions. In their earlier report (1976, but not 1977), they noted increases in the mean length of apposition in the SON from 1.58 μm in control animals to 2.62 μm after 24 hours of water deprivation. The average length of apposition in the PV-L was 1.73 μm, with no significant change following treatment (see Table 1).

One can only speculate on the functions of membrane appositions in the central nervous system. Theoretically, they could permit ephaptic (electrical) interactions between neurons (Bennett and Auerbach, 1969). Changes in appositions with dehydration could thus play some as yet undefined direct role in the electrophysiological responses of these nuclei to water deprivation. Changes in membrane apposition have been postulated to occur via the retraction and extension of fine astrocytic processes, since appositions changed in NC before neuronal cell size showed any increase (Tweedle and Hatton, 1976; Hatton, 1976). The mechanism of process retraction is not known. Actin-like proteins have been found in glial cells; it has been suggested that they function in glial cell migration (Groschel-Stewart, Unsicker, and Leonhardt,

1977). Likewise, the signal for glial retraction is unknown. Conceivably, neuronal activity could directly influence glial retraction via ionic or trophic substances. Other ionic or hormonal changes which accompany dehydration may also be involved. In response to increases in extracellular potassium, glia take up potassium and water and increase in volume (Bourke and Tower, 1966; Gill, Young and Tower, 1974). The putative transmitter glutamate also produces a dose-response glial swelling (Bourke and Tower, 1966). It might be postulated that glial cells accomplish this increase in volume by assuming a less convoluted shape; otherwise, surface area would have to increase by some mechanism. Therefore, increased neuronal activity could cause potassium levels to rise, which in turn may cause glial volume changes and process retraction. It has been proposed that glial uptake of potassium influences neuronal functioning by regulating extracellular potassium levels and membrane excitability (Orkand, Nicholls and Kuffler, 1966). Close appositions and the localized absence of glia may thus allow potassium levels to reach higher concentrations within restricted areas, altering membrane function. In frog cortex, stereological techniques have recently demonstrated changes in dendritic spine dimensions following the application of KCl (Trubatch, Loud and van Harreveld, 1977). The volume of the spine increased while the surface area did not change. Similar changes in spine morphology have been reported following electrical stimulation (see Trubatch, et al., 1977 for references). These workers suggested that changes in the shapes of neural elements in response to functional variation in potassium levels may be a structural mechanism for neuronal plasticity. Such a broad hypothesis obviously requires further testing.

It has been commonly reported that the metabolism of neurons in the hypothalamic magnocellular nuclei becomes more synchronized following enhanced secretion (Morris and Dyball, 1974; Zambrano and de Robertis, 1966 and others). In this regard, the overall rate of protein synthesis in guinea pig hippocampal slices has been shown to be dose-dependent on extracellular potassium levels within the physiological range of potassium concentration variation which accompanies neuronal activation (Lipton and Heimbach, 1977). Extracellular potassium levels could thus play some role in the enhancement of protein synthesis. The presence of membrane appositions, which may permit K^+ to accumulate, may contribute to the rapid synthetic changes which may occur in neurosecretory nuclei (for example, see Hatton, 1976). Little is known of the precise regulation of hormone synthesis in magnocellular neurosecretory cells. Hormones and intracellular factors may also contribute to the regulation of protein synthesis.

Several invertebrate experiments suggest electrical and metabolic changes in neurons which might be related to membrane appositions. The importance of extracellular potassium is also confirmed. By analyzing the laser light scattering from crab (Carcinus) pericardial organ in vitro, particle motion was found to be directly related to extracellular potassium levels (Englert and Edwards, 1977). The technique did not permit the identification of the particles which became more active. Morris and Steel (1977) examined ultrastructural changes in a small group of identified neurosecretory cells in the insect Rhodnius prolixus at selected times following feeding. The entire group of cells was easily identified and examined. Before feeding, the five cells of the group showed little indication of

hormone synthesis. In the first hours following feeding, dilation of rER, darkening of cytoplasm, and changes in the number of DCVs and MVBs were noted in many ways paralleling the response of activated hypothalamic neurosecretory cells. By seven days after feeding the number of lysosomes was elevated and synthesis appeared to be near its peak. It is for this discussion noteworthy that as the rate of synthesis seemed to slow in this system, numerous somato-somatic membrane close appositions appeared where glial processes had been previously found. It was proposed that the appearance of membrane appositions was in some way related to the termination of synthesis. Due to differences in the geometry of vertebrate and invertebrate neurons, it is not clear whether invertebrate somato-somatic appositions would be of direct electrophysiological significance. However, a role in metabolic regulation via ionic or other trophic substances seems possible.

Recent evidence suggests that ephaptic interactions do occur between apposed invertebrate processes (Alkon and Grossman, 1978; Ramon and Moore, 1978). In a series of neurophysiological experiments, Alkon and Grossman (1978) demonstrated in Hermissenda that two cells with naturally apposed processes continue to interact under various conditions of synaptic blockade. The mechanism appeared to involve the accumulation of extracellular potassium in periaxonal spaces. Ramon and Moore (1978) recorded from squid axons which had been experimentally placed in casual apposition. Ephaptic interactions were found and were greatly enhanced when extracellular volume was restricted by surrounding the apposition with oil. Thus there is evidence to support the biophysical calculations of Bennett and Auerbach (1969).

Dilation of Rough Endoplasmic Reticulum

There were apparent differences between cells in the dilation of rER (see Figure 4). No significant changes were found, however, with the treatment of up to 24 hours of water deprivation or with subsequent rehydration, in agreement with Kalimo's (1975) report of no "excessive" dilation with up to 6 days of water deprivation. Krisch (1974) did report some dilation in the PVN after 48 hours of water deprivation. It appears that the degree of dilation in the PV-L following brief or moderate dehydration is not great, if it exists. Ingestion of 2.0% NaCl for 3 days produced dilation of rER (Morris and Dyball, 1974), but that was a different and perhaps more extreme stimulus. They found that rehydration restored rER to the unstimulated state in the SON but not PVN. Cytoplasmic vacuoles were not found in the present study, although they have been reported by Yukitake, et al. (1977) in the PVN of untreated rats. In 24 animals, only one cell was encountered with dilation even approaching the stage of vacuolization (see Figure 4). In the SON and NC, dilation changes have been noted following dehydration. In their initial study, Tweedle and Hatton (1976) found significant increases in dilation in SON and NC at five days but not one day. Dilation was later seen in SON after 12 hours of dehydration but again not after 24 hours, thus suggesting some cyclicity of dilation (Tweedle and Hatton, 1977). In that experiment NC showed no dilation throughout 24 hours of dehydration or rehydration. Since Morris and Dyball did find changes in the dilation of rER with saline imbibition, it may be that water deprivation also produces such a dilation at some time which was not sampled in this study.

Dense Core Vesicles

Throughout the present study, the term dense core vesicle or the abbreviation DCV has been used instead of the functional label of neurosecretory vesicle, in order to preserve the traditional restricted meaning of neurosecretion. Because of the numerous projections from the PV-L to areas other than the neural lobe and median eminence, the contents of some of these vesicles appear to function as neurotransmitters or modulators just as similar substances are released from other neurons which are not typically labeled neurosecretory.

Small DCVs were clearly depleted from neuronal cytoplasm in the first 12 hours of dehydration (see Figure 10). Presumably, this indicates a net increase in transport versus synthesis of hormone(s). By 12 hours of dehydration, the rate of depletion was reduced, suggesting that hormone synthesis was probably by this time enhanced. Alternatively, net transport may have by then declined or the degradation of vesicles may have been reduced; reasons for the first possibility are unclear and there was not indication of cytoplasmic degradation of vesicles during the conditions of this experiment. The number of DCVs per unit of section area may be further complicated by the hypertrophy of neurons during dehydration and possible increase in somatic cytoplasmic volume, which reverses with rehydration. Using a karyometric analysis, Bandaranayake (1974) concluded that the PVN responded to dehydration with a greater latency and smaller magnitude when compared with the SON. Increases in cell size may contribute to the apparent decreased number of vesicles, and the temporal difference in cell size response between the PVN and SON may contribute to differences in the rate of change in the number of DCVs per unit of cytoplasmic section area.

In the SON and NC, small DCVs were reduced after 4 hours of water deprivation and had increased towards baseline by 12 hours (Tweedle and Hatton, 1977). Although the number of small DCVs was reduced in SON, NC and PV-L during brief dehydration, the enhancement of synthesis following this stimulus may have been less rapid in the PV-L. During rehydration after water deprivation for one day, DCVs continued to accumulate in the SON and NC toward baseline levels whereas they again declined in the PV-L. Likewise, the amount of somatic membrane in apposition per unit area also showed a cubic response in PV-L, increasing during initial dehydration, declining, then increasing again during rehydration. This may indicate that while the PV-L is slower to initiate its increased hormone synthesis to this stimulus, its synthetic activation and hormone transport may persist after the stimulus. This hypothesis is consistent with the findings of Morris and Dyball (1974) that 3 days of 2% saline imbibition produced a dilation of rER in the PVN which lasted through the rehydration period. It is apparently not known whether elevated numbers of cells with "bursting" (probably VP secreting) firing patterns appear earlier in the SON and NC but tend more to persist in the PVN. Further investigation of the correlations between physiological and morphological responses are needed. Little is presently known of the release of vasopressin or electrophysiological responses during slight, physiologically relevent degrees of dehydration (i.e., several hours after the last voluntary drink).

The possible continued activation of the PV-L might result from stress and the likely role that this nucleus plays in the response to stress. The experience of dehydration and/or rehydration may have resulted in an increased release of ACTH. Possibly aldosterone was

released during rehydration in order to prevent a mild hyponatremia from occuring. During rehydration, large numbers of DCVs may have been transported down axons for deposition into Herring bodies, since release as ADH would be paradoxical. The control of synthesis, transport and release of the hormones of these nuclei may instead differ for other unknown reasons. Since any of the measures of the cells in the PV-L, SON and NC may reflect changes in a population of cells with different hormones or projections, these measures may be composites which do not correspond to the activity of any particular cell type. In that case, the observed differences between the responses of the SON, NC and PV-L could be due primarily to differences in the combinations of cell types within the nuclei rather than due to differences in the responses of corresponding cell types which may be found in the several nuclei. The problem might be particularly serious if more than one cell type responded in these nuclei to a stimulus, and different cell types responded with different temporal patterns. The hormones OX, VP and enkephalin are found in the PVN (for example, see Defendini and Zimmerman, 1978; Sar, et al., 1978) and all are depleted from the neural lobe following dehydration (Jones and Pickering, 1969; Rossier, et al., 1978). Some of the same cells would probably respond to perturbation of fluid balance, preparation for lactation, the stresses of pregnancy and nonspecific stressors. Only quantitative examination of immunohistochemically identified neurons can clearly depict the temporal response of the cells in each nucleus which contain each hormone. Recent evidence (Armstrong, et al., 1979) suggests that descending projections from the PVN originate primarily from cells in the posterior rather than lateral magnocellular subgroup, thus activation

of cells with descending projections is not a likely explanation of the results during dehydration or rehydration.

Tweedle and Hatton (1977) investigated the number of smaller and larger DCVs in the SON and NC following dehydration and rehydration. Although many of the smaller vesicles were the result of sections through larger vesicles, the responses of smaller and larger DCVs were not parallel in these nuclei. These results suggest that vesicle size, like staining characteristics, may change with function or differ between hormones. Krisch's (1974) analysis of changes in mean vesicle size with dehydration and pregnancy reached equally uncertain conclusions. Her suggestion that OX-containing vesicles may be smaller than those containing VP is neither supported nor disconfirmed by the Tweedle and Hatton (1977) data. Large DCVs were depleted in the SON during four hours of dehydration in that study. These large vesicles were gradually replenished during further dehydration and rehydration. The large vesicles did not change in NC until rehydration when they increased to supranormal levels. There was no significant change in the number of large DCVs in the PV-L, although small vesicles did change as discussed above. Likewise, when small and large vesicles were combined, the results were not significant.

Morris and Dyball (1974) reported an increase in the ratio of the number of pale to dense granules in the PVN with dehydration. The counts of vesicles in the present study excluded pale vesicles, therefore the obtained decrease in the number of small dense DCVs with dehydration may be consistent with their result. However, during rehydration, they reported that the proportion of pale vesicles decreased and the total number of vesicles increased. Thus they must have

observed an absolute increase in the number of densely staining vesicles with rehydration, while small densely stained DCVs remained depleted in the PV-L. Kalimo (1975) observed that the number of DCVs seemed to be reduced after four to six days of dehydration. Differences in sampling within the PVN, definitions of DCVs, and the method and duration of dehydration make further comparisons between results difficult.

Lysosomes

No changes were detected in lysosomes in the PV-L. Definitions may again have been important in this regard. For consistency and ease of comparison with Tweedle and Hatton's (1977) data, lysosomes smaller than 4,000 Å were not counted due to their similarity to pale DCVs (which were also ignored). Tweedle and Hatton (1977) found that the number of lysosomes decreased in NC with dehydration and returned to normal with rehydration. In the SON, lysosomes were sharply depleted by four hours of deprivation, yet reappeared with further dehydration or rehydration. Three days of 2% saline imbibition reportedly depleted the cytoplasm of lysosomes in the PVN (Morris and Dyball, 1974). Lysosomes returned with rehydration in that study. Their counts included small lysosomes which were apparently distinguished from pale vesicles. Furthermore, they reported the reverse trends for MVBs. In the PV-L, MVBs were occasionally seen partially filled with a dense substance resembling the interior of lysosomes, apparent evidence of their conversion into lysosomes (see Peters, Palay, and Webster, 1976, pp. 38-39 for discussion). Therefore, large MVBs (>4,000 Å) were included with the counts of large lysosomes (>4,000 Å). The incidence of MVBs was much lower than the incidence of other large

lysosomes. The differences between these sets of results may be due to differences in sampling within the PVN, differences in stimuli, the small numbers of animals used in these studies, or differences in the definitions of lysosomes. The homozygous Brattleboro rat, as mentioned above, is chronically dehydrated. More lysosomes were found in the cytoplasm of the hypertrophied neurons of the homozygote than in non-dehydrated heterozygotes or normal rats (Tasso and Rua, 1978). The reason for the increased number of lysosomes in these animals is unclear. It may be that these cells require more lysosomes to cope with the strains of chronic cellular hyperactivity. However, the genetic defects of this strain might also directly influence lysosomal function or otherwise interact to produce an increase in lysosomal numbers.

Intranuclear Inclusions

Little is known of the function of intranuclear inclusions, either in neurons or in other cells (see Peters, et al., 1976, pp. 58-61 for discussion). Intranuclear fibrils exist in two basic forms, as rods or as sheets. The frequency of occurrence of these structures varies greatly across locations in the vertebrate brain and may also change in response to functional changes. For example, neurons in the cat stellate ganglion develop rodlets within 15 minutes after 10 minutes of electrical stimulation (Seite, Mei and Couineau, 1971). In the course of the present study, one intranuclear rodlet was observed (see Figure 14). Apparently, intranuclear rodlets have not been reported previously in magnocellular hypothalamic neurons.

Intranuclear membrane bound vacuoles were also found in the nuclei of magnocellular neurons in the PV-L. Similar forms were reported in 1-2% of the thin sections of nuclei in the SON (Tweedle

and Hatton, 1976). Remarkable variation was noted in the frequency of these bodies in the former nucleus. No such vacuoles were found in 14 of the 24 animals, yet as many as 30% of the nuclei (examined in thin section) contained these bodies in some animals. These structures may have been influenced by treatment, since dehydrated and nondehydrated animals differed in the presence or absence of any vacuoles (see Table 3). However, a more sensitive statistical analysis did not reveal a significant effect. The function of these bodies is entirely unknown. Tweedle and Hatton (1976) suggested that they may have contained lipid inclusions which were dissolved during histological processing. No change in the frequency of occurrence was detected in the SON or NC with up to five days of dehydration or with dehydration and rehydration (Tweedle and Hatton, 1976, 1977).

Neuronal Cilia

Cilia were encountered amongst the processes of the neuropil which is commonly seen surrounding the neurons of the PV-L (see Figure 13). In one section, a cilium was seen in longitudinal section emerging from a magnocellular soma. It is not yet known whether more than one cilium may extend from a single soma. Likewise, the proportion of cells possessing cilia (or the associated hormone(s) or projection(s)) is unknown. Intentional searches through grids of several animals revealed numerous cross sections of cilia per animal. In cross section, some cilia exhibited a 9 + 0 pattern of microtubules. In many cross sections, one pair of microtubules was displaced into an off-central position. Neuronal cilia with nine pairs of microtubules but lacking a central pair have been reported in the spinal cord,

hippocampus, lateral geniculate nucleus, neocortex, goldfish preoptic area (see Peters, et al., 1976, p. 42 for references) and elsewhere. In cilia found in these areas, 1 of the 9 peripheral pairs of microtubules was often displaced towards the central longitudinal axis in the distal cilia, yielding a modified 8 + 1 pattern. The functions of these cilia are unknown. They have, however, been suggested as sensory receptors or vestigial structures (see Peters, et al., 1976, p. 42-43).

Although function remains to be demonstrated for neuronal cilia, their comparison to known ciliated sensory cells is appealing and deserves further investigation. The suggestion of receptor cells within thalamic, cortical or spinal neuropil would necessitate major conceptual reevaluation of sensory functioning. However, the existence of receptors somewhere within the hypothalamus would be entirely consistent with present theories of hypothalamic function (see Hayward, 1977 and Hatton and Armstrong, in press, for reviews). The precise location, morphology and function of such hypothalamic receptive elements is yet unknown.

Comparative morphological evidence is suggestive of hypothalamic receptive function. Vigh and Vigh-Teichmann (see 1973, 1974 reviews) have analyzed several populations of ciliated neurons in the hypothalami of lower vertebrates. Ciliated neurons were found in the magnocellular preoptic nucleus of various fishes and amphibians. In teleosts, many processes from this nucleus extended through the ependyma into the third ventricle (Vigh-Teichmann, Vigh and Aros, 1976). Cilia with 9 + 0 patterns of microtubules projected from these processes which also contained DCVs. In the neuropil, cilia were also seen emerging from dendrites as well as somas. Between different species of teleost, much variation was seen in the number of CSF contacting dendrites as

well as cilia within the neuropil. Specifically, of the species which were examined, it seemed that more cilia were found in the neuropil of species having fewer CSF contacting magnocellular processes. As many as five cilia were seen emerging from a single soma and its dendrites. Furthermore, analysis of the reptile PVN has also revealed cilia in the neuropil (see Vigh-Teichmann, et al., 1976 for references). Vigh-Teichmann et al. (1976) theorized that these cilia may have evolved in the neuropil for the monitoring of extracellular fluid as direct contact with the ventricle was decreased. They concluded:

> If such ciliated perikarya--possibly derived from CSF contacting neurons--could also be found in the neurosecretory nucleus [sic] of higher vertebrates they could represent the physiologically supposed osmoreceptors which have not yet been demonstrated. Our further studies are aimed at elucidating whether the supraoptic and paraventricular nuclei of higher vertebrates contain ciliated neurosecretory cells.

These investigators have apparently not published again on this subject. Van den Pol has also observed cilia in the mammalian PVN (personal communication). Combined morphological-functional comparative analyses of vertebrate neurosecretory systems might contribute to the further understanding of the mammalian hypothalamus, in addition to less practical contributions. Curiously, Vigh-Teichmann et al. (1976) noted without comment the existence of "desmosome-like junctions" among neurons of the fish magnocellular preoptic nucleus. The similarity or difference between these junctions and the variable areas of close neuronal membrane apposition seen in mammals deserves investigation.

In an extensive analysis of the SON and NC of mature rats, Tweedle and Hatton (1976, 1977) have not observed cilia. However, cilia

were not previously observed by others in the PVN. Dellmann (personal communication) reports the presence of cilia and many neuronal membrane appositions in the SON of infant rats. Hatton (1976) proposed that the NC might function as an osmoreceptor. The probably absence of cilia in this nucleus need not reflect against its possible receptor status, since its specialized cytoarchitecture may make it especially responsive to chemical changes in the blood. Magnocellular cilia may exist more for monitoring extracellular fluid. It seems likely that multiple receptive mechanisms exist for the regulation of fluid balance.

Conclusion

The PVN was again implicated in water balance. Close appositions were found among neurons in the PV-L and their dimensions appeared to vary with dehydration. A larger sample of animals is needed to clarify the responses of this nucleus to dehydration. Comparing the data with that of Tweedle and Hatton (1976, 1977), there are apparent differences in the responses of the PV-L, SON and NC to dehydration and rehydration. These differences may reflect the presence and activity of cells containing hormones other than VP, real differences in the function of VP-containing cells in the different nuclei and/or sampling errors associated with heterogeneous nuclei and small groups. Differences in the functions of these nuclei, or reasons for their separate existence, are yet unclear. Various anatomical data suggest a more complex function for the PVN. The observation of cilia presents a further distinction between these nuclei. The function, distribution and frequency of these structures and the hormone(s) and projection(s) associated with them have yet to be determined.

APPENDIX A

(Supplies)

APPENDIX A

(Supplies)

Rats (Holtzman Co.) - $200 (estimates of approximate current prices for amounts utilized)

Sodium heparin, USP (Panheparin, Abbott) - $2

Ether for anesthetic - $3

Glutaraldehyde (Polysciences 25%) - $15

Sodium cacodylate (Electron Microscopy Sciences) - $30

Dimethyl Sulfoxide (Sigma) - $.50

Osmium tetroxide (Electron Microscopy Sciences) - $30

Absolute ethanol (Gold Shield) - $10

Plastic embedding media ingredient chemicals (Polyscience, Electron Microscopy Sciences) - $40

Xylene for cleaning glass - $4

Glass for sectioning knives (e.g., Sargent-Welch, from EM lab supplies)

Toluidine blue (Sigma) - $.25

Methylene blue (Sigma, from EM lab supplies) - $.25

Uranyl acetate - $3

Lead citrate - $1

200 mesh uncoated copper EM grids, 3.05" diameter (Electron Microscopy Sciences) - $20

Assorted adhesive tapes from EM supplies, primarily for thin sectioning

EM photographic film - 3.25" x 4", Kodak #4489 & 4463 - $160

Kodak D-19 developer, Rapid fix, Hypoclear, & Photoflo - $25

Print flattener solution (from EM lab supplies)

Agfa-Gaevert and Ilford Companies stabilization process activator and stabilizer solutions, and 8" x 10" single weight stabilization photographic paper - grades 1, 2, 3, & 4 - $180

Envelopes for storing EM and 35mm negatives - $5

1" x 3" glass microscope slides and assorted cover slips (e.g., Scientific Products) - $8

Protex mounting medium (Scientific Products from EM lab supplies)

Kodak Panatomic X, High contrast copy, and Direct positive (bulk) 35mm film (from Dr. Tweedle, EM lab and Department of Anatomy) - $10

Press on labels for micrographs (Paratype, Artype, Lettraset) - (from Dr. Tweedle, EM lab, Department of Anatomy and personal supplies)

Plastic slide boxes - $10

Liquid nitrogen (from EM supplies, for operation of electron microscope)

Paper and ribbons for Diablo machine - $15

Photocopying expenses (Neuroscience Program, Department of Anatomy, and personal)

Other miscellaneous supplies (e.g., soap, towels, NaCl, Parafilm, note paper, several index cards, marking pens and pencils, disposable gloves, disposable glass vials, etc. used from supplies in various labs)

APPENDIX B

(Equipment)

APPENDIX B

(Equipment)

Perfusion apparatus, surgical instruments - $100

Lab refrigerators, freezers, oven - $2,500

pH meter - $500

Magnetic stirrer with heater - $125

Millipore Milli RO4 water filtration system - $1,000

Bausch and Lomb dissecting microscope with stand - $900

Triple beam balance and Mettler precision balance (Hatton and EM lab) - $100, $1,200 respectively, each lab

EM molds - $10

Zeiss binocular microscope (Hatton)

Zeiss binocular microscope with built in 35mm camera (Retzlaff) - $8,000

Microscopic slide projector (Hatton) - $265

2 binocular microscopes (Leitz, Olympus) and 35mm camera attachment (EM lab) - $4,000

Dissecting microscope for trimming blocks (EM lab) - $700

Compensating polar planimeter (K + E 0005) - $150

Plan measure (Dietzgen 1719B, Tweedle) - $25

Meca minicomputer and associated equipment (Crano and Messe) - $3,500

Bausch and Lomb 7x measuring magnifier - $30

Fluorescent magnifying desk lamp - $100

Copy stand with 35mm camera (Kitai)

Unitech lettering set (Kitai) - $150

EM knife breaker (LKB) - $2,100

2 Sorvall MT-2B ultramicrotomes and miscellaneous other EM equipment - $15,000

2 hotplates (EM lab) - $50 each

Philips 201 electron microscope and ancillary equipment - ($130,000)

Arkay EM film development apparatus ($2,500) and film development darkroom

EM printing darkroom, including Durst S-45EM enlarger with point light source, Agfa-Gaevert and Kodak stabilization print processors, print washer and drier, dry mounting press, paper cutters, etc. - $15,000

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