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THE INFLUENCE OF THE TRIGEMINAL NERVE ON FACIAL GROWTH AND DEVELOPMENT

by

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GRADUATE STUDIES

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THE INFLUENCE OF THE TRIGEMINAL NERVE ON
FACIAL GROWTH AND DEVELOPMENT

Abstract

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ROLF GORDON BEHRENTS

For more than a decade Moss and his associates have argued that the growth of the craniofacial skeleton is governed by the growth of a variety of contiguous soft tissues and functioning spaces--the so-called "functional matrices." Recently, he has noted the existence of extensive data suggesting that the growth of the various matrices is controlled by "trophic" influences from nerves, specifically the trigeminal. If these two premises are correct, one conclusion is inescapable: nerves ultimately control facial skeletal growth. As judged from the literature, Moss's minor premise is tenable; however, his major premise--and, hence, his conclusion--has never been subjected to explicit experimental verification.

The present study was performed to evaluate the role of the trigeminal nerve in the regulation of facial growth and development in rats. Unilateral, electrolytic lesions of the trigeminal nerve were effected stereotaxically. Lesions were placed in one of three locations: (1) gasserian ganglion, (2) anterior to the ganglion, and (3) posterior to the ganglion.

The sample consisted of 119 experimental Long-Evans rats

(10-20 days of age), with twenty-six animals serving as controls.

Data were collected and analyzed in three categories:

1. General appearance, physical condition, and neurological condition;

2. Histological evaluation of the location of the lesion for the purpose of classification;

3. Cephalometric measurements of the experimental and control animals to determine the effect on facial growth of interrupting the trigeminal nerve.

Generally, the experimental animals showed no gross distortions or malformations of the facial structures.

Histological examination revealed 53 anterior lesions, 48 ganglionic lesions, 12 posterior lesions, and 6 controls.

Cephalometric measurements of the experimental animals showed cranial asymmetries resulting from a change in the magnitude and direction of growth. The greatest effect was noted in those animals which had suffered ganglionic lesions, followed, in order of severity, by posterior and anterior lesions. Deviation of the facial skeleton and reduction in size was noted on the ipsilateral side, except in the case of motor efferent destruction, which resulted in ipsilateral deviation of the mandible and contralateral deviation of the rostral portion of the skull.

The results of the study may be summarized:

1. Interruption of the trigeminal nerve in young rats produced a significant asymmetry of the facial complex; however, the magnitude of the change in the amount and direction of growth was small;

2. Cranial asymmetries were produced by both sensory and motor nerve deficiencies;

3. Animals with ganglionic lesions showed the greatest cranial asymmetry;

4. The changes seen are consistent with the assumption that the trigeminal nerve exerts a trophic influence on the craniofacial complex;

5. However, the conclusion that trophic effects constitute a major factor in the regulation of craniofacial growth cannot be supported.

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DEDICATED

TO

DR. and MRS. E. G. BEHRENTS

My parents, who made my education
and thus this paper possible.

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INTRODUCTION

> In proportion as the animal spirits enter the cavities of the brain, they pass thence into the pores of its substance, and from these pores into the nerve; where according as they enter, more or less, into this or that nerve, they have the power of changing the shape of the muscles into which the nerves are inserted. . . . Rene Descartes, 1664.

From the standpoint of organogenesis, the rates of growth and the final determination of size, shape and position of the various organs all seem to be carefully regulated. Something triggers the onset of growth, sustains it for a finite period, determines its magnitude, and retards it when enough new tissue has been produced (Morgan, 1906; Goss, 1972a, 1972b). This sequence holds also for regeneration. Here too, some regulator is involved in regeneration of lost limbs, organs, tissues, or cells, with differences noted at different locations on the phylogenetic ladder (Morgan, 1906).

Presumably, these basic processes are also involved in the growth and development of the face. Over the years, many theories have been advanced to explain not merely <u>what</u> happens when the face grows, but <u>how</u> it is accomplished. In trying to explain the local control mechanisms, many regulatory factors have been suggested: the genome, neurotrophic substances, pH levels, oxygen tension, temperature, function, bioelectric potentials, chalone-like inhibitors and stimulators, cyclic AMP, vasomotor control, nutrition, a multitude of enzymatic and localized hormonal factors, physiological demands, among others (Enlow, 1973).

Thus, it appears that in a complex structure such as the cranial and facial skeleton, many factors may be concerned with the determination of form. While it is known that certain parts of the facial skeleton depend on the degree of activity of the attached muscles for their full development, and that parts of the cranial skeleton depend on the growth of the brain for their full development, considerable uncertainty exists today regarding the role of nerves in determining the morphology of facial structure. It is well known that one aspect of neural activity, transmission at the neuromuscular junction, controls the activity of muscles and, pari passu, the extent of development and form of certain skeletal parts. Furthermore, there exists considerable evidence to suggest that nerves have another property which could directly regulate skeletal morphogenesis and possibly explain differences in growth within and among species. This mechanism is neurotrophism.

During the seventeenth century, the nervous system was believed to be a complex hydraulic machine with many fine tubes down which a mysterious vital substance flowed to the muscles. Although the analogies have progressed from a collection of liquid filled tubes, to a telephone exchange with wires and switches, to a collection of self-controlling systems and finally to a general purpose computer (Burns, 1968), the concept of "vital spirits" seems to still have some meaning in the light of present day theories of facial growth, specifically, those which presume a "trophic" influence from the peripheral and central nervous systems.

By definition, neurotrophism is described as a nutritional neural function which involves axoplasmic transport, provides for long-term interactions among neurons and the innervated tissues so as to regulate homeostatically the morphological, compositional, and functional integrity of the periphery (Gutmann, 1964), and is unrelated to the action potential. It is thought that the cell body forms a specific substance which flows down the axon by axoplasmic transport, is extruded at the axonic terminals, and, thereafter, serves to maintain the integrity of the innervated tissue.

In recent years neurotrophism has been the subject of considerable interest as it applies to facial growth (Moss, 1971a, 1972a, 1975). Unfortunately, there has been little relevant experimentation. The present investigation will attempt to explore both the functional and developmental relationships which, as hypothesized by Moss (1971a, 1972a, 1975), may exist between the neuromuscular, neurotrophic, and skeletal systems.

The experiment will involve the intracranial stereotaxic coagulation of various portions of the trigeminal nerve in developing rats. To determine both quantitatively and qualitatively the extent of neural regulation of facial growth, lesions will be placed at three locations--in the ganglion, posterior to the ganglion, and anterior to the ganglion. After a suitable amount of time, facial skeletal changes will be evaluated via measurements taken from dorsoventral cephalograms.

LITERATURE REVIEW

Neurotrophism

Early History

Although the concept of neurotrophism began in the nineteenth century (Skinner, 1961), to whom the credit goes is not really known. The concept that nerves are important to the integrity of non-neural tissue, was presented long ago as can be seen in the following statement by Prochaska (1784):

> As is known numerous physiologists, old and new, have maintained that nutrition is mediated by nerves. For example, Fr. Sylvius, Willis Glisson and others assumed two kinds of nerve fluids: a dense, protein fluid which serves nutritional processes; the other vaporizing, more liquid, diffused in the former and destined for eliciting motion and sensation.

Clinical Observations

Nerve section results in clinical symptoms which drew the attention of many clinical and experimental workers. As early as 1823, Mayo noted that, after cutting the trigeminal nerve, the conjunctiva of the eye became inflamed, the cornea ulcerated, and the face edematous. In 1824, Magendie observed keratitis following section of the trigeminal nerve. Similar observations were recorded by Novinska (1874) and Charcot (1876). In 1846, Romberg described facial hemiatrophy, but did not find changes in sensitivity and mobility.

Further clinical evidence was given by Mitchell in 1864, who encountered what were considered trophic disturbances accompanying nerve injuries suffered as the result of war wounds. Similar results were reported by Mitchell in 1895.

Experimental Observations

In 1866, Sechenov, in his investigation on the trigeminal and vagus nerves, characterized the trophic influences as:

> . . .processes which ensure the anatomical and functional integrity of tissues during life are generally designated as nutritional processes. These functions enable muscles, nerves, bone and other tissues in the organism to remain unchanged as far as their physiological properties are concerned.

It was Samuel in 1860, who clearly expressed in "Die Trophischen Nerven" that: ". . . der Grund der Ernahrung liegt in den Zellen, das Mass der Ernahrung in den trophischen Nerven." Bernard in 1865 and 1868, sectioned the trigeminal, vagus, and cervical sympathetic nerves, and reported that these nerves have some role in trophic processes mediated thrcugh specific trophic nerves and result in a constancy of the internal environment (the "milieu intérier").

"Trophic Nerves"

Heidenhain (1886) assumed the existence of trophic nerves to salivary glands and stated that they either secreted saliva or produced organic constituents of saliva. In 1890, 1898, and 1920, Pavlov was inclined to acknowledge the existence of trophic nerves

and hypothesized trophic reflexes which were responsible for various trophic disturbances, e.g., stomatitis, ulceration, etc. Pavlov stated:

> In this way every organ is under the influence of triple nervous control, i.e. functional nerves which either evoke or interrupt functional activity (muscle contraction, secretion in glands, etc.), vasomotor nerves regulating roughly the supply of chemical material (and removal of metabolic products) by increasing or decreasing blood circulation through the organ and finally trophic nerves, which control in the interests of the organism the exact amount of final utilization of this material in each organ.

These results, along with other confirmatory evidence, led the early investigators to conclude that besides the sensory, motor, and autonomic elements, there were associated fibers which were profoundly important in determining the metabolic activities of the tissues to which they were distributed. These fibers were supposed to originate in specific "trophic centers" within the brain, and specific "humours" were supposed to originate there, flow down the "trophic nerves" and maintain the integrity of the innervated tissues. Speranskii in 1935, however, rejected the hypothesis of a separate system of trophic function of the nervous system in the organism:

> The trophic function of the nervous system in the organism does not act in an isolated manner, but in connection with all other functions. . . . Every nervous process is followed by changes in the biophysical and biochemical state of the tissue, i.e. changes in its trophic state.

Recent Literature

Although primitive in nature and scope, the early experiments and observations form an historical basis for an understanding of

current day theories of neurotrophism. The early work along with concomitant advances in related fields have led scientists to discard some of the older theories (such as the concept that trophic substances were formed in specific centers in the brain and flowed down specific "trophic nerves"), in favor of believing that the organism is an autoregulatory system in which regulation of the "milieu intérieur" is by various well-known homeostatic mechanisms (Cannon, 1939), and by trophic influences which maintain the structure and integrity of the tissues in spite of the everchanging environmental conditions.

Growth of the Face

A relationship between neurotrophism and facial growth has been advanced by several contemporary workers. Moss (1971a, 1972a) has stated the neurotrophism is the control mechanism which regulates the growth of functional matrices, whose relation to facial growth is explained in numerous articles (Moss, 1960, 1962, 1969, 1971b, 1972b, 1972c; Moss and Rankow, 1968; Moss and Salentijn, 1969).

According to his method of "functional cranial analysis," Moss considers the head to be a region of the body which performs a variety of functions: respiration, speech, olfaction, etc. These individual functions are carried out by what he calls "functional cranial components", each of which consists of a "functional matrix" (those tissues necessary to carry out this function), and a "skeletal unit" (those skeletal tissues which protect or support the functional matrix). Skeletal units consist of "macro-skeletal units" (bones);

functional matrices are of two types--"periosteal matrices" (muscles, teeth, glands, etc.) or "capsular matrices" (brain, orbit, spaces, etc.).

Periosteal matrices act upon skeletal units directly by influencing deposition and resorption (a process Moss calls transformation) and in this way they alter the size and shape of their respective skeletal units. Capsular matrices act upon functional cranial components, as a whole, in an indirect manner. Growth of the capsular matrices, with the resultant increase in volume, causes a passive, outward movement of the embedded functional cranial components (a process Moss calls translation).

This view considers the functional matrices to be primary (as the inducer in a series of processes leading to growth of the face), and believes that the presence, size, shape, spatial position, and growth of any skeletal unit is secondary (or compensatory) and mechanically obligatory to changes in the size, shape, and spatial position of its related functional matrix.

Moss (1971a, 1972a) believes that neurotrophic processes are responsible for the growth and maintenance of the functional matrices and, accordingly, for the regulation of the growth of the craniofacial skeleton. He (1972a) concludes that:

> Oro-facial growth is now capable of being conceived of as a homeostatically controlled series of processes in which the neural center regulates the peripheral tissue and the periphery, in turn regulates the center.

Moss has inferred support for his hypotheses from computer analyses (Moss, 1962). In this study Moss speculated that the neurovascular or neurotrophic bundle seemed to be the core of the matrices.

Another author, Ricketts, supports the views of Moss and bases much of his theoretical argument for prediction of facial growth on "neurotrophic foci" (Ricketts, 1972a, 1972b, 1973c, 1972d). Ricketts feels that constructed points used in facial growth prediction coincidently support the view of Moss concerning the primary nature of neurotrophic foci. Ricketts justifies constructed points such as the foramen rotundum (polar center), mandibular foramen (Xi point), and some nutrient canals on the lingual surface of the mandibular ramus (point Eva), mainly based on the ideas of Moss. In describing the growth curve of the mandible (1972c), Ricketts states that:

> By teleological reasoning it could be speculated that a small series of foramina would provide trophic supply to the apparent growth aspect of the curve.

Ricketts also concludes (1972d) that:

> It appears therefore that the key to the rotation (of the mandible) as a central principle lay in the continued gnomic relation of the nerve supply.

His "gnomic" figures are triangular constructs whose apices are located at positions of supposed neurotrophic influence (Ricketts, 1972d).

Given this current interest in neurotrophism among craniofacial biologists, several questions would seem pertinent:

1. What is the neurotrophic substance?
2. How does a nerve cell manufacture and transport it?
3. What are its effects on innervated structures which are growing and differentiating?
4. What are the effects of the innervated tissue on the nerve?

Partial answers to some of these questions are available and will be presented in the following sections.

The Neuron

The basis for the theory of neurotrophism is the neuron. Although complex in nature, the neuron is basically the same as other cells. The generalized neuron consists of: (1) a dendritic zone (the receptor membrane of the neuron) either consisting of a set of tapering cytoplasmic extensions (dendrites) which receive synaptic endings of other neurons or differentiated to convert environmental stimuli into nervous activity (Bodian, 1962). Mitochondrial concentrations may also be present. (2) The perikaryon, or the cell body, is usually characterized by the presence of nuclear substance, nucleolus, nissl substance, and rough endoplasmic reticulum. (3) The axon, is a simple, often branched and usually elongated, cytoplasmic extension, which is known to conduct nerve impulses. It is characteristically uniform in caliber and ensheathed by neuroglia or neurilemma cells (Bodian, 1962). (4) Axon telodendria, the usually branched and variously differentiated terminals of axons related to synaptic transmission or neurosecretory activity. Mitochondrial concentrations, "synaptic vesicles," or secretory granules are commonly present in bulb-like terminals (Bodian, 1962). Ultrastructural studies of the axonal cytoplasm show that it contains a general protoplasmic ground substance plus mitochondria, vesicles, granules, filaments, microtubules, and sometimes secretory granules (Singer, 1964). Thus the

axon appears to have the machinery for synthesis and transport of secretory substances. However, it lacks rough endoplasmic reticulum, Golgi structures, and nissl substance (Singer, 1964).

Neurons show a wide variation of morphology and structure, and according to Wuerker and Kirkpatrick (1972), the variations of neurons do not represent differences in their conductive properties, but may reflect a difference in their metabolic or trophic function.

The neuron, when its axon is severed, behaves as other cells when a bit of cytoplasm is separated from the cell; it may waste and die. The remainder of the cell may regenerate (Singer, 1964; Boyd and Davey, 1968). The neuron, however, has the job of moving important substances great distances in the regeneration process. If the flow is stopped by block or section, the distal process contracts, forms folds and spirals and dies; the central portion swells as if under the influence of a turgor pressure (Young, 1944). Therefore, there must be essential substances which traverse the axon cytoplasm, which provides for the maintenance or regeneration of the axon. This view has been substantiated to a great extent by the development of the concept of axoplasmic transport.

Axoplasmic Transport

Axoplasmic flow is a term used to describe a cytoplasmic movement of substances within the cytoplasm of the axon (Schmitt, 1968), the cell body, and the dendrites (Wuerker and Kirkpatrick, 1972). The following discussion is intended to illustrate the relevance of the axoplasmic flow to the theory of neurotrophism and growth.

In addition, excellent reviews of the theory of axoplasmic transport can be found in Lubińska (1964), Dalhström (1971), and Wuerker and Kirkpatrick (1972).

Depending on the method used to study the process, there appears to be a wide variation in the nature of the flow and the material involved. Direct observations of axoplasmic flow were made by Cajal (1928) and Young (1942). Ligation and section of nerves causes various structural elements, particles, and enzymatic substances to accumulate both proximally and distally to the lesion (Pellegrino de Iraldi and De Robertis, 1968; Zelená, 1968). Radioactive substances have been used extensively to show that, when injected near the neuronal perikaryon, there is incorporation into neuronal cell macromolecules and subsequent passage down the axon (Weiss, Edds, and Cavanaugh, 1945b; Samuels et al., 1951; Ochs and Burger, 1958; Weiss, 1961; Droz and Leblond, 1962, 1963; Lubińska, 1964; Miani, 1964; Taylor and Weiss, 1965; Korr, Wilkinson, and Chornock, 1966, 1967; Ochs and Johnson, 1969; Ochs, Sabri, and Johnson, 1969).

Differential Flow Rate

The above techniques seem to show that axoplasmic transport consists of two main components: one is a slow flow at the rate of 1-4 mm/day, and the other is a fast flow at the rate of 10-20 mm/hour (Weiss and Hiscoe, 1948; Samuels et al., 1951; Ochs, Dalrymple, and Richards, 1962; Droz and Leblond, 1962; Lubińska, 1964; Miani, 1964; Taylor and Weiss, 1965; Kerkut, 1968; Ochs and Johnson, 1969; Ochs,

Sabri, and Johnson, 1969; Chou, 1970; Wuerker and Kirkpatrick, 1972; and many others). A range of reported flow rates seems to reflect a variation in materials and methods used, nerve, animal studied, and many other factors; thus intermediate rates are reported (Miani, 1964). Despite this, the data seem to indicate the existence of two basic flow rates.

The slow component was first demonstrated by Weiss and Hiscoe (1948) who showed that when a nerve is dammed by a narrow cuff the cytoplasm proximal to the cuff becomes blocked and swells. Distal to the cuff, the fibers narrow. Upon release of the cuff, the build-up subsides. Originally, the damming of axoplasm was taken as a sign of growing axon substances pressing distally thus indicating perpetual growth of nerves. However, it is now assumed that this centrifugal "supply stream" continues throughout the life of the mature neuron (Weiss, 1961). With respect to the slow component, the entire columnar mass of cytoplasm appears to move distally (Weiss, 1969). Many non-specific materials flow as part of the slow component. For a partial listing see Lubińska (1964) and Schmitt (1968). The slow component appears to move unidirectionally (Weiss, 1961, 1969), and appears to be responsible for the outflow of the fluid cytoplasm from the cut ends of sectioned nerve fibers (Young, 1942, 1945).

The fast component contains the structural, functional, and metabolic substances needed by the remote parts of the nerve (Hebb and Waites, 1956; Wuerker and Kirkpatrick, 1972). It appears that mitochondria, vesicles, and small particles utilize the fast component.

This component does not require continuity of the axon membrane because the particles appear to flow throughout channels in the axoplasm. Furthermore, the fast component is bidirectional (Miani, 1964; Zelená, 1968; LaVail and LaVail, 1972). This finding lends credence to the thought that some viruses flow through nerve fibers (Goodpasture, 1925; Speransky, 1943) as might tetanus toxin (Wright, 1955). Retrograde movements of various proteins from the axonal terminal to cell body, also suggests a possible mechanism by which neurons respond to their target areas (Kerkut, 1968).

Flow Mechanisms

Both the fast and slow components of axoplasmic transport require some physical or chemical mechanism to drive the substances which flow down the axons. It is estimated that cytoplasm equivalent to one to three times the volume of the nerve cell is synthesized per day for the metabolic maintenance of the remote parts of the axon (Weiss, 1961). Unaided diffusion down the length of the axon (which sometimes reach great length, are small in diameter, and have a cytoplasm which is colloidal in nature) would be too slow to be useful as a transport mechanism (Weiss, 1961). Although the mechanisms for the observed movement of substances have not yet been elucidated, various models and theories have been proposed. It has been shown experimentally that the transport is not brought about by an active force exerted from cell bodies or by diffusion (Ochs and Ranish, 1970). The mechanism seems closely dependent on oxidative metabolism (Ochs and Ranish, 1970).

Other mechanisms hypothesized for the movement of substances have been active constriction and distention of the axis cylinder (Abrams and Gerard, 1933; Ochs, 1965), a peristaltic wave pumping action (Weiss, 1950b, 1961; Weiss, Taylor and Pillai, 1962), and turgor pressure (Young, 1944).

Neurofibrils and Microtubules

A possible physical mechanism for the axoplasmic transport of essential substances could be the neurofibrils or microtubules which have been demonstrated throughout the nerve cell. A discussion of their discovery and description can be found in Barker (1906), Parker (1929), and Wuerker and Kirkpatrick (1972).

Neurofibrils, 100-200 Å in diameter, form an indefinately long, loose network oriented longitudinally inside the axon (Schmitt and Geschwind, 1957). Although they were first thought to conduct nerve impulses (Schmitt, 1950), they appear to be vital organelles of neurons made up of fibrous proteins. Several functions have been proposed for these structures: mechanical support and elongation, cytoplasmic flow, sensory transduction, ionic concentration, and secretory release (Wuerker and Kirkpatrick, 1972).

Pellegrino de Iraldi and de Roberts (1968) postulate that the neurotubular system is connected with the Golgi complex at the perikaryon and involved in the formation of vesicles. Parker (1929) concluded that:

> . . . The neurofibrillar system is concerned specifically with the conduction of the metabolic influences and not with the conduction of nerve impulses. These influences start in the region of the neuronic nucleus and spread

> over the lines of neurofibrils throughout the whole neurone. What the metabolic influences are it is impossible at present to say. . .they appear to be streams of material in the nature of a hormone, eminating from the region of the nucleus and percolating throughout the neurone.

More recently, Schmitt (1968) has hypothesized intimate contact between flowing materials and the microtubules in his "sliding vesicle theory." Similarly, Lasek (1970a), has postulated a "sliding filament" model involving neurofilaments.

Breakdown of the microtubules and neurofilaments, a process called neurofibrillary degeneration, occurs in aging and in some pathological conditions such as vitamin E deficiency, copper deficiency, retrograde and Wallerian degeneration, nerve ligation, and others. The common characteristic of these conditions is an accumulation of distorted neurofilaments which appears to be a morphological manifestation of a slowing of the axoplasmic flow (Wuerker and Kirkpatrick, 1972).

Factors Affecting Flow Rates

As the materials flow down the axon, it appears that they are altered by local metabolic activities (Clouet and Waelsch, 1961; Waelsch and Lajtha, 1961; Koenig, 1965a, 1965b, 1967a, 1967b). Substances also may enter the axon from the myelin sheath (Singer and Salpeter, 1966a, 1966b, 1966c; Singer, 1968a), which could possibly alter the flow of materials.

Various factors external to the myelin sheath also seem to influence the rate and content of the axoplasmic flow. There is an

acceleration of axoplasmic flow during functional activity (Gutmann and Hník, 1962). This is to be expected, inasmuch as functional activity demands continuous synthesis of new protoplasm by the perikaryon. A rapid turnover of proteins and nucleic acids had been shown to be the case during functional activity (Hydén, 1934). Transport is slowed by cooling, by the application of a local anesthetic, or by ensuring that the nerve is not stimulated electrically (Ochs, Dalrymple and Richards, 1962; Kerkut, 1968). The slow component of axonal protein transport in the neurons of growing rats (2-2.5 mm/day) was approximately three times faster than that in adult rats (0.6-0.9 mm/day, Lasek, 1970b). Presumably, growing animals would show increased neural synthesis of metabolites and flow rates. This correlates with an observation made by Ranvier (1878) in which he noted that, after nerve section, disappearance of neuro-muscular transmission happened faster in young animals. The axoplasmic velocity is also greater in regenerating neurons than normal ones, suggesting that the soma supplies additional proteins to growing axons (Lasek, 1970a). Whether or not there is any coupling between the action potential and transport is not known, however, fast transport of glutamate occurs only in stimulated nerves (Wheeler, Boyarsky and Brooks, 1966). Continued activity in severed nerves accelerates failure of conduction, and has been interpreted by Cook and Gerard (1931) as being related to a faster loss of metabolites, possibly through accelerated axoplasmic flow.

The significance of axoplasmic transport is great in view of the neurotrophic hypothesis. Wuerker and Kirkpatrick (1972) conclude that:

> The production, transport, and release of both trophic factors and neurotransmitters and their ability to control growth, metabolism, and excitability appear to be promising areas.

Similarly, Taylor and Weiss (1965) conclude that the axon appears to be:

> . . .a feeder column carrying major macromolecular systems from their exclusively near-nuclear production sites in the cell soma to distant peripheral sites, where they serve (a) within the axon, as replacements for catabolic losses, and (b) as transmitters, neurohumors, and perhaps trophic agents for terminal discharge.

It appears, therefore, that a mechanism exists which may carry substances down the axon to the periphery, and which is especially active during periods of increased function and growth. Whether or not the substances involved are causally related to growth remains to be proven.

Trophic Agents

If materials do in fact flow throughout the axon as the evidence indicates, the question next to be asked concerns the possible fate of these substances. One possibility is that they "percolate" through the axon surface, either through the sheath membranes or the synaptic membranes. If this is the case, the nature of the process of their introduction into various target tissues is not known. The nature of the substances involved also is not known,

however, various substances have been studied in this regard.

Nerve Growth Factor

In recent years the growth-promoting effects of a protein Nerve Growth Factor (NGF), which is isolated from some mouse sarcomas, snake venom, and mouse salivary glands, have been studied (Levi-Montalcini and Hamburger, 1951, 1953; Cohen and Levi-Montalcini, 1956; Levi-Montalcini, 1964, 1965; Cohen, 1965a; Levi-Montalcini and Angeletti, 1961, 1967). The nature of this substance has been reviewed by Levi-Montalcini (1955, 1967) who has shown that NGF stimulates the outgrowth of embryonic sensory, and embryonic and mature sympathetic nerve cells. The structure of NGF (and its antiserum) has been studied, but its exact nature is not known, nor is its exact physiological role. It does, however, appear to explain some of the effects of the periphery on growth of the nerves (Levi-Montalcini and Hamburger, 1951). Its effects on regeneration have also been studied, and it was shown that NGF produced a marked enhancement of regenerative growth (Scott and Liu, 1964; Weis and Weis, 1970).

Tissue Extracts

There is extensive literature on other substances concerned with the nervous system and growth. In extract studies, it has been shown that extracts of rat brain and embryos had growth stimulating effects on chicken fibroblasts grown in vitro (Hoffman, 1940; Hoffman, Tenenbaum and Doljanski, 1940). In similar studies, Trowell, Chir and Willmer (1938) showed that tissue extracts exhibited a loss

of growth-promoting power with age, that there was no correlation between the rapidity of an organ's growth and the growth promoting potential of the extract, and that brain or thymus extracts have the highest growth promoting potential. Neuroregenerative factor (NR), one of the substances presumably involved in the effect exhibited by brain extracts, has been isolated from calf and rabbit nervous tissue, and characterized by Koechlin (1955). This substance effects regeneration in the central nervous system. Another substance, Neurocletin, which is thought to be liberated from degenerating nerve fibers, and is responsible for evoking axon sprouting during regeneration has been studied by Hoffman and Springell (1951).

Hormones

Hormones and enzymes which control growth of the body in general and control the material available to it have been studied extensively. The interested reader is referred to Gerard (1932), Paschkis (1958), and Papaconstantinou (1967), for reviews of the effects of neurohormones on growth, differentiation, and regeneration.

Acetylcholine

Major emphasis has been placed on acetylcholine as the trophic substance. Welsh (1946) offered evidence favoring acetylcholine by placing Planaria in atropine sulfate and noting the disruption of sensory organs.

Drachman has written extensively on the subject, and has

argued that the acetylcholine released at the neuromuscular junction is the essential substance necessary to maintain the integrity of the muscle (Drachman, 1967). He was strengthened in his argument by the discovery of continual discharge of "quanta" of acetylcholine by motorneurons (Fatt and Katz, 1952). In experiments with botulism toxin, Drachman found varying degrees of atrophy, but was unable to determine that acetylcholine was the agent solely responsible for muscle maintenance (Drachman, 1968).

Evidence against the theory that acetylcholine is the trophic substance is plentiful and conclusive (Tower, 1935, 1939; Tower, Howe and Bodian, 1941; Denny-Brown and Brenner, 1944a; Singer, 1959; Miledi, 1959, 1960a). Essential to the definition of neurotrophism is the fact that it is unrelated to the action potential.

Action Potential

It has been claimed that muscle activity, as effected by intact nerves is essential to the maintenance of muscle tissue. Robert and Oester (1964, 1970a, 1970b) used local anesthetic to stop the impulses and, thus reduce the liberation of acetylcholine; however, they saw nothing characteristic of denervated muscles. Lømo and Rosenthal (1971, 1972) presented similar findings. Baker, Hodgkin and Shaw (1962), in experiments on a giant nerve fiber, found that the membrane alone was the essential structure needed for nerve propagation. The axoplasm thus was not needed for action potential propagation. Denny-Brown and Brenner (1944a, 1944b) interrupted nerve conduction by compression and noted no muscle

degeneration. Alternatively, it has been suggested that acetylcholine may function as a transmitter of the motor nerve's trophic influence (Drachman, 1968).

Summary of Theory

Neurotrophism, to summarize, is characterized by 1) a substance or substances flowing down the nerves by axoplasmic transport (probably related to the microtubules and neurofilaments), 2) no essential relationships to impulse conduction, and 3) the ability to modify or initiate the genetic expression of the target tissues. Although mostly supported by indirect evidence, this phenomenon appears to be a common characteristic of nerves in general, whether they be sensory or motor. As reported, the effects of neurotrophism are as varied as the tissues involved. The effects of neurotrophism on various target tissues will now be reviewed on the basis of the tissue or process affected--regeneration, epithelium, connective tissue, muscle, and the nerve itself.

Nerves and Regeneration

It is beyond the scope of this paper to discuss the field of regeneration. For a review of the subject the reader is referred to Goldfarb (1909), Singer (1952), Goss (1969), Simpson (1970), and Thornton (1970).

In an extensive series of experiments, Singer and his associates have given us much insight into the relationship between nerves and regeneration in a variety of species. His work has been

summarized in several excellent reviews, but the essential points will be brought out here along with works of other contributors.

In an attempt to understand why some vertebrates are able to regenerate body parts, Singer and others have characterized the conditions necessary for regeneration to occur. From the beginning, it was apparent that for regeneration to occur nerves must be present at the amputation site. Regeneration is normally dependent on the presence of a critical supply of nerves at a very early stage (Berrill, 1971), and does not occur if the stump is denervated at the time of amputation. Regeneration in the presence of an altered nerve supply leads to regenerates smaller, both in length and volume (Singer and Egloff, 1949). After regeneration has been initiated, if denervation is effected, further growth of the regenerate is stopped and atrophy begins (Singer and Craven, 1948).

Neurotrophic effects are elicited by somatic motor (Singer, 1945, 1946a), somatic sensory (Singer, 1943), or sympathetic nerves (Singer, 1942; Kamrin and Singer, 1955). Thus, the direction of action potential conduction is apparently unimportant to the effect of the nerve on regeneration. Moreover, central connections of the neurons are not needed for the neurons to influence regeneration (Sidman and Singer, 1951; Kamrin and Singer, 1953; Ashbaugh and Singer, 1957). Thus, all that is needed for the nerve to be effective is the cell body and axon. The effect is also independent of conduction. All parts of the nervous system independently, and some of the supporting tissues (Cox, 1968, 1969), are capable of inducing

regeneration, not just the peripheral neurons (Ashbaugh and Singer, 1957; Kamrin and Singer, 1959).

Besides the proximity requirement, a certain threshold of nerve material relative to the amount of tissue innervated is needed for regeneration to proceed (Singer, 1946b, 1947a, 1947b, 1952, 1968b; Van Stone, 1955). This threshold varies with the animal, location, and life cycle (Ashbaugh and Singer, 1957; Singer and Mutterperl, 1963; Peadon and Singer, 1965). The number of nerve fibers is unimportant, but rather the amount of nerve tissue per unit volume is important (Rzehak and Singer, 1966). Therefore, there is a qualitative and quantitative summation of nerve tissue which must be reached for regeneration to occur (Winick, 1952; Singer, 1964; Singer, Rzehak and Maier, 1967).

The threshold is not fixed, but depends on the relative amount of growth-promoting potential of the limb itself. It appears that tissue extracts are able to substitute in some degree for the trophic factor from the nerves (Deck and Futch, 1969). Therefore, nerve-dependency is not absolute in regeneration, instead, there seems to be a balance between nerve and tissue trophic factors (Thornton, 1954, 1970; Thornton and Thornton, 1970).

Regeneration does not occur normally in all life forms, save for a limited number of vertebrates and invertebrates. The reason for this, as hypothesized by Singer, is that the amputation stumps do not contain the needed threshold of nerve material to effect regeneration. This hypothesis appears to be tenable in view

of the results of a series of nerve augmentation experiments. Amputated limbs of normally non-regenerating animals have been made to regenerate by increasing the amount of peripheral nervous tissue. This has been accomplished in the frog (Singer, 1950, 1951, 1954; Rzehak and Singer, 1966a), lizard (Simpson, 1961, 1970; Singer, 1961; Zika and Singer, 1965), and opossum (Mizell and Isaacs, 1970), but was unsuccessful in the mouse (Bar-Maor and Gitlin, 1961; Rzehak and Singer, 1966b).

Nerves exert their effect on regeneration independent of conduction and the transmitter substance. Indeed, the trophic agent of the neuron is unknown despite various attempts to characterize it (Singer, Davis and Arkowitz, 1960; Lebowitz and Singer, 1970; Singer and Caston, 1972). The agent is probably chemical in nature, and is probably contained in vesicles which appear in a nerve as a response to regeneration (Van Arsdall and Lentz, 1968). The trophic substance must be ultimately connected with the metabolism of the regenerate as denervation decreases synthesis of RNA, DNA and protein in the blastema (Dresden, 1969). Apparently the trophic substance is formed in the cell body of the neuron, travels down the axon, is continually extruded from the nerve endings, bathes the target tissues, and activates and maintains the growth of the regenerate.

Nerves and Epithelium

Local Organizing Effects

The trophic influence of nerves on epithelium would seem to

be widespread. Local changes of the epithelium could possibly be due to different localized regulatory processes, as would seem to be indicated from several studies on the differential sites of mitosis. Overton (1950) showed that grafts of nervous tissue in the dorsal fin of Amblystoma produced an enlargement of the fin by stimulating mitotic activity in the epidermis overlying the graft. Overton (1965) also has shown that the epidermal mitotic index is elevated by homoplastic and xenoplastic grafts of spinal cord, cerebral cortex, and peripheral nerve, while pituitary tissue had no effect.

Bullough and Laurence (1960) suggested that the high mitotic activity in wounds of the epithelium may be controlled by some inhibitor substance. Bullough (1962) pointed out that in the healthy animal it is probable that no tissue is lacking in the basic nutrient materials or hormones that are necessary for cell growth and division. The mitotic index is influenced by hormones that prepare cells for mitosis, and is also influenced by tissue-specific substances, the nature of which are largely unknown.

Changes in the oral mucosa also could be due to localized regulatory processes (Henry et al., 1952; Meyer, Medak and Weinman, 1960). Beagrie (1970) found an increase in mitotic index for palate and tongue after injection of H^3-thymidine. It is postulated that this material might release the epithelium from inhibitors or speed up DNA synthesis. Yohro (1969), using colcemid and H^3-thymidine, reported that mitosis of epithelium was accomplished by a local

stimulatory mechanism.

Taste Buds

Neural influence on various receptor organs has been studied by several methods in many animal and experimental studies. An excellent review of the relationship between nerves and the receptor organs is given by Zelená (1964).

Although no attempt will be made to cover all these studies, an example of the argument for neurotrophic control of receptor organs, the relationship of the nerves to taste buds will be presented. For complete reviews of the subject, the reader is directed to Torrey (1922), May (1925), Zelená (1964), Bradley (1967) and Robbins (1967, 1970a, 1970b).

Taste buds are specialized, non-neural, gustatory receptor cells which depend on the integrity of the nervous system for their growth and maintenance. Taste buds begin to differentiate and grow when the lingual gustatory epithelium becomes invaded by the gustatory nerve fibers. This relationship can be partially explained by the organizing effects of the nerve on the epithelium alluded to in the preceeding paragraphs.

Transplantation Studies

In an attempt to characterize the presumed induction of taste buds by the gustatory nerves, several lines of investigation have been followed. One of these is the transplantation of the tongue to a non-gustatory environment. Torrey (1940) transplanted the tongues

of rats to the anterior chamber of the eye prior to the appearance of the taste buds (but after the tissue had been invaded by gustatory nerves), and found that taste buds developed normally. Stone (1933) performed a similar experiment in which the tongue was transplanted before it had been invaded with gustatory nerves. He found the same result, and concluded that in amphibians the gustatory nerves are not necessary for the formation of taste buds; however, he did not rule out the possibility of a foreign innervation from the transplantation site.

Denervation Studies

The dependence of taste bud maintenance on nerves is clearly shown by denervation in adult animals. Disruption of the innervation of taste buds leads to a degeneration of the terminal aborizations of the nerves, and a short time later to degeneration of the taste buds (mainly by arresting cell differentiation, and thus, failing to renew normally degenerating mature taste cells). This conclusion has been reached by many workers (Olmstead, 1920a, 1921, 1922; Whiteside, 1926, 1927; Torrey, 1936; Wagner, 1953; Guth, 1957, 1969) on many different animal models. Most--but not all--taste buds degenerate. This is attributed to nerve overlap or double innervation (Whiteside, 1927). Not only do taste buds degenerate, but the adjacent epithelium becomes thinner following gustatory nerve section (Guth, 1963). This indicates that there may be a neural influence governing epithelial thickness.

External Influences

The degenerative process was found to be slowed by decreased temperature (May, 1925), perhaps a function of a decreased axoplasmic flow rate. Electrical stimulation of the peripheral stump, on the other hand, hastens the degeneration process (Torrey, 1934). Thus, mere conduction of electrical impulses through the nerve to the taste buds does not maintain the taste bud.

Reinnervation

The process of atrophy can be arrested by reinnervation of the denervated taste bud by either the lingual or with a non-gustatory sensory nerve (Robbins, 1967). Once again, decreased temperature slows the regeneration process (May, 1925). By two lines of evidence, it has been shown that non-gustatory nerves can substitute for the normal innervation.

Transplantation

Following the transplantation of gustatory epithelium into a new environment, the "foreign" innervation induced regeneration (Mintz and Stone, 1934; Wright, 1951, 1955; Poritsky and Singer, 1963). Zalewski (1972) also found that homografted gustatory neurons can survive, and function, and induce taste bud regeneration.

Cross Innervation Studies

Experimentally crossed sensory nerves such as the glossopharyngeal (Guth, 1958), chorda tympani (Zalewski, 1969b), and vagus

(which is both sensory and motor), were all able to induce taste bud regeneration, while pure motor nerve crosses such as the hypoglossal (Arey and Monzingo, 1942; Guth, 1958) were not, despite previous claims of success (Olmstead and Pinger, 1936). Guth (1969) concluded that because only nerves known to contain gustatory fibers are capable of supporting taste buds, there appears to be some neural specificity in this example of trophic function. In inframammalian animals there appears to be a difference, in that either motor or sensory nerves are able to induce taste bud formation.

In functional terms, it appears that the gustatory nerve shows a considerable lack of specificity. Oakley (1969), in an experiment involving crossing the glossopharyngeal and chorda tympani nerves, found that the cross-regenerated nerve is changed in its relative responsiveness to different gustatory stimuli, that is, it responds like the nerve which normally innervates that particular region of the tongue.

Autonomic Relationships

The possible neurotrophic relations of autonomic innervation of the gustatory epithelium, has been demonstrated by Gabella (1969). The interruption of this innervation in the rabbit leads to degeneration of the taste buds. In this same vein, it is reported that in familial dysautonomia there is a taste bud deficiency (Dancis and Smith, 1966). The role of taste and disease has been explored by

Henkin (1967) with the thought that other neural syndromes have associated neurotrophic taste anomalies.

Central Relationships

As was the case in regeneration, the trophic influence on gustatory epithelium is independent of connections with the central nervous system. Kamrin and Singer (1953) found that transection of the roots of gustatory sensory ganglia is without effect on the taste buds, whereas transection of the peripheral process results in their disappearance. The trophic influence therefore, must be an intrinsic property of the neuron directly supplying the taste bud. Also it must be concluded that there is no relationship between the direction of the neurotrophic influence and the direction of the action potential, since in the gustatory sensory nerve the direction of supposed neurotrophic influence is opposite to that of the action potential. Similarly, Zalewski (1969d) found that either the central or peripheral processes from the vagal ganglia can induce taste bud regeneration.

Mechanisms

As with other "trophic" effects the mechanism by which the nerves influence the taste bud are essentially unknown. Robbins (1970a) has shown that the nerves are necessary for mitosis of taste cell percursors, and necessary for the maintenance of mature taste cells. This may be accomplished by control of DNA synthesis or some similar process (Robbins, 1970a, 1970b). May (1925) proposed a

neurochemical control theory. Zalewski (1968, 1969a, 1969b, 1969c) suggested a nerve-hormone interaction in which enzyme synthesis is under a neurotrophic control which is altered, but not controlled, by hormones.

Salivary Glands

Neurotrophic influence has also been implicated in the regulation, maintenence, growth, and differentiation of the salivary glands (Schneyer, 1972). Schneyer and Hall (1970) showed that loss of innervation to the developing rat parotid gland did not suppress the usual postnatal growth changes, but did decrease the magnitude of the change, as measured by a decrease in total cell number, DNA, cell size, gland size, and total RNA. As will be seen later, this also happens in muscle tissue. It has been found in humans that extirpation of the superior cervical ganglion results in underdevelopment of the submaxillary gland (Sicher, 1966). Disuse atrophy with intact innervation was simulated by feeding rats a liquid diet, but the results did not account for all the atrophy seen in denervation (Hall and Schneyer, 1973). Wells, Handelman, and Milgram (1961) suggest that the sympathetic nervous system is involved in the regulation of the normal growth and size of the salivary glands. Handelman and Wells (1963) suggest that the sympathetic nervous system regulates the metabolism of the RNA in salivary glands. In rabbits it has been found that transtympanic section, which interrupts parasympathetic innervation, results in atrophy of the parotid gland

(Wallenborn, 1968; Wallenborn, Jsu and Olinger, 1968).

Pathological Conditions

A variety of changes in the skin and underlying tissues are associated with sensory defects, and are assumed to be "trophic" in nature.

Hereditary Sensory Neuropathy

Turkington and Stiefel (1965) report on hereditary sensory radicular neuropathy, a familial neuropathological condition resulting in an apical sensory deficit in the lower limbs and associated with chronic perforating ulcerations of the feet and progressive destruction of the underlying bones.

Trigeminal Neuralgia

Similar ulcerations and underlying destruction are seen as complications associated with operations for the relief of trigeminal neuralgia. Several months to years following section or destruction of the trigeminal nerve, complications can arise which include: herpes simplex, neuropathic kerititis, corneal ulcers and opacity, iritis, conjunctivitis, dryness of the nasal mucous membranes, ulcerations of the skin corresponding to the trigeminal area affected, erosion of the cartilages of the ala nasi, paresthesia, and facial paralysis (Karnosh and Scherb, 1940; Friedman and Merritt, 1944; Freeman, 1961; Howell, 1962; Owen, Freeman and Knox, 1967). Similar lesions are seen after trauma involving the trigeminal nerve (Mitchell, Morehouse and Keen, 1864; Mitchell, 1865).

Experimental Pathoses

Experimentally, Mikhnev, Kolomitsev and Fedotov (1972) found that disruption of the sympathetic innervation of the accessory sinuses of the nose in rabbits causes morphological changes in their mucous membrane which was indicative of the development of chronic sinusitis.

Facial Malformation

It has also been argued that if epithelial growth is dependent on neurotrophic relationships, then in cases of facial hypoplasia or malformation, we should also anticipate a sensory defect which reflects the disturbed neurotrophic relationship (Moss, 1972a). Cited as evidence are soft tissue and skeletal changes noted in familial periodic paralysis (McArdle, 1956), cleft palate (Hochberg and Kabcenell, 1967), familial dysautonomia (Dancis and Smith, 1966), congenital insensitivity to pain (McMurray, 1950; Murray, 1957; Silverman and Gilden, 1959; Gorlin and Findborg, 1964; Swanson, Buchan and Alford, 1965; Bosma, Grossman and Kavanaugh, 1967; Rapoport, 1967; Zlatin, 1967; Henkin, Christiansen and Bosma, 1970), and related disorders. Attractive as this speculation is, a relationship among neurotrophism, malformation of growth, and sensory deficiency, remains to be proven.

Additional Neuro-Epithelial Relationships

Trigeminal neurotrophic mechanisms have also been hypothesized in the growth of antlers (Wislocki and Singer, 1946; Bubenik

and Pavlansky, 1965). In a similar manner, Kischer (1968) correlated the structure of down feathers with a neural trophic stimulus. A neurotrophic relationship can also be presumed to exist between the cochlear nerve and the hair cells of the cochlea similar to that shown for the taste buds (Howe, 1935).

Nerves and Connective Tissue

Fat

Lipid metabolism and turnover in response to nerve alteration have been examined by Östman, Efendié, and Arner (1969). It is thought that fat-mobilizing stimuli come from two sources: the nervous system and the blood. Nerve endings have been shown to be intimately associated with fat cells (Hausberger, 1964). Disruption of the nerve supply, be it sympathetic or mixed, decreases mobilization of fat (Beznak and Hasch, 1937). Sidman and Fawcett (1954) studied the effect of peripheral nerve section on fat deposits in the rat and concluded that changes in fat deposits were not due to changes in blood supply, hormonal changes, or impulse conduction of the nerves. They noted an increase of glycogen and lipid deposition in fat cells due to what they assumed to be a heightened sensitivity to circulating chemical substances.

Pathological Conditions

Loss of deposits of fat in the face has been noted in facial hemiatrophy and scleroderma and will be discussed later. The appearance of lipomas in areas formerly affected by neuritis and the growth

of symmetrical lipomas after concussion of the spinal cord are considered strong evidence for the influence of the nervous system on fat deposition (Hausberger, 1964). It has also been noted by Hausberger (1964) that paraplegic patients who die of starvation show considerable amounts of fat in the lower half of the body.

Bone

Bone has long been considered a relatively plastic tissue in relation to intrinsic growth and secondary muscular modeling. On the other hand, bone has been considered to be relatively inert and little affected by the nervous system. The neurotrophic theory, however, would view the process of bone growth and maintenance as intimately connected with neural control. De Castro (1925, 1930) showed that nerves are intimately associated with osteoblasts in growing bone. It was suggested that nerve was the property of controlling modeling of bone by neurohormonal or indirectly by reflex activities.

Denervation, pathological conditions and disuse may lead to changes in bone composition, physiological characteristics, and morphology. Experimental studies and clinical observations have focused on the problem of deciding whether bone changes, such as atrophy or relative shortness of limbs, are due to trophic influences, vasomotor upset, loss of sensation and thus the protection which pain affords, or changes as the result of disuse (Hurrell, 1937).

Denervation

Denervation leads to changes in bone composition, mass, and

physiological characteristics. Armstrong (1946) found that bone quantity and bone salt content were reduced significantly when compared against controls. Gillespie (1954), found that when denervation was compared against disuse, denervation caused a significant decrease in bone weight, ash weight, bending moment at the breaking point, and x-ray density. Coppo (1935) compared denervation against immobilization, stasis, ischemia, and muscle abscesses, and found that with denervation there was a decrease in mineral content, calcium, and magnesium. Immobilization resulted in stasis, ischemia, and muscle abscesses, but not a change in bone composition.

Bone Growth

Howell (1917) noted that it is a common observation that considerable growth takes place in a paralyzed arm, particularly in length, though it lags well behind the healthy arm in thickness. When a limb has an intact innervation, but is immobilized by a sling or cast so that no weight is born by the limb, growth in length is not affected to any great extent but its diameter is considerably reduced, relative to controls (Grey and Carr, 1915; Pottorf, 1916; Howell, 1917; Tower, 1937b; Gillespie, 1954). When a limb is denervated, thereby eliminating (in addition to the stress and strain due to weight of the body) the stress and strain due to muscle pull, it is found that growth in length is only slightly affected while the thickness of the bone is considerably reduced in comparison to controls (Grey and Carr, 1915; Pottorf, 1916; Howell, 1917; Tower, 1937b; Schiller, 1948; Gillespie, 1954). It was found that in growing animals the difference in length

is greater, but still relatively small in absolute terms (Howell, 1917; Pottorf, 1916). Thus, it appears that epiphyseal growth is not affected as much as appositional growth (Armstrong, 1946). However, over a long period of time, the increase in length of paralyzed limb bones lags far behind that of normal bones (Armstrong, 1946; Gillespie, 1954). This is evidenced by the malformations which occur in polio (Gillespie, 1954).

Spinal Column

Nervous tissue also has a direct effect in determining the shape and diameter of the spinal column. Holtzer (1953) showed that the diameter of the spinal column in urodele embryos is dependent upon the diameter of the spinal cord. A critical mass of neural tissue, approximately 25% of its normal regional value is a prerequisite for normal modeling of the vertebrae, a view strongly resembling the threshold values of Singer. It appears that the pre-cartilage cells were the cells affected by the neural mass (Holtzer, 1953). Grafts of spinal cord plus neural crest and overlying epidermis resulted in induction of a complete secondary axial system including vertebrae, ribs, and muscles (Holtzer and Detwiler, 1953).

Joints

The joints are also affected by nerve section, but it is not known whether this is due to trophic effects, disuse, or more probably, damage to joints in which there is no sensibility to pain (Eloesser, 1917; Brailsford, 1941).

Sympathetic Denervation

The effect of sympathetic denervation has been studied with inconclusive results. Sympathetic denervation was once the method of treatment prescribed to slow the growth of the normal leg to achieve a size consistent with the leg shortened by polio. In some cases bone length was found to decrease after sympathectomy (Harris, 1930; Harris and McDonald, 1936; Asher, 1937; Asher and Dirr, 1939), but remain unchanged in others (Cannon et al., 1929; Bisgard, 1931, 1933). Inconsistent results were noted by Gullickson (1951), who found that stimulation of the sympathetics served to decrease bone length. Sympathetic denervation was found to accelerate bone repair in some cases (Zollinger, 1933), and to have no effect or even to retard healing in others (McMaster and Roome, 1932, 1934; Key and Moore, 1933). Many of these effects were attributed to the effect of sympathectomy on the circulatory system. Sympathectomy caused an increase of the diameter of the vessels in the affected area (Zinn and Griffith, 1941), and an increase in the size of the haversian canals in the cat (Fell, 1949).

Pathological Conditions

Polio

Polio has often been studied with respect to bone growth and neurotrophic influences. One of the deformities which may follow poliomyelitis is a discrepancy in the length of the bones of the lower extremities. The age of onset of the disease appears

to have little effect upon the amount of shortening, and no correlation is seen between muscle strength of the thigh muscles and shortening of the tibia (Gullickson, Olson and Kottke, 1950). The effects of polio could possible be explained on the basis of some trophic disturbance within the cell bodies and axons since it has been found that the spread of the virus is by way of the nerve axons to the brain stem and cord (Fairbrother and Hurst, 1930).

Paraplegia

Soft tissue ossification is found in a large number of nervous disorders (Abramson, 1948). In paraplegia, soft tissue ossification occurs in a large variety of areas, but never above the level of the lesion, and often occurs in conjunction with bone atrophy (Abramson, 1948).

Clubfoot

Shortened and distorted limbs are also seen in clubfoot. Hamburger (1928) reported that, in the frog, a non-innervated hind limb exhibits a significant growth reduction. In studying chick limb transplants, Hamburger and Waugh (1940) came to the conclusion that innervation did not affect the form, but rather affected the length of the limb. Drachman and Coulombre (1962) were able to produce clubfoot experimentally, but attributed it to immobilization of the embryo under the influence of curare.

Facial Hemiatrophy

A central neurotrophic mechanism has been claimed to play some part in gross malformations of the face, namely facial hemiatrophy or Romberg's disease (Wyburn-Mason, 1950). This disease is characterized by a slow, progressive atrophy of the soft tissues, underlying fat, muscle, cartilage, and bones of half the face corresponding to the trigeminal field and, occasionally, atrophy of half of the body (Archambault and Fromm, 1932). The disease usually begins in the second decade, however, earlier onset has been reported (Rushton, 1951, 1956). The signs of the disease progress slowly for several years, then become stable for the remainder of life (Crikelair, Moss and Khuri, 1962; Gorlin and Pindborg, 1964). The disease appears related to scleroderma (Archambault and Fromm, 1932; Tower, 1937a). Vitiligo (Gorlin and Pindborg, 1964) and hearing impairments (Gowers, 1895) occasionally accompany the disease. Kyphosis of the cranial base has also been described, occasionally in relation to other corgenital malformations (Crikelair, Moss and Khuri, 1962).

Changes in the hair may be the first sign of the disease (Tower, 1937a; Archambault and Fromm, 1932). Poliosis or alopecia begin near the paramedian area and spread laterally, seemingly following the distribution of the trigeminal nerve. Commonly, the patient is afflicted with epilepsy, often late in the disease (Tower, 1937a; Archambault and Fromm, 1932). On the other hand, a trigeminal neuralgia and/or facial parathesia appears early in the course of the disease and may precede the rest of the changes (Wartenberg, 1945).

Sensory disturbances of the trigeminal nerve have also been noted (Wolff and Ehrenclou, 1927). The eye may be affected (Shumway, 1935), occasionally on both sides (Gorlin and Pindborg, 1964). The body and ramus of the mandible may be shorter, and the development of the angle on the involved side may be delayed (Rushton, 1951, 1956). The teeth may also be retarded in their development and eruption (Rushton, 1951, 1956).

Possible etiologic factors are: polio, encephalitis, trauma, heredity, irritation of peripheral trophic sympathetics, influenza, typhus, and diptheria (Gowers, 1895; Wolff and Ehrenclou, 1927; Tower, 1937a; Crikelair, Moss and Khuri, 1962). By unilateral cervical sympathectomy, Moss and Crikelair (1959) have produced a condition in rats which resembles human progressive hemifacial atrophy. However, Crikelair, Moss and Khuri (1962) were unable to find any comparable neural involvement in patients with the disease. Wolff and Ehrenclou (1927) stated that removal of the gasserian ganglion in adults does not result in hemiatrophy. In contrast, unilateral trophic changes sometimes occur in the face in cases of long standing trigeminal neuralgia (Tower, 1937a). Also Gowers (1895) described an experiment in which removal of the gasserian ganglion in dogs resulted in facial hemiatrophy.

Facial Hyperplasia

Unilateral gigantism has been reported in the literature with symptoms opposite those of facial hemiatrophy (Gutmann and

Hník, 1962). However, this condition is noticeable at or soon after birth (Rushton, 1937) and may affect the upper and lower jaws, alveolar processes, ears, lips, tongue, zygomatic arch, and teeth (Rushton, 1937, 1942).

It can perhaps be concluded that the gross form of individual skeletal elements is attained by intrinsic mechanisms. Such extrinsic factors as weight bearing and muscle pull can account for some of the discrepancies noted in experimental and clinical situations, however, neurotrophic influences may well influence skeletogenesis.

Nerves and Muscles

There are considerable data to support the contention that there is a degree of neurotrophic control over muscles. The extensive literature is beyond the scope of this paper, but some important aspects will be discussed. For further information, the reader is referred to excellent reviews by Gutmann and Hník (1962), Eccles (1964), Gutmann (1964, 1967, 1970), Ochs (1965), Katz (1966), Guth (1968, 1969, 1972), and Sandow (1970).

Development

There is no trophic interaction at an early stage of the development of nerve and muscle, since both develop independently (Harrison, 1904). Muscle spindles develop before the motor endplates and are entirely dependent on innervation by sensory fibers (Zelená and Hník, 1963; Zelená, 1964). Although embryonic muscle development is independent of motor nerve control, when the myoblast

stage is reached, motor innervation is accomplished and further development will not be continued unless innervation is maintained (Scott, 1957; Muchmore, 1964, 1968). Even after the formation of the neuro-muscular junction, it is remarkable that muscle has a considerable capacity to grow and differentiate though deprived of its nerve supply (Eccles, 1964); however, it grows more slowly and finally atrophies.

Nerve endings appear to have an inductive effect on both the morphological and chemical specialization of the neuromuscular junction (Gwyn and Aitken, 1966; Lentz, 1969; Veneroni and Murray, 1969). Like the muscle spindles, motor end plates will not form unless innervation is accomplished (Bornstein et al., 1968; Shimada, Fischman and Moscona, 1969; Peterson and Crain, 1970). The entire length of the fetal muscle fiber is uniformly sensitive to acetylcholine. Only after the development of motor end plates does this sensitive area shrink to the immediate vicinity of the end plate (Eccles, 1964).

Skeletal Attachments

The developing muscles are initially independent of their prospective skeletal attachments. If the skeletal units to which the muscle normally gains attachment are absent, the development of the muscle continues (Scott, 1957). Moreover, origins or insertions may be located in connective tissue with no apparent deleterious effects on the structure of the muscle (Scott, 1954, 1957).

Smith (1928) described, in the leg of a patient, a muscle, that had its origin and insertion on the same bone. The muscle performed no function, but had a normal nerve supply and structure.

Fast and Slow Muscles

In early fetal life, muscle fibers increase in number and size by both hyperplasia and hypertrophy; but, later in life, the cell population becomes stationary (as is the case also for nerve tissue) and growth of the muscle tissue is by hypertrophy of the individual fibers (Bardeen, 1906). At birth, limb muscles of the rat and cat are uniformly slow in their contractions, and it is not until later that development of fast and slow muscles can be differentiated (Buller, Eccles and Eccles, 1958, 1960a; Ridge, 1967; Gutmann et al., 1968). The importance of the nerve for the differentiation and maintenance of the fast and slow muscle types is shown by numerous experiments. Simply stated, experimental cross union of the nerves of fast and slow muscles results in a transformation of the fast muscle to a slow muscle type, and conversely, a transformation of the slow muscle to a fast type, even in the adult animal (Buller, Eccles and Eccles, 1960a, 1960b; Eccles, Eccles and Kozak, 1962; Drahota and Gutmann, 1963; Buller and Lewis, 1965; Romanul and Meulen, 1966, 1967; Dubowitz, 1967a, 1967b; Gutmann, 1967; Karpati and Engel, 1967a, 1967b, 1967c; Novak and Salafsky, 1967; Prewitt and Salafsky, 1967, 1970; Salafsky and Jasinski, 1967; Yellin, 1967, 1969; Elul, Miledi and Stefani, 1968; Guth, Watson and Brown, 1968; Buller, Mommaerts and Seraydarian, 1969; Close,

1969; Mommaerts, Buller and Seraydarian, 1969; Robbins, Karpati and Engel, 1969; Mann and Salafsky, 1970).

It may be concluded that embryonic muscle cells are somewhat self-differentiating and that the dependence on trophic influences from nerve fibers does not become significant until some time after motor end plates are established (Hamburger and Levi-Montalcini, 1950; Singer, 1952). It is possible that the expression of the genome of muscle tissue is not self-realized, but under neural control (Guth, Samaha and Albers, 1970; Samaha, Guth and Albers, 1970; Guth, Dempsey and Cooper, 1971).

Denervation

The trophic influence has been mainly studied by noting the effects of denervation on muscles. Detailed descriptions of the histological picture produced by denervation of muscle can be found in Langley (1915); Birks, Katz and Miledi (1960); Gutmann and Zelená (1962); and Miledi and Slater (1968). The problems inherent in studies of this sort have been to differentiate between disuse atrophy and denervation atrophy; between conduction and non-conduction effects. Besides denervation, several other experimental procedures have been used to test the neurotrophic hypothesis as it pertains to muscles. Attempts have been made to modify or suppress the conduction of impulses without destroying the nerves in order to preserve any trophic effects and still subject the muscle to disuse (Lipshütz and Audova, 1921; Tower, 1935, 1937a, 1937b, 1939; Eccles, 1941a, 1941b, 1944; Reid, 1941; Tower, Howe and Bodian, 1941; Solandt,

Partridge and Hunter, 1943; Denny-Brown and Brenner, 1944a; Gutmann and Young, 1944; Hines, Wehrmacher and Thompson, 1945; Helander, 1958; Sola and Martin, 1953; Jefferson, Ogawa and Necheles, 1958; Thesleff, 1960; Johns and Thesleff, 1961; Buller and Lewis, 1965; Fischbach and Robbins, 1969; Jirmanová and Zelená, 1970). What has been found by all these studies is that there are differences between the effects seen in denervation and other conditions, such as disuse, tenotomy, chemically induced nerve blockage, joint fixation, blockage of neuromuscular transmission and others. Differences, such as altered sensitivity to acetylcholine, development of fibrilation, changes in excitability, DNA content, nuclear characteristics, protein nitrogen, glycogen content, slowing of contraction, and many others are seen with denervation, but not in the other instances and are attributed by the various authors to neurotrophic effects. It would appear that many findings which cannot readily be explained are attributed to neurotrophism, thus much work remains to be done if the nature of the trophic influence of nerves on muscles is to be clarified.

Axon Length

Luco and Eyzaguirre (1955) showed that the length of severed motor nerves had a direct influence on the time of onset of acetylcholine sensitization and fibrilation, a finding which would suggest the spread of a trophic influence along the nerve at a velocity ranging from 1 to 2 mm per hour. This is approximately the axoplasmic flow velocity observed by Weiss and Hiscoe (1948). Histologically,

the intensity of post-denervation peripheral cell reaction depends on the relative length of the axon remaining (Cammermeyer, 1963). Slater (1966) also showed that the presence of the peripheral nerve stump delays the failure of neuromuscular transmission after nerve section. Similar results have been noted by Vial (1955), Emmelin and Malm (1965), and Emmelin, Nordenfelt and Perec (1966). Artificial stimulation of the peripheral stump did not prevent acetylcholine sensitization, and was even found to hasten atrophy (Langley and Kato, 1915; Fischer, 1939; Kosman, Wood and Osborne, 1948).

Biochemical Changes

A great deal of research has been conducted on the chemical and biochemical changes seen in muscles subsequent to denervation (Lissak, Dempsey and Rosenblueth, 1939; Nachmansohn, Steinbach and Machado, 1943; Johnson, McNabb and Rossiter, 1950; Mannell, 1952; Gutmann, 1959; Schapira and Dreyfus, 1959); however, it appears that changes in denervated muscle possibly do not differ significantly from those occuring in other types of muscle atrophy induced by disuse of tenotomy.

Sensory and Sympathetic Nerves

There is no evidence for an involvement of sensory or sympathetic trophic nerve fibers in the maintenance of the muscle fiber (Mott and Sherrington, 1895; Tower, 1931a; Weiss, 1934; Weiss, Edds and Cavanaugh, 1945b; Gutmann and Hník, 1962); motor neurons, only, are involved. When the ventral roots of the spinal cord are sectioned

the muscle atrophies, while dorsal root section produces no effect (Tower, 1931b).

Regeneration

On the other hand, there is clearer evidence that some trophic factors are probably involved in the maintenance of mature muscle. In regeneration, there is general agreement that the regenerating motor fibers indiscriminately make functional contact with any type of skeletal muscle fiber (Weiss and Hoag, 1946; Buller, Eccles and Eccles, 1960b; Bernstein and Guth, 1961; Eccles, Eccles and Kozak, 1962), although this is not true of preganglionic sympathetic fibers (Guth and Bernstein, 1961). Minced muscle tissue will form functional muscle if regeneration takes place in the presence of a motor nerve, but will only form fibrous tissue when there is no motor innervation available (Carlson, 1970, 1972).

Bone

If neurotrophic control of muscle differentiation and maintenance exists, then sectioning of a motor nerve would, by altering the form and function of muscle, be expected to have an effect on the structures to which they are attached, especially if the bones to which they are attached are in the process of growing. The effect of muscle on bone, especially the facial skeleton, has received much attention.

Facial Growth

Although the thickness and internal structure of mature bone is altered according to use and the direction of forces acting on it, the growth of bone is to a great extent independent of use (Scott, 1957), however, abnormal forces can effect a considerable distortion. Skull deformity can be produced by paralysis or removal of muscles (Washburn, 1946, 1947; Horowitz and Shapiro, 1955; Avis, 1959; Warner, 1969). The forces generated by the abnormal function do not produce a deformity directly, but cause a change in the direction of growth.

More specifically, the muscles of the face can affect the shape of the bones to which they are attached. Pratt (1943) removed the masseter and a portion of the zygomatic arch unilaterally and noted asymmetrical changes in the face and skull. Horowitz and Shapiro (1955), by a similar technique, noted severe alterations in the skull, jaws, and dental arches.

Washburn (1946), and Jarabak, Kamins, and Vehe (1949) by cutting the seventh cranial nerve in rat and rabbit, have shown that the facial muscles are sufficiently powerful to change the direction and extent of growth of the facial complex. Washburn (1947) also removed the temporal muscle in day-old rats and noted that after several months that there was a loss of secondary modeling of the skull and that the coronoid process was absent. Horowitz and Shapiro (1951) and Avis (1961) performed similar experiments, and reported similar findings. However, these experiments did not take into consideration the fact that the

surgical procedure compromised the blood supply to the coronoid process (Huelke and Castelli, 1965). Warner (1969) attempted to circumvent the problem of surgical scarring in the surrounding tissue and reduced vascular supply to the coronoid process by blocking the motor nerve to the muscles of mastication with alcohol at the level of the foramen ovale. His results confirmed the findings of the earlier investigators--that function alters bone.

Washburn (1947) believed that the coronoid process was an example of a structure which was self-differentiating, but required muscle function for full expression. Certain other osseous structures, such as the temporal line, nuchal crest, and mastoid crest, were believed to depend totally for their development on the degree of muscular function. This view was supported by Tower (1937b), Scott (1957) and Avis (1961). Local control was thought to be due to strictly mechanical function (Washburn, 1947), tension, or vascularization.

Neurocranium

The muscles of mastication, especially the temporal muscles, play an important role in determining the form of the cranial vault. Washburn (1947) believed that mechanical forces affected details of the superstructures, but not the general size and shape of the cranium as a whole. This view is supported in numerous articles by Moss, who believes that the outer cranial surface responds to muscle function while the inner cranial surface responds to the brain. A divergent

view is advanced by Warner (1969) who caused an alteration of the shape of the neurocranium by immobilizing the muscles of mastication, and concluded that the musculature can exert a profound influence on the cranial shape.

It would appear that certain bones (like muscles and nerves) are self-differentiating, but depend on a careful coordination of the related systems and other bones for full development of their primary or secondary characteristics. For example, the brain appears to control the size, shape and position of the cranial vault, while the attached muscles determine the shape of the secondary characteristics. Moreover, muscles do not seem to influence the general form of the cranial base, mandibular basal bones, the orbit, and nasal cavity (Scott, 1957), which are related to other organs and tissues with other controls--possibly neurotrophic.

Nerves

Since an extensive review of the subject of the presumed neurotrophic relationships among and within the nerves themselves does not pertain to the subject of neural effects on peripheral tissues (bone, muscle, etc.), only a general comment will be made concerning neurotrophic mechanisms within the central nervous system itself. Suffice it to say, the nerves themselves have a part in an overall neurotrophic theory with regards to growth of the nerve fibers, nourishment of distant axon processes, degeneration, and regeneration; nerves are, after all, the "trophic unit" of the

nervous system. There also appears to be a relationship which compliments the neural influence on the periphery--the influence of the periphery on the nervous system.

Trigeminal Nerve

All of the tissues mentioned thus far (muscles, epithelium, taste buds, bone, salivary glands, connective tissue proper, cartilage, nerves, fat, etc.) constitute the fabric of the face, and have at least one common denomination there--the trigeminal nerve. If, as has been suggested, these tissues are influenced by neurotrophic mechanisms individually (as the evidence would seem to indicate) then, in the facial region, any neurotrophic control over the growth and development of these tissues would come most probably from the trigeminal nerve. As Moss has stated (1971a): "The anatomic basis for oral neuroepithelial trophic interactions are established." Moreover, he has concluded that (1972a):

> Oro-facial growth is now capable of being conceived of as a homeostatically controlled series of processes in which the neural center regulates the peripheral tissues. . .

In the former statement Moss is referring to the trigeminal nerve, specifically with reference to the work by Humphrey (1966). Moss believes that the trigeminal nerve is intimately associated with the sensory and motor activity of the face during growth, development, maturity and senescence. Inasmuch as the neurotrophic effects Moss describes are supposedly brought about by the trigeminal nerve, a brief review of the pertinent anatomical and physiological

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data will be presented.

Anatomy, Embryology and Histology

For the embryology of the trigeminal system, the reader is referred to Hamburger (1961), Johnston and Hazelton (1967), Hogg and Bryant (1969), and Hill (1970). Humphrey (1966, 1971) gives a good description of the trigeminal innervation of the oral mucosa and facial skin and explores the functional relationships present during fetal life; however, emphasis is placed on what happens, rather than how it happens. The distribution of innervation can be found in Gaskell (1889), Dixon (1962), and Vij and Kanagasuntheram (1969). Sensory interaction and organization can be found in Kerr (1963b), Grossman and Hattis (1967), Jerge (1967), Storey (1967), Dubner (1970), and King (1970). The histology and growth of the gasserian ganglion is reported by Hoag (1918) and Nittono (1920). The somatotopic organization of the trigeminal ganglion in the rat is described by Gregg and Dixon (1973). It is clear from these reports that, as Moss suggests, the anatomic basis for neurotrophic control of the growth of the face is present in the trigeminal nerve. There also appears to be a relationship between the growth of the face, and the growth and maturation of the trigeminal nerve (Donaldson, 1924), in that the time-course of growth and development of the trigeminal nerve corresponds to the time-course of major events in facial growth.

Pathological Conditions

Injuries, and pathoses of the trigeminal nerve described in clinical and experimental literature may give some insight into possible neurotrophic influences of the trigeminal nerve. Injuries or tumors involving the ganglion and its branches usually result in varying degrees of anesthesia of the trigeminal distribution and paralysis of the masticatory muscles (Jefferson and Schorstein, 1955). Other possible complications include gustatory disorders, "trophic" destruction of the nose, xerostomia, and complete or partial Horner's syndrome (Gowers, 1895; Jefferson and Schorstein, 1955; Spillane and Wells, 1961; Knudsen and Kolze, 1972).

Trigeminal Neuralgia

Trigeminal neuralgia has been studied extensively, but as of now no definite cause has been elucidated. The symptoms are varied and the reader is referred to more specialized articles for details of the course of the disease (Sjöqvist, 1939; Harris, 1940; Stookey and Ransohoff, 1959; Hassler and Walker, 1970). Proposed etiologic factors are as varied as the modalities of treatment and include, among others, structural variations, varied pathologies, viruses, and trophic influences. Surgery is not directed at a cure, but toward relieving pain. This is usually done by tractotomy, section of the root, sectioning the nerve peripherally, or by chemical injection at varying locations along the length of the nerve complex. With surgery, common post-operative complications are herpes simplex, neuropathic keratitis, and "trophic" lesions of the

ala nasi, similar to those seen in leprosy (Spillane and Wells, 1961).

Facial Hemiatrophy

Facial hemiatrophy has been reported as a sequela of trigeminal neuralgia of long duration (Spillane and Wells, 1961). There are, however, no reports of facial hemiatrophy following section of the trigeminal sensory root (Spillane and Wells, 1961; Wolff and Ehrenclou, 1927). Gowers (1895) reported a single instance of facial hemiatrophy produced in dogs by dividing the fifth nerve within the skull.

Experimental Studies

Experimentally, Gregg (1971) demonstrated that trigeminal nerve lesions can result in a selective degeneration of the central processes of the secondary afferents. Kolomitsev, Fedotov and Miknev (1971) sectioned the trigeminal nerve in adult rabbits and found that this gives rise to ipsilateral chronic inflammation of the mucous membranes of the paranasal sinuses. Nittono (1925) sectioned peripheral branches of the trigeminal nerve of eighty day old rats, and studied the retrograde degeneration of the ganglion. Troyer (1972) sectioned the third division of the trigeminal nerve in two year old monkeys in order to study craniofacial changes, but noted only a few subtle alterations. This study was marred by a small sample, the use of qualitative assessment, and by the selection of animals whose comparative age in human terms was fourteen years. Frank and Karli (1962) destroyed the trigeminal nuclei in adult rats

and found elongation of the incisors, muscular atrophy, ulceration, hyperplastic lesions of the sclera, etithelial atrophy, salivary gland changes, and alterations in the temporomandibular articulation.

Ocular Defects

Ocular changes noted after nerve section have been studied and, for some, a neurotrophic mechanism has been suggested (Eayrs, 1951; Zander and Weddell, 1951). It has been found that interruption of the sympathetics to the eye results in less resistance to graded noxious stimuli (Asher and Dirr, 1939). Ocular lesions have been produced by fifth nerve injury in rats and cats (Sigelman and Friedenwald, 1954; Moses and Feldman, 1969; Saari and Kiviniemi, 1973). Ocular lesions have also been noted in conjunction with, and attributed to, nerve lesions (Segall, 1955).

Disturbances at an Early Age

Little attention has been paid to alterations in craniofacial growth as a result of trigeminal neuropathies. In cases of neurinoma and treatment for trigeminal neuralgia occuring in early life (14 cases, with ages from 16 months to 16 years), no growth discrepancies were reported (Harris, 1940, 1943; Knudsen and Kolze, 1972). However, there is one reported case of trigeminal section performed expressly in an attempt to slow the growth of one side of the face in a child with unilateral facial gigantism (Podolsky and Fields, 1954). However, the assymmetry and facial deformity persisted (Podolsky, 1974). Indeed, there are currently no data to justify such an attempt at controlling facial growth.

STATEMENT OF THESIS

There appears to be considerable evidence that nerves do in some way affect the differentiation and the growth of soft tissues. Whether this influence is by a strictly functional or strictly neurotrophic mechanism, or an inter-relationship combining the two, remains open to question; however, it appears from the experimental evidence that the latter may be true.

With this in mind, the question is raised as to the relation of nerves to hard tissues. In this case, there are several possibilities. One is that nerves can control differentiation and growth of the skeletal units directly by influencing the osteogenic cell through a neurotrophic mechanism. While clinical observations and anatomic intimacy give some credence to this theory, experimental verification is almost entirely lacking.

Another possibility is that the nerves control differentiation and growth of hard tissues by an indirect mechanism. In this case, nerves would induce or in some way affect growth of the soft tissues which in turn would, by some unknown mechanism, alter growth of the hard tissues. This view is held by Moss and his associates (1960) who believe that growth of the skeleton is secondary to the growth of soft tissues and "functioning" spaces:

> . . .Abundant data demonstrate that all growth changes in size, shape and spatial position and, indeed, the very maintenance in being, of all skeletal units are always secondary to temporally primary changes in their functional matrices.

Moss (1972a) believes that:

> All functional matrices are under regulation, a portion of which is a trophic process originating in the nervous system. . . .We hypothesize further that such neurotrophic process(es) is responsible, to a considerable extent, for the growth and maintenance of the functional matrices.

Moss (1971a) further states that:

> Because periosteal functional matrixes regulate the size and shape of specifically related skeletal units, it is apparent that the genetic control of the structural, chemical, and functional attributes of these same matrices cannot reside solely in the matrices themselves, but rather reflect constant, neurotrophically regulated, homeostatic control of the genome. It is also clear that similar trophic controls probably exist for capsular functional matrixes, which passively regulate the position of both skeletal unit and periosteal matrixes. Some degree of visceral neurotrophic control also is probable.

Moss (1971a) also states that: ". . .the anatomic bases for oral neuroepithelial trophic interactions are established." In this statement he is referring to the trigeminal nerve.

It will, therefore, be the purpose of the present investigation to study the behavior of the facial skeleton in the partial absence of trigeminal innervation. If the facial skeleton is grossly distorted or fails to grow, the neurotrophic-functional matrix theory of Moss would be sustained; if the facial skeleton is not distorted and continues to grow, then the result would indicate a need for re-evaluation of the neurotrophic-functional matrix theory and would favor more classical theories of craniofacial growth which do not accord so central role to nerves and soft tissues.

To examine this question, the trigeminal nerve will be destroyed both unilaterally and bilaterally in the growing rat, without disturbing any of the so-called "functional matrices." Three types of lesions will be employed. An attempt will be made to coagulate the nerve at the gasserian ganglion, anterior to the ganglion, and posterior to the ganglion. In this way the cell bodies of the gasserian ganglion would be destroyed, or left intact with the axon processes severed, or left intact with the axons intact but with the central connections severed. This design is based mainly on the work of Singer and his associates who have shown in numerous articles that neurotrophic influences are not dependent on central connections, are exhibited by both sensory and motor nerves, and require only a cell body in which to produce the trophic influence and an axon to transport it. Therefore, we would expect that:

1. in the ganglionic lesions, trigeminal neurotrophic influences would be interrupted and the growing face distorted;

2. in the anterior lesions, neurotrophic substances would be produced, but not transported and, thus, the face distorted; and

3. in the posterior lesions, the trophic substances would be produced and transported to the periphery, permitting the face to develop normally.

Anticipated (but not specifically desired) motor lesions would be expected to produce changes consistent with those seen by Washburn (1947) and others.

In this way we will be able to determine whether the alteration of the trigeminal nerve has any affect on growth of the cranio-

facial complex, and if so, whether the findings are consistent with the neurotrophic hypothesis based on findings in lower forms of animals.

METHODS AND MATERIALS

Sample

The present investigation employed 445 Long-Evans rats obtained from 48 litters. All animals were purchased from Blue Spruce Farms (Altamont, New York). The rats were ten to twenty days of age at the time of surgery. This age range was chosen for the following reasons:

1. A ten to twenty day old rat is approximately as mature as a ten to twenty month old human (Donaldson, 1924). This initial age permits the major portion of post-natal growth to be accomplished during the experimental period. Also, stereotaxic coordinates for the rat brain are known for these ages (Sherwood and Timiras, 1970);

2. The incisor teeth have erupted (8-10 days) and may be fixed in the stereotaxic headholder (Donaldson, 1924);

3. The trigeminal nerve is developed intracranially (Hill, 1970);

4. The rat is old enough to tolerate physically the surgical procedure;

5. The rat can be weaned early, and be expected to survive if the mother shows any tendency towards cannibalism after the operation;

6. The ears are open (2 1/2 to 3 1/2 days) and, accordingly, a stereotaxic headholder can be used (Donaldson, 1924);

7. The weight of the rats is 20-25 grams at ten days of age, and consistent within each age group from 11-20 days of age (Table I). Uniform size and age within each group are essential to the surgical procedure.

TABLE I

AVERAGE WEIGHTS

OF ANIMALS

Surgical Age (days)	Average Weight (grams)
10	22.50
11	25.05
12	24.40
13	24.50
14	29.25
15	31.00
16	28.33
17	30.43
18	33.60
19	35.66
20	46.35

8. The head of the young rat grows rapidly. Although cranial growth of the rat continues throughout life (Donaldson, 1924), by ten weeks it has reached adult proportions in its component parts and systems in regard to the dry weight of the skeleton (Lowrey, 1913) and the head weight (Jackson and Lowrey, 1912). According to Massler and Schour (1951) osteogenesis occurs on all the surfaces of the rat calvaria up until 60 days of age; after this time, growth occurs only in certain sites. According to Moss (1954) the growth of the neurocranium is completed by 34 days, while the visceral cranium continues well past that age. Baer (1954) states that the most rapid period of growth for individual bones is during the first 20 days of life. He also states that the face and cranial base showed the greatest increase in length from 29-50 days of age, whereas the largest incremental increase of the vault length occurs from 10-16 days of age. The experimental period covers a period of 10-50 days, and thus should include major events in the facial growth in the rat.

Originally it was planned to operate on the animals only at 10 days of age, but a preliminary study showed that 60% of the animals died within five days, postoperatively. Accordingly, the design was changed to employ animals from 11-20 days of age. Presumably, the mortality in young animals was due to an interference with the normal suckling reflex in the unweaned animals.

Experimental Design

Forty litters of from 8-14 animals each were used to test the effects of intracranial, unilateral section of the trigeminal nerve during the development of the facial complex. Bilateral section of the trigeminal nerve was attempted in three litters. The remaining four litters served as controls.

Each experimental animal underwent surgery at 10-20 days of

age. A stereotaxic technique was used to locate the trigeminal nerve intracranially, and the nerve was lesioned in one of three locations: (1) in the gasserian ganglion, (2) posterior to the ganglion (retrogasserian or proximal), and (3) anterior to the ganglion (distal). Lesions were accomplished using radio frequency electrical current delivered by a stereotaxically directed electrode. During the experimental procedure two desiderata were always kept in mind: (1) control over the amount of destruction, and (2) control over the locus of destruction (Brown and Henry, 1934). Table II illustrates the assignment of treatments to the various experimental litters. Appendices I-IV gives a breakdown of the treatment assignments in relation to the surviving animals.

Experimental Procedure

Stereotaxic Instrument

In order to reduce mutilation and undesirable tissue (functional matrix) damage which surgical exposure of the brain requires, a stereotaxic approach was used to locate and lesion the trigeminal nerve intracranially.

The stereotaxic method enables the operator mechanically to direct an electrode to a desired point within the cranium with reference to three established reference planes. The three intersecting reference planes are determined by prominent anatomical features of the skull which are reasonably constant, to which the neural structures of the brain bear a fairly constant relationship (Carpenter and

TABLE II

TREATMENT ASSIGNMENTS

(according to number of animals)

Age at Operation (in days)	Number of Litters	Size	Operative Side		Target Location		
			Right	Left	Ganglion	Anterior	Posterior
10	10	80	76	4	80	0	0
11	3	21	18	3	21	0	0
12	3	33	16	17	11	14	8
13	33	24	12	12	8	10	6
14	3	24	11	13	11	8	5
15	3	27	13	14	7	12	8
16	3	29	16	13	12	9	8
17	3	23	15	8	8	15	0
18	3	21	18	3	8	10	3
19	3	26	13	13	8	12	6
20	3	15	7	8	7	8	0
15[a]	3	33	33	33	12	11	10
X[b]	4	26	0	0	0	0	0
Total	47	382	248	141	193	109	54

[a]Bilateral lesions; [b]Control animals

Whittier, 1952). Distances of a specific neural structure from the point of intersection (0,0,0 point) of the reference planes constitute the stereotaxic coordinates of that structure. Coordinates have been worked out for the developing (Sherwood and Timiras, 1970) and adult rat (König and Klippel, 1963; Pellegrino and Cushman, 1967).

The stereotaxic instrument (Plates I and II) used in this experiment (Model #400, David Kopf Instruments, Tujunga, California) holds the rat on the table in a reproducible, fixed position using ear bars and a specially designed maxillary incisor bar (.039 inch wire) similar to the one suggested by Sherwood and Timiras (1970). The ear bars were used as furnished for the 13-20 day old rats, but for younger animals, the tips were modified with self-curing acrylic and equipped with an elastic "stop" (Alastic Modules, S-1, Unitek Corporation, Monrovia, California) to prevent over-insertion and damage to the relatively fragile skull. Plate III shows the modified incisor and ear bars.

The reference planes used to orient the 10-20 day old rat are those used by Sherwood and Timiras (1970) and are illustrated in Figure 1. The three planes are defined as follows:

1. The horizontal zero plane passes just below the base of the brain. It extends from the interaural line to the line along the dorsal surface of the toothbar.

2. The vertical or frontal zero plane passes through the interaural line and is perpendicular to the horizontal zero plane.

3. The sagittal zero plane is located midsagittaly and is perpendicular to the horizontal and vertical planes.

PLATE I

STEREOTAXIC INSTRUMENT

e--electrode; P--potentiometer

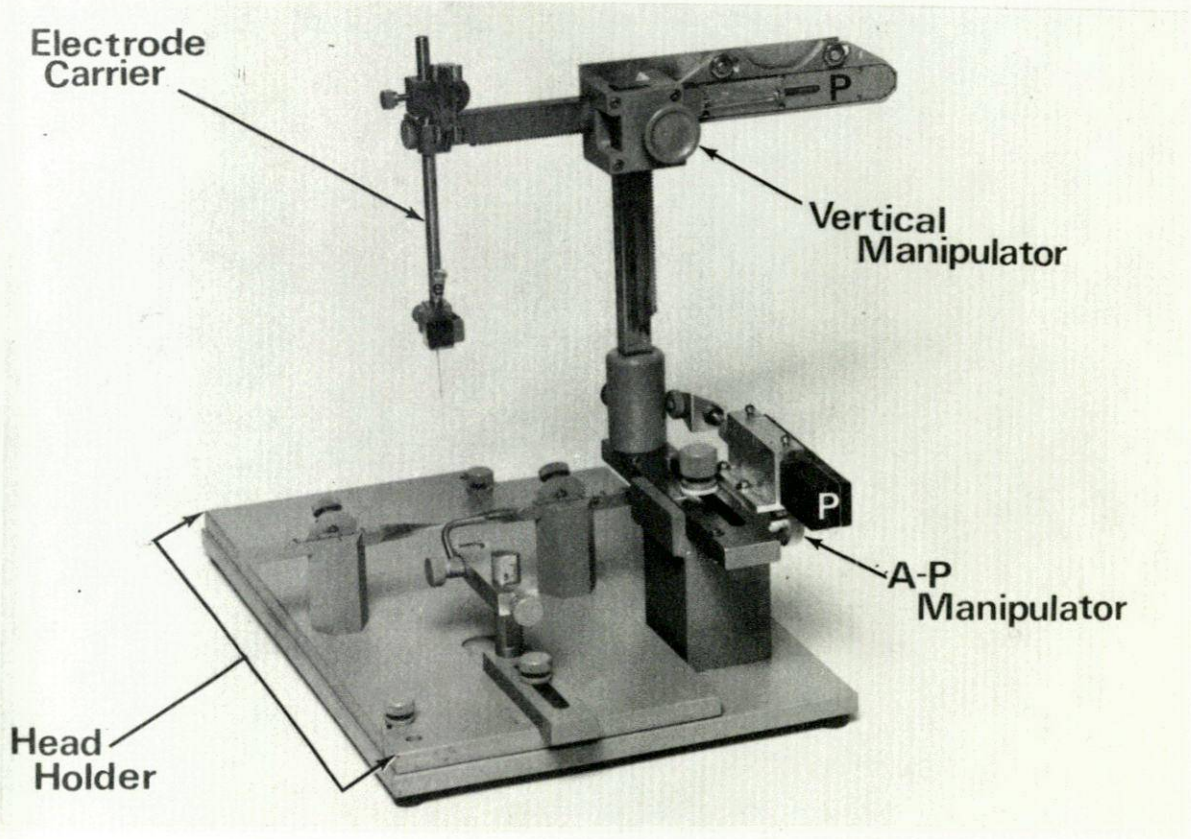

Electrode
Carrier
P
Vertical
Manipulator
P
A-P
Manipulator
Head
Holder

PLATE II

STEREOTAXIC SURGICAL SET-UP

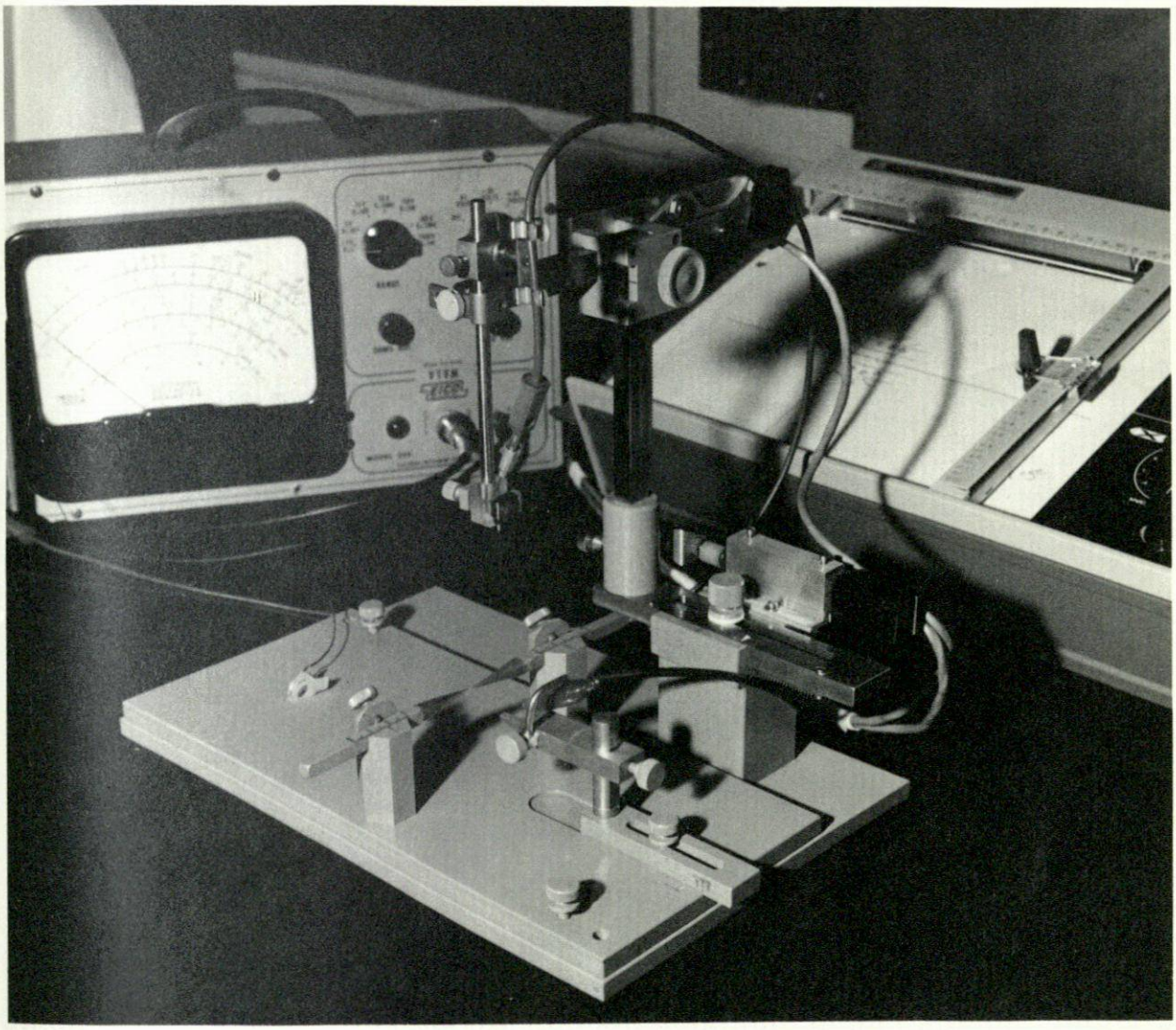


PLATE III

STEREOTAXIC HEAD HOLDER WITH MODIFIED
INCISOR AND EAR BARS

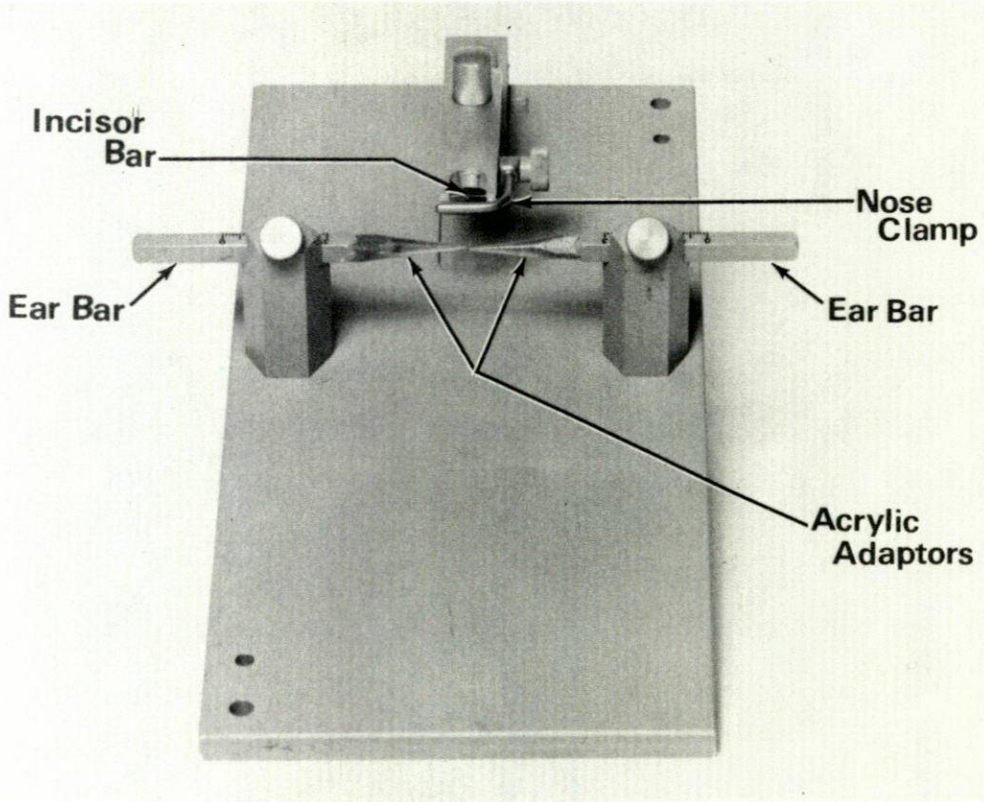
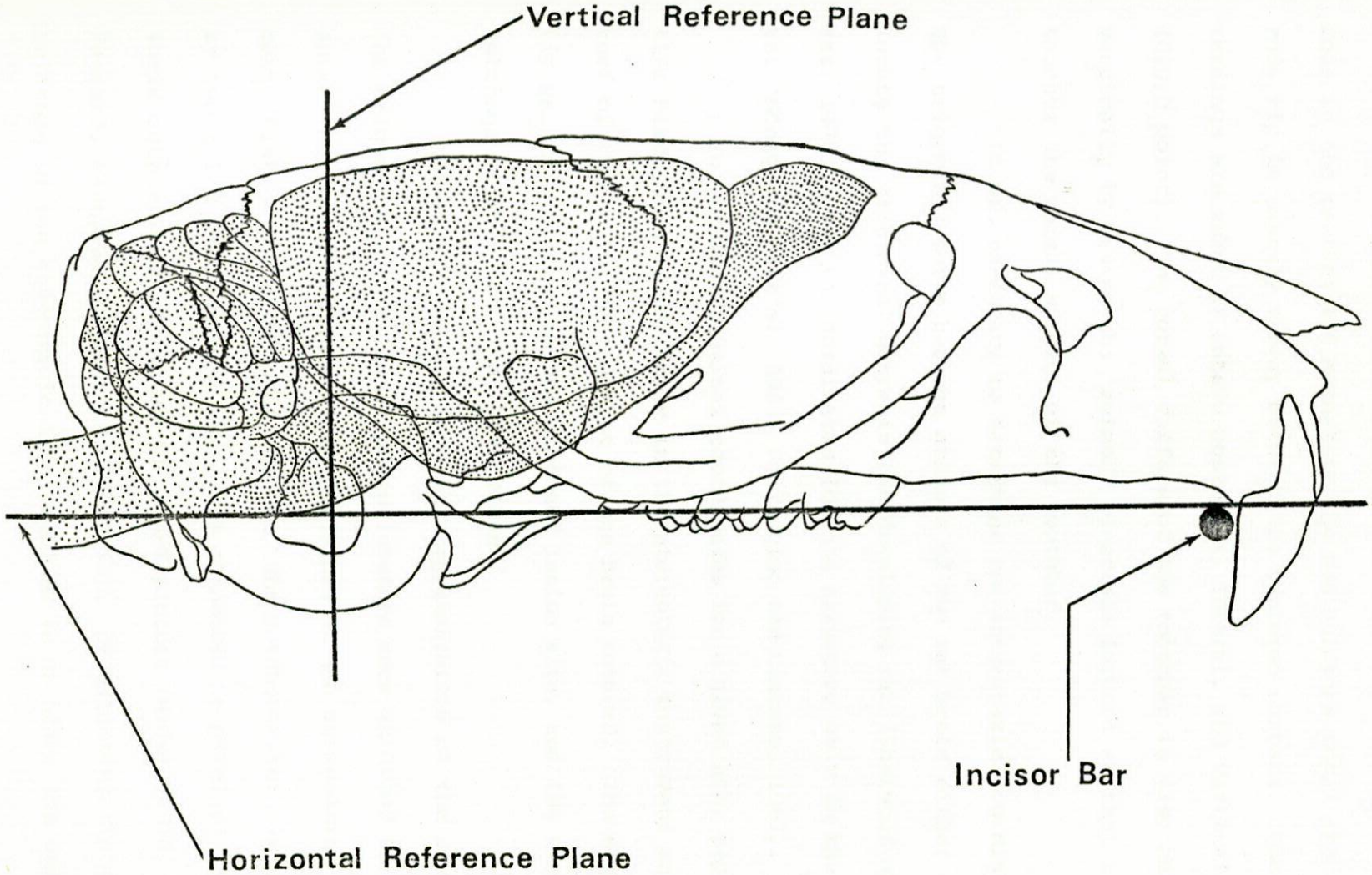

Incisor Bar
Nose Clamp
Ear Bar
Ear Bar
Acrylic Adaptors

Figure 1.--Stereotaxic reference planes.


Vertical Reference Plane
Incisor Bar
Horizontal Reference Plane

The stereotaxic instrument is "zeroed" by placing an electrode in the carrier and adjusting the manipulators until the electrode tip is exactly midway between the centered earbars. The vernier readings are taken as antero-posterior, lateral, and vertical zero (0,0,0 point). The dorsal surface of the toothbar is also calibrated vertically by moving the "zeroed" electrode forward so that it touches the dorsal surface of the toothbar.

It was necessary to determine the stereotaxic coordinates for the trigeminal nerve because atlases of the rat brain either do not locate the trigeminal nerve in the developing rat (Sherwood and Timiras, 1970), or give coordinates for the structure only in the adult rat (König and Kliffel, 1963; Pellegrino and Cushman, 1967).

To obtain trigeminal coordinates for a given age, representative heads were fixed firmly in the stereotaxic instrument and the roof of the calvarium and most of the brain removed. The electrode tip was then brought to the proposed lesion site, and the manipulator settings recorded for the three planes.

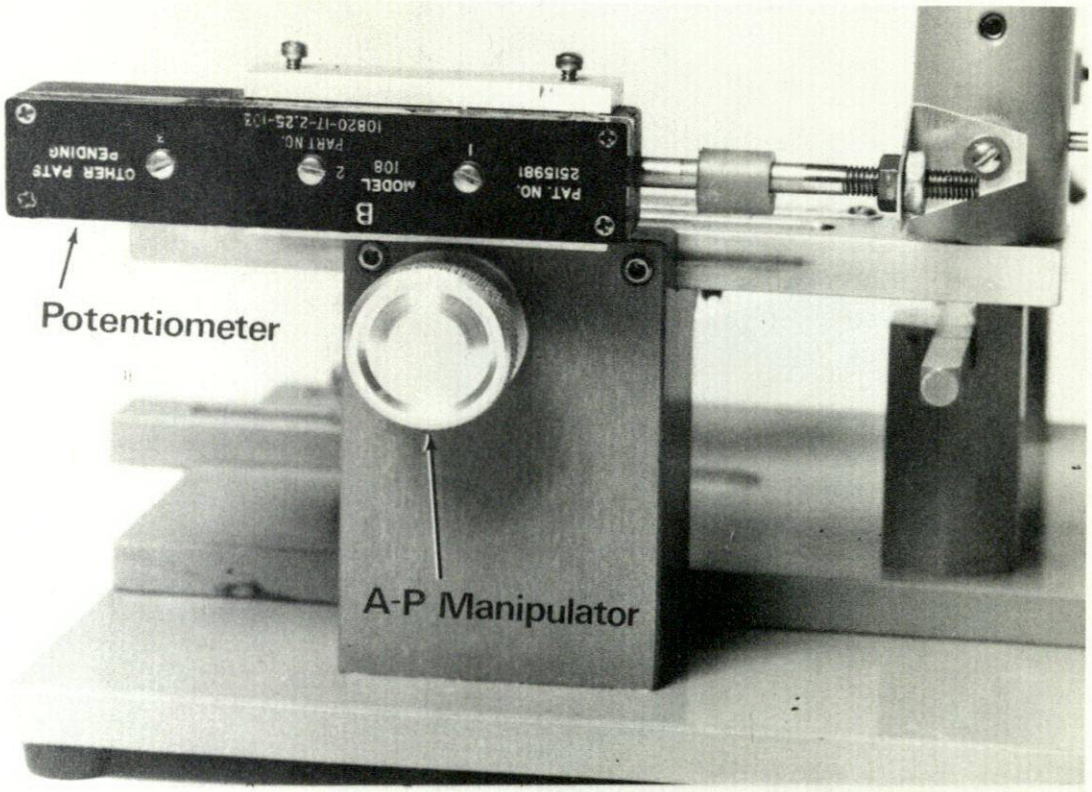
In order to obtain a visual representation of the course of the trigeminal nerve, linear potentiometers were attached to the antero-posterior and lateral manipulators of the stereotaxic instrument (Plates IV, V; Johnston, 1975). The potentiometers were driven by two 1 1/2 volt dry cell batteries connected in parallel, and their output leads connected to an X-Y plotter (Model 7035B, Hewlett-Packard, Pasadena, California; Plate VI). By adjusting the manipulators of the stereotaxic instrument so as to trace the outline of

PLATE IV

ANTERIOR-POSTERIOR POTENTIOMETER

PLATE V

LATERAL POTENTIOMETER


Potentiometer
A-P Manipulator

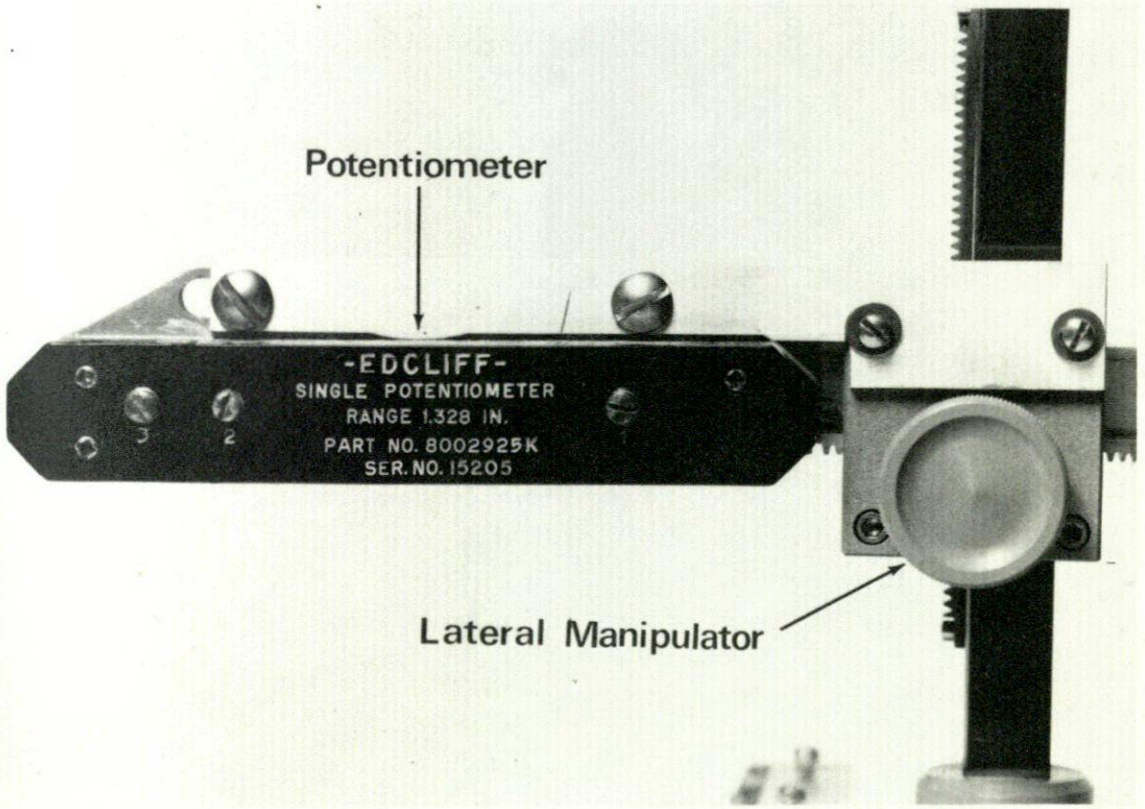

Potentiometer
-EDCLIFF-
SINGLE POTENTIOMETER
RANGE 1.328 IN.
PART NO. 8002925K
SER. NO. 15205
Lateral Manipulator

PLATE VI

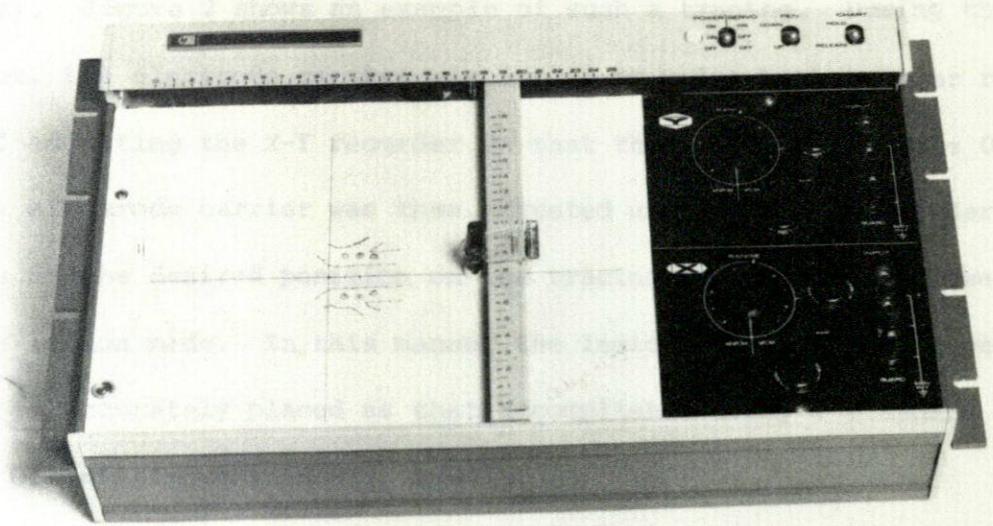
X-Y PLOTTER

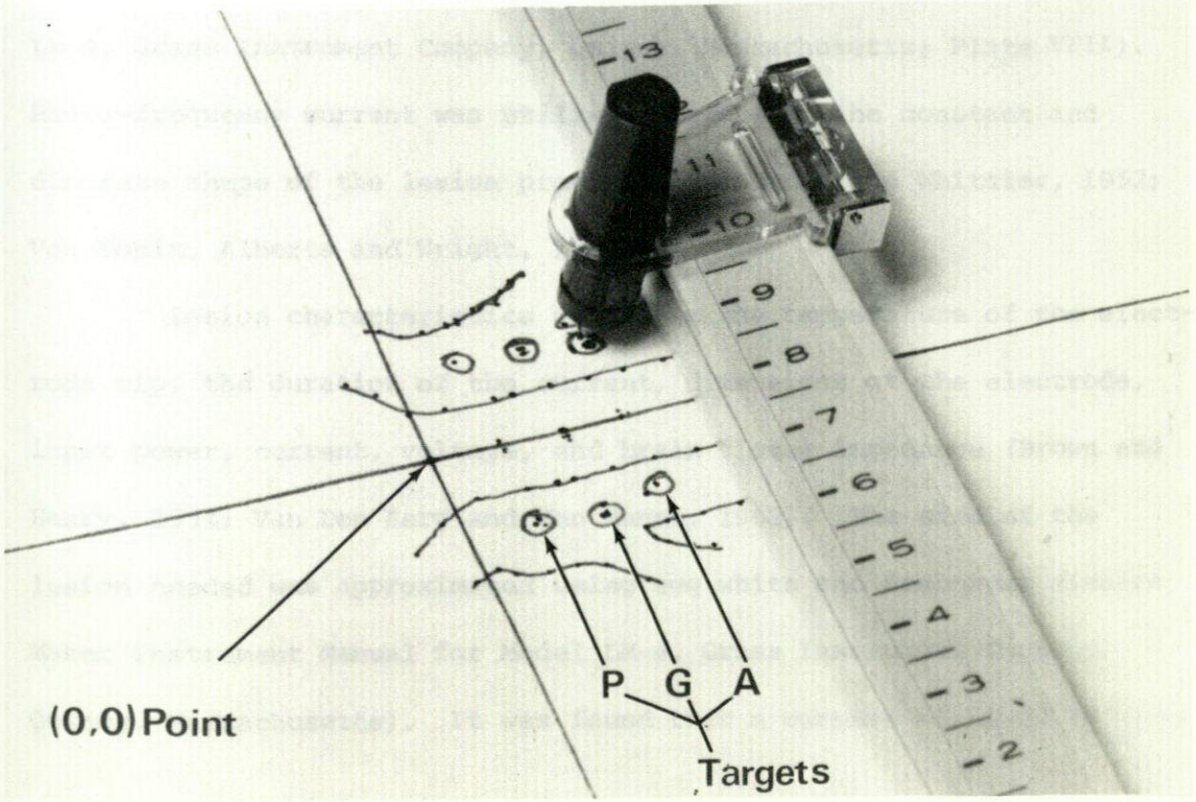
PLATE VII

NERVE TRACING

P--posterior; G--ganglion; A--anterior

X-Y Plotter





the nerve with the electrode, 6X, two-dimensional tracings similar to the maps of Kreig (1945) were produced by the X-Y recorder (Plate VII). Figure 2 shows an example of such a tracing. During the experiment, the electrode carrier was zeroed by using both vernier readings and adjusting the X-Y recorder so that the stylus was at the 0,0 point. The electrode carrier was then adjusted until the X-Y recorder stylus was in the desired position on the tracing, the electrode lowered, and the lesion made. In this manner the lesion site could be expected to be as accurately placed as that accomplished using a standard atlas (± 0.1 millimeter) (Hodge, 1964).

Lesion Generation

With the electrode in the appropriate location, trigeminal lesions were achieved with a radio-frequency lesion generator (Model LM-4, Grass Instrument Company, Quincy, Massachusetts; Plate VIII). Radio-frequency current was utilized because of the constant and discrete shape of the lesion produced (Carpenter and Whittier, 1952; Von Bonin, Alberts and Wright, 1965).

Lesion characteristics depend on the temperature of the electrode tip, the duration of the current, dimensions of the electrode, input power, current, voltage, and brain tissue impedance (Brown and Henry, 1934; Van Den Berg and Van Manen, 1962). The size of the lesion needed was approximated using egg white and hamburger (Lesion Maker Instrument Manual for Model LM-4, Grass Instrument Company, Quincy, Massachusetts). It was found that a current of 60-80 mA with

Figure 2.--X-Y plotter tracing (6X) showing the location of the trigeminal nerve (V), calvarial landmarks and gross outline of the rat head.

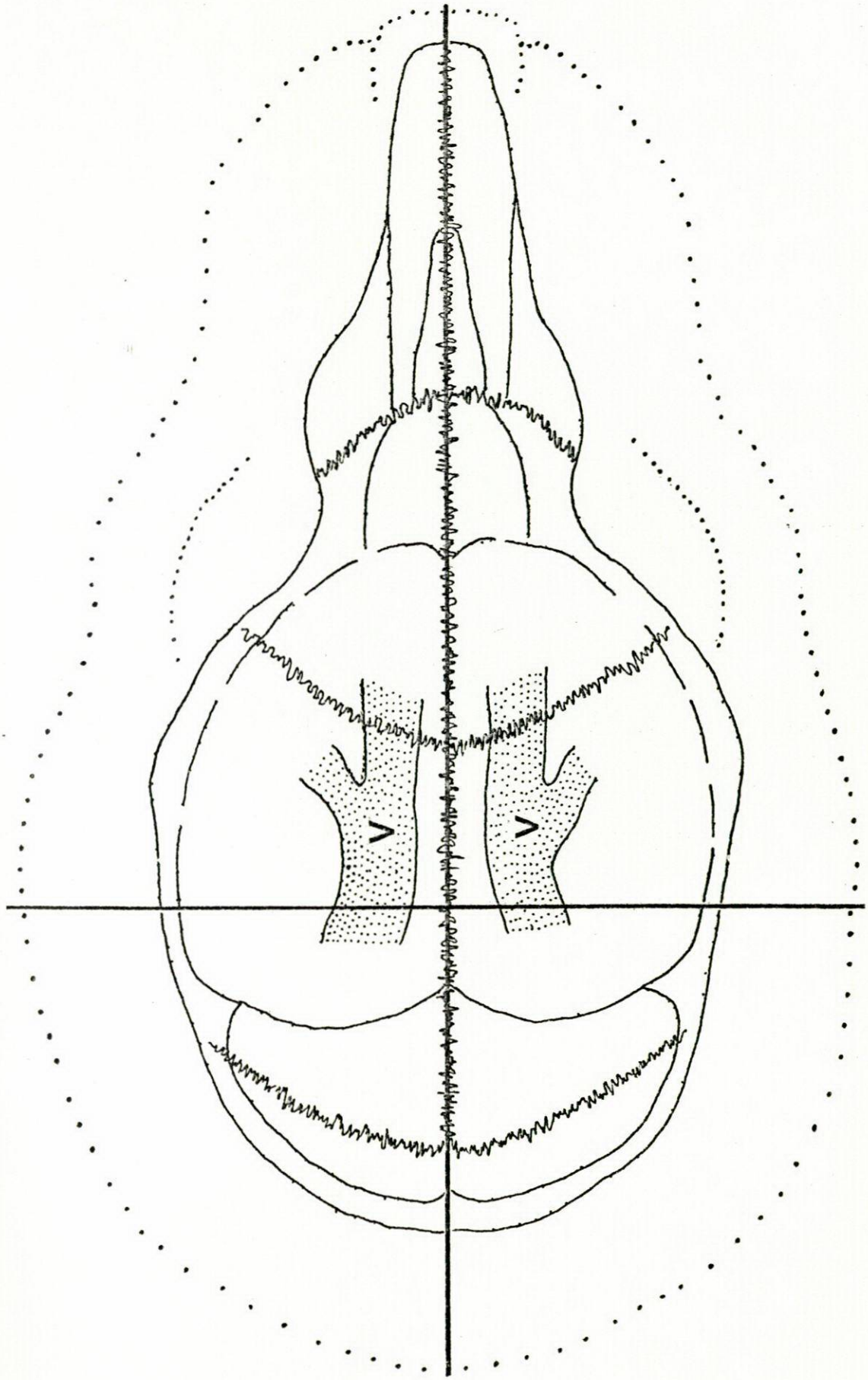


PLATE VIII

LESION GENERATOR AND VOLTMETER