The Morphology and Synaptic Organization of Neurons in the Metencephalic Reticular Formation in Turtles of the Genera Pseudemys and Chrysemys

Donald Newman
Loyola University Chicago

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THE MORPHOLOGY AND SYNAPTIC ORGANIZATION OF NEURONS IN THE
METENCEPHALIC RETICULAR FORMATION IN TURTLES
OF THE GENERA PSEUDEMYS AND CHRYSEMY

by

Donald B. Newman

A Dissertation Submitted to the Faculty of the Graduate School
of Loyola University of Chicago in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

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1976
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VITA

Donald B. Newman was born in Chicago, Illinois on January 1, 1948. He attended Brother Rice High School in Chicago, and was graduated with Honors in June, 1965. The following September he enrolled in Loyola University Lake Shore Campus, as a Biology major. He graduated with a Bachelor of Science in Biology in 1969.

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CHAPTER  I

INTRODUCTION

I. SUMMARY OF DISSERTATION

This dissertation represents a study of the organization of the metencephalic reticular formation (RF) in turtles of the genera Pseudemys and Chrysemys. The first part consists of a light microscopic study of the neuronal types in the metencephalic RF. The morphology and topographical distribution of reticular neurons as seen in Golgi and Nissl preparations are described. The second part of this dissertation consists of an electron microscopic study of the neurons of the metencephalic RF. The ultrastructural characteristics of the reticular neurons in the RF are described, and types, relative distribution and over-all density of boutons synapsing on them are characterized.

This study will argue that:

1) for the purpose of description and discussion, it has been found convenient to divide the metencephalic RF neurons into three groups -- small, medium, and large neurons.

2) each neuronal group or type tends to have certain morphological and cytological characteristics peculiar to that group.
3) Small and medium reticular neurons differ appreciably in their morphology from mammalian reticular neurons, in that the former are fusiform or tripolar leptodendritic neurons, whereas the latter are multipolar isodendritic neurons.

4) All three neuronal types in the metencephalic RF possess long, rectilinear dendrites which ramify predominantly in a transverse plane and which are essentially devoid of excrescences.

5) Two predominant bouton types, one tentatively linked with excitation, the other with inhibition, contact the somas and dendrites of the RF neurons.

6) On all three neuronal types, putative excitatory boutons outnumber putative inhibitory boutons on somas as well as dendrites.

7) Physiological predictions to the contrary, the density of synaptic covering on reticular neurons increases as a function of neuron size.

8) The density of synaptic covering is greater on reticular neuron dendrites than on the somas.
II. REVIEW OF THE LITERATURE

A. The Organization of the Mammalian Reticular Formation

The reticular formation is an area of the brain which was first described by the 19th century anatomists. They considered it to be a loosely organized melange of neurons of various size scattered among interweaving nerve fibers. The reticular formation was held to be a diffuse network of neurons which formed a core which extended from the rostral mesencephalon to the caudal medulla. It was maintained that the reticular core of the brainstem had little or no organization, especially when compared with well-organized nuclei such as the various cranial nerve nuclei, or the inferior olive.

This concept of the RF as an area of nervous tissue lacking organization was discredited in the late 1940's and early 1950's by a series of papers (e.g., Meessen and Olszewski, 1949; Olszewski and Baxter, 1954). These workers analyzed Nissl stained material from rabbits and humans and concluded that the mammalian RF could be subdivided in various nuclear groups with distinct cytoarchitectonics.

This impression was enhanced by various studies of other mammalian species, e.g., in the cat (Brodal, 1957), the guinea pig (Petrovicky, 1966) and the rat (Valverde, 1962). As it now stands, the mammalian reticular formation is considered to contain several nuclear groups. The caudal medulla, for
example, contains two distinct nuclear groups - a nucleus reticularis parvicellularis (nuc. R. pc.), consisting solely of small cells, and a nucleus reticularis ventralis (nuc. R.V.) which contains medium-sized cells as well as small cells. The former nucleus is located dorsolateral to the hypoglossal nucleus, whereas the latter nucleus lies ventrolateral to it. The nuc. R. pc. persists in the rostral medulla, but the nuc. R.V. is replaced by a rather extensive nucleus, which occupies the middle of a cross section through the rostral medulla and is known as the nucleus reticularis gigantocellularis (nuc. R. gc.). As its name implies, this nucleus contains very large cells (measuring up to 70 µ in soma length), as well as medium-sized and small cells. The nuc. R. gc. is situated medial to the nuc. R. pc. and just dorsal to the inferior olive. The nuc. R. pc. persists in the caudal pons as a small nucleus just lateral to a large nucleus which supplants the nuc. R. gc. and is known as the nucleus reticularis pontis caudalis (nuc. R.p.c.). Like the nuc. R. gc., this nucleus contains small, medium and large cells, and also like the nuc. R. gc., occupies the tegmental core of the pons. Whereas the nuc. R. pc. extends into the lateral portion of the rostral pons, the nuc. R.p.c. is supplanted by the nucleus reticularis pontis oralis (nuc. R.p.o.). This nucleus lies medial to the trigeminal complex and just lateral to the medial lemniscus. This nucleus contains a smaller
population of large cells than either the nuc. R. gc. or the nuc. R.p.c. The mesencephalic RF consists of a field of small and medium-sized cells lying dorsomedial to the red nucleus and ventrolateral to the periaqueductal gray. It is simply called the mesencephalic reticular field.

B. The Organization of the Reptilian Reticular Formation

Although as seen from the above, the mammalian RF has been the subject of several studies, relatively little has been done on that of reptiles. Relevant to this study is the fact that those papers dealing with the organization of the reptilian RF have often employed specimens of various genera of turtles. Van Hoevell (1911), for example, studied the reticular formation in both the crocodilian Caiman and the turtle Chelone. Regarding the organization of the brainstem RF in the latter, Van Hoevell remarked on the existence of two main nuclear groups; one in the myelencephalon, (which he designated the nucleus reticularis inferioris) and one in the metencephalon (which he termed the nucleus reticularis superioris). Although Van Hoevell did not give a detailed description of the various cell types present in these nuclei, he did note the presence of large polygonal neurons. In the myelencephalic RF, Van Hoevell observed, these large cells lie in or alongside the raphe rostral to the motor nucleus of
the vagus nerve. In the metencephalic RF, Van Hoevell noted, large cells extended from the level of the eighth nerve to the isthmus. The large cells were found to be most numerous at the level of the trigeminal nerve. The large cells of the metencephalic RF lie in the ventromedial quadrant of the brainstem.

Van Hoevell's rather cursory study of the RF in Chelone was superceded by a more extensive study of the brainstem RF in the turtle Pseudemys by Tuge (1932). Tuge subdivided the rhombencephalic RF into a: (1) nucleus reticularis diffusus, (2) nucleus motorius tegmenti pars superioris, (3) nucleus motorius tegmenti pars lateralis, (4) nucleus motorius tegmenti pars inferioris, and (5) nucleus reticularis lateralis. He characterized numbers 1 and 5 as nuclei which were situated laterally in the brainstem and which contained small and medium sized cells. The nucleus reticularis diffusus, Tuge noted, was found in the pretrigeminal area, whereas the nucleus reticularis lateralis was found in the myelencephalon. The nucleus motorius tegmenti was characterized by Tuge as containing very large cells, as well as medium-sized ones. The pars superioris extended from the pretrigeminal area to the level of the sixth nerve, whereas the pars inferioris extended from just behind the VIth nerve to the caudal myelencephalon. The pars lateralis occupied a lateral area in a gap between the pars superioris and pars inferioris. Like Van Hoevell
Tuge noted that large neurons in the metencephalic RF were most numerous around the level of the fifth nerve, whereas large neurons in the myelencephalic RF were most numerous around the level of the vagus. But, whereas Van Hoevell considered both the metencephalic RF and myelencephalic RF to be continuous fields of reticular neurons from the raphe to the lateral aspect of the brainstem, Tuge (1932) did not. He subdivided these fields into a medial large cell portion, the nucleus motorius, and a lateral small-celled portion, the nucleus reticularis, with the idea that the medial portion of the brainstem tegmentum subserved motor functions, whereas the lateral portion subserved sensory correlation.

A more systematic analysis of the reptilian brainstem RF was that of Cruce and Nieuwenhuys (1974). On the basis of a topographical and topological analysis of the brainstem of the tortoise Testudo, these workers subdivided the RF into clearly discernable subnuclei, using the terminology of Kappers, Huber and Crosby (1936). Starting caudally, these nuclei are:

(a) the nucleus reticularis inferiorus -- this nucleus, extending from the obex to the caudal pole of the sixth nerve, contains mostly medium-sized and some large cells. It corresponds to Tuge's nucleus motorius tegmenti pars inferioris. (b) the nucleus reticularis medius--this nucleus, which extends from the rostral VI nerve nucleus to the rostral metencephalon, contains medium-sized cells and many large cells. It corresponds
to the caudal portion of Tuge's nucleus motorius tegmenti pars superioris. (c) the nucleus reticularis superioris--this nucleus, which lies rostral to the trigeminal motor nucleus, also contains medium-sized and large cells. It corresponds to the rostral portion of the nucleus motorius tegmenti pars superioris of Tuge; (d) the nucleus reticularis isthmi, which is situated in the mesencephalic tegmentum, contains small cells. It corresponds to Tuge's nucleus reticularis diffusus. In this paper, Cruce and Nieuwenhuys' terminology will be adopted to refer to the various reticular nuclei in Pseudemys and Chrysemys.

C. Afferents to the Mammalian Reticular Formation

One of the best documented sources of afferents to the mammalian reticular formation is the spinal cord. Neurons in the intermediate gray matter of the cord give off large bundles of spinoreticular fibers which ascend in the ventrolateral funiculi. This massive spinoreticular pathway has been documented in the cat (Rossi and Brodal, 1957; Anderson and Berry, 1959), in the monkey (Mehler, Feferman and Nauta, 1960), and in man, using Marchi and Nauta degeneration techniques (Bowsher, 1957, 1962; Mehler, 1962). These spinoreticular fibers are distributed, at least in the cat and monkey, to most of the reticular subdivisions of the brainstem RF (Johnson, 1954; Mehler, 1960), although some areas are sites of maximal termination. The nucleus reticularis ventralis and nucleus reticularis
gigantocellularis of the medulla, for example, receive the densest projection to the brainstem (Bowsher, 1957; Mehler, 1960). The nucleus reticularis pontis caudalis and rostral part of the nucleus reticularis pontis oralis also receive an abundant input from the cord. A projection from the spinal cord to the mesencephalic RF has been demonstrated in the cat (Rossi and Brodal, 1957; Anderson and Berry, 1959), in the monkey (Mehler, et al., 1960), and in man (Bowsher, 1962).

Afferents to the RF from the spinal cord have also been demonstrated physiologically. Pompeiano and Swett (1962), for example, elicited reticular-induced cortical arousal or synchronization (to be discussed in detail below) by stimulating cutaneous nerves.

This activation of reticular mechanisms induced by peripheral stimulation has been observed by several other workers (e.g., Scheibel et al., 1955; Duensing and Schafer, 1957; Wolstencroft, 1964; Magni and Willis, 1964b). Pompeiano and Swett (1962) found that the majority of reticular neurons which could be activated cutaneously were in the nucleus reticularis gigantocellularis—a fact which dovetails with anatomical findings (mentioned above) showing the gigantocellularis to receive the densest spinoreticular projection. On the other hand, pontine and mesencephalic reticular units have also been activated by cutaneous or afferents (Rossi and Zanchetti, 1957; Hayashi and Yoshi, 1966; Bowsher, 1969 and 1970).
Afferents to the mammalian RF also come from a wide variety of other sources. For example, several cranial nerves send fibers into the RF—the vagus, glossopharyngeal, and trigeminal nerves all send primary afferents into the RF (Torvik, 1956; Clarke and Bowsher, 1962; Kerr, 1962). In addition, the spinal nucleus of the trigeminal sends second order sensory fibers into the RF (Stewart and King, 1963; Darian-Smith, 1973). Afferents to the RF also come from the ascending acoustic pathways (Scheibel and Scheibel, 1958). Visual information is routed into the RF via pathways from the superior colliculus (Kawamura et al., 1974; Peterson et al., 1971). The RF also receives input from the vestibular nuclei (Ladpli and Brodal, 1968; Wilson, 1972; Spyer et al., 1974).

An important point to be made here is that reticular neurons exhibit both heterotopic and heterosensory convergence. A single unit, for example, can be driven by a variety of sensory modalities, such as tactile stimuli, auditory clicks, or vagal stimulation (Rossi and Zanchetti, 1957; Scheibel and Scheibel, 1958; Potthoff et al., 1967). As an example of heterotopic convergence, Bowsher (1970) found that 77% of the caudal bulbar reticular units responded to single shocks applied to all four limbs.

In addition to inputs from a wide variety of sensory sources, the mammalian RF receives input from various "motor" sources. For example, the fastigial nucleus of the cerebellum
sends afferents to the RF (Walberg et al., 1962; Eccles et al., 1975) as does the hypothalamus (Nauta, 1958) and pallidum (Johnson and Clemente, 1959).

A large contingent of corticoreticular fibers has been demonstrated both physiologically (Rossi and Zanchetti, 1957; Magni and Willis, 1964a) as well as anatomically by numerous workers. Rossi and Brodal (1956), for example, showed that fibers originating from the sensorimotor cortex terminated in the nuc. R. gigantocellularis and the nuc. R. pontis caudalis and oralis of the cat. Valverde (1966) found similar termination of cortico-reticular fibers in the rat.

All of the above-mentioned afferents with the exception of the uncrossed tectoreticular path, terminate in the medial two-thirds of the RF. The nuc. R. pc. apparently receives no direct afferent projections.

D. Afferents to the Reptilian Reticular Formation

Whereas numerous workers have elucidated the nature of afferent fibers to the mammalian RF, relatively little is known about afferents to the reptilian RF.

One source of afferents to the RF which has been documented fairly well in reptiles is the spinal cord. Goldby and Robinson (1962) studied spinal input to the RF in the lizard Lacerta. They reported inputs from the cord chiefly to the medullary RF. Ebbesson (1967) studied spinal input to the RF of the tegu lizard,
Tupinambis. He reported that spinal fibers terminate throughout that part of medullary RF known as the nuc. R. inferioris. The degeneration was especially heavy in the caudal and lateral areas of the medullary RF. He also observed spinal fibers terminating in the caudal metencephalic RF (the nuc. R. medius), although these were fewer in numbers compared to the medulla. Finally, Ebbeson noticed a rather sparse spinal input into the lateral portion of the nucleus reticularis superioris in the rostral metencephalon. Thus, it would appear that, based on Ebbeson's findings, the reptilian RF, like that of mammals, receives a spinal projection. It differs, however, in that spinoreticular fibers in reptiles are largely confined to the medulla, whereas those of mammals reach the mesencephalic RF.

Another documented source of afferents to the reptilian RF is the optic tectum. Foster et al. (1973) studied descending projections from the optic tectum in Iguana and found two tectoreticular pathways—one crossed pathway which terminates in the medial portion of the metencephalic RF, and another uncrossed pathway which terminates in the lateral portion of both the metencephalic and myelencephalic RF. Ulinski (1976a) has found projections from the optic tectum to the brain stem RF in the snake Natrix, and Ten Donkelkarr (1975) has observed two tectoreticular pathways similar to those observed by Foster in the turtle Testudo.
Hall and Ebner (1974) have found projections in *Pseudemys* from the forebrain to the RF. They have observed one pathway which emanates from the dorsal cortex and descends via median forebrain bundle (MFB) to the mesencephalic RF. Another descends via both the MFB and the lateral forebrain bundle to the mesencephalic RF. Both pathways terminate in the lateral two-thirds of the full rostral-caudal extent of the mesencephalic RF. Hall and Ebner found no direct cortical projections below the level of the midbrain RF.

E. Efferents from the Mammalian Reticular Formation

The reticular formation has been found, through the combined efforts of several biological disciplines, to influence many widely separated areas of the CNS. One of the best known projections of the RF is to the spinal cord. The existence of reticulospinal pathways has been known since the turn of the century. Kohnstam (1899) and Van Ghueten (1903), among others, studied the origin of reticulospinal fibers using retrograde degeneration techniques, whereas Probst (1899), Tschermak (1898) and Papez (1926) studied reticulospinal pathways using Marchi techniques.

Prior to the 1950's, however, the RF was considered to have no subnuclei, and so results of work before this decade concerning the precise origin of reticulospinal fibers were desultory at best. The enlightening cytoarchitectural studies of
Meessen and Olszewski (1949) and Olszewski and Baxter (1954) paved the way for more definitive studies regarding the precise origin of reticulospinal fibers. Using retrograde degeneration techniques rather than orthograde ones (which tend to destroy fibers of passage and thus give erroneous results), Torvik and Brodal (1957) presented a comprehensive study of the origin of reticulospinal fibers in the cat. They found that both the medullary and pontine reticular formation give rise to descending tracts. Medullary nuclei giving rise to reticulospinal pathways include the nuc. R. gigantocellularis, the nuc. R. ventralis, and the nuc. R. lateralis. Pontine nuclei giving rise to reticulospinal pathways include the nuc. R. pontis caudalis and the caudal portion of the nuc. R. pontis oralis. Whereas pontine reticulospinal fibers descend homolaterally in the ventral funiculus, medullary fibers descend bilaterally in the ventral portion of the lateral funiculus. Torvik and Brodal found that giant, large, medium and small reticular neurons were all involved in reticulospinal projections.

Information regarding the sites of termination of reticulospinal fibers in mammals was rather scanty until the work of Nyberg-Hansen (1965). Using orthograde degeneration methods (sp. the Nauta method) Nyberg-Hansen found that, in the cat, medullary reticulospinal fibers descend bilaterally in the lateral funiculus to terminate, in all segments of the cord, chiefly in lamina VII with some termination in lamina IX.
Pontine reticulospinal fibers, Nyberg-Hansen found, descend ipsilaterally in the ventral funiculus and terminate in laminae VII and VIII.

These patterns of reticulospinal terminations have been found to obtain in other species of mammals, e.g., the monkey (Kuypers et al., 1962) as well as metatherian mammals. In the opossum, for example, Martin and Dom (1971) and Beran and Martin (1971) found the pontine reticulospinal tract to descend largely homolaterally in the ventral funiculus and terminate in laminae VII and VIII, with some input into lamina IX at certain levels.

The medullary reticulospinal pathway, they found, descended chiefly in the ventrolateral funiculus to terminate in laminae VII and VIII. In this sense, the medullary reticulospinal pathway of the opossum differs from that of eutherian mammals, as Nyberg-Hansen found little medullary reticulospinal input into lamina VIII of the cat. In addition, Martin and Dom found that the mesencephalic RF of the opossum projected to the spinal cord.

Anatomical evidence that the RF influences the spinal cord is supported by a voluminous literature from physiological experiments, probably the most famous of which is the classical paper by Magoun and Rhines (1946). These workers found that stimulation of the rostral medullary RF (especially the nuc. R. gigantocellularis) could cause a generalized inhibition of muscle tonus. Rhines and Magoun (1946) subsequently found sites in the lower pontine RF from which muscle tonus could be facilitated.
It appeared for a while as if the lower brainstem RF contained two distinct centers which had opposite effects on muscle tonus; one center, in the nuc. R. gigantocellularis, produced inhibition of extensors and facilitation of flexors (Lundberg and Voorhoeve, 1962; Rossi and Zanchetti, 1957), whereas the other center, in the pontine RF, produced facilitation of extensors and inhibition of flexors (Rossi and Zanchetti, 1957; Brodal, Pompeiano and Walberg, 1962).

This concept of two clear-cut opposing reticular centers was challenged through studies by several workers who showed, for example, that the lower pontine RF can facilitate flexor motoneurons as well as extensor motoneurons (Grillner and Lund, 1968; Wilson and Yoshida, 1968). Recent research has demonstrated that the medullary RF chiefly inhibits extensor motoneurons (Jankowska, Lund, Lundberg and Pompeiano, 1964; Llinas and Terzuolo, 1964), whereas the pontine RF excites extensors of the back (Wilson, Yoshida and Schor, 1970), as well as hip and toe extensors, while inhibiting knee and ankle extensors (Grillner, Hongo and Lund, 1971).

In addition to modulating muscle tonus and motor activity, the ventrolateral reticulospinal pathways can influence the level of sensory transmission through the spinal cord. Primary afferent depolarization, a phenomenon associated with presynaptic inhibition, can be elicited by stimulating many areas in the brainstem RF (Carpenter et al., 1962, 1966; Lundberg,
1967). Recently, it has been shown that certain areas of the RF can also cause primary afferent hyperpolarization in the cord, a phenomenon associated with pre-synaptic facilitation (Chan and Barnes, 1972).

In addition to modulating the inflow of sensory data into the cord, the RF can depress activity in certain reflex pathways. Eccles and Lundberg (1959) and Holmqvist and Lundberg (1959) discovered a reticulospinal pathway which depresses transmission through the polysynaptic flexor reflex afferent (FRA) pathways. This pathway originates from the ventromedial part of the lower pontine and upper medullary RF (Holmqvist, 1961) and courses in the dorsolateral funiculus.

The RF also can exert pressor effects through descending reticulospinal input to the sympathetic pre-ganglionic cell column in T1-L2 (Alexander, 1946).

Another important projection of the RF besides the spinal cord is to the thalamus. Based on Golgi studies, Scheibel and Scheibel (1958) reported that many neurons in the rostral medullary and lower pontine RF send their axons into several of the intralaminar thalamic nuclei (e.g., the centromedian, parafascicularis, centralis lateralis, etc.) as well as the thalamic reticular nucleus. These results were confirmed by Nauta and Kuypers (1958) using orthograde degeneration techniques in the cat. These workers found massive degeneration in the centromedian, parafascicular, paracentralis and centralis
lateralis nuclei (all members of the intralaminar group) following lesions in the lower brainstem RF.

The intralaminar nuclei, in turn, have been found to give off a diffuse projection to widespread areas of the cerebral cortex (Jones and Leavitt, 1974). Thus, solely on the basis of anatomical grounds, it can be seen that the reticular formation, acting through the thalamic intralaminar nuclei, can influence the cerebral cortex. That the RF does indeed influence cortical activity has been known through numerous physiological studies starting with the classical paper of Moruzzi and Magoun (1949). These workers were able to arouse chloralose-anesthetized cats from synchronized, slow-wave sleep into a state of mental alertness (cortical desynchronization) by high-frequency electrical stimulation of the brainstem RF. Jasper (1960) speculated this cortical arousal was mediated by the reticular-intralaminar thalamic system. It has subsequently been shown that stimulating peripheral nerves at various frequencies can arouse a sleeping animal, apparently by activating the spinoreticulo-reticulo-thalamocortical pathway (Pompeiano and Swett, 1962). On the other hand, rhythmic stimulation of peripheral nerves using other frequencies can cause the RF-induced cortical synchronization associated with slow-wave sleep (Weiss, 1961). It appears then, that the RF gives off an ascending reticular activating system which is driven by sensory stimuli and which tonically either maintains consciousness or depresses it. Empirical proof of the
existence of this system comes from findings that lesions which transect the reticulothalamasic pathways result in a permanently synchronized EEG and behavioral sleep (Bremer, 1935; Lindsley et al., 1950; French et al., 1952).

In addition to spinal and thalamic projections, the RF has been shown to project to numerous cranial nerve nuclei (Scheibel and Scheibel, 1958), various forebrain areas such as the hypothalamus and septal region (Nauta and Kuypers, 1958) and even to the cerebellum. Indeed, evidence is accumulating that the RF may be the major source of climbing fibers to the cerebellum (Rivera-Dominguez, Noback and Mettler, 1974).

F. Reticular Formation Efferents in Reptiles

As in mammals, some of the best known efferent pathways from the RF in reptiles are the reticulospinal pathways. Robinson (1969), for example, demonstrated several reticulospinal pathways in the lizard Lacerta viridis using retrograde and orthograde techniques. He found that the myelencephalic RF gave off a reticulospinal tract which descended ipsilaterally in the lateral funiculus, whereas the metencephalic RF projected to the cord via both ipsilateral and contralateral pathways, both of which descend in the lateral funiculus. Reticulospinal fibers were also found to descend in the anterior funiculus.

Cruce (1975), using retrograde techniques, has demonstrated the existence of reticulospinal projections in the lizard Tupinambis.
Very little work has been done regarding the physiological effects of reticulospinal pathways in reptiles, but it is presumed that they modulate motor activities in the cord. Schapiro and Goodman (1969), for example, were able to elicit circling movements in the alligator Caiman by stimulating the optic tectum, a neural center which is known to project to the RF. Thus, the circling movements observed by Schapiro and Goodman could have been mediated by a tectoreticulospinal pathway. Shapovalov (1975) induced EPSPs in lumbar motoneurons of turtles by stimulating the medial rhombencephalic RF.

G. Pharmacology of the Reticular Formation

As mentioned above, a voluminous literature exists concerning anatomical and physiological aspects of the RF, particularly in mammals. On the other hand, relatively little is known regarding the pharmacology of the RF—i.e., what neurotransmitters are utilized by the various subsystems of the RF. From evidence to date, it appears that various neuronal groups within the RF use several different neurotransmitters. For example, Dahlstrom and Fuxe (1964) found adrenergic neurons within the pontine tegmentum of the rat, using histofluorescence techniques. These neurons were located lateral and dorsal to the superior olive in the caudal pons, and ventral to the superior cerebellar peduncle in the rostral pons. In addition to adrenergic cells, Nobin and Bjorklund (1973) discovered serotonergic cells in the
lateral RF of the mesencephalon and pons of the human fetus. Finally, a large percentage of neurons in the mammalian RF seem to utilize acetylcholine as a neurotransmitter. Palkovits and Jacobowitz (1974), for example, found ACH-containing cells in the nuc. R. pontis caudalis, nuc. R. gigantocellularis, and nuc. R. ventralis.

In the turtle *Chrysemys*, Parent (1973) reports the presence of adrenergic nerve endings in the lateral portion of the brain-stem RF, and serotonergic endings in the medial portion.

H. **Morphology of Individual Reticular Neurons**

While a good deal is known regarding the numerous functions of the RF in general, knowledge as to whether these various physiological roles can be ascribed to certain neuronal subtypes within the RF is rather scanty. Most studies of reticular neurons (to be discussed in more detail below) were light microscopic studies describing morphological characteristics of reticular neurons in general (e.g., Valverde, 1961a; Scheibel and Scheibel, 1958; Ramon-Moliner and Nauta, 1966), and concentrated on mammalian material. Studies on the reptilian reticular formation (e.g., Tuge, 1932) yielded even less information regarding the morphology of individual reticular neurons. Electron microscopic studies of reticular neurons, which might reveal differences in morphology and synaptic patterns among reticular neurons, thus giving clues as to their functions, are represented
solely by two papers on the RF of the cat (Bowsher and Westman, 1970 and 1971). These papers yielded a relatively small amount of quantitative data regarding dimensions of and synaptic patterns on the neurons of the reticular formation.

No electron microscopic studies have been done on the reptilian reticular formation. An extensive, combined light and electron microscopic study of reticular neurons, with the objective of distinguishing various subtypes on the basis of morphology and/or synaptic patterns, might do much to elucidate the mechanisms behind the multiplicity of functions performed by the RF. This paper, it is suggested, represents a step in that direction.
CHAPTER II

LIGHT MICROSCOPY

INTRODUCTION

This chapter will deal with the morphology of the metencephalic reticular neurons of *Pseudemys* and *Chrysemys* as seen with the light microscope. In the first part, the appearance of reticular neurons in Nissl-stained material will be reported. Nissl stains, such as cresyl violet, are useful for determining the relative distribution and numerical density of various neuronal types, as every neuron is stained. Such dyes stain the neuronal soma, and thus yield useful information regarding its size and shape, as well as the amount and disposition of Nissl substance. As Nissl stains do not stain dendrites and axons, however, they do not reveal a neuron's total morphology. Thus, the second part of this chapter will report on the appearance of reticular neurons as seen with Golgi stains. These solutions, while staining only a few neurons in a given nucleus, usually stain the entire neuron (although sometimes not its axon). The morphology of the reticular neurons as seen with Golgi and Nissl stains will be compared with studies by others.
on the reticular formation, and the physiological significance of these findings will be discussed.

II. MATERIALS AND METHODS

Ten adult specimens of the turtles *Pseudemys scripta* or *Chrysemys picta* were anesthetized with Nembutal and processed for either the Golgi-Cox or Golgi-Kopsch technique.

The Cox technique used was Ramon-Moliner's (1970) modification. Slices of fresh brain were immersed in an aqueous solution of potassium dichromate, mercuric chloride and sodium tungstate for either 30 or 60 days. The blocks were then dehydrated in ethanol, embedded in celloidin, and sectioned on a sliding microtome at 120 µ.

The Kopsch technique used was Colonnier's (1964) modification. The animals were perfused with 5% PO₄-buffered glutaraldehyde. The brains were then removed, cut into blocks, and placed in an aqueous solution of one part 25% glutaraldehyde and four parts 2.5% potassium dichromate for five to six days, after which they were placed in 0.75% silver nitrate for five to six days. The brains were then dehydrated in acetone and embedded in paraffin. Sections were cut on a sliding microtome at 120 µ.

Most of the brains processed by either technique were cut in the transverse plane, although two specimens, treated with either the Cox or the Kopsch technique, were sectioned in the
sagittal plane. The rationale behind this preference for sectioning in the transverse plane is that the dendrites of reticular neurons have been shown through Golgi studies to ramify in a transverse plane (Scheibel and Scheibel, 1958; Valverde, 1961a), and so it was felt that transverse sections would display the dendritic arborization of RF neurons in Pseudemys to their fullest extent.

With regard to the success of the Golgi methods employed, it was noted that the Golgi-Kopsch technique produced an impregnation of the brainstem RF which was superior to the Cox method. The latter method produced an extremely sparse impregnation of reticular neuronal somata, although axons were well impregnated. The Kopsch method, on the other hand, achieved more consistent, acceptable results, in that at least 8 or 9 reticular neurons per section were impregnated. It produced an excellent impregnation of cell bodies and dendrites, whereas axons were usually impregnated only in their proximal portions.

Successfully impregnated neurons were traced on graph paper which was ruled into 1 mm squares, using a Leitz microscope equipped with a camera lucida. The neurons were drawn using the 40X objective at a total magnification of 500 X, so that five 1 mm squares on the tracing paper spanned a length of 10 μ. In this way, the length of neuronal perikarya and dendrites could be measured directly from the paper on which they were drawn. As a check, at least 30 Golgi-impregnated neurons were
photographed with a Zeiss Photomicroscope I. These neurons were then measured with a calibrated plastic scale and the measurements compared with those derived from the drawings of the same neurons.

In addition to Golgi impregnated material, Nissl stained material was also at hand. Three adult specimens of *Pseudemys* were perfused intracardially with a solution of 10% P0₄-buffered formalin. The brains of these turtles were then removed and placed in the fixative for several days. They were then removed and placed in the fixative for several days. They were then dehydrated in ethanol, embedded in paraffin, and sectioned on a rotary microtome at 15 μ. The sections were stained with cresyl violet, mounted on glass slides, and coverslipped.

The sections were photographed with a Zeiss Photomicroscope I, and photos of neurons were enlarged to a uniform magnification of 1,280 X and measured with a calibrated plastic scale.

III. RESULTS

A. Distribution of Neuronal Populations in the Reticular Formation

The reticular formation in *Pseudemys* consists of fields of diffusely organized, scattered neurons of various sizes.
occupying the ventrolateral quadrant of the brainstem and extending from the caudal medulla to the rostral midbrain. It contains small neurons, medium neurons, and large neurons. The cytological characteristics of reticular neurons of a given size category will be discussed below. With regard to the distribution of the reticular neurons in the brainstem RF, the following has been determined.

Small neurons are present at all levels of the brainstem RF. In the caudal medulla, 15-30 small neurons are usually scattered through the reticular field in a given hemisection of the brainstem. As one proceeds rostrally through the myelencephalon, the number of small cells increases, reaching a maximum in the rostral medulla. At the level of the rostral pole of the vagal motor nucleus, for example, there are as many as 65 small RF neurons per hemisection. Rostral to this region, the number of small reticular neurons declines as one proceeds rostrally until by the time one attains the level of the trigeminal motor nucleus in the rostral metencephalon, only 20-30 small neurons are seen in the RF per hemisection.

Medium cells have a similar distribution in that they also increase in number as one proceeds rostrally through the medulla, and, after achieving a maximum density in the rostral myelencephalon, decrease in number as one proceeds through the metencephalon. In the caudal myelencephalon, for example, there are 4 or 5 medium neurons per hemisection. In the rostral
medulla medium neurons achieve a density of 8-10 cells per hemisection. In the rostral metencephalon, on the other hand, medium neurons have declined to 5-7 per hemisection. At all levels, both small and medium cells appear to be scattered randomly through the mediolateral and dorsoventral extent of the reticular field.

Large reticular neurons, in contrast to small and medium neurons, are present only at certain levels of the brainstem. There are no large cells, for example, in the caudal myelencephalic RF (Figure 1A). In the rostral myelencephalon, on the other hand, large cells are clustered along the raphe and occupy the most medial portion of the reticular field. This colony of large cells spans the myelencephalic-metencephalic junction, extending from a level just rostral to the vagal motor nucleus to a level just caudal to the facial motor nucleus. There are usually 3-6 cells per hemisection (Figure 1B). Rostral to the facial nucleus, there is a gap in the brainstem RF in which no large cells are seen. At the level of the lateral vestibular nucleus, large cells once again make their appearance. They lie scattered through the ventrolateral and ventromedial quadrant of the brainstem, although the majority are seen in the medial portion of the RF (Figure 1C). Large cells continue to be seen in the RF until the rostral metencephalon (Figure 1D), whereupon they once again disappear. Thus, in the metencephalon, they form a population
that extends from the level of the 8th nerve to a level just rostral to the trigeminal motor nucleus. There are usually 4-8 large neurons in a given hemisection.

An initial impression gained from the study of Nissl and electron microscopic preparations of the turtle RF was that there appeared to be three sizes of reticular neurons; small, medium, and large neurons. Neurons of all three sizes, as will be discussed below, tend to possess certain distinguishing morphological and ultrastructural characteristics. Small reticular neurons, for example, tend to exhibit certain morphological characteristics which are not possessed by neurons which clearly lie in the medium size range. Similarly, neurons which are clearly in the middle or upper end of the large size range tend to exhibit certain morphological characteristics which are not shared by medium-sized reticular neurons. On the basis of these data, it was initially suggested that there were three distinct populations of neurons within the metencephalic RF of the turtle. When a sample of toluidine-blue-stained, plastic-embedded reticular neurons were analyzed with regard to soma length, however, it appeared that reticular neurons are distributed over a size continuum. For example, a light microscopic profile length histogram (Figure 2) appears to demonstrate that small reticular neurons and large reticular neurons merely represent extremes of a unimodal population of neurons. An analysis of soma lengths of reticular neuronal
profiles observed with electron microscopy (EM) expressed as a profile length histogram (Figure 12) also failed to demonstrate a clear-cut trimodal distribution of reticular neuronal soma lengths. However, there appeared to be two separate peaks in this histogram towards the smaller end of the size range, indicating that small and medium reticular neurons might comprise distinct size groups. As the EM neuronal sample available for size analysis was considerably larger than the light microscopic sample available (191 vs. 93 somas, respectively) it might be construed that the data obtained from the EM profile analysis might have more significance. In addition, the fact that the EM neuronal profiles can be measured more accurately than light microscopic neuronal profiles also tends to lend more reliability to an EM soma profile analysis. On the other hand, when considering a size analysis of an EM sample of neuronal profiles, a sampling factor must be considered; namely, the fact that large neuronal profiles are only partially included in an ultra-thin section, or if completely present, are often obscured by grid bars (slot grids were not used for technical reasons). Therefore, the number of measurable neurons in the larger end of the size range observed in an EM sample is less than that seen in a sample of neurons observed with the light microscope.

It is concluded on the basis of the data available in this study that it cannot be positively determined whether or not
there are three, distinct size categories of neurons in the RF of the turtle. For purposes of description and discussion, however, it has been found convenient to divide the metencephalic RF neurons into three groups—small, medium, and large neurons.

B. Cytology of Neuronal Types

1. Large Neurons

Large neurons possess polygonal perikarya ranging in length from 27-60 µ, based on the light microscopic profile length histogram (Figure 2). They have a large, oval nucleus containing a large, prominent nucleolus. Their cytoplasmic area is large, and contains numerous coarse clumps of Nissl substance, which is also found in the massive primary dendrites often seen emerging from the perikaryon (Figure 3A).

2. Medium Neurons

Medium neurons possess oval or polygonal perikarya 19-26 µ long, based on the above mentioned histogram (Figure 2). The cytoplasmic area relative to the size of their nucleus is moderate, and contains small clumps of Nissl substance. These cells usually have 2 or 3 primary dendrites (Figure 3B).
3. Small Neurons

Small neurons possess an oval or spindle-shaped perikaryon 10-18 µ long. These cells have a thin rim or shell of cytoplasm, with no visible Nissl substance, surrounding an oval nucleus (Figure 3C). The primary dendrites emerge at the poles of the perikaryon.

Thus, in Nissl material, there appear to be three neuronal types, each tending to possess certain morphological characteristics. Large neurons 27-60 µ long, medium sized ones 19-26 µ long and small ones 10-18 µ long.

C. Appearance of Neurons in Golgi Preparations

As with Nissl-stained material, three neuronal types are seen in the metencephalic RF of Pseudemys and Chrysemys in Golgi-stained sections.

1. Small Neurons

In Golgi-Kopsch preparations, small neurons appear as fusiform cells (Figure 4) with oval perikarya 10-18 µ long. The somas of these cells often appear flattened on one side and give off two slender, wavy, usually unbranched dendrites from the poles of their perikaryon. Based on a sample of twenty-eight dendrites, these dendrites average 123 µ in length. Thin, beaded axons emerge from either the soma or a primary dendrite, and course wavily through the neuropil,
without a preferred direction, before disappearing from the plane of focus (Figure 5).

Occasionally, the primary dendrites of small neurons branch into secondary dendrites (Figure 6). The dendrites of small neurons average 2 µ wide and remain essentially untapered as they course through the neuropil. They ramify almost exclusively in a plane parallel to a cross-section of the brainstem—i.e., in a transverse plane. Within that plane, some dendrites run obliquely through the neuropil, while others course horizontally or vertically. As can be seen in Figures 5 and 6, both primary and secondary dendrites of small neurons have smooth contours, being essentially devoid of excrescences.

2. Medium Neurons

In Golgi-Kopsch preparations, medium neurons appear as either fusiform neurons bearing two primary dendrites (Figure 8) or as tripolar neurons with polygonal perikarya bearing three primary dendrites (Figure 9). The somas of these neurons range in length from 19 µ to 26 µ. Their dendrites, like those of small neurons, are slender and wavy, but differ in that they usually branch into secondary dendrites (Figure 7). Based on a sample of 57, the dendrites of medium cells average 230 µ in length, but some course through the neuropil for distances of up to 500 µ. The dendrites of medium
neurons average 4 microns in width, and like those of small neurons, are essentially untapered. The dendrites of medium neurons ramify, like those of small neurons, predominantly in a transverse plane. The dendrites of medium neurons are also like those of small neurons in that they do not bear any spines or excrescences (Figures 8 and 9).

Like small cells, medium cells give off thin, beaded axons, from either the soma or a proximal dendrite, which course wavyly through the neuropil until they leave the plane of focus (Figure 9).

3. Large Neurons

The large neurons of the metencephalic RF are readily identified by their very large, polygonal perikarya, which range in length from 27 μ to 60 μ. These neurons bear three to five massive primary dendrites, ranging up to twenty microns in width, which in turn give off very long, straight secondary dendrites averaging 10 μ wide. The primary dendrites usually emerge from the ventral surface of the perikaryon, and their secondary branches are more highly branched than those of small and medium neurons, often giving off tertiary dendrites (Figure 10). The dendritic arborizations of large neurons extend for enormous distances in the transverse plane, sometimes spanning half the dorsoventral width of the brainstem—a distance of over 600 μ. The dendrites
of large neurons resemble those of the other populations of reticular neurons in that they are devoid of spines or excrescences, and also in that their dendrites ramify predominantly in a transverse plane.

IV. DISCUSSION

A. Distribution of Neuronal Populations in the Reticular Formation

As mentioned above, the cytoarchitecture of the RF in the turtle was previously studied by Van Hoevell (1911) in Chelone, by Tuge (1932) in Pseudemys and by Nieuwenhuys and Cruce (1974) in Testudo. While saying little or nothing regarding the distribution of small and medium neurons, these authors did give fairly detailed descriptions of the distribution of large neurons within the turtle brainstem RF. All of the above-mentioned studies found, as did this study, that large neurons are extant in the myelencephalon as a group of neurons clustered in or along side the raphe, chiefly at the level of the vagal motor nucleus and immediately rostral to it. Similarly, this research concurs with these earlier workers in that large reticular neurons are sparse or absent in the caudal metencephalon, but are present as a distinct population in the central tegmental field of the mid and rostral metencephalon, reaching a maximum density at the level of the
trigeminal motor nucleus.

Comparing the distribution of large neurons in the RF of Pseudemys with that of other representative reptilian genera, Newman (1974) found that the pattern of distribution of large neurons in the RF of four-legged reptiles is essentially similar—Caiman, Iguana and Pseudemys possess a colony of large neurons in the rostral myelencephalon which are confined to a cluster in and alongside the raphe. In the metencephalon, on the other hand, the large neurons in the RF of these reptiles are scattered throughout the central tegmental area.

Newman (1974) reported that snakes present a somewhat different situation from four-legged reptiles with regards to the distribution of large reticular neurons. Whereas four-legged reptiles have a small population of large neurons in the myelencephalon, confined chiefly to the raphe, snakes such as Natrix and Constrictor possess a large population of large reticular neurons in the myelencephalon; they are scattered throughout the central tegmental area.

The distribution of medium sized and small reticular neurons is also similar among the four-legged reptiles studied by Newman (1974) and again different from that in snakes. In Caiman, Iguana, and Pseudemys, both medium sized and small cells are fairly numerous in the caudal myelencephalon, achieve their greatest population density in the rostral myelencephalon and decline to moderate numbers in the metencephalon. In snakes,
on the other hand, small and medium sized neurons are most dense in the caudal myelencephalon and decline in number as one proceeds rostrally through the brainstem.

From a survey of the literature it would appear that the mammalian RF, like that of reptiles, possesses large, medium, and small neurons. Large neurons in the mammalian RF seem to be concentrated in the rostral medulla and caudal and mid pons (i.e., in the nuc. R. gigantocellularis and nuc. R. pontis cuadalis) (Brodal, 1959; Beran and Martin, 1971), whereas medium and small neurons are found throughout the RF (with the exception of the nucleus reticularis parvicellularis).

B. Cytology of Neuronal Types

As discussed above, while a good deal has been written about the distribution of large reticular neurons, both in reptiles and mammals, relatively little mention has been made regarding the distribution of small or medium sized reticular neurons. Even less has been written concerning the cytological characteristics of the individual neuronal types of the RF as seen in Nissl material, either in reptiles or in mammals. Cruce and Nieuwenhuys (1974) reported medium sized reticular neurons to average 25 µ in length in the RF of Testudo, whereas large neurons averaged 40 µ in length. Beran and Martin (1971) reported medium sized neurons in the opossum to range in length from 20-30 µ, whereas large neurons ranged in length from
30-60 μ. These figures are in good agreement with the measurements of reticular neurons reported in this study.

C. Appearance of Neurons in Golgi Preparations

Aside from this study, the only other Golgi study done on the turtle RF, or for that matter, that of any reptile, is that of Tuge (1932). Tuge studied Golgi-impregnated sections of the brainstem of the turtle *Chrysemys*. While he only gave a detailed description of the large metencephalic neurons, his work is of interest in that this author concurs with his impressions regarding the morphology of these neurons. He described the large neurons as giving off coarse, sparsely-branching dendrites which radiated out in several directions, but chiefly towards the ventral surface of the brainstem. Tuge's camera lucida drawings show these large neurons giving off long, rectilinear dendrites which span the dorso-ventral width of the brainstem (qv. Figure 30), as reported in this study.

Unlike studies on the reptilian RF, studies on the mammalian RF are somewhat more numerous, and profess considerable detail of observation. Valverde (1961a), for example, studied reticular neurons in dogs, cats and rats, and found three main cell types: modioliform, multipolar, and round or oval. The first two types were large or medium-sized and possessed four to six sparsely-branching dendrites, up to eight hundred micra in
length, which ramified in a transverse plane. These large or medium sized cells were mainly confined to the medial two-thirds of the RF. The third cell type was smaller and possessed an oval perikaryon with two sparsely branching dendrites which emerged from the poles of the perikaryon to ramify in a transverse plane. These fusiform or spindle-shaped neurons predominated in the lateral one-third of the RF.

Leontovich and Zhukova (1963) studied Golgi-stained reticular neurons in cats and dogs, and like Valverde, found them to be characterized by "their sparse, long, poorly-ramified dendrites." Leontovich and Zhukova emphasized the simple dendritic ramification of reticular neurons by juxtaposing Golgi drawings of them with drawings of neurons from various brainstem sensory and motor nuclei (qv. Figure 2).

Perhaps the most discerning study of the morphology of mammalian RF neurons is that of Ramon-Moliner and Nauta (1966). Based on a Golgi-Cox study of the brainstem in cats, they described three types of reticular neurons: large, medium and small. All of the large neurons, and most of the medium ones, possess long dendrites which, in their words, are "relatively rectilinear, show little tendency to branch, and, when they do branch, the resulting segments are, as a rule, longer than those from which they take origin." Whereas the large neurons (and most of the medium-sized ones) bear 3-6
primary dendrites which radiate out from the soma in a multi-polar fashion, small cells, and some medium ones, are usually fusiform or spindle-shaped, in that they possess an oval perikaryon from whose poles emerge two essentially unbranched dendrites. Ramon-Moliner and Nauta's figures (1966) (e.g. Figures 7-11) show that the dendrites of all three RF neuronal types ramify in a transverse plane.

Scheibel and Scheibel (1958), while not specifically describing the dendritic morphology of reticular neurons, show in their Golgi material from cats, dogs, mice and rats, reticular neurons with long, sparsely branching dendrites. They, too, report that reticular neuronal dendrites ramify in a transverse plane.

As reported above, the three neuronal types described in this study on the RF of Pseudemys and Chrysemys also possess long, sparsely branching dendrites, which ramify in a transverse plane. Newman (1974) reported that reticular neurons in the snake Natrix also possess simple dendritic arborizations, again confined to a transverse plane.

As judged from the above data, it would appear that the trend across phylogeny seems to be for the reticular formation to be characterized by neurons with rather generalized dendritic patterns, and with dendritic arborizations confined to the transverse plane. Ramon-Moliner and Nauta (1966) emphasize the simple ramification pattern of reticular neurons, especially
when compared with that of motoneurons, and even more so, with the complicated branching pattern of sensory neurons. They term the latter "idiodendritic," because a neuron in a given sensory nucleus will have a complex dendritic branching pattern peculiar to that cell type. Motoneurons, with their less elaborate dendritic arborization, were called "allodendritic." Reticular neurons, with their uniformly long, rectilinear, sparsely-branching dendrites, were called "isodendritic." Ramon-Moliner and Nauta, in fact, suggested that the RF would be more accurately called the isodendritic core of the brainstem.

The question naturally arises as to the physiological significance of the generalized dendritic pattern exhibited by neurons of the reticular formation. Ramon-Moliner and Nauta (1966) point out that the long, radiating dendrites of reticular neurons might be suited for sampling on-going activity in neighboring or more remote territories. Reticular neurons in general have been shown to exhibit a good deal of heterotopic and heterosensory convergence. This has been demonstrated both anatomically (Scheibel and Scheibel, 1958; Valverde, 1961a) and physiologically (Scheibel and Scheibel, 1955; Pompeiano, 1973). Their long, rectilinear dendrites are crossed by a multiplicity of fiber tracts from ascending and descending systems. As such, they are ideally suited, as Ramon-Moliner and Nauta (1966) point out, for integrative functions, in that
they can receive a wide variety of input from both major sensory and motor systems, and thereupon influence both supraspinal functions such as consciousness (Magoun and Rhines, 1946) as well as spinal functions such as modulation of muscle tone (Grillner, Hongo and Lund, 1968; Chan and Barnes, 1972; Wilson and Yoshido, 1968; Grillner and Lund, 1968) and modulation of sensory input (Pompeiano, 1973). Reticular neurons of Pseudemys and Chrysemys appear to be capable of these functions as well in that they also possess long, rectilinear dendrites which potentially could intersect any major ascending or descending system passing through the brainstem. That turtle reticular neurons could indeed be a focus for polysensory convergence was demonstrated anatomically by Tuge (1932), who described the dendrites of large metencephalic reticular neurons as being contacted by (a) the tecto-bulbo spinal system, (b) the spinal lemniscus system, (c) the cerebello-tegmental system, (d) arcuate fibers from the acoustic area and (e) the neuropil of the raphe complex. In observations from this laboratory, Golgi-Kopsch material from turtles also shows the dendrites of reticular neurons to intersect a multiplicity of morphologically variegated axonal systems.

In addition to having isodendritic branching patterns, it was pointed out above that the dendrites of reticular neurons (both those of mammals and reptiles) ramify predominantly in a transverse plane, whereas neurons in other brainstem
subsystems may ramify in the sagittal plane (e.g. hypoglossal motoneurons, Scheibel and Scheibel, 1958), or several planes. The significance of this confinement of reticular dendritic arborizations to the transverse plane is not quite clear, but Scheibel and Scheibel (1958) venture the speculation that this arrangement might allow for segregations of input-output systems to a certain level of the brainstem—in other words, the transverse orientation of reticular neuronal dendrites might allow for a functional segmentation of the RF, so that a given reticular subsystem might be confined to a certain rostro-caudal level of the hindbrain.

Although reticular neurons of the turtle generally resemble mammalian reticular neurons in that they possess a generalized dendritic pattern and transversely-ramifying dendrites, it should be pointed out here that they also differ somewhat. For example, whereas mammalian reticular neurons are reported to possess 3-6 primary dendrites which radiate from the soma in a multipolar fashion (Valverde, 1961a; Leontovich and Zhukova, 1963, etc.), turtle small and medium reticular neurons are usually fusiform or spindle-shaped, i.e., they possess only two primary dendrites emerging from the poles of an oval perikaryon. These fusiform neurons, exhibiting a dendritic pattern even simpler than most reticular neurons, were termed "leptodendritic" by Ramon-Moliner (1969). Spindle-shaped reticular neurons possessing only two primary dendrites
are occasionally seen in the mammalian RF (Valverde, 1961b), as well as the mammalian periventricular areas of the brainstem and diencephalon (Ramon-Moliner and Nauta, 1966).

Ramon-Moliner and Nauta (1966) state that spindle-shaped neurons are numerous in lower species. As they put it, "One significant difference between the neurons of the mammalian nervous system and the average neuron of the brainstem of lower forms seems to lie in the number of dendrites per cell. It is generally less in the latter forms; spindle-shaped cells are relatively numerous in the brainstem of the shark." On the other hand, preliminary Golgi studies of the snake RF (of *Natrix* and *Constrictor*) show their reticular neurons to be multipolar neurons resembling those seen in the mammalian RF. As turtles are thought to have evolved much earlier than snakes in the course of prehistory, and also earlier than mammals, it might be argued that the many fusiform neurons seen in the turtle RF points to their having retained the primordial neuronal morphology of the more primitive vertebrates.

This line of thinking, however, can be questioned on several points. First, although it is true that turtles as an order did evolve considerably earlier than snakes (turtles are generally considered to have appeared in the early Triassic period, whereas snakes first appeared in the late Cretaceous, Romer, 1962), this does not imply that the central nervous systems of existing species of turtles necessarily resemble
those of their prehistoric ancestors. In fact, paleontological evidence would seem to suggest that modern genera of turtles such as *Pseudemys* have evolved as recently as the Oligocene period, about 50 million years ago. As the first turtles appeared approximately 225 million years ago, it is obvious that millions of years of selective changes could have occurred in the brains of Chelonians since they began to radiate as an order.

Secondly, as Hodos (1970) points out, the fact that the class or order to which a given genus belongs has evolved earlier than the class or order of some other genus does not imply that members of the former have more primitive brains than members of the latter. It simply means the genus or species in question has evolved to fit into an ecological niche, and having done so, has escaped selective pressure to evolve further. In other words, the brain of a turtle is not necessarily more primitive than the brain of a mammal—rather, it is perfectly matched to allow the animal to function within its ecological niche. Hodos (1970), for example, presents a synopsis of various learning experiments in which it has been shown that pigeons surpass rats, cats, squirrels, and even several species of primates in certain learning curves—yet, bird brains are allegedly more "primitive" than even the lowest mammalian brain.
Washburn and Harding (1970) point out that tests designed by humans to test for intelligence in animals are biased towards human, visually-cued stimuli. Thus, animals with poor vision do not reveal their true intelligence on these tests, whereas an animal, such as the rat, with a keen sense of smell might manifest high intelligence on a test with olfactory cues. As they put it, "rat intelligence is based on the sense of smell, touch, and proximity, rather than on vision and distance. It would be easy to devise tests in which the sense of rats were favored; the intelligence of pigeons and men would then be rated as low." "From the point of view of evolution, to ask which is more intelligent is meaningless." "Both represent successful ways of life, and differences cannot be measured by any simple psychological test."

In the context of the discussion at hand, then, to consider turtle reticular neurons as being more "primitive" than either snake reticular neurons or those found in mammalian brains might be a serious error—instead, it might be safer to say that modern genera of turtles have evolved leptodendritic, rather than multipolar, reticular neurons because the former have conferred a selective advantage. As to why leptodendritic reticular neurons might subserve a turtle's neural functions more efficiently than isodendritic ones, speculations would be fruitless at this point. The answer to this question awaits further scientific investigation of the physiology of
reticular neurons in general, and those of the turtle in particular.

A second way in which RF neurons differ from those of mammals is that, whereas accounts of mammalian reticular neurons show their dendrites to be covered by irregular excrescences or spines, the dendrites of turtle reticular neurons are smooth and spineless. Leontovich and Zhukova (1963), for example, described mammalian reticular neurons as being covered by long, stick-like spines, and Valverde (1961a) reported rat reticular neurons to be covered by short, irregular, protuberances.

Both Tuge (1932) and this study, however, found turtle reticular neurons to be devoid of excrescences. This apparent discrepancy in morphology between mammalian and turtle reticular neurons might be resolved if one considers the recent finding by Scheibel, Davies, and Scheibel (1973) that the dendrites of reticular neurons of newborn animals are covered with spines, which disappear by the third or fourth month after birth. In the adult condition, the dendrites of mammalian reticular neurons are smooth and spineless. As Valverde (1961a) and Leontovich and Zhukova (1963) both used newborn animals in their studies, it is apparent that the spines they observed in their material were really the neonatal "protospines" described by Scheibel et al. (1973). Furthermore, as the turtles used in this study were adult specimens, the spineless
dendrites observed in the RF conform to the Scheibel's findings.

On the other hand, the possibility that turtle reticular neurons do possess spines, which simply failed to impregnate, must not be overlooked. Powell (1967) reported that spines of neurons in the olfactory bulb were poorly impregnated with Golgi methods following section of the olfactory filaments. Similarly, Westrum (1966) found that spines of neurons in the pyriform cortex did not impregnate after cutting the olfactory tract, even though they were still visible with the electron microscope. Both Powell and Westrum concluded that an altered biochemical status of the neurons following deafferentiation rendered their spines refractory to Golgi impregnation. Perhaps turtles used in this study were nutritionally deficient (although fed with fish ad libitum), and thus, their neurons might have been in an abnormal metabolic state. This in turn might have precluded a normal Golgi impregnation of their spines. One fact that lends credence to this hypothesis is the finding that reticular neuron dendrites of other reptilian species (e.g. Natrix, Caiman, and Iguana) do appear to be covered by short, irregularly-spaced excrescences, as reported in an earlier Golgi-Cox study by this laboratory (Newman, 1974). On the other hand, electron microscopic findings to be discussed below show that the dendritic profiles of reticular neurons are devoid of excrescences of any kind. As the sample size of
dendrites analyzed was fairly large (N=97), the probability that dendritic spines were missed as a sampling phenomenon is low.

Therefore, the possibility exists that reticular neurons of adult turtles do not possess spines. If so, in what way are they limited physiologically? Dendritic spines have been the source of many speculations regarding their physiological roles. One theory holds that spines are extensions of the dendritic surface which "capture" the appropriate afferents from among the myriad fibers passing through a neuron's dendritic arbor (Peters and Kaiserman-Abramof, 1970). The Scheibels (1968) noted that the frequent pairing of presumed excitatory and inhibitory synapses on dendritic spines points to the latter subserving an integrative function, i.e., excitatory synapses might interact with inhibitory synapses situated more proximally on the spines (a phenomenon reported by Jones and Powell, 1969).

Diamond, Gray and Yasargil (1970) suggest that dendritic spines provide a post-synaptic region which is isolated from the synaptic "noise" generated by neighboring axon terminals, allowing for a clearer signal input from a given synapse.

Finally, Rall and Rinzel (1971) suggested that the necks of spines may vary in diameter in vivo, which would in turn alter their resistance to a given post-synaptic potential. As they put it, "fine adjustments of the stem resistances of many
spines ... could provide an organism with a way to adjust the relative weights of the many synaptic inputs received by such neurons; this could contribute to plasticity and learning of a nervous system."

If one holds to several or all of these theories regarding the function of dendritic spines, it would appear that turtle reticular neurons, being devoid of spines, are unable to integrate, or modify, the relative weight of synaptic inputs in the same way that spine-bearing neurons (e.g., the pyramidal neurons of the cerebral cortex) do. Exactly how turtle reticular neurons integrate excitatory and inhibitory synaptic input, or modify the relative importance of a given synaptic system, can only be answered after further anatomical and physiological investigations of the nature of their synaptic input have been accomplished.

V. SUMMARY

In summary, then, it would appear that, as seen in both Nissl and Golgi preparations, neurons within a given size range possess certain morphological characteristics. Large reticular neurons in Pseudemys resemble those seen in mammalian material in that they are multipolar neurons with polygonal perikarya and long, rectilinear dendrites which ramify in a transverse plane and which bear no spines. Medium and small
neurons in the turtle RF, on the other hand, are spindle-shaped or fusiform neurons with two or at most three sparsely ramifying dendrites. They are thought to resemble the lepto-dendritic neurons which are characteristic of lower vertebrates.
CHAPTER III

ELECTRON MICROSCOPY

I. INTRODUCTION

This chapter will deal with the ultrastructural morphology of the neuronal types in the metencephalic RF of Pseudemys and Chrysemys, as well as the synaptic patterns found on those neurons. It will first consider the ultrastructural characteristics of each of the three neuronal types of the metencephalic RF. It will then argue that there are four distinct types of boutons synapsing on the reticular neurons, and that each bouton type comprises a variable percentage of the total bouton population, depending on its location on the cell surface, and the type of reticular neuron on which it is synapsing.

Finally, it will be argued that the overall synaptic density varies from one neuronal type to another, and also from soma to dendrite of a given cell type.

II. MATERIALS AND METHODS

Eight adult turtles of the genera Pseudemys and Chrysemys were anesthetized intraperitoneally with Nembutal and then
perfused intracardially with a 0.15 molar phosphate buffer containing 4% sucrose. This was followed immediately by a perfusion with 2,000 ml of fixative consisting of 4% paraformaldehyde and 5% glutaraldehyde in a phosphate buffer containing 4% sucrose. The final pH of this solution was between 7.2 and 7.4. The turtles were then placed in a sealed plastic bag and left in the refrigerator overnight. The following morning, their brains were removed and placed in the perfusing fixative for two hours.

The brainstems were then blocked in the transverse plane and placed in the fixative for another hour. They were then washed in 4% sucrose-phosphate buffer and placed in 2% OsO₄-sucrose-phosphate buffer for two hours. The blocks were then rinsed in buffer, dehydrated in ethyl alcohol, and transferred through propylene oxide into Epon 812. After curing, the blocks were thick-sectioned at 2 µ on a Porter-Blum MT-1 ultramicrotome. The faces of the block were large enough so that each section represented an entire hemisection of the brainstem, cut transversely. The sections were collected on glass slides and stained with 1% toluidine blue. The rostral metencephalic reticular field was taken as that area of cells medial to the trigeminal motor nucleus and lateral to the medial longitudinal fasciculus (M.L.F.), and midway between the dorsal and ventral surfaces of the brainstem. The block face was trimmed into a small trapezoid encompassing this area alone (Figure 11). Ultra thin sections were then made on a Reichert OM-U2V ultramicrotome. The sections
were stained with uranyl acetate and lead citrate, and examined and photographed with a Zeiss EM-9 electron microscope. In this study, a total of eight blocks, representing left or right halves of the brainstem, were examined in eight animals. Two blocks from two different animals were selected for formal sampling. Approximately 500 sections from the metencephalic reticular field were analyzed.

III. RESULTS

A. Morphology of Neurons

In the previous light microscopic study of the metencephalic reticular formation in *Pseudemys* and *Chrysemys*, three types of neurons were identified, both on the basis of size and dendritic morphology. Large neurons have angular perikarya, ranging in length from 27-60 µ, which give off 3 to 5 massive, sparsely-branching primary dendrites. These course through the neuropil for a distance of up to 600 µ. Large neurons are multipolar in shape. Medium neurons are fusiform or tripolar; they have oval or triangular perikarya, 19-26 µ long, from whose poles emerge two or three wavy dendrites which average 400 µ in length. These dendrites are essentially unbranched. Small neurons are also fusiform, but are characterized by having both smaller perikarya (10-18 µ long) and
shorter dendrites (200 μ long). The dendrites of all three cell types are essentially devoid of excrescences.

With the electron microscope, three types of neurons can also be distinguished on the basis of size and cytoplasmic characteristics. A size histogram (Figure 12) of the somal lengths of 191 reticular neuronal EM profiles shows that, as is also seen in light micrographs, small neurons range in size from 10-18 μ, medium neurons from 19-26 μ and large neurons from 27 to 60 μ.

A typical small neuron can be seen in Figure 13a, which is a toluidine-blue stained, plastic section light micrograph. These cells possess oval or round perikarya 10-18 μ long, and are characterized by a pale, oval nucleus surrounded by a thin rim of pale cytoplasm in which basophilic granules can be seen. At the ultrastructural level, small neurons possess round or oval perikarya often surrounded by oligodendrocytes in a satellite position. They have an oval nucleus, containing homogeneous karyoplasm and a prominent nucleolus. The nucleus is surrounded by a sparse cytoplasmic rim (Figure 13b). Dispersed elements of rough endoplasmic reticulum (ER) can be seen in the cytoplasm, as well as free ribosomes. The dust-like particles of rough ER are often condensed into aggregates resembling classical Nissl bodies. These are usually found towards the periphery of the cytoplasm. Mitochondria are observed in the cytoplasm as either long, slender rodlets or
circular profiles. Elements of Golgi apparatus, consisting of dilated tubules and round vacuoles, are usually seen arrayed circumferentially around the nucleus. Dark, irregularly-shaped lysosomes and round gray lipid droplets are also seen in the cytoplasm of small cells.

Primary dendrites are often seen to emerge from the poles of the perikaryon and course wavily through the cytoplasm (Figure 14). A histogram (Figure 18) based on a sample (N) of 17 dendrites showed these dendrites to range in width from one to four μ, with a mean value of 2.08 and a standard deviation(s) of ± 0.66. As these primary dendrites sometimes taper initially as they emerge from the soma, their width was measured at a distance of at least twenty microns from the soma. These proximal dendrites contain most of the organelles associated with the perikaryal cytoplasm; the mitochondria, however, appear as rodlets which are even longer than those in the soma. Parallel arrays of neurofilaments and, especially, neurotubules are seen in these proximal dendrites. The surface of these dendrites are devoid of excrescences.

Based on the aforementioned dendritic width histogram, it was felt that an isolated dendritic profile with a width ranging from one to three microns seen coursing through the neuropil probably represented a small cell dendrite. These putative small cell dendrites (Figure 19a) have a wavy appearance and contain, like more proximal dendrites, long tubular
mitochondria and parallel arrays of neurotubules. Ribosomes and other organelles are less numerous in these dendrites than in the soma of the cell, giving the cytoplasm of these dendrites a clear appearance.

Medium-sized neurons, as seen in light micrographs of toluidine blue-plastic sections (Figure 15a), possess oval or polygonal perikarya 19-26 μ long. Their cytoplasm is darker than that of small cells and occupies a greater area relative to the size of the nucleus than that of small cells. Dispersed elements of Nissl substance, as well as mitochondria, can usually be identified.

At the ultrastructural level, medium cells possess oval or polygonal perikarya with a relatively larger cytoplasmic area than small cells. Like small cells, they possess round nuclei containing homogeneous karyoplasm and a prominent nucleolus. The cytoplasm of medium cells contains rough ER which is aggregated as distinct clumps of Nissl substance. Free ribosomes are also scattered throughout the cytoplasm (Figure 15b). The Golgi apparatus consists of prominent elements arranged in a wreath around the nucleus. Each element consists of stacks of irregular cisternae and rounded vesicles. Dark, irregular lysosomes and round gray lipid droplets are present through the cytoplasm in moderate numbers.

Medium cell profiles are seen to give off primary dendrites which the dendritic width histogram (Figure 18) showed
to range in width from 1.5 to 6 µ, with a mean value of 4.21 µ and an s of ± 1.10, based on an N of 37 dendrites. These primary dendrites usually emerged from the poles of the peri-karyon, giving medium cells an appearance similar to that seen with the light microscope (Figure 16). The proximal dendrites of medium cells take a curving (occasionally wavy) course through the neuropil. They contain long tubular mitochondria, free ribosomes, clumps of rough ER, and lysosomes. Neurotubules and neurofilaments are seen dispersed among the other cytoplasmic elements. Based on the aforementioned dendritic width histogram (Figure 18), an isolated dendritic segment was judged to represent a medium cell dendrite if it ranged in width from 3-6 µ. These isolated dendrites (Figure 19b) course wavyly through the neuropil. Prominent features of these dendrites include long, rod-like mitochondria and dense arrays of parallel neurotubules and neurofilaments, as well as a paucity of other cellular organelles. The dendrites of medium cells are lacking excrescences of any kind.

Toluidine-blue plastic section photos of large neurons (Figure 17a) show them to possess polygonal perikarya ranging in length from 27-60 µ. They contain dark basophilic granules, dense clumps of Nissl substance, and an oval nucleus with a very prominent nucleolus. As seen in Figure 17a, large cell profiles are often seen to give off massive (15 µ wide) primary dendrites.
As seen in low power electron micrographs, large cells (Figure 17b) possess polygonal perikarya with a comparatively vast cytoplasmic area. They have a large oval or round nucleus containing homogeneous karyoplasm and a large, round nucleolus. Their cytoplasm contains an abundance of various organelle types; Golgi apparatus and lysosomes, for example, are scattered throughout the cytoplasm. The rough ER consists of dense stacks of cisternae studded with ribosomes; free ribosomes are also scattered throughout the cytoplasm in small clusters. The most distinguishing characteristic of large cells, apart from their great size, is the fact that their cytoplasm is densely packed with the round gray lipid droplets seen in moderate numbers in medium and small reticular neurons. In addition, small, rounded spines are occasionally seen to protrude from the somas of large neurons.

Large cells, as determined by the dendritic width histogram (Figure 18), give off massive primary dendrites 7-14 μ wide with a mean value of 11.83 and an s of ± 4.09, N=6. These contain dense clumps of rough ER, as well as lysosomes, neurotubules, and round gray lipid droplets.

Isolated dendrites of large cells, appearing as short segments (Figure 19c) in the neuropil, are easily identified by their great width (usually around 10 μ wide) and the abundance of rough ER and round gray lipid droplets within them. Like small and medium cell dendrites, those of large cells
also contain long, rod-like mitochondria and bundles of neurotubules weaving among the other organelles. Large cell dendrites also resemble small and medium cell dendrites in that they are devoid of excrescences of any kind.

B. Classification of Terminals

The somata and dendrites of metencephalic reticular neurons in *Pseudemys* and *Chrysemys* are contacted by two predominant bouton types classified according to vesicle shape and the morphology and width of their synaptic cleft.

1. Round asymmetric terminals (RA) are ellipsoidal in shape and contain numerous round or spheroidal synaptic vesicles 500 Å in diameter (Figure 20A). They are associated with a wide (200 Å) synaptic cleft and a thick (260 Å) post-synaptic density. A variant or subtype of RA terminal possesses a more abundant vesicle population and a post-synaptic dense body with post-junctional bodies (also known as Taxi bodies, after Taxi, 1965) (Figure 20B).

2. Pleomorphic symmetric terminals (PS) are ovoid or conical and contain pleomorphic vesicles measuring 650 Å x 250 Å (Figure 21A). These terminals were associated with a rather narrow (130 Å - 200 Å) synaptic cleft and little or no post-synaptic dense material (Figure 21B).
In addition, two other bouton types were occasionally seen contacting RF neurons, but in all instances they comprised a rather small percentage of the total bouton population. They are:

1. Round symmetric terminals (RS) Figure 22A), which contain round spherical vesicles larger than those of RA terminals. They average 520 Å in width. They are associated with a 260 Å wide cleft and little or no post-synaptic dense material.

2. Pleomorphic asymmetric (PA) terminals (Figure 22B), which contain pleomorphic vesicles and a wide cleft (260 Å) with a fairly prominent post-synaptic density.

C. Relative Distribution of the Various Synaptic Types

To ascertain the relative distribution of the various synaptic types, high power electron micrographs of boutons were pooled according to the type of cell with which they were seen to synapse, as well as their location on the cell surface. The relative numbers of a certain type of bouton observed on either the soma or dendrites of a given cell class was expressed as a percentage of the total number of bouton profiles photographed on that particular area of a given cell type. The relative percentages of given populations were expressed as histograms. Figure 23 shows the relative distribution of
synaptic types upon somas of the three cell types. On large cell somas RA terminals comprise 59% of the total bouton population, and PS terminals comprise the remaining 41% (based on an N of 29 boutons). On medium cell somas, RA terminals comprised 54% of the bouton population, whereas PS boutons comprised 44%; the remaining 2% consisted of RS terminals (N=50). On small cell somas, RA boutons comprised 68% of the total population, PS boutons 20%, and RS boutons 12% (N=25).

Figure 24 shows the relative distribution of synaptic types on dendrites of all three cell classes. Fifty-nine % of the boutons on large cell dendrites were RA terminals; 39% were PS, and 2% were RS, (N=46). On medium cells, 65% of the terminals were RA, 21% were PS, 9% were RS, and 5% were PA (N=182). Finally, on small cell dendrites, 72% of the bouton population were RA boutons, 22% were PS, and 6% were RS (N=71). The above data are summarized in Table 1.

While these data appear to show that, on all three neuronal types, the percentage of the total bouton population comprised by a given bouton type varies when comparing the somatic distribution with that on the dendrites, Chi-square tests showed these differences in distribution of a given bouton type to be statistically insignificant. One conclusion which can be gleaned from these data, however, is that RA boutons outnumber PS boutons on both the somas and the dendrites of all three neuronal types.
D. Density of Synaptic Covering

While attempting to calculate synaptic density on an actual, three-dimensional neuron based on measurements of two-dimensional profiles seen in electron micrographs has its obvious limitations, other workers have deemed it a worthwhile undertaking (Conradi, 1969; Lemkey-Johnston and Larramendi, 1968). The method used here to calculate the total synaptic density on a given profile is similar to that of Conradi (1969).

A rolling map-distance measure was used to find the total membrane length of a given profile. The same instrument was then used to measure the total bouton surface contacting the given profile. The mean ratio of total bouton length to profile membrane length was taken as a two-dimensional expression of the percentage of the somatic or dendritic surface covered by boutons, (191 somas were used in this analysis, as well as 97 dendrites). The mean values of ratios of bouton length to profile membrane length were compared and plotted to make a histogram showing the relative synaptic density on the various parts of the three cell types. This histogram, shown in Figure 25, revealed the following information:

(a) small neurons--small neuron soma profiles had a mean bouton coverage of 5% of the profile surface, with an s of ± 0.06, based on an N of 75 profiles. Small neuron dendritic profiles had a mean bouton coverage of 19% of the profile surface, with an s of ± 0.19, based on an N of 32 profiles.
(b) medium neurons—medium neuron soma profiles had a mean bouton coverage of 11% of the profile surface, with an s of $\pm$ 0.09, based on an N of 106 profiles. Medium neuron dendritic profiles had a mean bouton coverage of 21% of the profile surface, with an s of $\pm$ 0.14, based on an N of 53 profiles.

(c) large neurons—large neuron soma profiles had a mean bouton coverage of 16% of the profile surface with an s of $\pm$ 0.11, based on an N of 10 profiles. Large neuron dendritic profiles had a mean bouton coverage of 27% of the profile surface, with an s of $\pm$ 0.10, based on an N of 12 profiles. These data are summarized in Table 2.

The data presented above show that the mean percentages of the soma and dendritic profile surfaces covered by boutons increases when comparing medium neurons with small neurons. Two-tailed Student's T-tests revealed these differences in synaptic density between small and medium neurons to be statistically significant at the 0.001 level. Similarly, the data presented above show that the mean percentages of the soma and dendritic profile surfaces covered by boutons increases when comparing large neurons with medium neurons. Two-tailed Student's T-tests revealed these differences in synaptic density between medium and large neurons to be statistically significant at the 0.001 level. These data appear to demonstrate, therefore, that the percentage of the neuronal surface covered by boutons increases as a function of cell size and
that this increase is statistically significant.

The data presented above also show that, on all three neuronal types, the percentage of the dendritic surface covered by boutons is greater than the percentage of the somatic surface covered by boutons. Two-tailed Student's T-tests revealed that the increments in synaptic density observed when comparing dendrites with somas are statistically significant at the 0.001 level (for small and medium neurons) or the 0.05 level (for large neurons).

E. Mean Bouton Width

The question arose as to whether the observed increase in bouton coverage as a function of cell size and spatial location could be explained by an increase in bouton size, or whether it was due instead to an increase in the number of boutons contacting a given profile. To ascertain the former possibility, high magnification photos of all the boutons observed on a given part of a particular cell type (e.g., on the dendrites of medium cells) were pooled and measured with a calibrated plastic scale. The longest diameter through the terminal was taken as its width. The mean values of these widths were then compared. It was found that, based on a total sample of approximately 520 boutons, the mean widths of boutons on the soma and dendrites of the three cell types did not vary appreciably, ranging from 1.6-1.9 µ (see bouton width histogram, Figure 26).
The increase in bouton coverage as a function of surface location or cell size, therefore, could not be explained on the basis of any increment in bouton size.

F. Bouton Number per Profile

It followed logically that the increment in bouton coverage observed as a function of neuronal size and surface location had to be explained by an increase in the mean number of boutons per profile and this was found to be the case. Small neuron soma profiles, for example, had a mean of 1.2 boutons per profile, with an s of ± 1.39, based on an N of 75 profiles. Small neuron dendritic profiles had a mean of 3.7 boutons per profile, with an s of ± 3.06, based on an N of 32 profiles. Medium neuron soma profiles had a mean bouton covering of 4.2 boutons per profile, with an s of ± 4.34, based on an N of 106 profiles. Medium neuron dendritic profiles had a mean bouton covering of 7.4 boutons per profile, with an s of ± 4.9, based on an N of 53 profiles. Finally, large neuron soma profiles had a mean of 10.0 boutons per profile, with an s of ± 8.0, based on an N of 10 profiles. Large neuron dendritic profiles had a mean of 12.9 boutons per profile, with an s of ± 6.54, based on an N of 12 profiles.

These data show, therefore, that the number of boutons per profile increases as a function of neuronal size. In addition, on all three neuronal types, the mean number of boutons observed
on dendritic profiles is greater than that observed on soma profiles. These data are summarized in Table 3. On the basis of two-tailed Student’s T-tests, it was concluded that, with regard to both small and medium cells, the differences between the mean bouton numbers on the somas and the mean bouton numbers on the dendrites were statistically significant at the 0.001 level. It was also found, perhaps due to the small sample size available, that the mean bouton number on large cell somas did not differ significantly from the mean bouton number on large cell dendrites.

Two-tailed Student T-tests also revealed that the difference between the mean number of boutons on the somas of small cells and the mean number of boutons on the somas of medium cells was statistically significant at the 0.001 level, as was the difference between the mean number of boutons on medium cell somas and the mean bouton number on large cell somas.

Finally, T-tests demonstrated that the difference between the mean bouton number on small and medium cell dendrites, as well as the difference between the mean bouton number on medium and large cell dendrites, were both statistically significant at the 0.001 level.
IV. DISCUSSION

A. Identification of Cell Types

As mentioned above, Golgi studies show tentatively that, three types of neurons exist in the metencephalic RF of Pseudemys, both with regard to size and to dendritic morphology. The electron microscopic observations lend credence to this impression. Large, medium and small cells tend to exhibit ultrastructural features peculiar to their size range.

The only other ultrastructural studies of the reticular formation to date consist of one on neurons in the medullary nuc. reticularis gigantocellularis of the cat (Bowsher and Westman, 1970), and another on the cat mesencephalic nucleus of the posterior commissure and medullary nucleus R. caudalis (Bowsher and Westman, 1971). In both these studies, Bowsher and Westman reported finding only two populations of neurons; large "polydendritic" neurons and smaller "oligodendritic" neurons.

Their large neurons resembled those found in the turtle RF, in that both are large (30-70 µ) polygonal neurons whose cytoplasm contains an abundance of various organelles. Their electron micrographs, for example, (Bowsher and Westman, 1971, p. 25, fig. 2) show their large cells to contain numerous dense clumps of rough ER, arrays of Golgi apparatus, mitochondria and lysosomes. In addition, they often observed their large
cell perikarya to bear short rounded, somatic spines, as were observed on large cells in this material (see above). In general, the size range and ultrastructural features of these large reticular cells in the cat and turtle are similar to those reported by other workers studying large cells in the CNS; e.g., the large cells of the feline lateral vestibular nucleus (Mugnaini et al., 1967), the large cells of the monkey red nucleus (King et al., 1971), those in the rabbit deep cerebellar nuclei (O'Leary et al., 1972) and cat deep cerebellar nuclei, (Angaut and Sotelo, 1973) and the large alpha motor-neurons in the spinal cord (Conradi, 1969; McLaughlin, 1972). All these neurons are large, polygonal cells with dense clumps of Nissl substance (rough ER), as well as numerous examples of other organelles. It should be pointed out here, however, that only the large reticular neurons in Pseudemys contain the abundant round gray lipid droplets reported above.

A point of variance between the results of this study and those of Bowsher and Westman's is that, apart from the large cells, two other types of neurons appear to be extant in the turtle, whereas there is only one in the cat. That is, whereas this study showed the existence of medium and small neurons, Bowsher and Westman describe only small ones. Closer scrutiny of their results, however, gives evidence that their single population of small, oligodendritic neurons might encompass the small and medium neurons observed in this material.
For example, at the EM level, small and medium neurons in the turtle and the oligodendritic neurons in the cat possess oval or rounded perikarya, pale cytoplasm with a moderate number of organelles, and an oval or enfolded nucleus. On the other hand, a picture of an oligodendritic neuron in the nucleus reticularis gigantocellularis (Bowsher and Westman, 1970, p. 26, fig. 3) shows it to resemble a small reticular cell in Pseudemys, in that it has a rather sparse cytoplasmic area and small size, whereas a picture of one in the nucleus reticularis caudalis (Bowsher and Westman, 1971, p. 448, fig. 4) shows it to resemble a medium reticular neuron in Pseudemys, in that it has a larger cytoplasmic area and more abundant organelles than the smaller oligodendritic cell. Bowsher and Westman (1971) report small cells to range in size from $14-34 \mu$, based on the analysis of 24 profiles. Had they performed a size histogram based on a large sample size, as was done in this study (N=191), they might have encountered a somewhat bimodal distribution in small neuron perikaryal lengths, as was seen in the turtle. Also, concomitant Golgi studies, as were performed in this report, might have done much to elucidate whether the class of small oligodendritic neurons in the cat RF really contained distinct subpopulations.

In considering the categorization of isolated dendritic profiles seen coursing through the neuropil as belonging to one cell population or another, it is felt that such classifi-
cations can be made with some degree of confidence. First, as mentioned in the results, a dendritic width histogram revealed that the proximal dendrites of the three types of reticular neurons fell into three rather distinct size ranges; small cell dendrites ranged from 1-4 μ, medium cell dendrites ranged from 1.5-6 μ, and large cell proximal dendrites ranged from 7-14 μ. Golgi studies of the metencephalic RF of the turtle, discussed above, reveal that the dendrites of small and medium neurons do not taper until their very tips. While very distal dendrites of large cells do taper somewhat, these are usually located near the vertral surface of the brainstem, and thus were not included in the sections analyzed for electron microscopy in this study. Thus, it is felt that isolated dendritic segments coursing through the neuropil represent segments of dendrites of widths similar to those of dendrites which are seen to be continuous with a given type of neuron. The possibility that isolated dendritic segments represent dendrites from non-reticular cell types has not been excluded, but Golgi studies do not show any particular nucleus of cells which possess dendrites that cross through the medial metencephalic reticular field which has been studied here. The dendrites of trigeminal motoneurons, for example, can be seen to be segregated in large loose bundles which course towards the ventrolateral corner of the metencephalon.
B. Classification of Bouton Types

As seen above, there appear to be two predominant bouton populations in the turtle metencephalic RF: one with round or spherical vesicles, a wide cleft, and a prominent post-synaptic density, and another with pleomorphic vesicles, a narrow cleft, and little or no post-synaptic density. These were termed Round-Asymmetric (RA) and Pleomorphic-Symmetric (PS) respectively, after the terminology of Colonnier (1968), who described synaptic clefts as being either symmetric or asymmetric in the cat visual cortex. Colonnier found that boutons with round vesicles were almost invariably associated with a wide cleft and an asymmetric membrane thickening, whereas boutons with pleomorphic vesicles were usually associated with a narrow cleft and a symmetric thickening. The same correlation between vesicle shape and cleft width has been substantiated by numerous workers in various areas of the CNS in a variety of species, notably, cat spinal cord (Bodian, 1966), cat thalamus (Ralston and Herman, 1969), mouse and cat cerebellum (Lemkey-Johnson and Larramendi, 1968; Uchizono, 1967), cat reticular formation (Bowsher and Westman, 1970), turtle cortex (Ebner and Colonnier, 1975) and snake dorsal ventricular ridge (Ulinski, 1976b).

Recently, freeze-etch studies of fixed and unfixed tissue (Pfenninger, 1973; Landis and Reese, 1974) have corroborated the concept that there are at least two distinct populations of boutons in the CNS of vertebrates. One type of bouton
contains a rather wide synaptic cleft, a prominent post-synaptic density, and spherical vesicles averaging 480 Å in diameter. The other type of bouton possesses a somewhat narrower synaptic cleft, little or no post-synaptic density and spherical vesicles averaging 390 Å in diameter (Pfenninger, 1973). The riddle as to why terminals with symmetric synaptic clefts contain pleomorphic vesicles in aldehyde-fixed material, and spherical ones in freeze-etched material was solved by Valdivia (1971), who showed that vesicle flattening was induced (in terminals with symmetrical clefts) by the high osmotic pressures generated by the aldehyde fixatives commonly used to perfuse nervous tissue. Valdivia found that when employing aldehyde fixatives with a lower osmotic pressure, boutons with symmetric clefts contain predominantly small round vesicles similar to those seen in freeze-etched material. It is thus concluded that the flattened vesicles seen in PS terminals in this material are in reality a by-product of the osmotic pressure produced by the glutaraldehyde fixative, and in reality are spherical vesicles which are smaller than those seen in RA boutons.

Concerning the general dimensions of various elements of the RA and PS boutons observed in this material, it seems that the measurements dovetail with those observed by other workers using various species. Regarding the diameter of synaptic vesicles seen in RA boutons, for example, both Conradi (1969)
and McLaughlin (1972) found RA terminals (S bulbs) in cat spinal cord to contain vesicles ranging in width from 300-700 Å. Pfenninger (1973) reported these vesicles to average 480 Å. Those seen in this material range from 400-500 Å. Similarly, Conradi and McLaughlin found PS terminals (F bulbs) to possess pleomorphic vesicles measuring either 200 x 600 Å (Conradi) or 350 x 650 Å (McLaughlin). Both Conradi (1969) and Pfenninger (1973) found RA boutons to be associated with synaptic clefts averaging 150-160 Å wide, and PS boutons to be associated with synaptic clefts averaging 90-130 Å. RA boutons in this material had clefts averaging 200 Å, whereas PS boutons had clefts averaging 130 Å.

Post-synaptic densities seen in this material were also similar in morphology and dimensions to those observed by other workers. Pfenninger (1973), for example, reported RA boutons to possess post-synaptic densities ranging in width from 200 Å to 500 Å. RA boutons in the turtle had post-synaptic densities 260 Å wide.

As mentioned above, a variant of the RA terminal was associated with post-junctional, or Taxi bodies, after Taxi (1965), who first described them. A preliminary impression gained from the study of turtle material is that those RA boutons associated with post-junctional bodies had a somewhat thicker post-synaptic density than the standard RA bouton; Conradi (1969), who considered RA boutons with post-junctional
bodies to be a distinct population (called T bulbs) also found this to be the case. He found that T bulbs had a post-synaptic density which was as much as 100 Å thicker than the normal RA bouton. (S bulb in his terminology).

The mean widths of boutons observed in this material ranged from 1.6 to 2 μ, and thus were in the same size range (1-5 μ) as reported by numerous studies of mammalian material (Conradi, 1969; McLaughlin, 1972; Ralston and Herman, 1969; Bowsher and Westman, 1971).

As seen above, it appears that the turtle CNS, like that of most vertebrates, contains two principle bouton populations; one characterized by spherical vesicles, a wide cleft, and a prominent post-synaptic density, and the other characterized by pleomorphic vesicles (as seen in aldehyde-fixed material), a narrow cleft, and little or no post-synaptic density.

It would now be in order to consider whether this morphological dichotomy in bouton types is associated with a physiological dichotomy as well—in other words, do RA boutons and PS boutons subserve different and perhaps opposite neurophysiological functions? In the mammalian cerebellum, for example, neuronal circuits which have been proven to be excitatory (such as the mossy fiber-granule cell contact, or climbing fiber-Purkinje cell contact (Eccles, Ito, and Szentagothai, 1967) have been shown by Uchizono (1967) to be associated with axon terminals possessing round vesicles and an asymmetric cleft,
whereas known inhibitory circuits (e.g., the basket cell-purkinje cell synapse, or the Golgi cell-granuel cell contact (Eccles et al., 1967) have been shown by Uchizono to possess axon terminals having pleomorphic vesicles and a symmetric cleft. As another example, in the mammalian hippocampus, the synapses of basket cells on pyramidal cell somas, which was shown by Anderson et al. (1964) to be inhibitory, have been shown by Gottlieb and Cowan (1972) to be characterized by terminals containing flattened vesicles and an asymmetric cleft. Atwood et al. (1972) have shown a similar correlation between PS terminals and inhibition in the crayfish. Also, Hajdu, Hassler, and Bok (1973) have shown that axon terminals of the rat strio-nigral pathway (a known inhibitory pathway) contain pleomorphic vesicles and symmetric clefts. Recent autoradiographic studies have shown that axon terminals with round vesicles concentrate acetylcholine, a putative excitatory neurotransmitter (Marchbanks, 1969), whereas terminals with flattened vesicles concentrate glycine (Matus and Dennison, 1971) or GABA (Hokfelt and Lundahl, 1972) both of which are putative inhibitory transmitters.

On the other hand, the fact that acetylcholine (ACH) has been found to be associated with axon terminals containing round vesicles, and possessing an asymmetric cleft, does not mean that RA terminals are invariably excitatory. Similarly, evidence pointing to an association between GABA and glycine
and flattened vesicles and symmetric clefts does not imply that PS terminals are of necessity involved in inhibitory functions. Instead, a body of evidence is accumulating which suggests that the effect produced by an axon terminal upon a post-synaptic element is a function of properties of the post-synaptic membrane, rather than of the type of neurotransmitter released by the pre-synaptic terminal. It appears, in other words, that a given neurotransmitter can evoke either an excitatory or inhibitory post-synaptic potential, depending on membrane properties of the post-synaptic structure. ACH, for example, is usually thought of as being an excitatory neurotransmitter, and indeed, has been implicated as such in several CNS circuits; e.g., in the afferents to Renshaw cells (Eccles, Fatt and Koketsu, 1954), in the ascending reticular activating system (Phillis et al., 1967) or in the afferents to deep pyramidal neurons of the cerebral cortex (Krnjevic, 1969). Conversely, ACH has been shown to produce inhibition in neurons in the RF (Bradley et al., 1966), in the medial geniculate (Tebecis, 1968) and in the caudate nucleus (Bloom, Costa and Salmoiraghi, 1965). Similarly, norepinephrine, while normally considered to be solely an excitatory neurotransmitter, has been shown to produce excitation of some neurons, and inhibition of other neurons of the following systems: brainstem RF (Bradley and Wolstencroft, 1965), cerebellar cortex (Yamamato, 1967) and lateral geniculate (Phillis et al., 1967b).
GABA is another putative neurotransmitter which has paradoxical effects. In the cat cerebellar cortex (Krnjevic and Phillis, 1961), in the lateral vestibular nucleus (Obata et al., 1967) and in the cat cerebral cortex (Krnjevic and Schwartz, 1967) GABA has been shown to produce the hyperpolarization characteristic of post-synaptic inhibition. In the cuneate nucleus, on the other hand, GABA has been shown to depolarize the terminals of primary afferents (Davidson and Southwick, 1971) in the course of eliciting a pre-synaptic inhibition of those fibers.

It appears then, that even if one can tentatively associate round vesicles with ACH (or perhaps glutamate) or pleomorphic vesicles with GABA or glycine, this does not necessarily imply that RA terminals are always involved in excitation; or conversely, that PS terminals are invariably mediating inhibition.

That notwithstanding, certain synapses which have been demonstrated physiologically to be excitatory have been shown to be associated with round vesicles, and others proven to be inhibitory have been associated with pleomorphic vesicles. As no examples demonstrating the converse have yet been documented, one can speculate tentatively that RA terminals are generally associated with excitation, whereas PS terminals are usually involved in inhibition. One might use this line of evidence, although circumstantial at best, to suggest that in
the RF of *Pseudemys* RA terminals are also involved in excitation, whereas PS terminals are mediating inhibition.

As mentioned in the results, boutons with round vesicles and a symmetric cleft (RS boutons) comprise a small but somewhat consistent population of boutons in the metencephalic RF of *Pseudemys*. While the majority of classical papers detailing bouton morphology have not reported the existence of such terminals (Colonnier, 1968; Bodian, 1966; Conradi, 1969) Ebner and Colonnier (1975) feel they exist as a distinct, albeit small, population in turtle cortex. The fact that the RS boutons in this material possess round vesicles larger than those of RA boutons (520 Å versus 500 Å) and a symmetric cleft points to the possibility that they subserve a physiological role different from RA boutons. Palay and Chan-Palay (1974), for example, speculate that terminals in the cerebellar cortex with round vesicles and symmetric clefts probably mediate inhibition.

C. **Differences in Placement of Boutons of Different Types**

A general conclusion gleaned from the analysis of synaptic distribution on the reticular neurons of *Pseudemys* is that axon terminals with round vesicles and asymmetric clefts are relatively more numerous, on both somas and dendrites, than axon terminals with pleomorphic vesicles and symmetric clefts. On small neurons, for example, RA terminals comprised 68% of the
total bouton population on the soma, and 72% on the dendrites. On medium neurons, RA terminals comprise 54% of the bouton population on the somas, and 65% on the dendrites. On large neurons, RA terminals comprise 59% of the total bouton population on the somas, and 59% of the total bouton population on the dendrites (See Table 1). The fact that RA terminals are relatively more numerous than PS terminals on the somas of all three reticular neuronal types is of interest in that these results are at variance with the majority of the data available regarding synaptic distribution in the mammalian CNS--i.e., the majority of ultrastructural studies of synaptic distribution on mammalian neurons found that RA boutons were relatively less numerous than PS boutons on neuronal somas. Conradi (1969) for example, found that RA terminals comprised only 34% of the total bouton population of feline spinal motoneuron somas. Gottlieb and Cowan (1972) found that RA terminals comprised only 25% of the total bouton population on the somas of hippocampal pyramidal neurons. In several areas of the mammalian CNS, RA terminals seem to be entirely lacking on neuronal somas--for example, pyramidal neurons in the feline striate cortex have no RA terminals on their somas (Colonnier, 1968), nor do Purkinje cells in the feline cerebellar cortex (Uchizono, 1967); neither do neurons in the feline lateral geniculate nucleus (Guillery and Scott, 1971). On the other hand, there appear to be some instances in the mammalian CNS where RA
boutons, like those in the turtle RF, outnumber PS boutons on neuronal somas. For example, Lemkey-Johnston and Larramendi (1968) reported that RA terminals comprised 80% of the total bouton population on basket cell somas of the mouse, and 92% of the total bouton population on stellate cell somas.

A second conclusion gleaned from the analysis of synaptic distribution on the reticular neurons of Pseudemys is that the percentage of the total bouton population comprised by PS boutons does not vary appreciably when comparing dendritic distribution with soma distribution. While the relative percentage of PS terminals on large neurons does drop slightly when comparing dendrites with somas (39% on dendrites vs. 41% on somas) and even more so on medium neurons, (21% on dendrites vs. 44% on somas) these differences in PS terminals were shown by Chi-square tests to be statistically insignificant.

On the other hand, studies dealing with mammalian material have generally shown that the percentage of the total bouton population comprised by PS terminals decreases when comparing dendritic distribution with somatic distribution. As mentioned above, for example, Conradi (1969) found S bulbs (RA terminals) to comprise 34% of the bouton population on motoneuron somas, and 46% of the population on dendrites. He found F bulbs (PS terminals), on the other hand, to comprise 58% of the synaptic population on the soma, and 48% on dendrites. McLaughlin (1972) also studying motoneurons in cat spinal cord, obtained
similar results in that RA terminals comprised 42% of the population on somas and 53% on dendrites, whereas PS terminals comprised 47% of the synaptic population on somas and 39% on dendrites.

A similar distribution of synaptic types has been found in the cerebellar cortex. In the mouse, for example, as mentioned above, Lemkey-Johnston and Larramendi (1968) found that the percentage of the total bouton population comprised by RA terminals on the soma of basket cells was 80%, whereas their percentage of the total on dendrites was 90%. PS terminals, on the other hand, comprised 20% of the total population on basket cell somas, and only 10% on dendrites. On stellate cells, RA terminals comprised 92% of the population on somas and 100% on dendrites, whereas PS terminals comprised 8% of the population on somas and 0% on dendrites. In the hippocampus of the cat, Gottlieb and Cowan (1972) found the ratio of PS to RA boutons on pyramidal cell somas to be 3 to 1, whereas 95% of the synapses on dendrites were of the RA variety.

The tendency for the proportion of PS terminals to increase when comparing the somas with the distal dendrites is carried to extremes in some instance. Colonnier (1968), for example, found PS terminals to comprise 100% of the synapses on the somas of pyramidal cells in cat striate cortex; Uchizono (1967) reached similar conclusions regarding Purkinje cell somas in cat cerebellar cortex, as did Guillery and Scott (1971),
regarding somas of small neurons in the cat lateral geniculate nucleus. In turtle cortex, Ebner and Colonnier (1975), found that most of the synapses on neuronal somas were of the PS type.

If one adheres to the argument that boutons with pleomorphic vesicles and symmetric clefts are inhibitory, as discussed above, it seems clear that the general trend, in many areas of the mammalian CNS is for inhibitory synapses to be concentrated on the cell soma, whereas excitatory synapses are relatively more numerous on the dendrites. These findings are consistent with physiological speculation, as well as proven evidence. Anderson, Eccles and Loyning, (1964) for example, theorized that inhibitory synapses located on the cell soma are in an optimal site for controlling discharge down the axon triggered by the electrotonic spread of either EPSPs down the dendrites or dendritic spikes. Rall (1967a) proved experimentally that inhibitory synapses become increasingly more effective in decreasing the size of EPSPs, recorded at the soma, as they are placed nearer the soma. An inhibitory synapse on a proximal dendrite, for example, will decrease on EPSP by only 43%, whereas one on the soma will decrease on EPSP by up to 60%. Burke, Fedina, and Lundberg (1971) demonstrated that two of the main inhibitory systems to spinal motoneurons, namely, Renshaw cells and Group Ia inhibitory interneurons, synapse solely on somas and proximal dendrites of spinal motoneurons.
On the other hand, the persistence of PS boutons on distal dendrites, although in reduced numbers, cannot be overlooked. Llinas and Terzuolo (1965) theorized, purely on physiological evidence, that descending inhibitory reticulo-spinal paths to flexor alpha motoneurons in the cat activate interneurons which generate IPSPs on distal dendrites. They speculate that inhibitory synapses on distal dendrites might provide for a smoothly graded, tonic inhibitory control over the neuron. Similarly, Cook and Cangionia (1972) demonstrated that a slow, tonic inhibition of Ia input to spinal motoneurons was mediated by inhibitory systems synapsing on distal dendrites.

As mentioned above, this study appears to show that, contrary to synaptic patterns seen in the majority of mammalian studies, putative excitatory boutons (RA terminals) appear to out-number putative inhibitory boutons (PS terminals) on neuronal somas as well as dendrites, although this pattern has also been observed on stellate and basket cells in the cerebellar cortex of the mouse. These findings have interesting physiological implications. Due to electrotonic properties of cell membranes, excitatory axon terminals situated on neuron somas tend to elicit different membrane voltage changes (EPSPs) than those located on dendrites. As Shepherd (1974; pgs. 72 and 73) puts it, "When the synaptic locus is on the soma, the EPSP at the site has an immediate and sharp rise and a rapid decay."
For the middle dendritic input, the soma transient is reduced in amplitude and has a slower time course. This effect becomes more extreme with the peripheral input site. "The general conclusion from this type of analysis is that local inputs provide for rapid responses, whereas distant inputs provide for slower and more sustained activity. The rapid responses may have the function, in some neurons, of triggering impulses (as in the motoneuron of the spinal cord)."

One might use this line of thinking to argue that turtle reticular neurons (as well as mouse cerebellar basket and stellate cells) are able to sustain rapid-firing phasic activity, as they possess an inordinately high number of putative excitatory (RA) boutons on their somas. The inhibitory synapses on their somas may be concerned with blocking this phasic excitatory activity, whereas those on the dendrites may be concerned with mediating a more tonic inhibition.

D. Synaptic Density

1. As a Function of Cell Size.

When comparing the synaptic density on the three neuronal types of the turtle RF, it becomes apparent that the percentage of the cell surface covered by boutons varies between the cell types. Small reticular neurons in *Pseudemys*, for example, had a bouton coverage of 5% on the soma, and 19% on the dendrites, whereas medium neurons had a bouton
coverage of 11% on the soma, and 21% on the dendrites. Large cells had 16% of the soma covered by boutons, opposed to 27% of the dendrites.

It would thus appear that in the turtle RF, the percentage of the neuronal surface covered by boutons increases as a function of cell size. This same trend appears to occur in the mammalian CNS. Small neurons (in the 6-18 µ range) of the mammalian CNS have been found to have a relatively sparse synaptic coverage. For example, 15% of the soma surface of rat dentate gyrus neurons are covered by boutons (Blackstad and Dahl, 1962) and only 10% of the surface of somata of small neurons in lamina V of the cat spinal cord are covered by boutons (Bowsher and Westman, 1971). Nakamura (1975) found that small neurons in the feline red nucleus had a synaptic coverage on the soma of only 6.1%. Medium sized mammalian neurons (18-30 µ) have been found to have a somewhat greater synaptic coverage. Bowsher and Westman (1971), for example, found that medium neurons in lamina V of the cat spinal cord had 30% of the soma covered by boutons, and 74% of the dendrites. Medium sized neurons in the cat RF had 22% of the soma covered by boutons (Bowsher and Westman, 1971), and medium neurons in the feline red nucleus had 16.6% of the soma surface covered by boutons (Nakamura, 1975).
Finally, large mammalian neurons have been found to have a rather large percentage of the cell surface covered by boutons. In the cat lateral vestibular muscles, for example, Mugnaini et al. (1967) found that up to 66% of the surface of giant Deiter's cells somas were covered by boutons. Westman (1971) found 42% of the neuronal soma surface and 48% of the dendritic surface to be covered by boutons in the lateral cervical nucleus of the cat. Nakamura (1975) found that 61.5% of the soma surface of large neurons in the cat red nucleus were covered by boutons. Conradi (1969) found 47% of the soma surface and 72% of the dendritic surface to be covered in cat spinal motoneurons. In the RF of the cat, Bowsher and Westman (1971) found as much as 80% of the surface of large neuronal somas to be covered by boutons.

The observation that synaptic density increases as a function of neuron size, both in the turtle and various areas of the mammalian CNS, agrees with known physiological findings. For example, Henneman et al. (1965) showed that, in the cat spinal cord, small motoneurons were the first to fire off in response to a Ia fiber burst induced by a phasic stretch, whereas the largest motoneurons fired off last. They concluded that the firing threshold for small motoneurons is considerably less than that for larger neurons. Claman et al. (1974) substantiated this firing rank in cat motoneuron
pools, and Tanji and Kato (1973) and Milner-Brown et al. (1973) found a similar motor neuron firing order in human muscle activity.

This greater excitability of small neurons as opposed to large ones can be explained in light of the fact that, since the dendritic tree of small neurons offers a greater input resistance, a relatively small synaptic input current will produce a large voltage change (EPSP). Conversely, a large neuron will need a greater synaptic input to effect a voltage change sufficient to depolarize it to threshold.

As a given axon terminal releases a limited number of quantal packets of neurotransmitter, usually 1 to 3 (Eccles, 1973), it follows logically that the total number of boutons synapsing on a large neuron must be relatively large to deliver sufficient synaptic excitation to discharge the cell.

2. Synaptic Density as a Function of Distance from the Soma

The analysis of synaptic patterns on reticular neurons in Pseudemys has shown that the percentage of the cell surface covered by boutons increases when comparing the soma coverage with that of the dendrites. Small cells, it will be recalled, have 5% of their somas surface covered by boutons, and 19% of their dendrites' surface. The bouton coverage on medium cells is 11% on the soma and 21% on the dendrites. Sixteen % of the somas of large cells is covered by boutons, whereas the dendrites have a bouton coverage of 27%.
Several workers studying mammalian material have also reported that the bouton coverage on neuronal dendrites in some areas of the CNS is greater than that of the soma. Bowsher (1971), for example, found that the somas of neurons in lamina V of the cat spinal cord had a synaptic coverage of 30-40%, whereas 50-74% of the surface of dendrites were covered by boutons. O'Leary et al. (1972) found that 40-60% of the somas of rabbit cerebellar nuclei were covered by boutons, whereas 80% of the dendrites were covered. Lemkey-Johnston and Larramendi (1968) found that basket cells had a bouton coverage of 30% on somas and 36% of dendrites; they also found stellate cells to have a soma coverage of 18% and dendritic coverage of 36%. Westman (1971) found that, on neurons in the lateral cervical nucleus in the cat, 42% of the soma surface was covered by boutons and 48% of the dendritic surface. Conradi (1969) found that 47% of the surface of somas of cat spinal motoneurons was covered by boutons, and 72% of the surface the dendrites.

The observation that neurons in the CNS of both turtles and mammals have a greater synaptic density on the dendrites than the soma might be explained if one considers a study by Rall (1967b) on electrophysiological aspects of cat spinal motoneurons. Rall found that, as recorded from the axon hillock, both EPSPs and IPSPs generated on the soma have a faster time course and steeper rate of rise than PSPs generated
on dendrites. The latter, especially those on distal dendrites, have a rather slow time course and rate of rise. Thus, synaptic terminals located on somas are suited more towards inducing fast, phasic firing patterns, whereas those on dendrites are perhaps given to sustaining tonic neuronal activity. In the context of this observation, it might be argued that RF neurons are chiefly involved in sustained, tonic activity. It will be recalled, however, that putative excitatory boutons are inordinately numerous on the somas of reticular neurons, indicating a potential on their part for phasic, rapid bursts of activity. It is suggested that reticular neurons may receive somatic afferents from one source and dendritic afferents from another source. In this way, they could operate in either phasic or tonic modes, depending on which afferent source is driving them.

V. SUMMARY

In summary, then, it is argued that the data presented above establishes the following points:

(1) As seen with the electron microscope, the three types of reticular neurons each have ultrastructural characteristics peculiar to that cell type; small cells, for example, have a thin rim of cytoplasm with a paucity of organelles, whereas large
neurons have abundant organelles, especially rough ER, and round gray lipid droplets.

(2) There are two predominant bouton types synapsing on reticular neurons in the turtle: RA and PS boutons.

(3) On all three neuronal types, RA boutons are relatively more numerous on the soma and on the dendrites. The ratio of RA to PS boutons on a given locus, however, varies from one neuronal type to the next.

(4) The overall density of synaptic terminal coverage increases when comparing the dendrites with the soma, on all three classes of reticular neurons.

(5) The overall synaptic density increases as a function of cell size. Medium neurons have a greater synaptic coverage than small cells, but less than large neurons.
CHAPTER IV

CONCLUSIONS AND GENERAL DISCUSSION

The data presented in this study have led to the following conclusions regarding the organization of the metencephalic reticular formation in turtles of the genera *Pseudemys* and *Chrysemys*:

(1) On the basis of morphological and cytological characteristics, three types of neurons appear to exist, although soma length analyses available suggest they belong to a unimodal population of neurons. For purposes of description and discussion, it has been found convenient to divide the metencephalic RF neurons into three groups: small, medium, and large neurons.

(2) Small and medium reticular neurons differ appreciably in their morphology from mammalian reticular neurons in that the former are fusiform or tripolar leptodendritic neurons, whereas the latter are multipolar, isodendritic neurons.

(3) All three neuronal types in the metencephalic RF possess long, rectilnear dendrites which ramify in a transverse plane and which are essentially devoid of excrescences.
(4) Two predominant bouton types, one tentatively linked with excitation, the other with inhibition, contact the somas and dendrites of the RF neurons.

(5) On all three sizes of reticular neurons, putative excitatory boutons outnumber putative inhibitory boutons on somas as well as on dendrites.

(6) Physiological predictions to the contrary, the density of synaptic coverage on reticular neurons increases as a function of cell size.

(7) The density of synaptic coverage is greater on the dendrites of reticular neurons than on their somas.

While points (2) through (7) have been discussed above, certain facets of point (1) have yet to be considered. For example, the question arises as to why there might possibly be three neuronal populations within the metencephalic RF of Pseudemys and Chrysemys. It is suggested that the different sizes of reticular neurons may, perhaps, subserve different physiological roles. Small neurons, for example, may serve as interneurons involved solely in local circuits. Preliminary Golgi-Kopsch observations in this laboratory demonstrate that axons of small reticular neurons do not travel any great distance. They could, perhaps, be considered then as intrinsic neurons. While the general concensus is that most reticular neurons have long,
highly branched axons (e.g., Scheibel and Scheibel, 1958), Valverde (1961b) reported small neurons with short axons in the RF of the rat. Beran and Martin (1971) found that small neurons in the RF of the opossum are at least not involved in spinal projections, although an earlier study of the cat RF (Torvik and Brodal, 1957) did demonstrate caudally-projecting small neurons. In any case, it is offered as a speculation that small turtle reticular neurons might function as interneurons involved in short association, or even more probably, inhibitory pathways. In many subsystems of the central nervous system where several size classes of neurons exist, the smaller neurons are usually found to be involved in inhibitory functions, e.g., in the thalamus (Famiglietti and Peters, 1972), in the cerebellum (Eccles, Ito and Szentagothai, 1967) or in the olfactory bulb (Shepherd, 1974). Medium reticular neurons may receive a good deal of the afferent input to the RF in the turtle. Golgi-Kopsch studies from this laboratory show that the long, radiating dendrites of medium neurons are crossed by many axons of various shapes and sizes. In addition, medium reticular neurons, unlike small neurons, may influence distant centers. Golgi-Kopsch studies in this laboratory demonstrate that axons of medium reticular neurons often travel fairly long distances before leaving the plane of section. Golgi studies on mammalian material show axons of medium-sized reticular neurons traveling for considerable distances along the rostrocaudal extent of the brainstem (Scheibel
and Scheibel, 1958). Studies on both the RF of the cat (Torvik and Brodal, 1957) and that of the opossum (Beran and Martin, 1971) demonstrate that medium-sized reticular neurons project to the spinal cord, while another study of the RF of the cat and rat show that medium-sized reticular neurons project to the thalamus (Scheibel and Scheibel, 1958). It is suggested, then, that medium-sized reticular neurons in the turtle might serve as projection neurons, influencing either rostral or caudal levels of the neuraxis.

Large neurons of the turtle RF are almost certainly involved in afferent projections to the RF. Tuge (1932) reported that the long, radiating dendrites of large RF neurons in the turtle were crossed by numerous sensory and motor systems, such as the acoustic and cerebello-tegmental system. Observations in this laboratory tend to corroborate Tuge's (1932) observation in that the long, radiating dendrites of large RF neurons virtually span an entire hemisection of the brainstem, and are seen to be crossed by numerous axons. Furthermore, Bowsher and Westman (1970) report that spinal afferents to the feline nuc. R. gc. terminate almost exclusively on the somas and dendrites of large reticular neurons.

On the other hand, large neurons in the turtle RF are probably involved in efferent projections as well. Tuge (1932) reported that large neurons in the metencephalic RF give off axons which enter the M.L.F., which is the main rostrocaudal tract in the reptilian brainstem. Cruce (1975) has determined
that large reticular neurons in the tegu lizard project to the spinal cord. Studies on the feline RF (Torvik and Brodal, 1957) and that of the opossum (Beran and Martin, 1971) show that, in these animals, large reticular neurons project to the spinal cord (Torvik and Brodal's study also demonstrated that large neurons project rostrally).

It must now be asked whether medium and large reticular neurons in the turtle might serve the same physiological roles, since they are probably both involved in efferent projections from the RF. It is suggested that large reticular neurons might project to regions more distant than those influenced by medium reticular neurons. Brodal (1969), for example, argues that there is a relationship between the size of a neuron and the length of its axon. He cites evidence which shows that the largest pyramidal neurons in area 4 of the human cerebral cortex send their axons to the lumbosacral cord. One might use this line of thinking to suggest that large reticular neurons of the turtle may simply be larger editions of medium reticular neurons. The former may influence activity in caudal segments of the spinal cord, whereas the latter may influence rostral levels of the cord or, perhaps, the thalamus. Further speculation would be fruitless, however, regarding the physiological roles of the RF neurons.

Clearly, much experimental work must be done. To elucidate whether or not the neurons in the RF do indeed comprise distinct populations and if so, whether each cell type performs a different function, several experiments are proposed.
First, it must be clarified as to whether the neurons in the metencephalic RF of *Pseudemys* and *Chrysemys* comprise three distinct populations according to size or rather are part of a unimodal population. To determine which is so, a soma length analysis should be carried out on a sample of at least 1000 reticular neurons in Nissl-stained, paraffin embedded material. Second, it should be determined whether small, medium, and large reticular neurons all receive afferents from the same sources. To determine if this is so, EM degeneration studies should be done. Lesions should be placed in areas which are known to project to the RF in reptiles such as the optic tectum (Foster et al., 1973; Ulinski, 1976a) or the spinal cord (Ebbesson, 1967). Then, the presence or absence of degenerating boutons on all three neuronal types following transection of a given afferent source should be noted. It might be determined, for example, that the optic tectum projects to medium RF neurons, but not to large ones. Such data would lend credence to the idea that there is more than one population of neurons within the turtle RF.

Finally, it should be determined whether small, medium, and large reticular neurons all project to the same targets. To ascertain this, both retrograde chromatolysis and horseradish peroxidase (HRP) retrograde transport techniques could be used. Using the former method, a lesion could be made in an area suspected of receiving RF projections (such as the spinal cord) and the presence or absence of chromatolysis in small, medium, and
large cells noted. Using the retrograde transport technique, HRP would be injected into an area suspected of receiving RF projections, and the presence or absence of the HRP reaction in all three sizes of the RF neurons would subsequently be noted.

Using these two methods, it could be determined whether all three sizes of reticular neurons project to the same areas. If it were found, for example, that medium neurons project to the thalamus, whereas large neurons do not, further credence would be lent to the hypothesis that each of the three sizes of reticular neurons in the turtle subserves separate physiological roles.

It is the intention of this researcher to employ the procedures outlined above to elucidate the organization of the RF in turtles, and thereafter, to determine whether the RF of other orders of reptiles is organized in a similar fashion. In this manner, a clear picture of the physiological roles of the RF in reptiles will emerge.
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### TABLE 1

Relative Distribution of Bouton Types

<table>
<thead>
<tr>
<th>Neuron Size</th>
<th>RA</th>
<th>PS</th>
<th>RS</th>
<th>PA</th>
<th>N*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Somata</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small</td>
<td>68%</td>
<td>20%</td>
<td>12%</td>
<td>0</td>
<td>25</td>
</tr>
<tr>
<td>Medium</td>
<td>54%</td>
<td>44%</td>
<td>2%</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Large</td>
<td>59%</td>
<td>41%</td>
<td>0</td>
<td>0</td>
<td>29</td>
</tr>
</tbody>
</table>

| Dendrites   |      |      |     |     |     |
| Small       | 72%  | 22%  | 6%  | 0   | 71  |
| Medium      | 65%  | 21%  | 9%  | 5%  | 182 |
| Large       | 59%  | 39%  | 2%  | 0   | 46  |

*N=number of boutons sampled.*
TABLE 2
Mean % of Cell Surface Covered by Boutons

<table>
<thead>
<tr>
<th></th>
<th>Small Cell</th>
<th>Medium Cell</th>
<th>Large Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somas</td>
<td>N = 75</td>
<td>N = 106</td>
<td>N = 10</td>
</tr>
<tr>
<td></td>
<td>$\bar{x} = 0.05$</td>
<td>$\bar{x} = 0.11$</td>
<td>$\bar{x} = 0.16$</td>
</tr>
<tr>
<td></td>
<td>SD = 0.06</td>
<td>SD = 0.09</td>
<td>SD = 0.11</td>
</tr>
<tr>
<td></td>
<td>SE = 0.01</td>
<td>SE = 0.01</td>
<td>SE = 0.04</td>
</tr>
<tr>
<td>Dendrites</td>
<td>N = 32</td>
<td>N = 53</td>
<td>N = 12</td>
</tr>
<tr>
<td></td>
<td>$\bar{x} = 0.19$</td>
<td>$\bar{x} = 0.21$</td>
<td>$\bar{x} = 0.27$</td>
</tr>
<tr>
<td></td>
<td>SD = 0.19</td>
<td>SD = 0.14</td>
<td>SD = 0.10</td>
</tr>
<tr>
<td></td>
<td>SE = 0.03</td>
<td>SE = 0.02</td>
<td>SE = 0.03</td>
</tr>
</tbody>
</table>

S.D. = Standard Deviation; S.E. = Standard Error; N = Number of profiles sampled.

TABLE 3
Mean Bouton Number per Neuron

<table>
<thead>
<tr>
<th></th>
<th>Small Cell</th>
<th>Medium Cell</th>
<th>Large Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somas</td>
<td>N = 75</td>
<td>N = 106</td>
<td>N = 10</td>
</tr>
<tr>
<td></td>
<td>$\bar{x} = 1.2$</td>
<td>$\bar{x} = 4.2$</td>
<td>$\bar{x} = 10.0$</td>
</tr>
<tr>
<td></td>
<td>SD = 1.39</td>
<td>SD = 4.34</td>
<td>SD = 8.0</td>
</tr>
<tr>
<td></td>
<td>SE = 0.16</td>
<td>SE = 0.42</td>
<td>SE = 2.0</td>
</tr>
<tr>
<td>Dendrites</td>
<td>N = 32</td>
<td>N = 53</td>
<td>N = 12</td>
</tr>
<tr>
<td></td>
<td>$\bar{x} = 3.7$</td>
<td>$\bar{x} = 7.4$</td>
<td>$\bar{x} = 12.9$</td>
</tr>
<tr>
<td></td>
<td>SD = 3.06</td>
<td>SD = 4.9</td>
<td>SD = 6.54</td>
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<tr>
<td></td>
<td>SE = 0.54</td>
<td>SE = 0.67</td>
<td>SE = 1.89</td>
</tr>
</tbody>
</table>

S.D. = Standard Deviation; S.E. = Standard Error; N = Number of profiles sampled.
PLATE 1

EXPLANATION OF FIGURES

Figure 1A. A low power, transverse section through the mid-myelencephalon of *Pseudemys* at the level of the obex. Cresyl Violet. 10X Objective.

Figure 1B. A low power transverse section through the rostral myelencephalon of *Pseudemys* at the level of vagal motor nucleus. Cresyl Violet. 10X Objective.

Figure 1C. A low power, transverse section through the caudal metencephalon of *Pseudemys* at the level of the lateral vestibular nucleus. Cresyl Violet. 10X Objective.

Figure 1D. A low power, transverse section through the rostral metencephalon of *Pseudemys* at the level of the trigeminal motor nucleus. Cresyl Violet. 10X Objective.
Figure 2. A histogram of the perikaryal lengths (long axis of the soma) of a sample of 93 metencephalic reticular neurons. Taken from light micrographs of 2 micron, Toluidine-Blue stained plastic sections. Neuronal soma lengths appear to be dispersed along a continuum, suggesting that turtle reticular neurons constitute, at least with regard to size, a unimodal population.
Figure 3A. A large neuron of the metencephalic RF of *Pseudemys*. Coarse Nissl bodies can be seen in the cytoplasm. Cresyl Violet. 40X. Oil Immersion Objective. X 1,401.

Figure 3B. A medium neuron of the metencephalic RF of *Pseudemys*. Cresyl Violet. 40X. Oil Immersion Objective. X 1,401.

Figure 3C. A small neuron of the metencephalic RF of *Pseudemys*. Notice the thin rim of cytoplasm around the nucleus. Cresyl Violet. 40X. Oil Immersion Objective. X 1,401.
Figure 4. A small neuron of the metencephalic RF. Notice the fusiform soma and wavy dendrites. Camera Lucida drawing, Golgi-Kopsch Preparation. 40X. Oil Immersion Objective. X 250.
PLATE 5

EXPLANATION OF FIGURE

Figure 5. A small neuron of the metencephalic RF. Notice the unbranched wavy dendrites (D), one of which is giving rise to a thin, beaded axon (ax). Golgi-Kopsch Stain. 40X. Oil Immersion Objective. X 1,401
Figure 6. A small neuron of the metencephalic RF. One dendrite of this neuron is branched (arrow). Notice the spineless, wavy configuration of the dendrites (D). Golgi-Kopsch Stain. 40X. Oil Immersion Objective. X 1,401
Figure 7. A medium neuron of the metencephalic RF of *Pseudemys*. Notice the fusiform soma and wavy dendrites (D), which branch to a greater degree than those of small neurons. Camera Lucida Drawing. Golgi-Kopsch Stain.

40X. Oil Immersion Objective. X 250
Figure 8. A Medium Neuron. This neuron is fusiform in shape, with wavy, smooth dendrites (D). Golgi-Kopsch Stain. 40X. Oil Immersion Objective. X 1,401
Figure 9. A medium neuron. This neuron is polygonal in shape. Three wavy dendrites can be seen emerging from the corners of the perikaryon. Notice the axon (ax) emerging from one corner of the perikaryon. Golgi-Kopsch Stain. 40X. Oil Immersion Objective. X 1,401.
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Figure 11. Low power photograph of a transverse section through the rostral metencephalon. Trapezoid outlines area, encompassing metencephalic reticular field, which was trimmed for thin sectioning. The trigeminal motor nucleus (TM) can be seen near the corner of the ventricle. Nissl Stain. 10X Objective.
Figure 12. A histogram of the perikaryal lengths (long axis of the soma) of a sample of 191 metencephalic reticular neuronal soma profiles. Taken from low power electron micrographs. A bimodal distribution of soma lengths can be seen, indicating the presence of two and perhaps three neuronal populations.
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EXPLANATION OF FIGURES

Figure 13a. A small neuron of the metencephalic RF. 2 micron, Toluidine Blue-stained plastic section. 40X. Oil Immersion Objective. X 1,401

Figure 13b. A small neuron of the metencephalic RF. Dust like particles of rough endoplasmic reticulum and Golgi apparatus (GO) can be seen in the sparse cytoplasmic rim. An oligodendrocyte (O) abuts against the cell membrane. Low Power Electron Micrograph. X 6,660
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Figure 15b. A medium neuron of the metencephalic RF. Numerous examples of Golgi apparatus (GO) and rough endoplasmic (RER) can be seen in the cytoplasm. Notice the bouton (arrow) synapsing on the cell's soma. Low Power Electron Micrograph. X 6,660
Figure 16. A medium neuron giving off a proximal dendrite (D). A bouton can be seen synapsing on the dendrite of this neuron (arrow). X 6,660
PLATE 17

EXPLANATION OF FIGURES

Figure 17a. A large neuron of the metencephalic RF. A massive primary dendrite (D) can be seen emerging from the perikaryon. 2 micron, Toluidine-Blue stained plastic section. 40X. Oil Immersion Objective. X 1,401

Figure 17b. A large neuron of the metencephalic RF. Nissl Bodies (NB) and round gray lipid droplets (L) can be seen in the cytoplasm. Low Power Electron Micrograph. X 6,660
Figure 18. A histogram showing the range of widths of proximal dendrites of RF neurons, based on a sample of 17 small neuron dendritic profiles, 37 medium neuron dendritic profiles, and six large neuron dendrites.
Figure 19a. A putative isolated dendritic segment of a small neuron. Notice its wavy configuration. Low Power Electron Micrograph. X 6,660

Figure 19b. A putative isolated dendritic segment of a medium neuron. Parallel arrays of neurotubules (NT) can be seen in the cytoplasm. Low Power Electron Micrograph. X 6,660

Figure 19c. A putative isolated dendritic segment of a large neuron. Numerous ribosomes (R) and lipid droplets (L) can be seen in the cytoplasm. Low Power Electron Micrograph. X 6,660
Figure 20A. A bouton with round vesicles and an asymmetric cleft (RA bouton) can be seen emerging from its parent axon (a). Higher Power Electron Micrograph. X 35,200.

Figure 20B. An RA bouton synapsing on a neuronal soma. Post-junctional bodies (PJ) can be seen below the postsynaptic density. Higher Power Electron Micrograph. X 35,200.
PLATE 21

EXPLANATION OF FIGURES

Figure 21A. A bouton with pleomorphic vesicles (PS) synapsing on a dendrite. Next to it is a bouton with round vesicles and an asymmetric cleft (RA). Higher Power Electron Micrograph. X 35,200

Figure 21B. A bouton with pleomorphic vesicles and a symmetric cleft (PS) synapsing on a neuronal soma. X 35,200
Figure 22A. A bouton with round vesicles and a symmetric cleft (RS). Higher Power Electron Micrograph. X 35,200

Figure 22B. A bouton with pleomorphic vesicles and an asymmetric cleft (PA). Higher Power Electron Micrograph. X 35,200
Figure 23. A histogram showing the relative percentage of the total bouton population on the somas of the three neuronal types in the RF comprised by a given bouton type. RA = round, asymmetric boutons; PS = pleomorphic symmetric; RS = round, symmetric; N = number of boutons in sample.
Figure 24. A histogram showing the relative percentage of the total bouton population on the dendrites of the three neuronal types in the RF comprised by a given bouton type. RA = round, asymmetric boutons; PS = pleomorphic, symmetric; RS = round, symmetric; PA = pleomorphic, asymmetric; N = number of boutons in sample.
Figure 25. A histogram comparing the density of synaptic coverage among the somas and dendrites of the three types of neurons in the metencephalic RF. The hatched bars represent the mean percentage of the somas covered by boutons, and the clear bars represent the mean percentage of the dendrites covered by boutons. S=small neurons; M=medium neurons; L=large neurons.
EXPLANATION OF FIGURE

Figure 26. A histogram showing the range in widths of boutons synapsing on the three neuronal types of the RF. S = somatic boutons; D = dendritic boutons; N = number of boutons measured.
Figure 27. A histogram comparing the mean number of boutons per profile among the somas and dendrites of the three neuronal types. $S$ = the mean number of boutons per soma profile, and $D$ = the mean number of boutons per dendritic profile. $N$ = the number of profiles analyzed.
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The dissertation is therefore accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Date: 1/8/75

Director's Signature: [Signature]