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Neuroanatomical and chemical changes within the nucleus accumbens paralleling patterns of behavioral activation in the developing rat

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NEUROANATOMICAL AND CHEMICAL CHANGES WITHIN THE NUCLEUS ACCUMBENS PARALLELING PATTERNS OF BEHAVIORAL ACTIVATION IN THE DEVELOPING RAT

by

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A dissertation submitted to the Graduate Faculty in Psychology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University New York.

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Abstract

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Nina Lauren Goodless

Advisor: Dr. Gordon A. Barr

Various lines of evidence suggest that in the neonate certain stimuli elicit a constellation of oral-motor ingestive and non-ingestive responses, expressed as behavioral activation. The stimuli that activate pups include stroking, oral infusion of milk, and electrical stimulation of forebrain limbic structures. As the animal matures these stimuli become less reliable in eliciting these activating behaviors. The cause of the progressive decline in activation in older animals is not understood. Recent research from our laboratory and others have implicated a role for the Nucleus Accumbens (NAc) in behavioral activation. The present study explored the changing temporal and topographic patterns of stimulation-bound activitiy elicited by electrical stimulation of the NAc. Because the NAc is diversely innervated by several neurotransmitters systems, the second goal of the present study was to investigate the differential maturational patterns of the neuroanatomical and the neurochemical systems that may subserve the age related changes in behavioral activation.

This investigation explored the normal postnatal development of serotonin (5-HT), methionine enkephalin (met-Enk)- and γ–aminobutyric acid (GABA)-like immunoreaactivity in tissue sections prepared using Sternberger's unlabeled antibody peroxidase-antiperoxidase technique. To relate the development of these

neurotransmitters to morphological and metabolic characteristics of the NAc, the ontogenetic pattern of Nissl stained cells, Bodian silver stained fibers, and cytochrome C oxidase activity was examined.

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Finally, the last section of my thesis to be written, and, at last, a section of my thesis that can be written without regard to content, grammer or spelling.

I consider myself very fortunate to have been surrounded by a collection of people who have all possessed wonderful senses of humor and expressed confidence in my intellectual abilities throughout this long and arduous process of completing my thesis. These qualities were extremely helpful at times of utter exhaustion, frustration and desire to spend entire afternoons eating lunch at 103.

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others that I would like to name specifically, but the space limitaions do not allow me to do so.

Harold, thanks for putting up with all this shit, I love you.

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aca	Anterior Commissure
BNT	Bed Nucleus of the Stria Terminalis
cc	Corpus Callosum
cg	Cingulum
CL	Claustrum
CPu	Caudate/Putamen Complex
DBB	Diagonal Band of Broca
DBBT	Tract of the Diagonal Band of Broca
ec	External Capsule
f	Fornix
HA	Hippocampus, pars anterior
HT	Hippocampocortical tract
ICj	Islets of Calleja
ic	Internal Capsule
lo	Lateral Olfactory Tract
LS	Lateral Septal Nucleus
LV	Lateral Ventricle
mfb	Medial Forebrain Bundle
MS	Medial Septal Nucleus
NAc	Nucleus Accumbens
ON	Olfactory Nucleus

OTV	Olfactory Tract, Ventral
P	Preoptic Nucleus
PC	Piriform Cortex
SHBT	Septohabenularis tract
SHi	Septohippocampal nucleus
ST	Stria Terminalis
Tu	Olfactory Tuberculum

Introduction

" He who sees things grow from the
beginning will have the finest view of them."

Aristotle

THE DEVELOPMENTAL PARADIGM

A developmental strategy is one approach that can be used to study behavior. In using this strategy, a behavioral pattern is observed and manipulated at different stages during its maturation in order to trace its assembly and organization. A critical assumption here is one of continuity; the same behavioral system is assumed to be present at successive stages of development. This suggests that the infantile behavioral form is gradually elaborated into the adult form (Hall and Williams, 1983). This assumption is not, however, always borne out by experimental observation. Developmental strategies have been applied to the study of feeding behavior in the rat, for example; and this has resulted in the failure of the above assumption. Feeding behavior is present at birth and continues into adulthood, however, suckling, the normal early form of feeding, is not the same ingestive behavior used by adults. The question is, then, is suckling the appropriate behavior to utilize as the starting point for an analysis of the ontogeny of ingestive behavior? Hall and Williams (1983) present compelling differences in physiological controls and neural substrates of suckling and later ingestion and conclude that, "While suckling is the primitive ingestive response of the infant and serves to get the infant food, this functional similarity is not in itself confirmation that the suckling system of the infant and the feeding system of the adult are the same system" (p. 220).

Within this developmental framework, then, either the infantile form can be continuous with the later adult form and be based on the same underlying neural structures and organization, with the infantile form undergoing changes that turn into the adult form. Or, behavioral patterns may appear early in development but differ as

a function of the ontogenetic process when finally elaborated into the adult form. The adult form may be built from separate systems that have little in common with, and are distinctly different from the infantile form. Additionally, experimental manipulation may elicit potential forerunners of behavioral patterns in the neonate, well in advance of their normal occurrence and are not useful to the organism until later in development. Interpreting the role and significance, then, of such behaviors occurring during infancy becomes a difficult task inherent within the developmental framework.

BEHAVIORAL ACTIVATION

One behavioral pattern in which one can utilize a developmental framework is behavioral activation. Behavioral activation is operationally defined by a constellation of specific stimulation-bound behaviors that occur together in space and time which is observed in maternally and nutritionally deprived pups. These include ingestive and non-ingestive oral-motor behaviors including mouthing, licking, pawing, probing, rooting, stretching, as well as, rolling, wall climbing, grooming, and locomoting (Hall, 1979 a and b). This behavioral excitation has been described as the affective component of the feeding response in young animals (Hall, 1979 b). Hall speculated that this state, "represented the functioning of an undifferentiated reward or reward system ...." and this could allow access to a "primitive system in which to explore the development of motivation." (p.338). These behavior patterns and postures, Hall (1979 b) asserts further, may be considered the building blocks or subroutines of later complex motivational acts. In these movement patterns one can see fragments of other species-specific behaviors, (such as sexual responses, lordosis: Hall, 1979 b; grooming: Fentress, 1977; and locomotion: Pijnenburg and Van Rossom, 1973). Behavioral activation appears to be tightly coupled to reinforcement during development (Bulut and Altman, 1974; Moran et al., 1981; Pederson et al., 1982). Those stimuli that function as reinforcers elicit behavioral activation.

The infant may become behaviorally aroused by a variety of naturally reinforcing stimuli. These stimuli include oral infusion of milk (Johanson and Hall, 1979; Brake 1981), warm ambient temperature (Hall, 1979 a), maternal stimulation

(Hofer, 1975), deprivational status (Johanson and Hall, 1981), and home cage odor (Bulut and Altman, 1974; Johanson and Hall, 1981). Electrical stimulation of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus (Moran et al., 1981; Moran et al., 1983) and pharmacological manipulation of catecholamines (CA) (Campbell and Mabry, 1973) also produce profound activation. Data gathered in our laboratory suggests that electrical stimulation of the nucleus accumbens (NAc) elicits activation in the neonatal rat pup (Lee, 1988). The magnitude and topography of the activating effects elicited are stimulus-specific and the time course is age-dependent and influenced by contextual variables.

During development, pups undergo several changes in the way they respond to reinforcing stimuli. There is a transition in response repertoire from 3 to 10 days of age, when stimulating electrodes are directed towards the medial forebrain bundle. Three day old rat pups emit rapid shifts from oral consummatory responses to integrated sexual responses. Behavioral responses of 10 day old rat pups are more temporally segregated to response type. That is, sensory input from goal objects, such as food or water are necessary to elicit behavior. Regardless of the type of stimulation, behavioral activation abates as the animal matures. The specific behaviors that characterize behavioral activation become less prevalent and, by the end of two weeks, activation is no longer recognizable and subsequently disappears (Hall, 1979 b; Johanson and Hall, 1979; Moran et al., 1983).

The uniqueness of behavioral activation to infancy raises major conceptual issues. It suggests that behavioral activation may have important adaptive significance critical for the neonate's survival. It has been posited, (Camp & Rudy, 1987) that behavioral activation may contribute to the formation of infant-maternal bonds. For the infant pup, behavioral activation appears to have rewarding properties that ensures that contact with the mother will be reinforcing. They suggest further, that for the mother, the dynamic situation sets up by the display of activity by the neonate may provide cues that elicit various forms of maternal care.

The first objective of the proposed research was to further characterize the time course of behavioral activation by pinpointing its time of offset as well as to describe the loss, inhibition, or replacement of each of the behaviors that defines behavioral activation in response to NAc stimulation. In addition, the present reseach hoped to determine the significance of this dramatic form of behavior that is

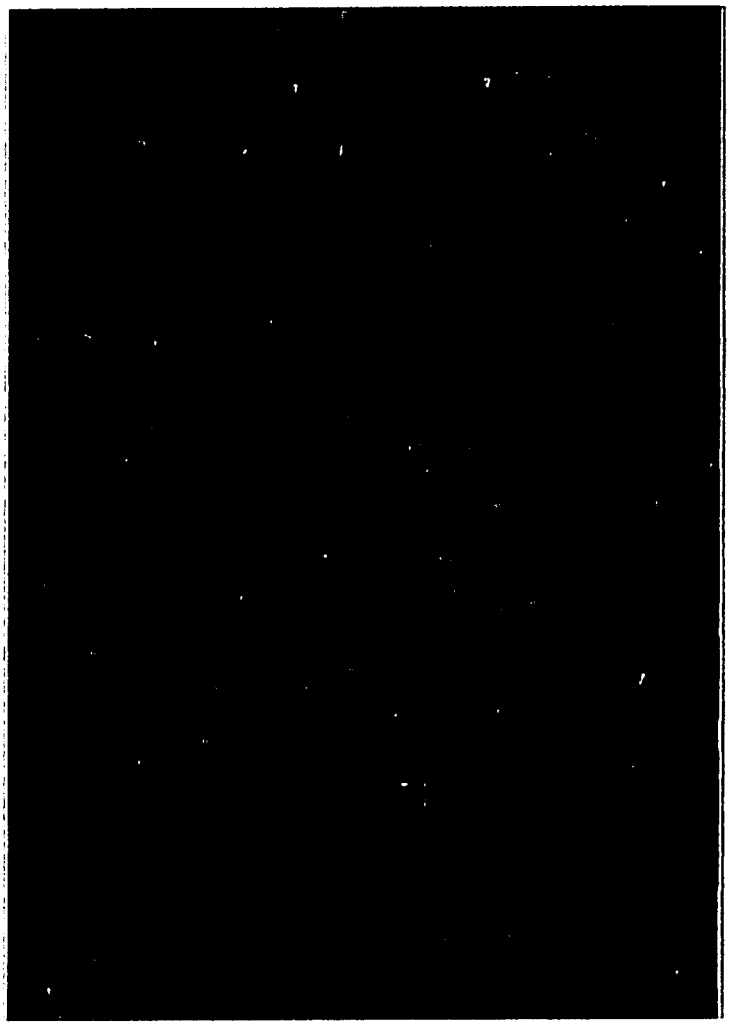
observed early during ontogeny. Based upon these findings, the second goal of the present research was to demonstrate developmental changes in the neuroanatomy and neurochemistry of the NAc that is correlated with patterns of behavioral activation in the preweanling rat. The normal postnatal development of serotonin (5-HT)-, methionine enkephalin (met Enk)-, and gamma-aminobutyric acid (GABA)- like immunoreactivity was examined. To relate the development of these neurotransmitters to morphological and metabolic characteristics of the NAc, the ontogenetic pattern of Nissl stained cells, Bodian silver stained fibers, and cytochrome C oxidase activity was examined.

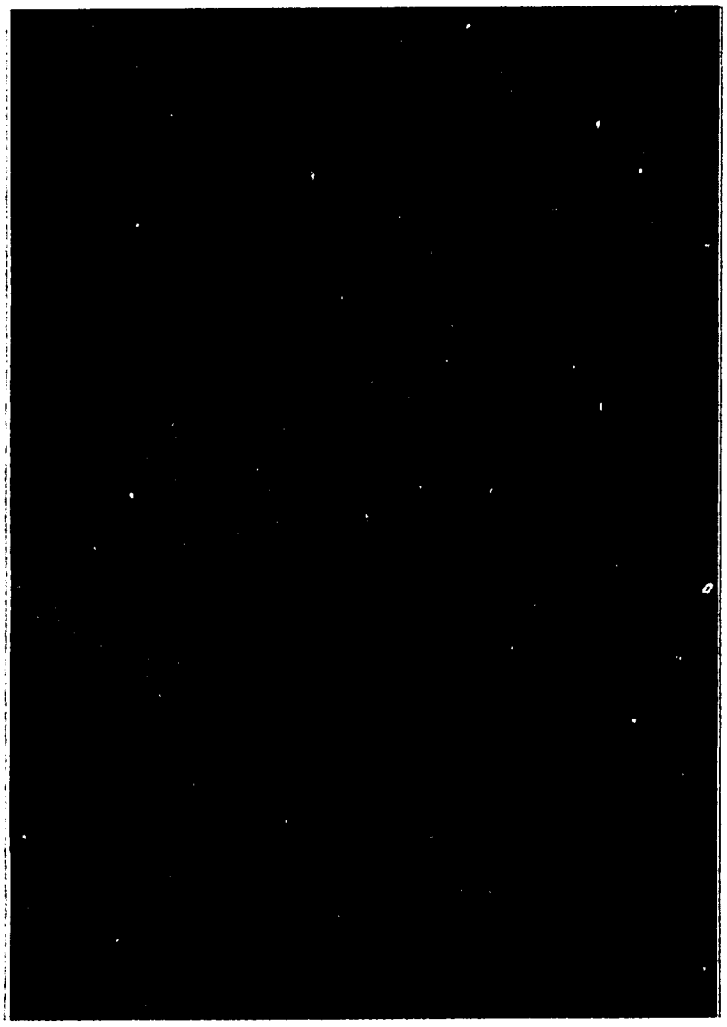
ANATOMY

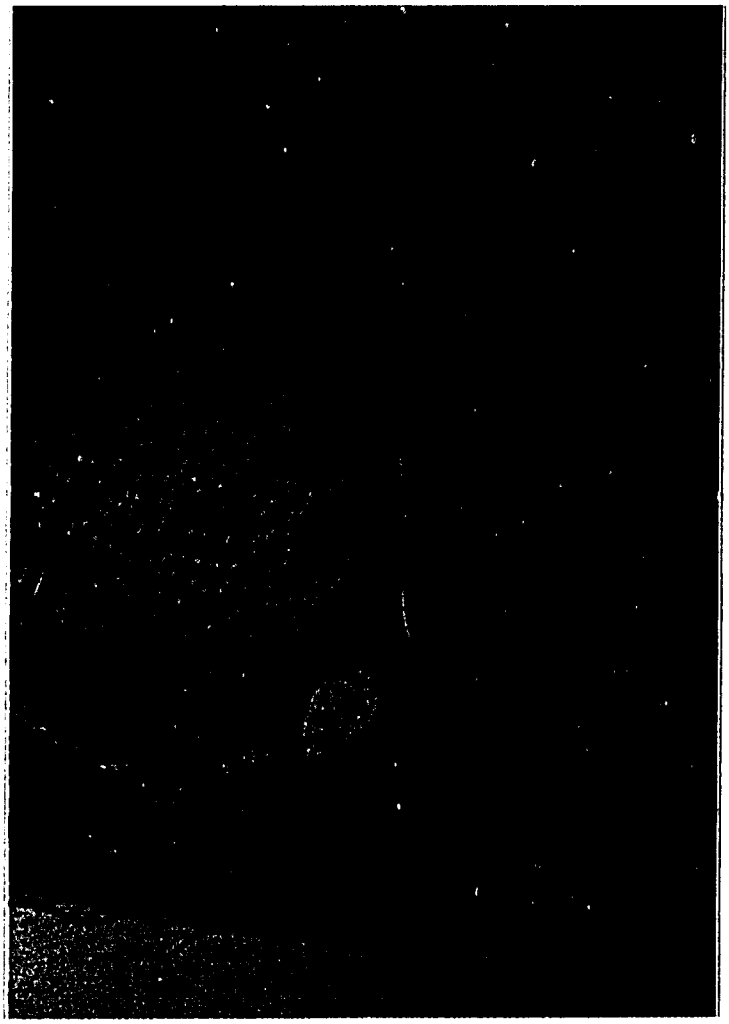
The study of the NAc, its anatomy, physiology and functional significance has received increased attention in the literature. However initial speculation about its functional significance dates back more than 100 years. The Austrian anatomist-psychiatrist, Meynert described an extension from the caudate nucleus into the midline forming the septipellucidi (1872). The nucleus accumbens was given its present name by Ziehen (1897) 20 years later. Anatomists are still characterizing this nuclear mass with caution, (Koikegami et al., 1967) due to its close proximity to other structures. The topographic location and delineation of its boundaries still remains a difficult and somewhat controversial task. Discrepancies in the anatomical definition of the NAc may reflect differences in the techniques employed (e.g. axonal degeneration, radioautography, electrophysiological, and immunohistoflourescence) or interspecies differences (e.g. rat, rabbit, cat, and monkey). Hence, words such as "boundaries" and "borders" are used with hesitation .

Described as a fairly large nucleus, the nucleus accumbens is a basal forebrain structure. The nucleus is traversed by the anterior portion of the anterior commissure and lies ventral to the anterior horn of the lateral ventricle. This provides a very pronounced border with the septal region for most of its rostral-caudal extent. Rostrally, the NAc is bordered by the posterior aspect of the olfactory nucleus and the intermediate olfactory tract. Caudally, it appears continuous with the bed nucleus of the stria terminalis. It contacts the olfactory tuberculum and the MFB ventrally. Medially it borders the septum, the medial septal nucleus (MSN), the tract and nucleus of the diagonal band of Broca (DBB). Laterally and dorsally the NAc appears to blend with the caudate nucleus (Domesick, 1981) (refer to Figure 1).

Figure 1. Three Nissl stained photomicrographs of representative coronal sections through the nucleus accumbens. These sections shows the continuity between the nucleus accumbens and the caudatoputamen. (4x)



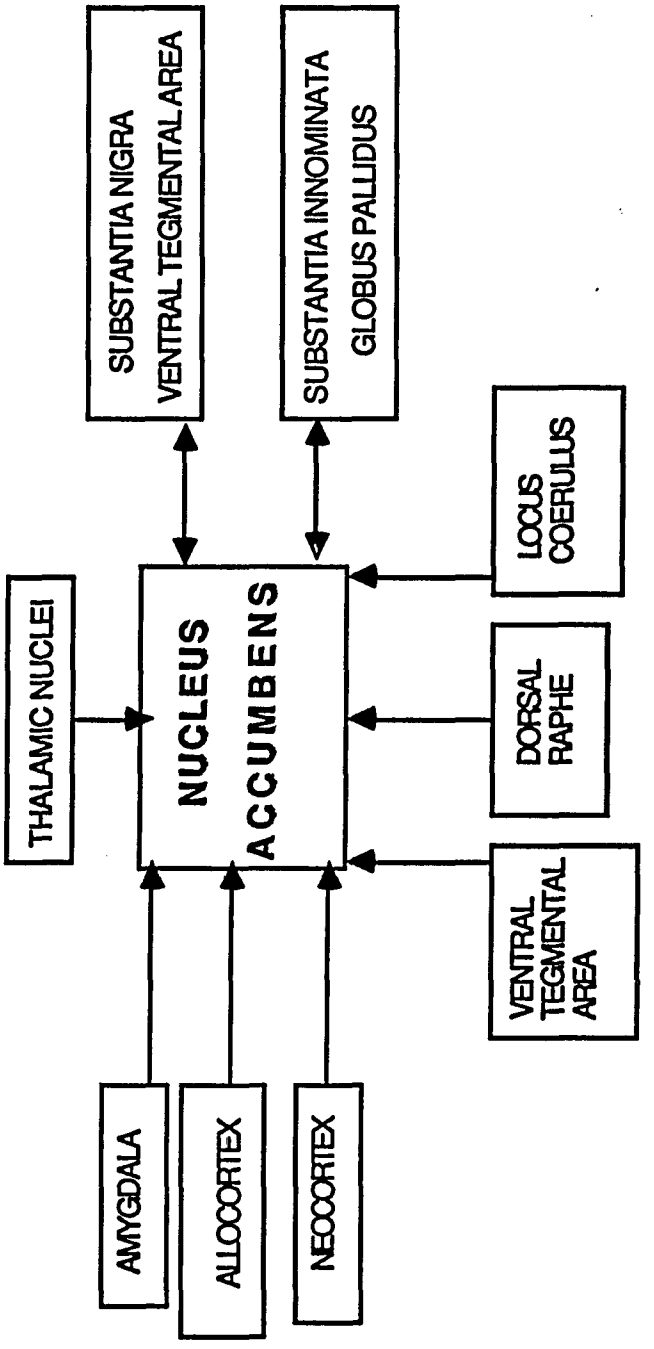




The NAc is located strategically at the interface between limbic structures and the basal ganglia (Graybiel, 1976; Mogenson et al., 1980). Recent studies of both afferent and efferent connections reveal that the accumbens may receive projections from limbic structures and sends projections to extrapyramidal structures. Limbic afferents arise from the septum (Powell, 1963; Swanson and Cowan, 1975) and hippocampus (DeFrance and Yoshihara, 1975; Melbach and Siegal, 1977; Swanson and Cowan, 1977; DeFrance et al., 1980; Groenewegan et al., 1980). Projections are also received from the amygdala (Cowan et al., 1965; Powell and Leman, 1976 ; Groenewegan et al., 1980), cingulum (Koikegami et al., 1967; Swanson and Cowan, 1975), and from the limbic thalamic nuclei (medial, anteromedial, anterolateral, reunions and gelatinosus) (Cowan and Powell, 1955; Jones and Leavitt, 1974; Nauta and Domesick, 1976; Herkenham, 1978). In return, the NAc sends efferents to the septum (Conrad and Pfaff, 1976; Williams et al., 1977), hippocampus (Conrad and Pfaff, 1976; Powell and Leman, 1976), hypothalamus (Conrad and Pfaff, 1976; Nauta et al., 1978), amygdala (Nauta et al.,1978) and limbic thalamic nuclei (Powell and Leman, 1976; Nauta et al., 1978). The striatal component of the NAc is designated by its projection to the globus pallidus (Pycock and Horton, 1976; Williams et al., 1977; Jones and Mogenson 1980; Weiss and Haber, 1987) to the ventral caudate-putamen complex, by reciprocal connections with the ventral tegmental area (VTA) (Carter and Fibiger, 1977; Williams et al., 1977; Chronister et al., 1980; Swanson, 1982; Kalivas and Miller, 1984), to the substantia innominata (Austin and Kalivas, 1987), and the pars compacta and reticulata of the substantia nigra (Conrad and Pfaff, 1976; Williams et al., 1977; Fallon and Moore, 1978; Nauta et al., 1978).

These anatomical data suggest a possible functional linkage between these three components-the limbic system, the NAc, and the extrapyramidal system (Yim and Mogenson, 1982). In 1976, Graybiel suggested that the NAc acted to translate motivation into action by gating signals from limbic structures to the basal ganglia (see Mogenson et al., 1980; Mogenson and Yim, 1981 for a review and, refer to Figure 2). However, at present the value of this rather general hypothesis regarding motivation, in explaining the functional role of the NAc in mediating behavioral activation in the neonate is still unknown.

Figure 2. A tentative model suggesting that the nucleus accumbens may be a functional interface between the limbic and motor systems. Gating inputs tune the transmission of signals from the limbic stuctures through the nucleus accumbens to the motor system.



MORPHOLOGY AND CYTOARCHITECTURE

The NAc appears as a collection of neurons with a unique position and cytoarchitecture. In Nissl stain preparations certain regions appear to contain a higher than normal density of medium sized cells.

The rostral NAc is characterized by several constant features: predominantly small neurons with a few scattered large cells that exhibit a fairly uniform cellular appearance. Cells of the NAc at the level of the anterior hippocampus are similar to those found at the rostral pole. The small-sized cell zone which is pronounced near the dorsal cell layer extends from a small dorsal cap. These cells are more darkly stained are characterized by a clumping of Nissl substance, and vary in size. The most ventral portion of the NAc at this level gives rise to the less dense, polymorphic zone. At this level, the densely packed small-celled dorsal neuron zone is discernable. The cells lose their dense packing and a rim of Nissl substance surrounding the nucleus is visible. In many of the cells at this level, the Nissl substance extends into the dendrites. The larger cells are localized to the dorsal aspect of the NAc. Also at this level are clusterings of medium-sized cells. These cells tend to surround a larger cell layer.

At more caudal levels, the most dramatic feature of the NAc appears. This structure is the large Islets of Calleja, identified on the dorsal medial border. It consists of dense clusters of small cells with associated larger cells. The larger cells have very dense chromatin and congregate around the periphery of the Islet. These cells appear to be appendages of the MFB. Lateral to the Islet, the diffuse region of the NAc develops. This region has small to medium sized neurons dorsally, while ventrally, the neurons are slightly larger. The NAc tapers drastically, caudally to the Islets. At this level, the NAc exists solely as an aggregation of neurons at the tip of the lateral ventricle that becomes continuous with the bed nucleus of the stria terminalis and the lateral septal complex.

The Golgi method demonstrates two neuronal profiles: spiny and aspiny cells. Spiny cells are most numerous. This cell type can be further subdivided into

three categories based upon dendritic or spine distribution. The first category includes small to medium sized, densely spined neurons with a spine-free initial dendritic segment and very short dendrites. Chronister and DeFrance, (1981) have described the appearance of this first, most numerous type. Most have cylindrical to spherical somas with both apical and basilar dendrites. Spines appear to be absent from both the initial segment and cell body. Only at the primary dendritic bifurcation do the spines become numerous. This dense distribution of spines occurs in clusters on bulbous swellings near the end of the shaft. The dendrites of these cells often end with thin filamentous extensions. Myelinated axons leave the cell at the soma or from the spine free segment of the dendrite.

The second subdivision of spiny neurons, characterized by delicate dendrites, appears to be localized throughout the total extent of the NAc, but become most dense in the dense cell portion. These cells have smaller spherical cell bodies and the dendrites begin spine-free. At bifurcations, the distribution increases but not as extensively as seen in the "robust-dendritic variety". Neonatal golgi preparations suggest that these cells may be interneurons of the NAc (Chronister et al., 1981).

The third type of spiny neuron appears to be the least frequent. Their distribution of spines are sparse. These cells differ from the other cell types because of the presence of spines on the cell body and the initial segment.

The second neuronal profile demonstrated by the Golgi method are the aspiny neurons. The dendrites of the aspiny variety are not completely devoid of spines. However, the few spines that are present tend to cluster on swellings or varicosities. Another distinguishing feature of this type is their large arborizing dendritic fields. A second type of aspiny neuron has been described in the NAc. This type has a major dendritic extension which can be difficult to distinguish from the soma. The axon typically leaves from the shaft of the dendrite and has many collaterals. The final type of aspiny neuron identified is small with delicate dendrites. Like the other cells of the aspiny type, they have extensive dendritic arborizations. Unique to the accumbens is the previously mentioned large Islets of Calleja. This cell cluster is dramatically stained in rapid Golgi preparations. Tissue prepared by Nissl stain reveal two cell types: small, tightly clustered cells and large densely stained cells. The smaller cells have fewer dendrites with scattered spines with complicated axonal plexuses. Some axons have a curious beading with an occasional very large

varicosity. The large cells of the Islet have their cell bodies in the core of the NAc, but their dendrites enter the Islets. Such cells are generally pyramidal to polymorphic in appearance. These cells are typically embedded within the latticework of the densely beaded axons of the smaller cells.

DEVELOPING NAc

The anatomical placement of the NAc is a difficult task in the prenatal organism as well because of its continuous relationship with adjacent olfactory and striatal structures. It has been demonstrated (Bayer, 1981) that the NAc has strong neurogenetic and morphogenetic links to the olfactory system. She suggests that the NAc and the olfactory bulb granule cells share the same germinal source-the subependymal zone, as well as the late-forming neurons of the olfactory system. Futhermore, the neurons of the olfactory tubercle and Islets of Calleja may also be located within the subependymal zone. The neuroblasts then migrate through the differentiating cell zone of the NAc *en route* to their final adult destinations. The subependymal zone, (the most likely of the two germinal zones), giving rise to the NAc, also continues along the lateral wall of the lateral ventricle into the primordium of the caudate-putamen complex. This suggests a strong developmental link to the striatum.

Between embryonic day 19 (E19) and postnatal day one (P1), neuroblasts are actively being produced and rapidly accumulating in the primordial NAc. Regardless of location, the cells appear primitive. Cytodifferentiation is delayed beyond this primitive state until all neurons have migrated to their final location. Differentiation then proceeds rapidly throughout the nucleus. Neurogenesis is completed by P4, which is several days later than the neurogenesis of nearby structures, such as the septal nuclei, caudate, and bed nucleus of the stria terminalis (Bayer, 1981).

By P5, the processes that sprout initially appear to be dendrites. The most intensive period for dendritic lengthening occurs from P5 to P10. At P10, spines are present and at P20 their density approximates adult levels (Bayer,1981).

CHEMICAL NATURE OF NAc

The concept of chemical transmission within the central nervous system is not new, but only recently have anatomical connections been mapped by their chemical nature. The aim of the following section will be to present the data which address the localization of the classical neurotransmitters and modulators within the NAc, detail those that may contribute to the complex behavior under investigation, and suggest a rationale for their utilization within this developmental framework. The complexity of the NAc is best illustrated by the results of immunocytochemical experiments that have shown that a number of neurotransmitters and peptides are distributed heterogeneously throughout this structure.

Classical Neurotransmitters

Acetylcholine

Immunohistochemical analysis reveals that there are high levels of choline acetyltransferase (ChAt), acetylcholinesterase (AChE), and substantial amounts of acetylcholine in the NAc, particularly in the medial part. This would suggest that this brain locus has a high density of cholinergic neurons (Lewis and Shute, 1967; Jacobowitz and Palkovitz, 1974; Cheney et al., 1975; Lehmann and Fibeger, 1978; Palkovits et al., 1979; Meredith, 1987).

Catecholamines

Presently, immunohistochemical procedures are available for the localization of biosynthetic enzymes of the catecholamines, ie., tyrosine hydroxylase (TH), aromatic l-amino acid decarboxylase (AADC), dopamine-B-hydroxylase (DBH), and phenylethanolamine-N methyl transferase (PNMT) (Hartman, 1972; Hokfelt et al., 1973l; Pickel et al., 1977). TH is a marker for all catecholaminergic neurons, whereas the enzyme DBH is specific for noradrenaline-adrenaline-containing neurons.

The levels of dopamine (DA) within the NAc are among the highest in the brain. Anatomical identification of DA terminals within the NAc have been verified

using a variety of methods, particularly immunofluorescent visualization of antibodies to TH (Hokfelt et al., 1977) and AADC (Cuello, 1978). Histochemical fluorescence has also positively identified DA nerve endings (Ungerstedt, 1971; Linvall and Bjordlund, 1978). The dense dopaminergic projection to the NAc, derives from cells localized within the ventral tegmental area (VTA). (Fallon and Moore, 1978; Beckstead et al., 1979). Fibers from these cells project as part of the medial forebrain bundle (MFB) (Berger et al., 1976). This system is called the mesolimbic A10 system (Dalstrom and Fuxe, 1964). These afferents project to the medial border of the accumbens. Other monoamines, such as, noradrenalin, (NE), also seem to be present at comparatively high concentrations (Versteeg et al., 1976; Lindvall and Stenevi, 1978), although only a few DBH-positive nerve fibers were localized to this area (Hokfelt et al., 1977 b). The NE pathway arises in the locus ceruleus, A6 regions (Dalstrom and Fuxe, 1964). The locus ceruleus is located in the upper pons of the medulla and projects the majority of NE fibers rostrally which ascend to join the MFB to find their termination primarily along the ventromedial border of NAc (Koslow et al., 1974; Farley and Hornykiewicz, 1977).

Indoleamines

Antibodies have been raised against the two enzymes involved in the biosynthesis of 5- hydroxytryptamine (Serotonin, 5HT), tryptophan-hydroxylase (TrH) and aromatic amino acid decarboxylase (AADC). Steinbusch (1981) identified sites of serotonin immunoreactivity in the rat brain by immunofluorescence using an antibody raised against serotonin. The raphe nucleus dorsalis, situated in the midline of the brainstem and the medulla, is believed to be the origin of these serotonin fibers (Bobillier, et al., 1976). The NAc is innervated by these fibers, which comprise the dorsal raphe forebrain tract. The results of this work showed mainly a low density innervation of the NAc area with scattered medium and high density islands on the medial border, and ventrolateral to the anterior commissure. Positively stained cell bodies were not reported (Bornstein and Paldvats, 1974).

Amino Acids

Amino acids functioning as neurotransmitters, glutamate (or aspartate) and γ-aminobutyric acid (GABA) have been biochemically localized within the NAc (Fonnum et al., 1977; Walaas and Fonnum, 1979). Intrinsic glutameric neurons, as well as afferents from the subiculum and frontal cortex were found in the NAc (Fonnum et al., 1977). Glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, is found in measurable amounts in the inhibitory neurons that utilize GABA as their chemical transmitter. GAD is considered a reliable endogenous marker for GABAergic neurons (Mugnaini and Oertel, 1985). The GAD-immunoreaction reveals a low to medium density of fine, weakly to moderately stained fibers and cell bodies scattered in the neuropil. The density was the highest in the dorsal aspect of the posterior NAc (Perea et al., 1981). In contrast, Fonnum and Walaas (1981) obtained a relatively high level of immunoreactivity to GAD, particularly in the medial part. Local circuit (Walaas and Fonnum, 1981) and GABA-containing efferents project to the ventral globus pallidum and nucleus Basalis (Jones and Mogenson, 1980; Austin and Kalvas, 1987) and to the substantia nigra and VTA (Walaas and Fonnum, 1980; Gale and Casu,1981).

Neuroactive Substances

Peptides

The NAc is equally rich in neuroactive peptides. Peptides differ in several respects from the classical neurotransmitters. Their mode of synthesis differs from neurons using amino acids and monoamines. The peptides are synthesized in the perikaryon directed by messenger RNA on ribosomes and packaged into neurosecretory granules, transported to the nerve terminal for storage and subsequently released.

Substance P

Substance P, an undecapeptide, is present in small amounts in neuronal systems in many parts of the CNS (Cuello and Kanazawa, 1978; Ljungdahl et al., 1978). According to Ljungdahl and coworkers, single immunoreactive, medium and occasionally large-sized cells were detected in the medial portion of the NAc. A weakly immunofluorescent plexus of both medium and highly dense nerve fibers were localized in rostral sections. At more caudal levels, medium densities of nerve fibers were found. The most caudal tip contained low density immunofluorescence.

Opioid Peptides

Unlike the pro-opiomelanocortin (POMC) precursor which contains only one opioid peptide, several opiate-active peptides are derived from proenkephalin. These are (Leu) enkephalin, (Met) enkephalin and potentially several larger opioids. Met Enk and Leu Enk differ in their amino acid sequence with regard to the C-terminal amino acid residue (Bloom, 1983). The NAc has been found to have high concentrations of enkephalinergic terminals, a large number of cell bodies, and receptors.

The distribution of mu (μ), delta (δ), and kappa (κ) receptor subtypes has been revealed using *in vitro* autoradiography (Mansour et al., 1987, Unterwald et al., 1987) in the NAc. Very dense patches of μ binding was detected throughout the nucleus. Approximately one third of μ opioid receptors in the NAc appear to be located on either the presynaptic DA terminals or on the neurons upon which they impinge; κ binding is also dense in this area, specifically at the most ventral aspect of the NAc. However the equally dense δ binding was more diffusely distributed throughout the NAc. These two receptor types are not located on presynaptic DA terminals.

The enkephalin-immunoreactivity appears to be more homogeneous with a preferential distribution to the ventrolateral area. The patchy distribution appears in more rostral sections. The most dense fluorescence, is seen in sections adjacent to the anterior commissure (Elde et al., 1976; Hong et al., 1977; Sar et al., 1978; Goodman et al., 1980; Pickel et al., 1980; Wamsley et al., 1980).

The distribution of the dynorphins has recently been elucidated by both biochemical (Zamir et al., 1984; Cone et al., 1983) and anatomical (Fallon et al., 1985) techniques. More recently, Fallon and Leslie (1986) mapped the distribution of dynorphin and enkephalin peptides in rat brain. Dynorphin B, Dynorphin A (1-8), and the proenkephalin precursor met-enkephalin-arg-gly-leu (MERGL) immunoreactivity was localized in cell bodies within the NAc. Staining was moderate compared to adjacent areas. Medially and dorsally, heavy-stained fibers in the region of the striohypothalamic tract and the diagonal band, ventrally, were located. This dense distribution of labled fibers swept laterally to the large Islets of Calleja to meet the lateral and ventral septum and the bed nucleus of the stria terminalis.

Somatostatin

Isolation and purification studies (Brazeau et al., 1973) have characterized this molecule as a tetradecapeptide, with disulfide bridges between Cys 3 and Cys 14. Both cell bodies, along the entire rostral-caudal extent, and nerve fibers are present at the level of the NAc in moderate amounts. Immunoreactive nerve fibers are localized in the middle and caudal regions of the nucleus. A patchy appearance characterized by a high immunoreactivity mixed with medium dense areas are found in rostral areas. At its most caudal extent, somatostatin immunoreactive fibers are observed (Finley et al., 1978; Kobayashi et al., 1977; Bennett-Clarke et al., 1980).

Cholecystokinin

Sequenced, synthesized and found to contain 33 amino acid residues, this gut hormone, cholecystokinin (CCK) has also been identified in brain. Using immunohistochemical techniques Loren et al., 1979, localized a narrow band of CCK nerve fibers to the ventral and medial part of NAc. In addition, Hokfelt et al., (1980), found a dense CCK innervation in the caudal region, with the most dense labeling in the dorsal medial aspect. These fibers, Hokfelt and coworkers suggest, coexist together with the DA mesolimbic neurons arising in A10 and innervate, *inter alia*, the NAc.

Cytochrome C Oxidase

Another important property by which the nervous system can be described, is by the localization of endogenous mitochondrial enzymes in neurons. This system is associated with neurons' metabolic machinery, which is related to levels of neuronal activity. Among the energy-deriving enzymes, the cytochromes are responsible for electron transport and oxidative phosphorylation which yields adenosine triphosphate (ATP). Adenosine triphosphate is needed for vital processes such as rapid axoplasmic transport, protein synthesis and maintenance of the resting potential within neurons. Seligman et al., (1986) and Wong-Riley (1979) have exploited these metabolic characteristics of neurons in a simple yet highly specific histochemical paradigm. Wong-Riley has reasoned that a more "active" neuron would engage more vigorously in the above processes and thus would have a more developed cytochrome system. It could also be reasoned that different neuronal groups with varied functional demands or perhaps at different developmental stages may exhibit different levels of cytochrome oxidative activity and that such levels may change when the degree of maintained activity changes.

RATIONALE

To attribute a single neurotransmitter to a behavior as complex as behavioral activation is simplistic, when it has been revealed that (in the adult), this neuroanatomical site utilizes and produces an impressive compliment of neurotransmitters and modulators. The aim of this section is to suggest possible neurochemical substrates for electrical stimulation-induced behavioral activation. A few criteria have been employed in the selection of the particular neurotransmitters used in this research to generate their ontogenetic profiles. It was hoped that the chemical maturation of these systems within the NAc contribute to the decline of behavioral activation seen as the pup matures.

It is possible that the dissappearance of behavioral activation may be due to either neural reorganization, the "uncoupling" of neural circuits, the development of inhibitory systems or the appearance of new and competing behaviors (Moran et al., 1986). Thus not only were the major inhibitory neurotransmitters chosen, but also neurotransmitters that have been previously implicated in subserving behaviors resembling or comprising those behaviors that define behavioral activation.

The nucleus accumbens is a major terminal field of the mesolimbic DA projection system. Evidence indicates that the pars reticulata of the substantia nigra is involved in the motor control of oropharyngeal musculature (Lytle and Kantak, 1987). And it is now established that the mesolimbic DA system also plays an integral role in the regulation of locomotor responses (Anden and Johnels, 1977; Hornykiewicz, 1978; Jones et al., 1981; Joyce et al., 1981) and a parallel may exist between the stimulation induced behavioral activation observed in the neonate and the functional role of mesolimbic system in the adult. When administered in low or moderate doses in rat, d-amphetamine (d-amp) augments locomotor activity and stereotyped behavior (see Cole, 1978 for a review). This d-amp-induced locomotor activation has been suggested to be mediated by an increase in DA neurotransmission, localized to the NAc (Kelly et al., 1975; Pijenburg et al., 1976; Costall et al., 1977). Direct injection of DA agonists into the NAc (Jackson et al., 1975; Makanjiola et al., 1980), as well as stimulation directed towards the NAc (Costall, 1984) produces strong locomotor activation. The locomotor-stimulant effect of d-amp is antagonized by neuroleptics locally injected into the NAc (Jackson et al., 1975; Pijnenburg et al., 1975).

In a number of studies, the mesolimbic DA pathway was damaged or blocked by pharmacological procedures. Injections of the DA antagonist, spiroperidol, into the NAc resulted in oral-motor deficits (Jones and Mogenson, 1979). Injecting spiroperidol into the NAc was also reported to attenuate locomotor activity (Mogenson and Yim, 1981).

Additionally, a number of studies utilizing infant rats suggest a role for the DA system in the mediation of behavioral activation (Sobrian et al., 1975). The stimulation of the mesolimbic component of the MFB at the level of the lateral hypothalamus, reliably elicited behavioral activation in the neonate, but failed to do so in the adult (Moran et al., 1983). Stimulation of the NAc but not the caudate elicits behavioral activation in 3-day-old pups; both DA agonists and antagonists modulate the stimulation-induced behavioral activation (Lee, 1988).

A role for 5-hydroxytryptamine (5-HT, serotonin) has also been suggested in the mediation of locomotor activity as well as oral-motor behaviors. Fibiger and Campbell (1971) have presented evidence for a neural inhibitory system mediated by 5-HT (in addition to one mediated by acetylcholine) in the modulation of arousal in the adult. Depletion of brain 5HT was found to produce a marked and prolonged increase in locomotor activity. This outcome could be reversed by the administration of 5-hydroxytryptophan, the precursor of serotonin. These findings were confirmed by Mabry and Campbell (1984), who demonstrated that, in the neonate, decreases in serotonin first enhanced locomotor behavior at around 15 days of age, suggesting that a serotonergic inhibitory system becomes functional as the brain matures.

Ontogenetic differences in attenuation of mouthing behavior was observed in the neonate as a result of treatment with serotonergic antagonists (Enters and Spear, 1985). In 3-4 day old pups, the 5-HT agonist, quipazine increased mouthing, whereas, methysergide decreased mouthing. At 10-11 days postpartum, both pharmacological agents decreased milk-induced mouthing.

There is good evidence to support the notion that GABA is the most widespread inhibitory amino acid transmitter localized within the mammalian nervous system. Early maturing GABAergic systems have been suggested to play a role in behavioral

inhibition in the young organism (Murphy et al., 1979; Reitzel et al., 1979). In order to delineate whether signs of GABAergic inhibition of behavior are seen in the preweanling pup, Spear and coworkers (1986) investigated the direct action of the antagonist, picrotoxin. They report a significant induction of behavioral activation to picrotoxin and an attenuation in mouthing behavior after administration of muscimol, a GABA agonist. These data support their suggestion that the GABAergic inhibition of behavior is functional at early postnatal periods. These data, along with the 5-HT data, suggest to these authors that there may be a GABAergic inhibitory component as well as a serontonergic (5-HT) facilitatory system modulating mouthing behavior during the early postnatal period.

Endogenous opioid peptides have been implicated in the physiological processes that are responsible for the regulation of ingestive behaviors. Administration of opioid receptor agonists or antagonists produce relative elevation or attenuation of food intake, respectively. The κ receptor has been implicated in the physiological processes that are responsible for the regulation of ingestive behaviors (for review see Morley et al., 1983; Sanger, 1983; Cooper et al., 1985). In addition, a possible role of the NAc in mediating alterations in locomotor activity, produced by various opioids has been suggested. Locomotor depression and catalepsy may be mediated by opioid receptors of the μ-type, whereas locomotor stimulation appears to be mediated by the δ-type receptors (Havemann and Kuschinsky, 1985).

A remaining unresolved issue is the relationship between behavioral activation in the preweanling rat and locomotor activity and facial reflexes involved in feeding in the adult. Behavioral activation may represent the components of as-of-yet unorganized motivational systems. With increasing age, however, these behaviors become bound to sensory input from a goal object (MacDonnel and Flynn, 1966). Behaviors that are lacking in organization at the height of activation may come to resemble the response patterns of adults. Locomotor activity may be one of the fundamental components of food procurement, nest building and other biologically significant behaviors. Accordingly, it might be expected that the NAc contributes to drinking, thermoregulation and other goal directed behaviors.

There is another aspect of ingestive behavior in which the NAc maybe involved, the consummatory phase. This phase includes such motor movements as lapping, chewing and swallowing (Craig, 1918). These motor movements are similar

to those constituents that help define behavioral activation in the neonate. These behaviors may, in the adult comprise the sequential stages of response initiation, procurement of food or water, followed by the consummatory phase. Evidence to support this suggestion comes from those studies in which 6-hydroxydopamine or spiroperidol injections were made towards the NAc. Measurements of tongue extension and lap volume were made. Both treatments significantly reduced these behaviors (Mogenson and Kucharczyk, 1978).

In addition to the proported contribution that these neurochemicals may play in the mediation of this behavior, a second question arises, that is; how do these neurotransmitters interact to express this behavior?

The proposed research attempted to trace and compare the normal progessive neurochemical and anatomical changes in the infant to those in the adult by exploring the development of innervation patterns of 5-HT-, met Enk-, and GABA-like immunoreactivity, Cytochrome C Oxidase activity, and Bodian Silver stain.

As well, the proposed research explored the changing temporal and topographic patterns of stimulation-bound activity that may be the primitive anteceedent to goal directed exploratory behavior seen in adults. That the NAc continually functions at birth to adulthood, as a "motivational-emotional" director of behavior remains to be investigated (Mogenson and Yim, 1981). This sequence of experiments was an initial attempt to study changes in oxidative metabolism, cytoarchitecture and neurotransmitter distribution of the NAc during the time that this locus becomes unresponsive to stimulation.

MATERIALS AND METHODS

SUBJECTS

Experimental subjects were the offspring of Long-Evans hooded rats mated and bred in our colony. Litters were housed in plastic tubs with stainless steel wire lids on a substrate of pine shavings. The colony was maintained on a 12 hour light/dark cycle, under constant temperature and humidity. Purina rat chow and water was available *ad libitum*. Cages were checked twice daily. When pups found, they were termed 0 days of age. Three days following parturition, litters were culled to 12.

BEHAVIORAL ANALYSIS

Pups aged 3, 5, 7, 10, and 14 days served as subjects. An individual litter was used at one age and contributed only two pups. An average of nine pups per age group was tested. Mean weights for each age was 3-d-o: 9.87 g; 5-d-o: 13.79 g; 7-d-o: 17.88 g; 10-d-o: 22.81 g; and 14-d-o: 27.96 g. Thirty minutes prior to surgery, experimental pups were removed from their mothers, weighed, numbered and placed with littermates in an incubator maintained at 32 ^{o} C.

ELECTRODE IMPLANTATION

Teflon-coated stainless steel wires (.125 mm, Plastic Products Company) bared at their tips served as chronic bipolar electrode units. The bipolar electrodes were mounted in plastic frames (8 mm in length). Prior to surgery, electrodes were precut to the proper dorsal-ventral coordinates for each age. All rats were anesthetized by methoxyflurane (Metofane; Pitman-Moore Inc.) inhalation.

Following a midsagital scalp incision to expose the skull, a hole was drilled through the skull, but not the dura, for the electrode. The pups were placed in a stereotaxic device modified for neonatal surgery by the addition of a neonatal head holder (Heller et al., 1979). The apparatus held the head by means of a mouth bar anteriorly and a recurved needle inserted into the foramen magnum posteriorly. The head was maintained in the horizontal position with lambda and bregma at equal heights. Electrodes were aimed at the NAc. All placements were made to the left side of the brain. For operating coordinates, the anterior-posterior coordinate (AP)

refers to the distance from bregma, the medial-lateral dimension (ML) refers to the distance from the midline, and the dorsal-ventral (DV) refers to the distance ventral to the dural surface. The stereotaxic coordinates for each age were day 3; +0.1 mm AP, +0.7 mm ML and -2.7 mm DV; day 5; +0.2 mm AP, +0.8 mm ML and - 4.3 mm DV; day 7, +0.2 mm AP, +0.9 mm ML and -4.8 mm DV; day 10, +0.3 mm AP, +1.1 mm ML and -5.0 mm DV; day 14, +0.4 mm AP, +1.3 mm ML and -5.3 mm DV (modified from the atlas of Sherwood and Timaris, 1970)). The electrode was lowered after the dura was gently incised. The unit was secured with cement (caulk grip cement and standard dental acrylate) to the skull.

Following surgery, pups were placed in individual plastic containers and placed in an incubator. Temperature in the incubator was maintained at 32 ^{o} C. The pups appeared fully recovered the day after surgery. All pups were tested approximately 18 hrs after electrode implantation. Stimulation was delivered at approximately the same time each day to minimize the variation due to circadian periodicity.

TEST PROCEDURES

Pups were individually tested 18 to 24 hrs postoperatively in a felt-covered open-topped Plexiglas box (17.5 x 17.5 x 8.5 cm) housed within the test chamber maintained at 32 ^{o} C by a heat lamp. The stimulation apparatus administered stimulation trains of 480 msec pulsed biphasic current. A train consists of 30 pulses, 2 msec in length. Current was determined by the voltage drop across a 10 k resistor. The current that was administered was 60 μA. Pups that did not respond at this current were not used. The stimulation parameters (such as pulse frequency, train duration, stimulus intensity and stimulation schedule) used in this study were similar to those found effective in self-stimulation paradigms and stimulation induced activation studies (Moran et al., 1981; Lee, 1988). After a 5 min adaptation period and a 2 min baseline observation period, behavior was observed over three one-minute stimulation periods. Stimulation was delivered once every 10 sec during the stimulation minute. Each of these three periods was followed by a one-minute non-stimulation period. The occurrence of all behaviors was recorded every 10 sec.

The behavioral categories included those behaviors that define the state called behavioral activation (Hall, 1979 a and b). The categories were:

mouthing -- rapid oral movements, including chewing during which there was notongue extension
licking -- extension of the tongue outside of the mouth
pawing -- moving the forepaw across the snout or head
probing -- extension of the head with the neck flexed
locomotion -- movement of at least two limbs in any direction
stretch -- extension of the forepaws and hindpaws
rolling -- turning on the side or back, and then returning to the upright position

Age and session effects (ie., baseline, stimulation and interstimulation minutes) were compared by means of a two way factorial analysis of variance for each behavior for all ages. If the overall tests for main effects were significant, then further manipulation of the data to find the source of that effect was done. The multiple comparison post hoc test of all means posed by Tukey was used (Kirk, 1968).

ELECTRODE VERIFICATION

Following behavioral testing, all pups were sacrificed by an overdose of metofane. The brains were perfused via intracardiac infusion of 0.9% saline and 10% cold formalin. Saline and fixative flowed at a constant rate with a perfusion pump (Cole-Parmer Instrument Co.). Brains were blocked just rostral and caudal to the NAc and were immersed in the fixative and 30% sucrose solution for at least 24 hrs. Cryostat sections of frozen tissue (30 μm thick; -20 ^{o} C) were stained with cresyl violet. Histological analysis to verify electrode tip placement was made independent of the behavioral data.

HISTOLOGICAL and HISTOCHEMICAL TECHNIQUES

Rat pups were anesthetized by metofane inhalation and adults were anesthetized with 14% chloral hydrate and then perfused through the heart with

buffered 0.9% physiological saline (pH. 7.4) followed by cold 4% paraformaldehyde sodium phosphate buffer. All perfusions were done with a perfusion pump (Cole-Parmer Instrument Co.). Brains were removed and immersed overnight in the same fixative containing 30% sucrose. Brain sections (30μm thick) were cut on a cryostat at -20 ^{o} C. Two adjacent sections were placed in test tubes containing phosphate buffer saline (PBS; 0.1M, pH. 7.2) in preparation for immunostaining. The next adjacent section was stained with cresyl violet for nuclear identification and every fourth section was mounted and stained for neurofilaments (Bodian) or placed in PBS for cytochrome oxidase.

BODIAN SILVER STAIN

The bodian silver stain procedure is quite selective for axons. The intensity of the staining is correlated with the density of neurofilaments as a result of specific staining for neurofilament polypeptides (Gambetti, et al., 1981).

Animals were perfused according to the previously described procedures. Slide mounted tissue sections (30 μm) were incubated for 24 hrs at 37 ^{o} C in a solution of 1% silver albumose (Protargol-S; Sterling Organics) and copper foil (6g/100ml). Following rinsing, preparations were stained according to the following schedule: reduced for 15 min in a 1% hydroquinone/10% commercial Formalin solution; toned in 0.2% gold chloride for 10 min; developed in a 2% oxalic acid solution; fixed in 5% sodium thiosulfate for 10 min; and counterstained with Van Gieson's solution. Rinses were in at least 6 changes of distilled water and all steps were at room temperature. Localization of neurofillaments was made at the light microscopic level.

CYTOCHROME C OXIDASE

The fixation procedure for this technique was identical to that for immunocytochemistry. Staining was done on sections (30 μm) free floating in PBS. Brain tissue was incubated in a solution (S) of 2.0g of sucrose, 16.7 mg of Cytochrome C (Sigma, type III), and 3.3 mg of .03% 3.3' diaminobenzidinetetrahydrochloride (DAB; Polysciences) in 50 ml of PBS at 37 ^{o}C for 1 to 6 hrs. When the desired staining intensity was reached, sections were

mounted on chrome alumgelatine slides, dehydrated, rinsed in xylene, and coverslipped. Localization of metabolic activity was made at the light microscopic level.

IMMUNOCYTOCHEMISTRY

For all immunoreactions the unlabelled antibody peroxidase-anti-peroxidase (PAP) technique of Sternberger was used (1974). Sections were washed in PBS for 5 min and subsequently incubated for 30 min in PBS containing 3% normal goat serum (GS) and 0.4% Triton-X-100 (T-X-100). The sections were incubated for 24 hrs in the primary antisera diluted 1:20,000 (5-HT, Immuno Nuclear group); 1:500 (Enk, Immuno Nuclear Group); and,1:5000 (GABA; Chemicon). Using 2 washes of 1% GS-PBS-T-X-100 between incubations the tissue slices were incubated consecutively for 30 min in each of the following: goat-anti-rabbit serum (GAR, 1:100, Antibodies Inc., p4 fraction) and PAP (1:200, Sternberger Myer). All incubations were performed in the presence of 1% GS-PBS-T-X-100. After incubations, all sections were rinsed in straight PBS (omitted for GABA) for 10 min, followed by 0.05 M Tris buffer (pH. 7.6), for 10 min, and then stained with 0.03% 3.3' diaminobenzidinetetrahydrochloride (DAB; Polysciences) in 0.05 M Tris buffer and 0.003% H_20_2 for 18-20 min or until the desired intensity was achieved.

The fixation protocol for GABA differed from the standard procedure described above in the following way. Following anesthesia, rats were perfused with 0.9% physiological saline, (pH.7.4) followed by 0.5% glutaraldehyde in 4% paraformaldehyde. Brains were left *in situ* for 1 hr before being removed from the skull. The brains were immersed in the same fixative and 30% sucrose for at least 24 hrs. Sections 30 µm thick were cut on a cryostat and collected in PBS (pH. 7.2). Sections were incubated for 30 minutes in 1% sodium borohydride in PBS at room temperature to erase non-specific immunostaining (Kosaka, et al., 1986). In addition, all reagents containing T-X-100 were omitted to enhance cell body staining. At this point, GABA tissue was subjected to the standard PAP procedure.

All incubations were performed at room temperature during vigorous shaking. Following the labeling reaction, the sections were washed in PBS followed by alcoholic gelatin, mounted on glass slides coated with chrome alumgelatin to prevent detachment of the sections during the dehydration procedures. Sections

were dehydrated through graded ethanols rinsed in xylene, and coverslipped. Localization of immunoreactive neurons was made at the light microscopic level.

SPECIFICITY CONTROLS

To control for immunocytochemical specificity, GS and buffer (1% GS-PBS-T) was routinely substituted for specific antisera in all experiments, Blocked (absorbed) antisera was also examined in order to evaluate the selectivity of the immunostain for Enk and 5-HT. Anti-Enk or Anti-5-HT, at their working dilution was incubated with met-Enk (100 μM) or 25 μM for 5-HT overnight at 4 ^{o}C and the ability of the antisera to stain tissue was tested.

Control sections for Cytochrome C Oixdase, from comparable levels, were incubated in S with 0.1M potassium cyanide (KCN) at 37 ^{o}C for approximately 2 hrs in the dark. Potassium cyanide is an inhibitor of mettalloenzymes, especially the heme-enzymes. After incubation, control and normal sections were rinsed in PBS and alcoholic gelatin, mounted on chrom alumgelatin subbed microscope slides, dehydrated, rinsed in xylene, and coverslipped (Wong-Riley, 1976).

Different ages were run at the same time to ensure that the absence of staining was only a function of age and not any methodological flaws. For all procedures and all ages, anatomically-matched sections were selected at 2 levels along the rostral/caudal extent of the NAc. All tissue was qualitatively rated for immunoreactivity and oxidative activity.

PHOTOMICROSCOPY

There are technical limits to these procedures as there are with most methods. The photomicrograph comes close to an actual representation of specimen-fixed slides (J. Gordon, personal communication). A proportion of information is lost from the photomicrograph as the image to recover is passed through several optical systems. This lost value can be calculated from the optical transform function: First, information must pass through the physical components of the eye; causing an initial loss of information. This is further compounded when the optical transform function of the camera is considered. Moreover, the process of black and white photomicroscopy has an additional appropriate optical transform function. Thus,

while the photomicrograph is a routinely accepted and respected anatomical procedure, it represents a somewhat less than ideal guide to evaluating results. Therefore data was generated by analyses of the actual slide mounted sections.

RESULTS

BEHAVIORAL ACTIVATION

Electrical stimulation directed towards the NAc elicited a dramatic and reliable change in behavior in 3-, 5-, 7-, and 10-day old pups. In response to stimulation, pups reliably emitted those behaviors that define the state of behavioral activation. An overall activity score was generated for each age group and is presented in Table 1. The prominent behaviors that were observed were mouthing, licking, pawing, probing and locomotion.

Table 1. Behavioral activity scores for pups with and without locomotion.

	ACTIVITY	
	WITH LOCOMOTION	WITHOUT LOCOMOTION
AGE	MEAN ± SEM	MEAN ± SEM
3-d-o	9.4 ± 1.13	6.5 ± 0.92
5-d-o	10.22 ± 2.69	8.1 ± 2.42
7-d-o	9.87 ± 1.34	4.5 ± 1.10
10-d-o	13.90 ± 2.20	11.0 ± 2.02
14-d-o	7.70 ± 1.14	0.0 ± 0.0
	F(4,42)=1.55 p<.205	F(4,42)=7.49 p<.001

MOUTHING

Two way ANOVA revealed significant main effects for both variables of interest, age: $F(4,42)=6.20, p<.0005$ and session: $F(2,84)= 63.29, p<.0001$, as well as significant interaction effects, $F(8,84)= 4.23$, $p< .0003$. The data are presented in Figure 3 and were analyzed by post hoc tests. Three-, 5-, 7-, and 10-day -old pups exhibited significantly more mouthing behaviors than did 14-day-olds during stimulation minutes.

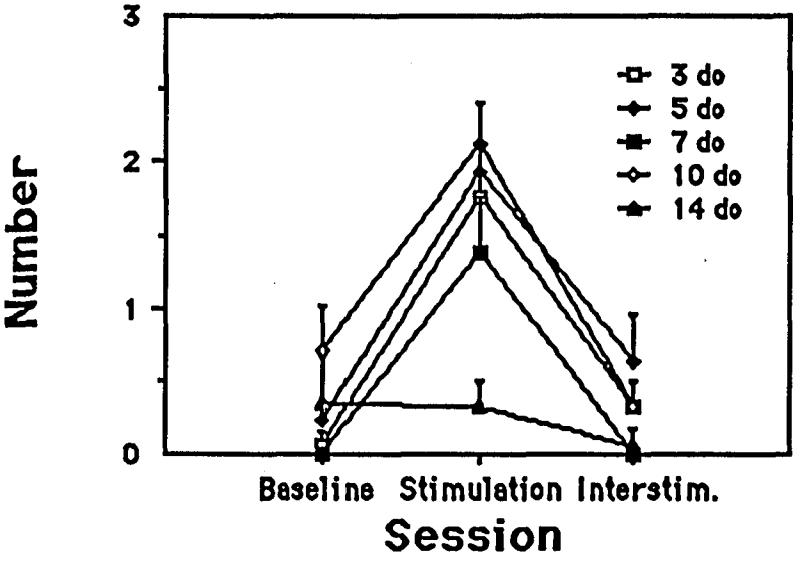
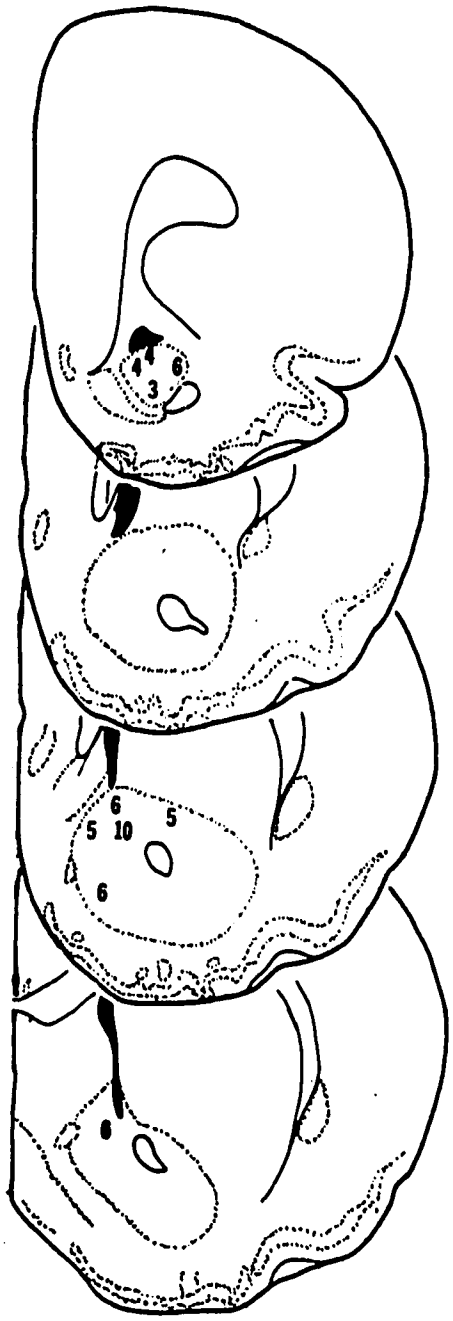


Figure 3. Mean and + SEM of the number of occurrences of mouthinng behavior plotted as a function of session

Figures 4 to 8 represent the number of mouthing behaviors observed in response to the 3 stimulation minutes for each animal at each age. Each number represents a single electrode placement for that animal. This presentation of the data reveals no site specificity for this behavior.

Figure 1. Diagramatic representation of stimulation effects on mouthing behavior in 3-day-olds. The number to the right of the coronal sections corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the number of mouthing behaviors observed from that electrode placement.



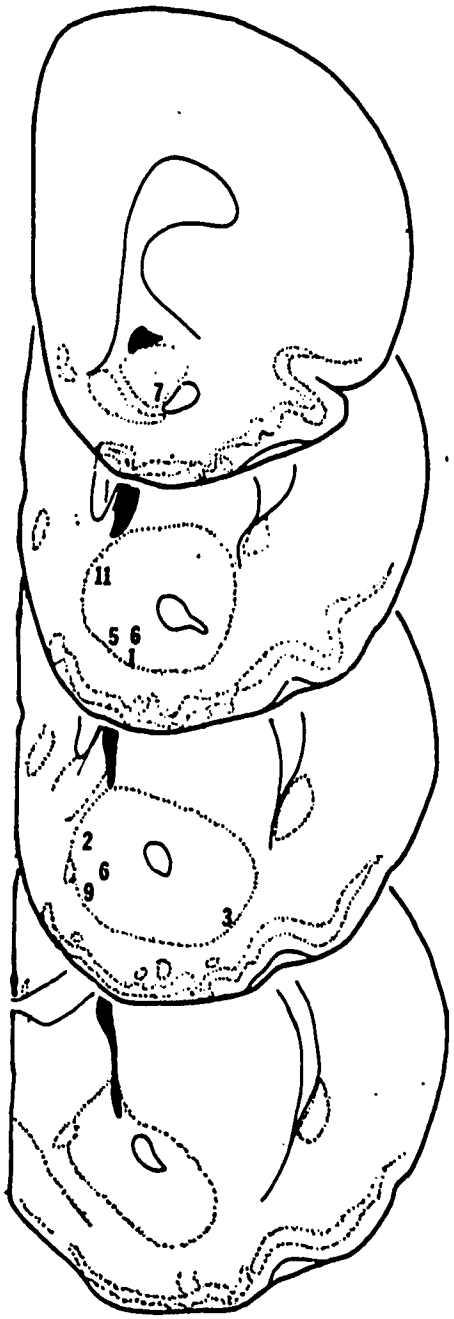
7.0

6.5

6.2

5.9

Figure 5. Diagramatic representation of stimulation effects on mouthing behavior in 5-day-olds. The number to the right of the coronal sections corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the number of mouthing behaviors observed from that electrode placement.



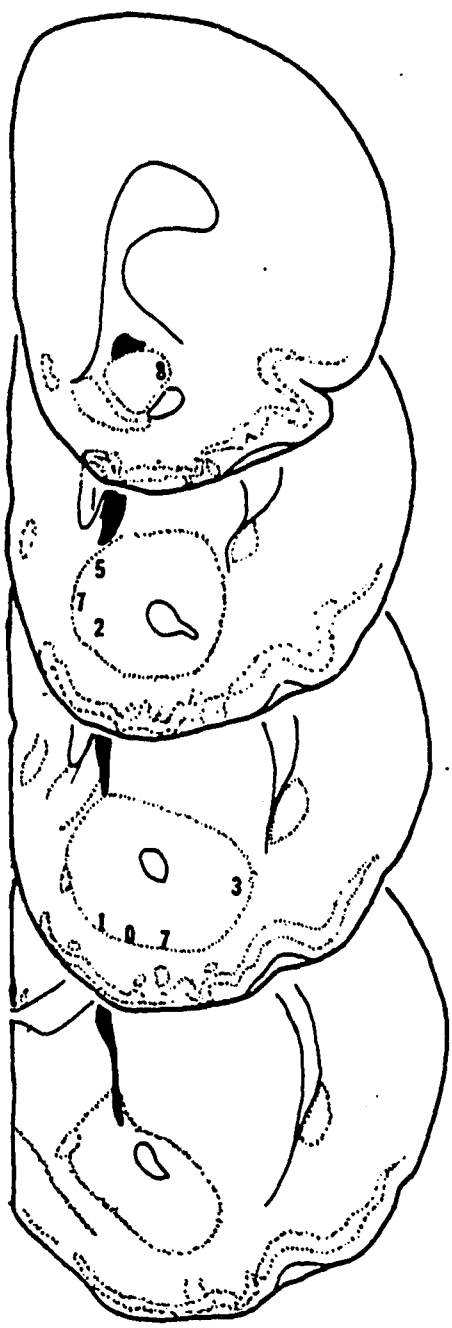
7.0

6.5

6.2

5.9

Figure 6. Diagramatic representation of stimulation effects on mouthing behavior in 7-day-olds. The number to the right of the coronal sections corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the number of mouthing behaviors observed from that electrode placement.



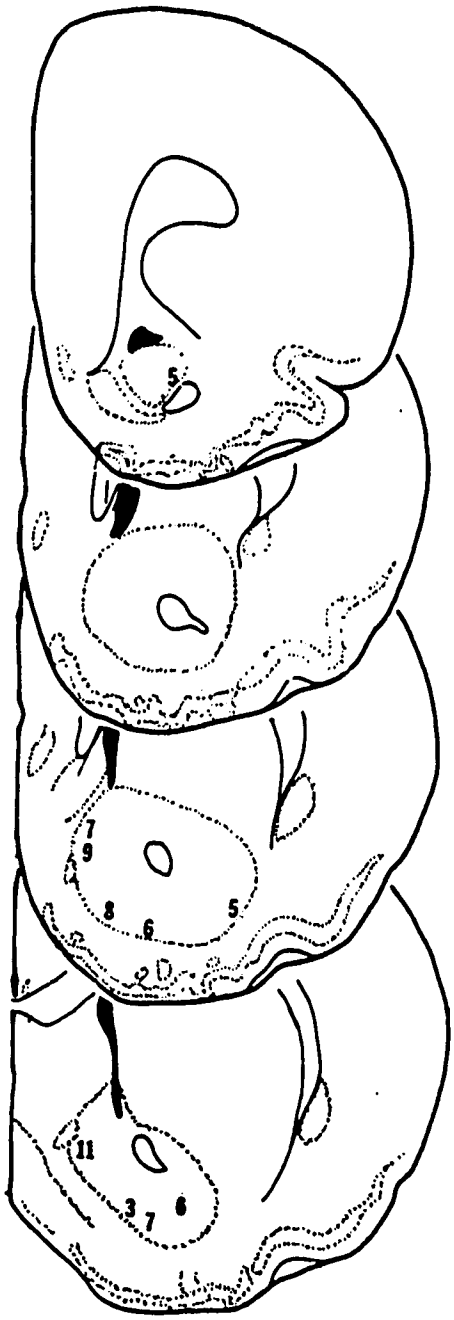
7.0

6.5

6.2

5.9

Figure 7. Diagramatic representation of stimulation effects on mouthing behavior in 10-day-olds. The number to the right of the coronal sections corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the number of mouthing behaviors observed from that electrode placement.



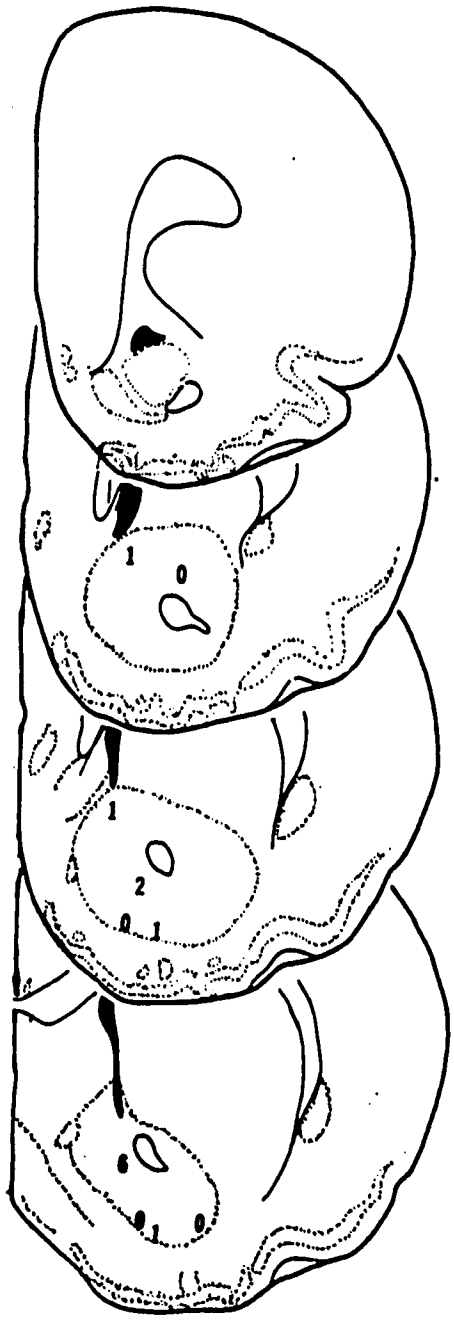
7.0

6.5

6.2

5.9

Figure 8. Diagramatic representation of stimulation effects on mouthing behavior in 14-day-olds. The number to the right of the coronal sections corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the number of mouthing behaviors observed from that electrode placement.


7.0
6.5
6.2
5.9

LICKING

The data for licking are presented in Figure 9. There were no significant age effects but there was a significant session effect, $F(2,84)=5.82, p < .0043$. Post hoc tests revealed significant differences between stimulation and baseline minutes.

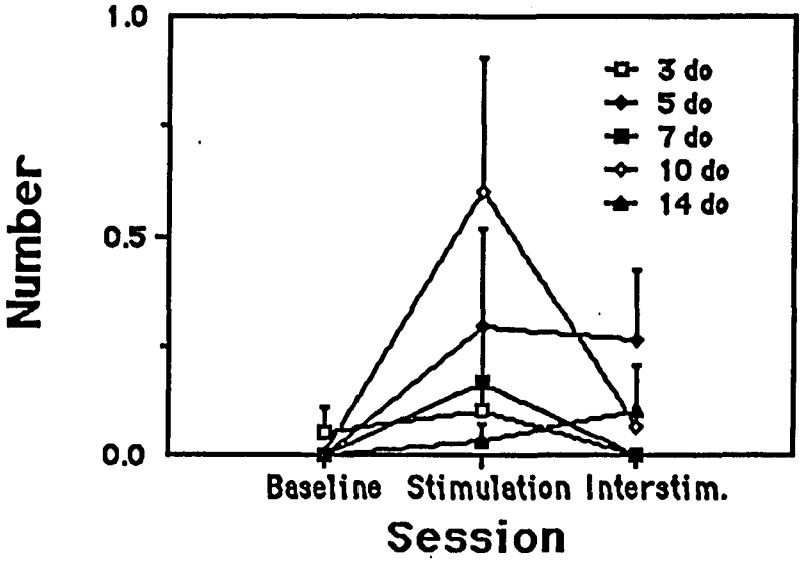


Figure 9 . Mean and +SEM of the number of occurrences of licking behaviors plotted as a function of session.

PAWING

The pawing data are presented in Figure 10. Analysis of variance revealed significant age, $F(3,35)=4.83 p<.006$ and session effects $F(2,70)=6.34, p<.0029$, as well as significant interaction effects, $F(6,70)=2.81, p < .016$. Post hoc analysis of the data indicates that there are no session effects for 3- and 14-day-olds. Five- and 10-day-olds did show significant session effects.

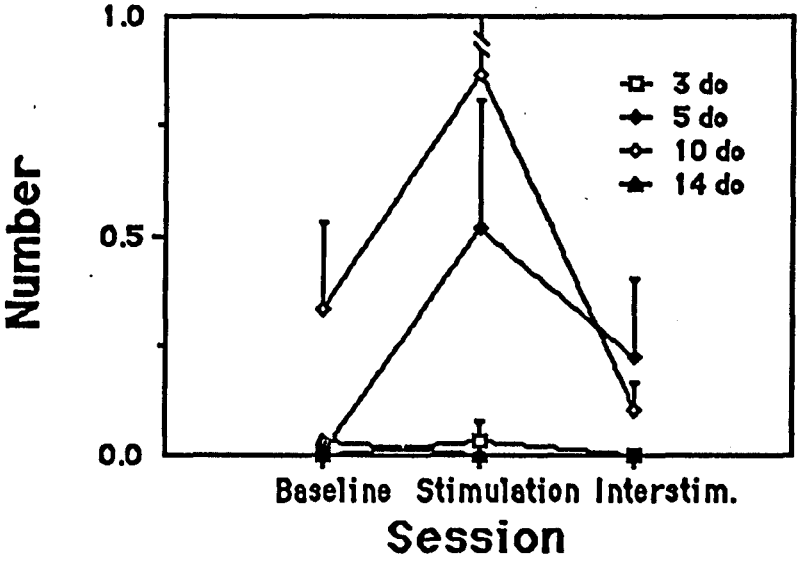


Figure 10. Mean and +SEM of the number of occurrences of pawing behaviors plotted as a function of session.

PROBING

Data for probing behavior are presented in Figure 11. Analysis of variance on these data reveals a significant session effect, $F(2,70)=7.62, p<.001$. Post hoc analysis reveals significant differences between stimulation, interstimulation and baseline minutes.

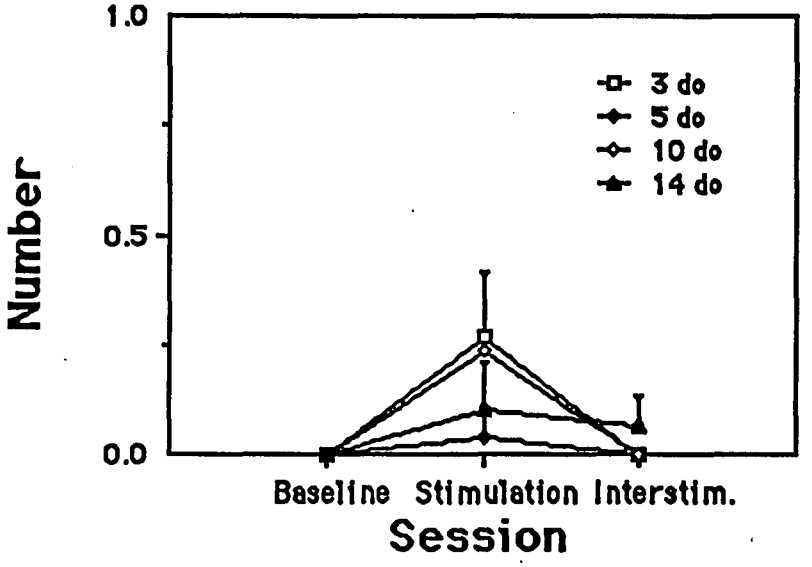
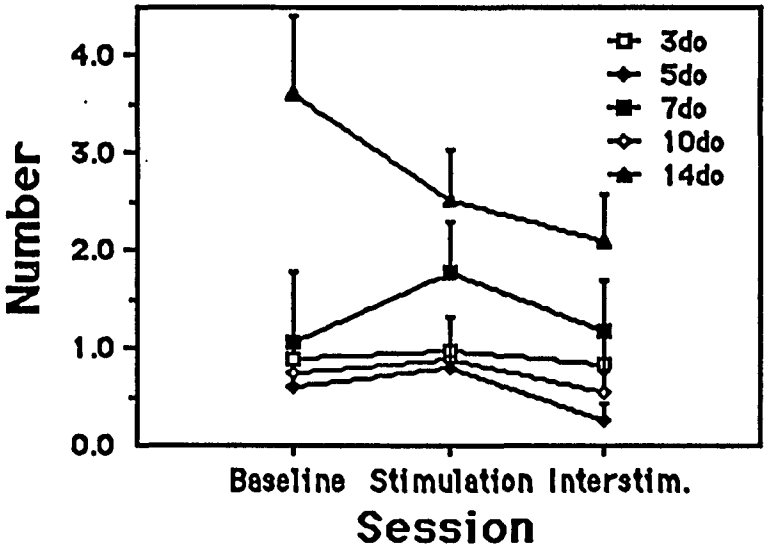


Figure 11. Mean and +SEM of the number of occurrences of probing behaviors plotted as a function of session.

LOCOMOTION

Figure 12. shows the locomotor data. In contrast to the other behaviors analyzed, 14-day-olds exhibited higher rates of locomotor activity over all three sessions. Analysis of variance ($F(4,42)= 7.56$, $p<.0001$)) show significant age effects with the data for 14-day-olds significantly different from 3-, 5-, 7-, and 10-day-olds. Analysis of variance also revealed session effects, $F(2,84)=3.72$, $p< .02$. Further analysis of the data with Tukey's post hoc tests reveals that the differences are found between baseline and intersimulation minutes.



Fiigure 12. Mean and +SEM of the number of occurrences of locomotion behavior plotted as a function of session.

Pups 14 days of age were unresponsive to stimulation administered to NAc. Grooming was the predominant behavior at this age and did not appear to be concordant with stimulation. During testing sessions, 14 day old pups would either explore the test box, try to escape from the test box and/or groom. Grooming was not broken down into its components , ie. mouthing licking and pawing (refer to Table 1).

HISTOLOGICAL ANALYSIS

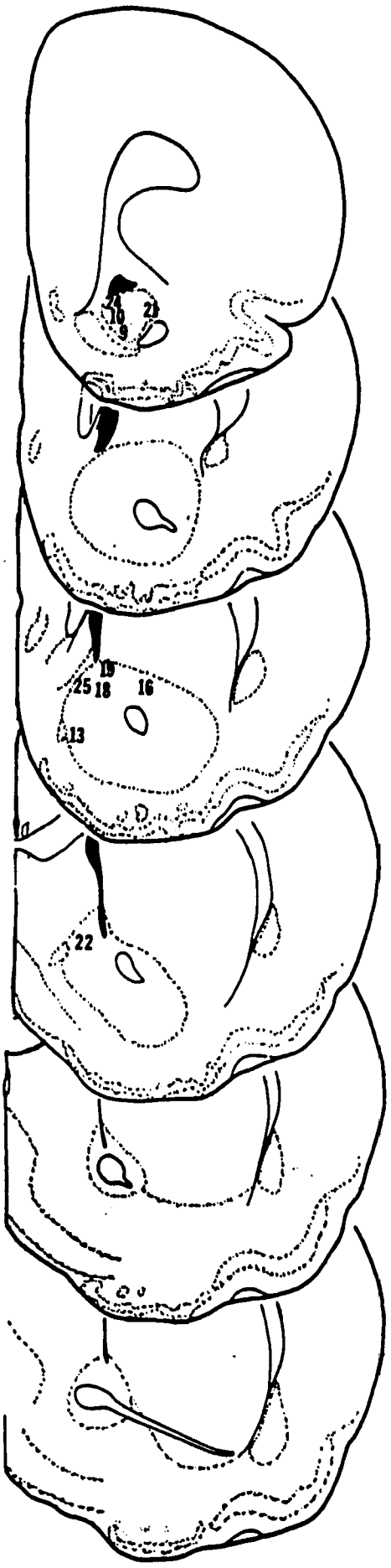
Histological analysis of electrode placement revealed a highly localized distribution of sites within the NAc. As depicted in Figures 13 to 17, the electrode tract was typically situated medial-ventral to the lateral ventricle. Pups that did not respond during stimulation trials were generally found to have electrode placements outside of the NAc, with electrode tract termination sites usually being too far rostral.

NISSL STAIN

The cytoarchitectonic organization of the NAc in the developing rat is illustrated in the series of photomicrographs of Nissl-stained coronally sectioned tissue in Figure18 through 27.

At all ages a dense aggregation of cells was seen throughout the rostral/caudal extent. At very rostral sections there was a very densely stained cap of cells over the NAc. The pattern of Enk-like immunoreactivity followed this Nissl cell-dense organization. At caudal levels the Islets of Calleja appeared as a dense collection of tightly packed cells. There was a distinct abscence of cell bodies along the medial ventral border, the zonula limitan of Johnston, separating the septal nuclei, DBB and the OT form the NAc. The boundary between the NAc and caudate/putamen was much more difficult to discern. Staining in the one day old was very similar, except for the absence of the Islets of Calleja at caudal levels, which first apppeared at 3 days of age.

Figure 13. Histological summary of electrode placement in 3-day-old rat pups. The number to the right of the coronal section corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the terminal area of the electrode tract when stimulation was behaviorally activating.



7.0

6.5

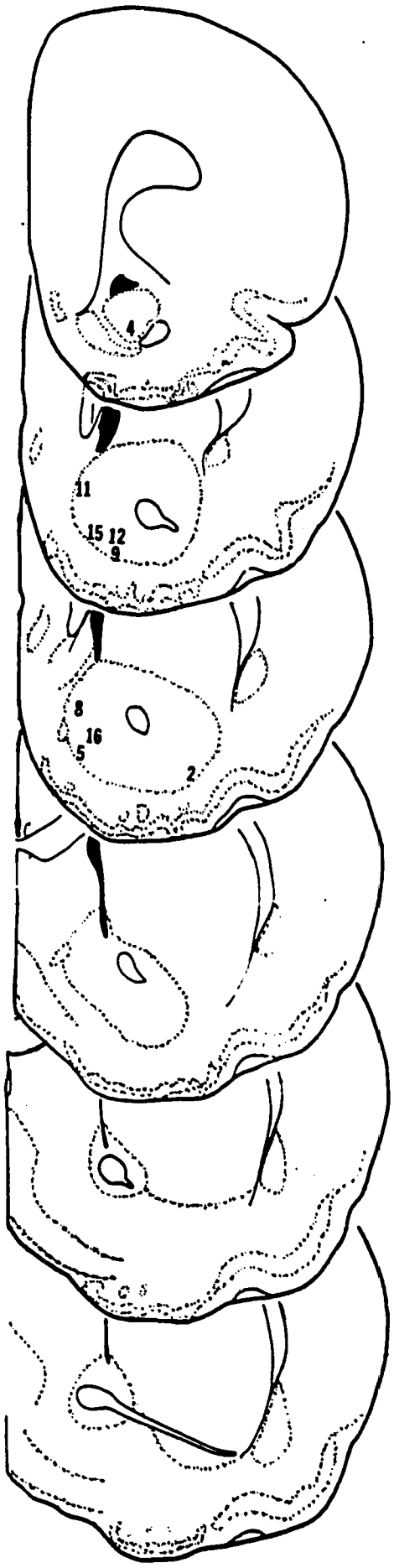
6.2

5.9

5.6

5.3

Figure 14. Histological summary of electrode placement in 5-day-old rat pups. The number to the right of the coronal section corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the terminal area of the electrode tract when stimulation was behaviorally activating.



7.0

6.5

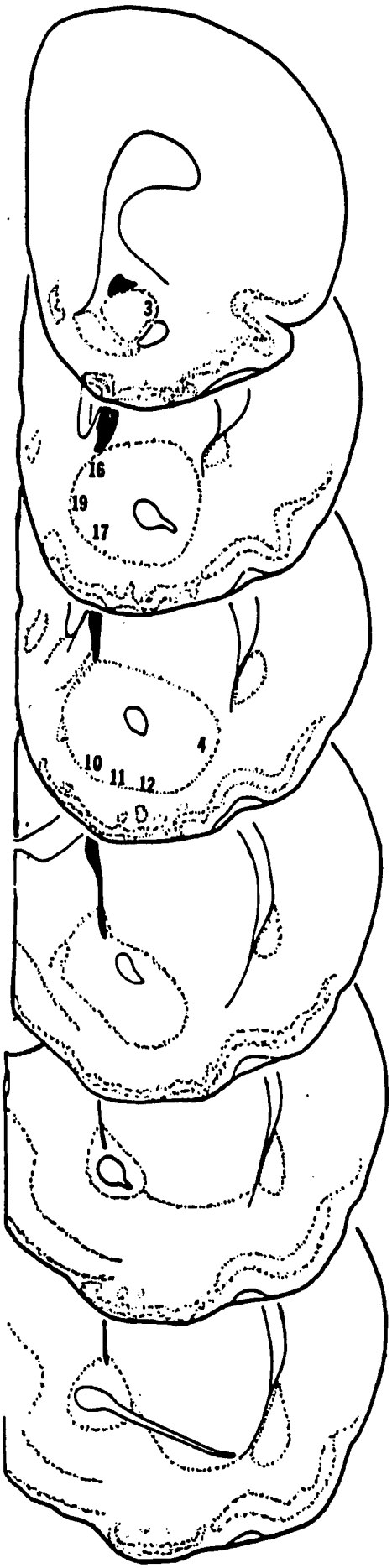
6.2

5.9

5.6

5.3

Figure . Histological summary of electrode placement in 7-day-old rat pups. The number to the right of the coronal section corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the terminal area of the electrode tract when stimulation was behaviorally activating.



7.0

6.5

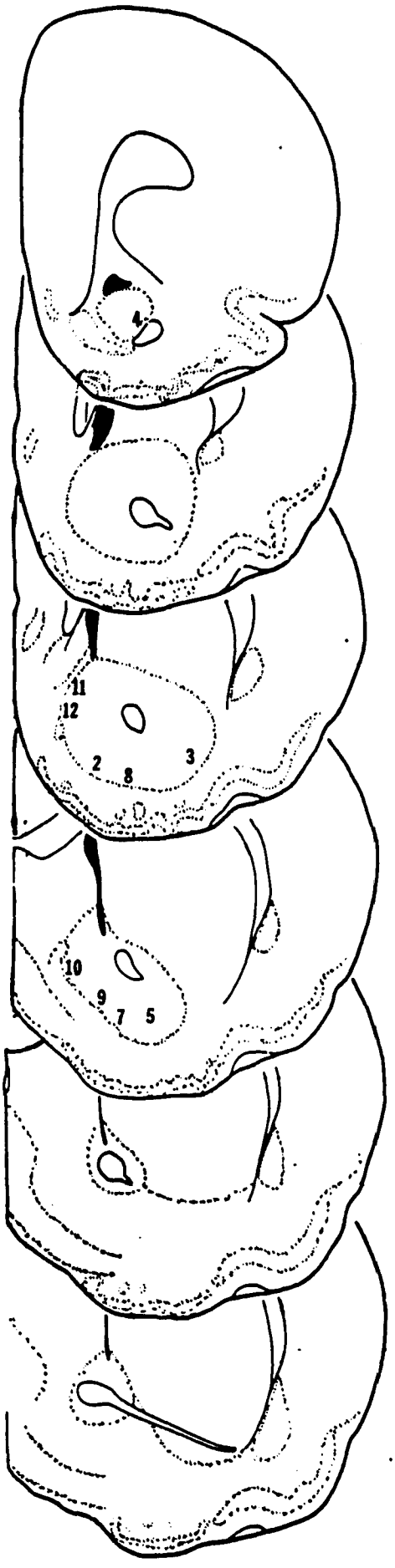
6.2

5.9

5.6

5.3

Figure 16. Histological summary of electrode placement in 10-day-old rat pups. The number to the right of the coronal section corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the terminal area of the electrode tract when stimulation was behaviorally activating.



7.0

6.5

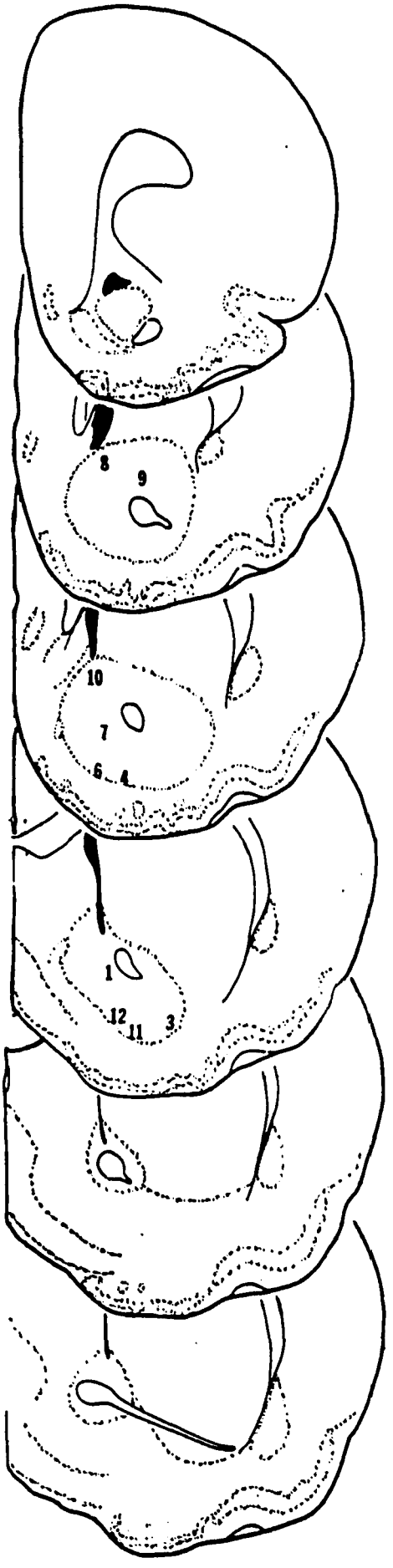
6.2

5.9

5.6

5.3

Figure 17. Histological summary of electrode placement in 14-day-old rat pups. The number to the right of the coronal section corresponds to the anterior-posterior coordinates from the Sherwood and Timiras atlas for 10-day-olds. The number located within the coronal section represents the terminal area of the electrode tract when stimulation was behaviorally activating.



7.0

6.5

6.2

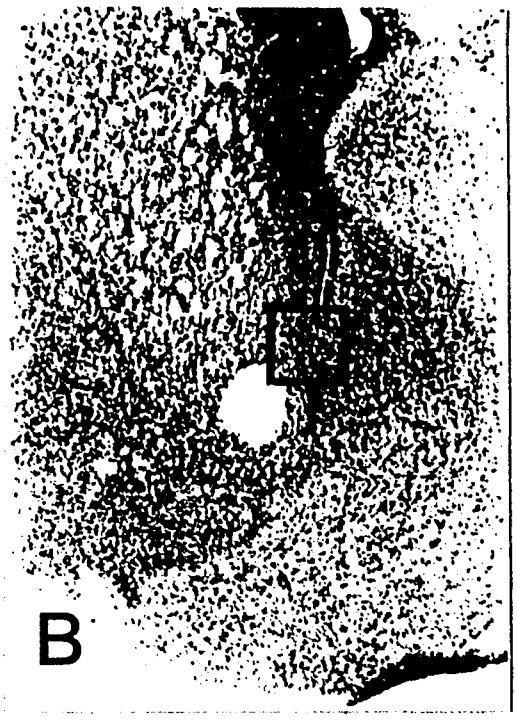
5.9

5.6

5.3

Figure 18. Photomicrographs of 1-day-old Nissl stained coronal sections through the NAc taken through rostral (A) and caudal (B) levels. Bar = 500μm. The framed area in (B) is shown at higher magnificacion in (C). Bar = 200μm.


A


B


C

Figure 19. Photomicroghraphs of 3-day-old Nissl stained coronal section through the NAc taken through rostral (D) and caudal (E) levels. Bar = 500μm. The framed area in (D) is shown at hegher magnification in (F). Bar = 200μm.

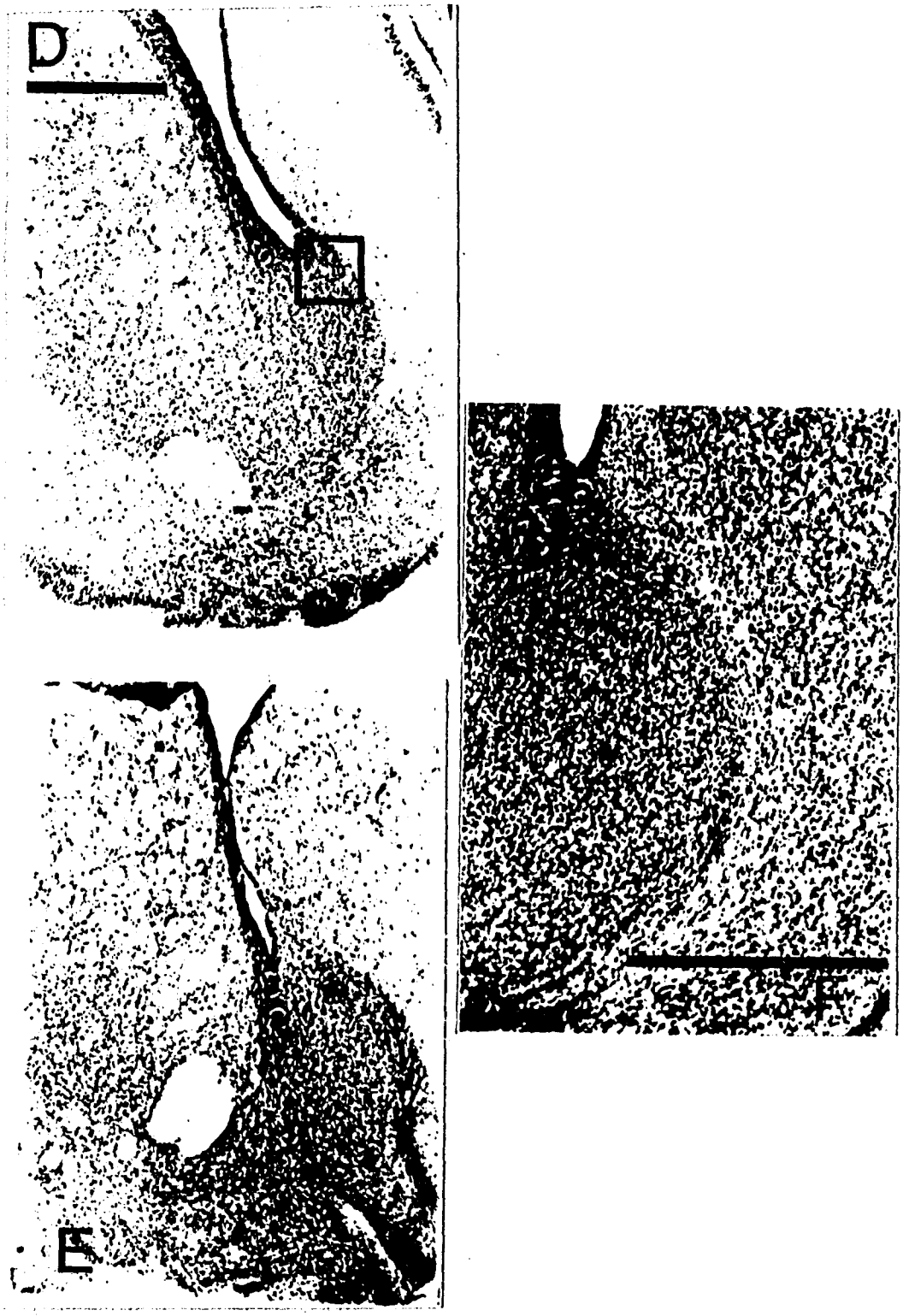

D
E
F

Figure 20. Photomicrographs of 5-day-old Nissl stained coronal sections through the NAc taken through rostral (G) and caudal (H) levels. Bar = 500μm. The framed area in (G) is shown at higher magnification in (I). Bar = 200μm.

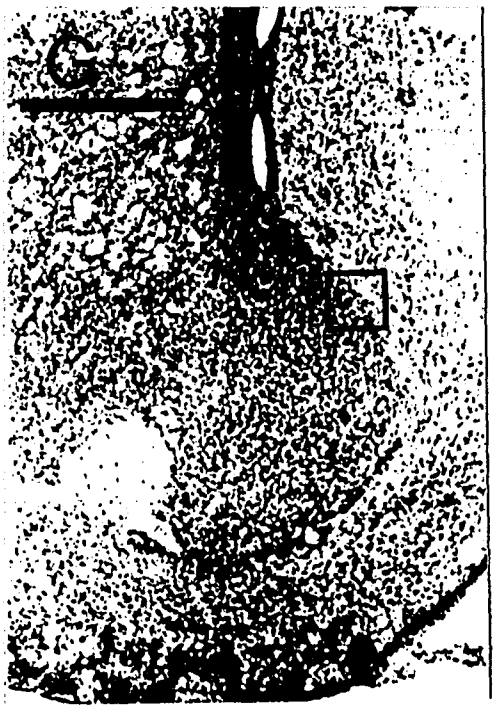
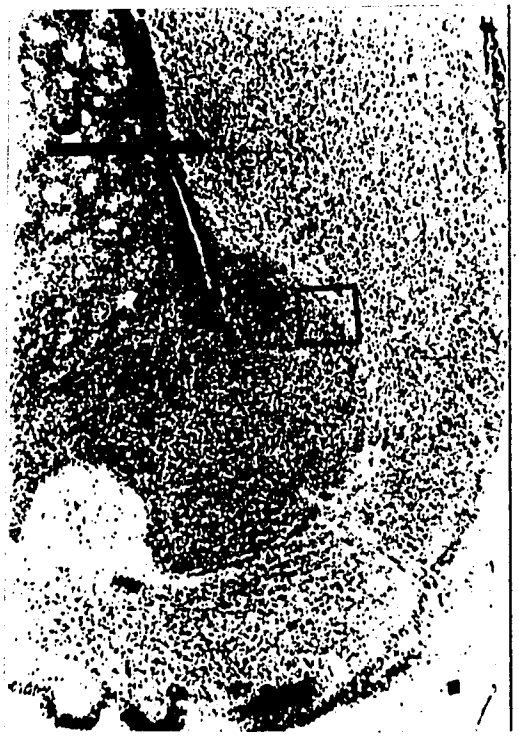
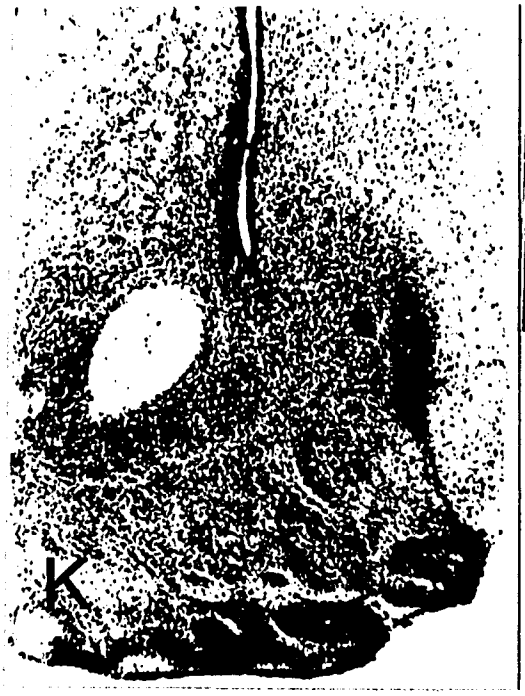


Figure 21. Photomicrographs of 7-day-old Nissl stained sections through the NAc taken through rostral (J) and caudal (K) levels. Bar = 500μm. The framed area in (J) is shown at higher magnification in (L). Bar = 200μm.




K

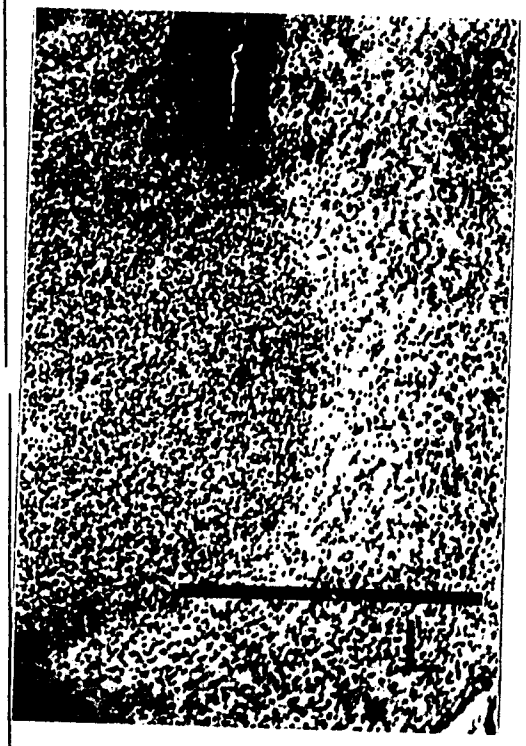


Figure 22. Photomicrographs of 10-day-old Nissl stained coronal sections through the NAc taken through rostral (M) and caudal (N) levels. Bar = 500μm.


M


N

Figure 23. Photomicrographs of 14-day-old Nissl and Van Gieson's stained coronal sections through the NAc taken through rostral (O), medial (P) and caudal (Q) levels. Bar = 500μm. The framed area in (P) is shown at higher magnification in (R). Bar = 200μm.

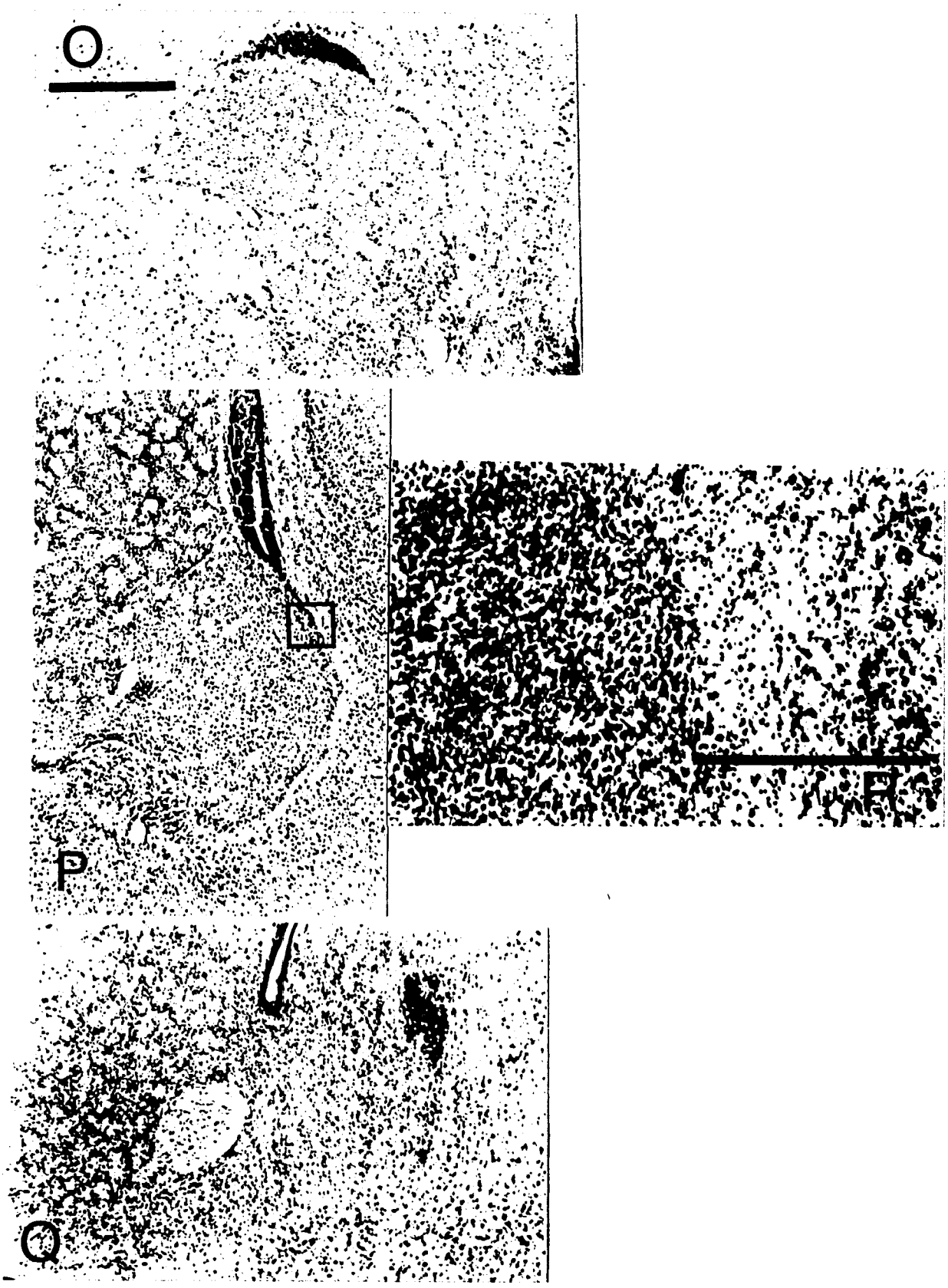
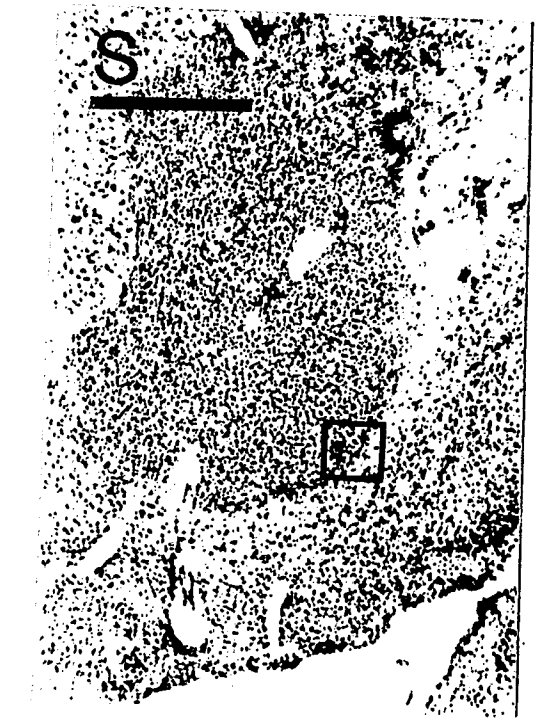

O
P
R
Q

Figure 24. Photomicrographs of 20-day-old Van Gieson's stained coronal sectons through the NAc taken through rostral (S) and caudal (T) levels. Bar = 500 μm. The framed area in (S) is shown at higher magnification in (U). Bar = 200 mm.


S


T


U

Figure 25. Photomicrographs of 20-day-old Nissl stained sections through the NAc taken at the level of the Islets of Calleja (V). Bar = 500 μm. (W) represents a photomicrograph of the framed area in (V) at a higher magnification. Bar =200 μm.

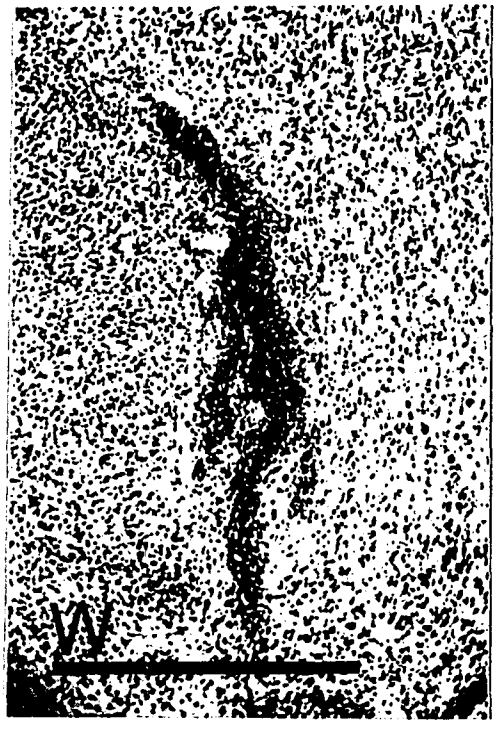
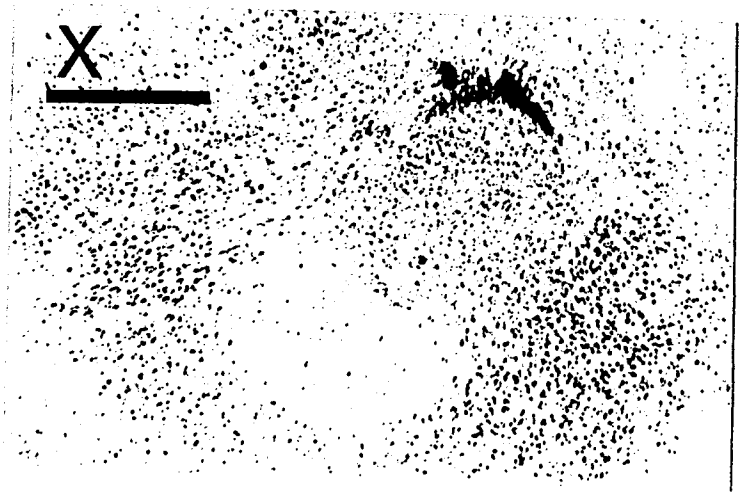


Figure 26. Photomicrographs of Adult Nissl stained coronal sections through the NAc taken through rostral (X) and caudal levels. Bar = 500μm.


X

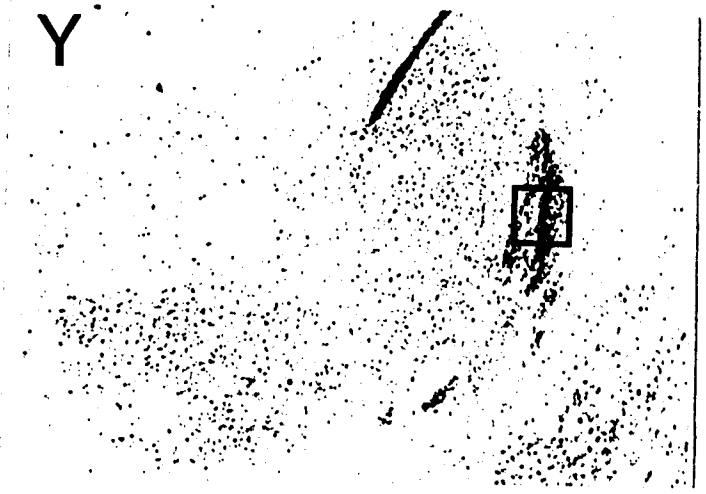
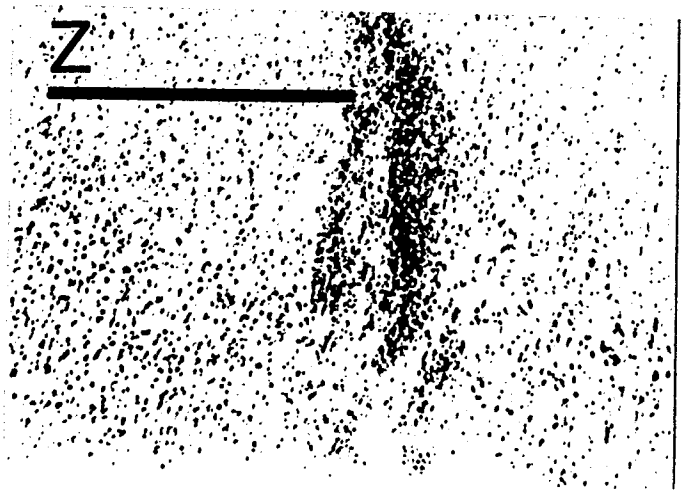
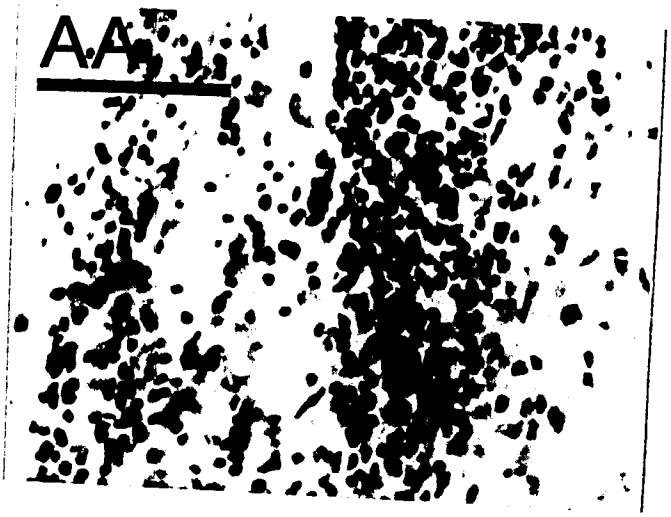

Y

Figure 27.(Z)is a photomicrograph of the framed area in (Y) shown at higher magnification of the Islets of Calleja. Bar = 200μm. (AA) represents a photomicrograph of the framed area in (Y) at higher magnification. Bar = 50μm


Z


AA

BODIAN SILVER STAIN

In the adult, as well as the 20 day old, the Bodian silver stain method revealed a dense tangled network of axons with fibers coursing around the ventral anterior commissure in a medial lateral direction (Figures 28 through 36). Throughout the rostral/caudal extent of the nucleus, patches of axon fibers from the internal capsule were seen. The number of fibers increase moving caudally throughout this structure. At the level of the Islet of Calleja, axons appear to exit this structure and enter the NAc. There was the obvious cell poor band, the zonula limitans of Johnston, along the medial-ventral edge of the nucleus at rostral levels.

Tissue at 14 days old presented with considerably more sparse distribution of axon fibers and patches of axons from the internal capsule. At more caudal levels, there were patches of the internal capsule interdigitatd within the NAc. The exiting axons from the Islets of Calleja were not present prior to this age (Figure32 and33).

Prior to 7 days of age, there were few lightly stained axons within the NAc. At higher magnifications a delicate lattice-work pattern was observed within the NAc, but lateral to the cell poor regional much more dense fiber system was present (Figure30 and 31).

During the first week, no axon were stained within this structure. Dense cell staining and axons could be localized in the corpus callosum in these sections.

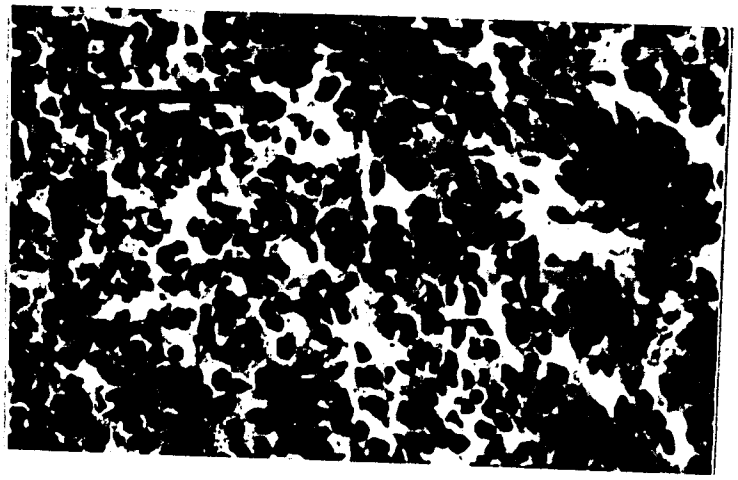
CYTOCHROME C OXIDASE

The normal rat NAc exhibits a differential developmental pattern of histochemical staining for the mitochondrial enzyme cytochrome oxidase. Cytochrome oxidase activity (CO), when visualized by the DAB reaction, appeared as a brown reaction product within the tissue sections. Frequently, the boundaries between regions of high and low CO activity are sharply defined (DiFiglia, et al., 1987).

Figure28. (A) is a photomicrograph of 5-day-old Bodian stained coronal sections through the NAc. Bar = 500μm.


A

Figure 29. Photomicrograph (B) represents the framed area in (A) at higher magnification (□). Note the abscence of fibers within the NAc. Photomicrograph (C) represents the framed area in (A) at higher magnification (△). Note the lack of fibers in the NAc, the cell poor band, and the dense fiber plexus medially to the NAc. Bar = 50μm.



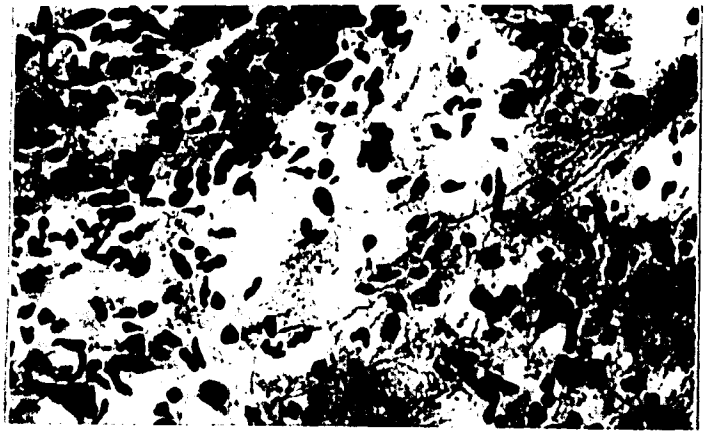


Figure 30. (D) is a representative photomicrograph of 7-day-old Bodian stained coronal sections through the NAc. Bar = 500μm.

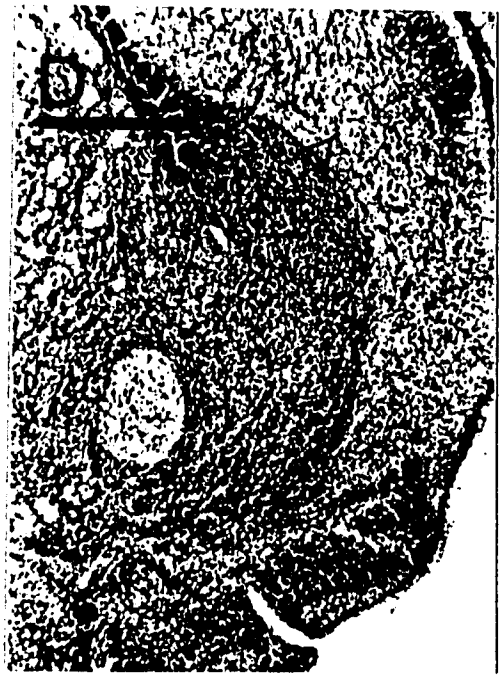
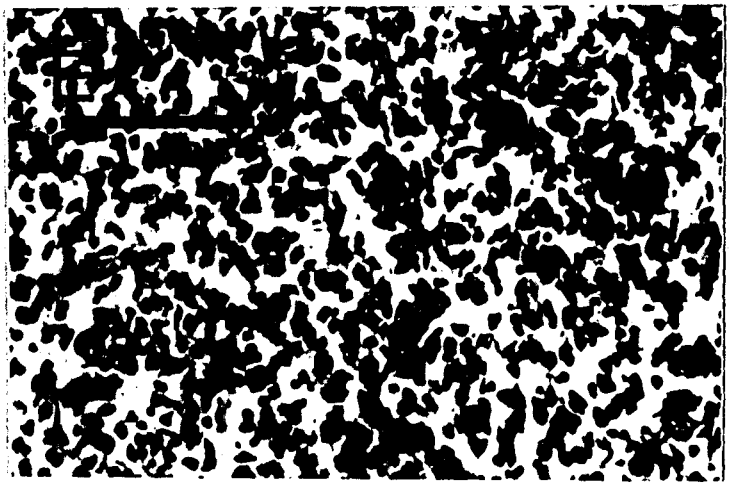


Figure 31 . Photomicrograph (E) represents the framed area shown in (D). (E) represents a higher magnification of an area within the NAc (□). Note that there are no fibers in the NAc. (F) represents the framed area in (D) (△). Note that there are no fibers in the NAc, the cell poor area, and the dense fiber plexus medial to that. Bar = 50μm.



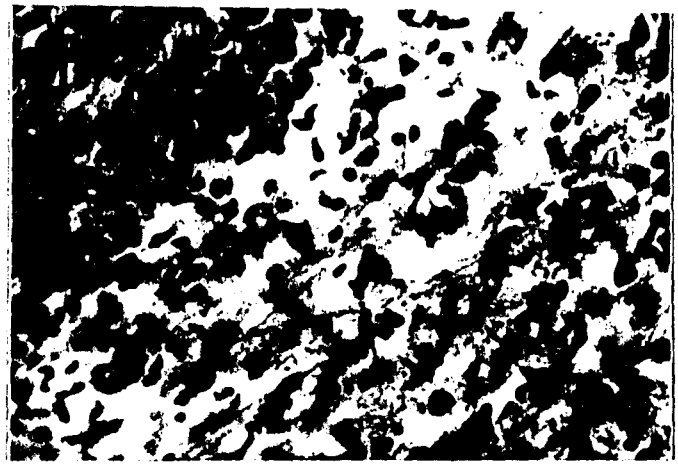
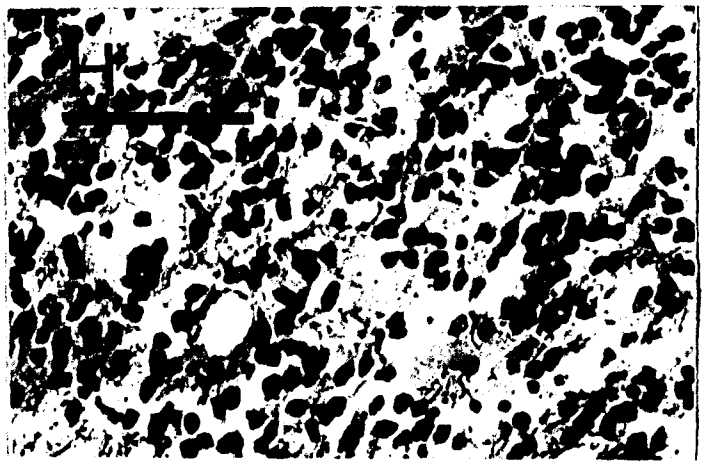


Figure 32. (G) is a representative photomicrograph of 14-day-old Bodian stained coronal section through the NAc. Bar = 500μm.


G

Figure 89. .Photomicrograph of Framed areas in Figures (G). (H) represents an area within the NAc at high magnification (□). There is the presence of fibers within the NAc. (I) represents the framed area in Figure (G) at higher magnification (▵). Fibers are present in the NAc, in the cell poor region and at the medial edge. Bar = 50μm.



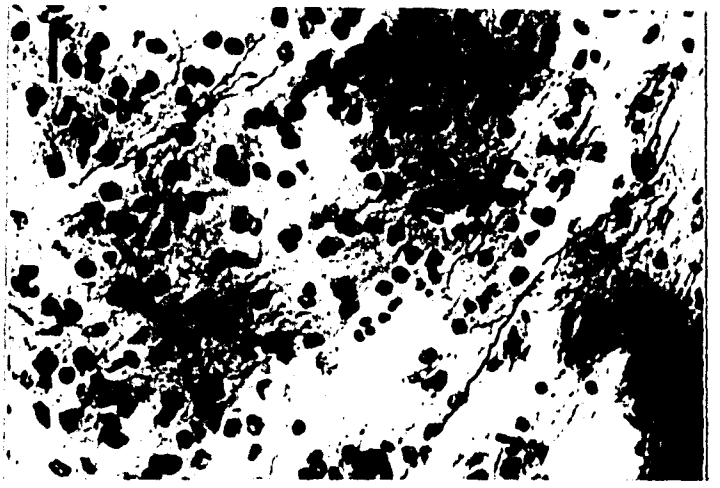


Figure 34. (J) is a representative photomicrograph of 20-day-old Bodian stained coronal sections trough the NAc. Bar = 500μm.


J

Figure 35. Photomicrographs of the framed areas in (J). (K) represents the framed area in (J) (□) at higher magnification. Note the dense plexus of fiber within the NAc. (L) represerns the framed area in (J) at higher magnification (△) Similarly, ther is a tangled network of fibers within the NAc and the surrounding areas. Bar = 50μm.

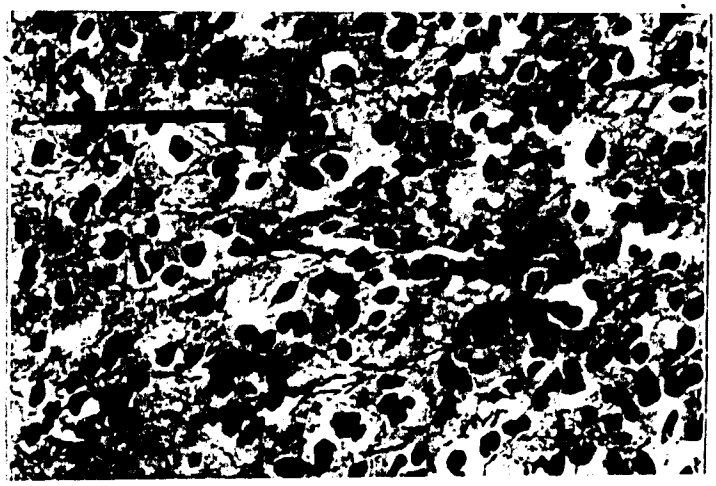
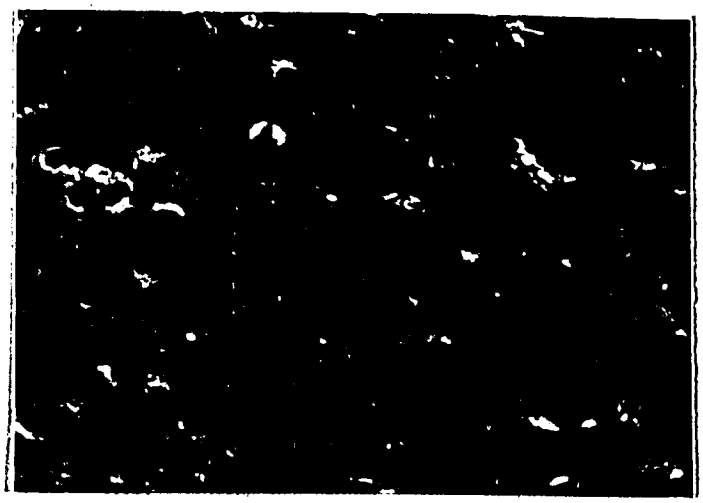


Figure 36. (M) represents a photomicrograph of Adult Bodian stained section through the NAc. Bar = 500μm. (N) represents the framed area in (M) taken at higher magnification. Bar = 200μm.(□) (O) represents the framed area in (N) taken at a higher magnification. Bar = 50μm.



This was illustrated in Figure37 through 42, for each age, which show CO processed coronally sectioned through the NAc.

In adult tissue the density of the DAB reaction product in the NAc was darker when compared to the lighter adjacent areas, such as the MS and the MFB. At rostral levels there was moderate to high levels of CO activity as compared to adjacent areas. Reaction product was concentrated mainly within the medial quadrant of the nucleus in caudal sections. This pattern of staining was constant throughout.

The level of CO activity in the 20 day old was quite similar to the adult when examining the distribution of intensity along the rostral/caudal extent. This pattern of staining was confined to neuropil.

In 14 day old tissue, CO reactivity was concentrated to neuronal perikarya, with intense staining within the medial NAc. There was a conspicuous edge along the medial/ventral border just outside of NAc which separates it from the MSN in which many CO reactive neurons were identified. Dark staining patterns were localized in the Islets of Calleja. At caudal sections CO reactivity was localized to neuronal perikarya; but again, the MS and DBB appear most intense.

Rostral and midlevel sections of 7 day old tissue exhibit a more uniform pattern of staining. There was a similar abrupt edge along the medial extent of NAc. Caudal sections reveal patches of intense staining similar to that seen in the adult. Dark staining was again seen distributed within the cell bridges and in the Islets of Calleja.

Similarly, the 3 day old treated tissue revealed the same pattern of staining but at a somewhat lighter intensity. No reactive cell bodies were visible at this age. There were identifiable cells in the MS but their distribution was less dense. There was an abrupt medial edge at this age, and the Islets of Calleja ere somewhat darker, as well.

The presence of KCN in the incubation medium (S) greatly retarded the histochemical reaction or completely eliminated the visualization of CO activity.

Figure 37. Photomicrograph of 3-day-old Cytochrome C oxidase (COX) treated coronal sections through the NAc taken through rostral (A) and caudal (B) levels. Bar = 500μm.

Figure 38. Photomicrograph of 7-day-old COX treated coronal section through the NAc taken at rostral (C) and caudal (D) levels. Bar = 500μm.


C


D

Figure 39. Photomicrograph of 10-day-old COX treated sections taken through medial level of section. Bar = 200μm.


E

Figure 40. Photomicrograph of 14-day-old COX treated coronal sectio through the NAc taken at rostral (F), medial (G) and caudal (H) levels. Bar = 500μm.

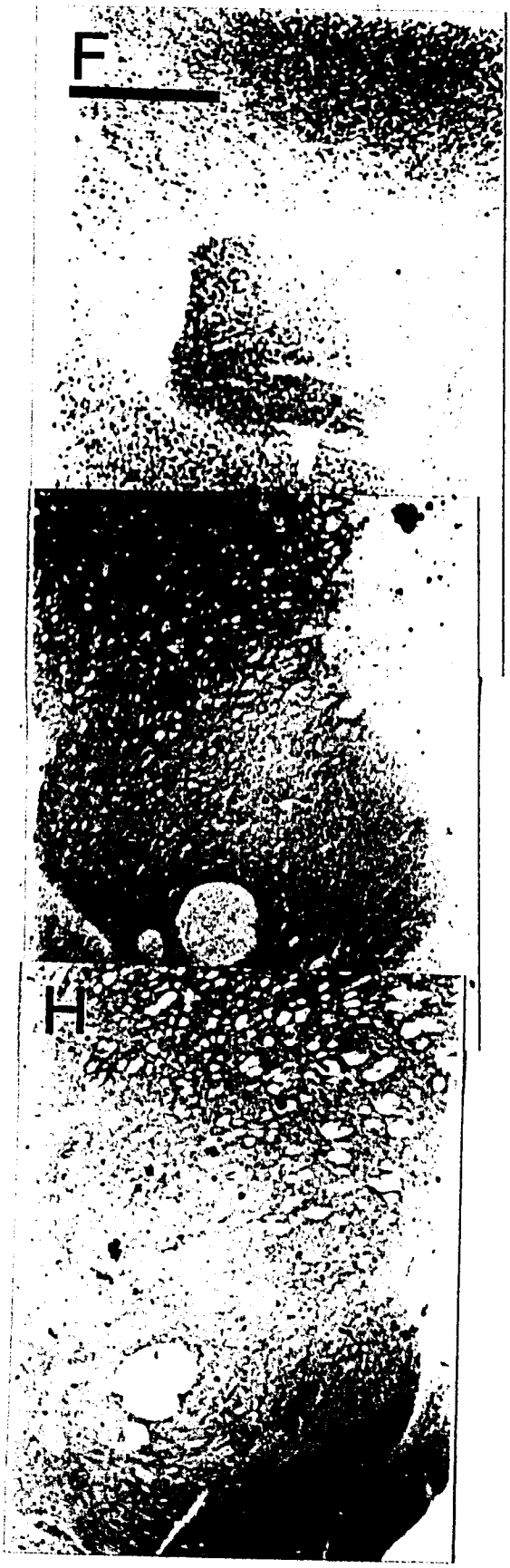

F
H

Figure 41. .Photomicrograph of 20-day-old COX treated coronal section through the NAc taken at rostral (I), medial (J) and caudal (K) levels. Bar = 500μm.

Figure 42. Photomicrograph of Adult coronal sections through the NAc at rostral (L), medial (M) and caudal (N) levels. This tissue was treated for COX. Bar = 500μm.


L
M
N

IMMUNOCYTOCHEMISTRY

SEROTONIN

The present findings reveal the differential expression of serotonin-like immunoreactivity in developing rat brain (Figures 43 through 46). The adult pattern of 5-HT, at rostral levels, was confined to the medial-ventral perimeter of the NAc where varicose fibers were present. Fibers were also localized to the medial septal nucleus (MS) and coursed around the medial margin of the NAc.

Similarly, at caudal levels, at the level of the Diagonal Band of Broca (DBB), there were long delicate fibers in the lateral septal nucleus and the medial septal nucleus and fibers were located along the lateral border.

There was intense immunoreactivity along the medial aspect of the nucleus. Caudal level of section was characterized by the presence of an aggregation consisting of delicate, thin fibers with many varicosities. This level was also characterized by dense plexus of varicose fibers medial to the anterior commissure.

During the second week, at caudal levels, the degree of immunoreactivity was lower than that seen in the adult. There was a delicate lattice-work of fibers throughout the nucleus. The abundant number of fibers within the medial septal nucleus and along the lateral edge, seen at this age was similiar to that seen in the adult.

The pattern of staining in the 5 day old was similar to the older animals, but the intensity and density of immunoreactive fibers was less. There were few varicose fibers throughout the nucleus. However, a dense network of fibers were localized along the medial border of the NAc and in the MS. At caudal levels the pattern of staining was similar-homogeneously light. This was contrasted to the heavier staining of fibers in the MS.

Staining was not present in the 1 day old. There were very few fibers. This lighter staining pattern was offset when compared to the heavy staining seen in the same section, within the cingulate.

ENKEPHALIN

In contrast, Enk-like immunoreactivity in the 1-day-old (Figure 47), mimicked the staining pattern and intensity that was seen in the adult NAc (Figure 48).

In Enk-prepared tissue, at rostral levels a cap of intense staining forms over the anterior commissure which becomes the dorsal aspect of the NAc. At this level staining appears within cell bodies. At caudal levels, the intensity remains the same, however the most intense areas of immunoreactivity for Enk has moved ventral to the anterior commissure.

GABA

GABA-like immunoreactivity (Figures 49 through 54), was moderate in the adult and at 21 days. There was a moderate density of staining of both cell bodies and terminal boutons throughout the rostral/caudal extent of the NAc. Neuronal perikarya were identifiable at medial levels of section. At more caudal levels, the density of immunoreactivity was at the medial ventral aspect of the nucleus.

The NAc, in 14 day old demonstrated light staining with patches of neurons ventral and medial to the anterior commissure. Many immunoreactive cells were identified in the olfactory tubercle (OT) and the periform cortex.

GABA-like immunoreactivity was moderate to light at 10 and 7 days of age, and could be described by the few cell bodies along the dorsal medial surface. A few delicate processes were also noted. A dense plexus of perikarya within the MS and DBB was consistently present. At rostral levels, the entire NAc was lightly stained. This was in contrast to the intense GABA-immunoreactivity localized to its

neighbors: the septal nuclei and OT. There were few immunoreactive cell bodies throughout the medial sections. Along the ventral margin, there was a dense network of neurons stained.

In 5 day olds, rostral levels of the NAc were much more lightly stained when compared to the OT, but there were a few scattered cell bodies. Cell bodies were localized around the anterior commissure and the ventral medial border.

The NAc of three day old's were also lightly stained with more reaction product deposited in neuronal perikarya at the ventral tip of the lateral ventricle in caudal sections. The medial and ventral boarders of the NAc are clearly delineated by the abscence of staining.

There was a homogeneous pattern of very light staining throughout the 1 day old NAc, with few reactive cells.

Figure 43. (A) is a representative photomicrogrph of 1-day-old coronal section throgh the NAc at caudal levels. This tissue was assayed for 5-HT-like immunoreactivity. Bar = 500μm.

Figure 44. Photomicrograph of 5-day-old coronal sections taken through the NAc at rostral (B) and caudal (C) levels, processed for 5-HT-like immunoreactivity. Note the abscence of staining within the NAc. Bar = 500μm.


B
C

Figure 45. (D) is a representative photomicrograph of 14-day-old coronal section through the NAc stained for 5-HT-like immunoreactivity. Bar = 500μm. (E) represents the framed area in (D) at higher magnification. Bar = 200μm. (F) represents the framed area at higher magnification. Bar = 50μm.

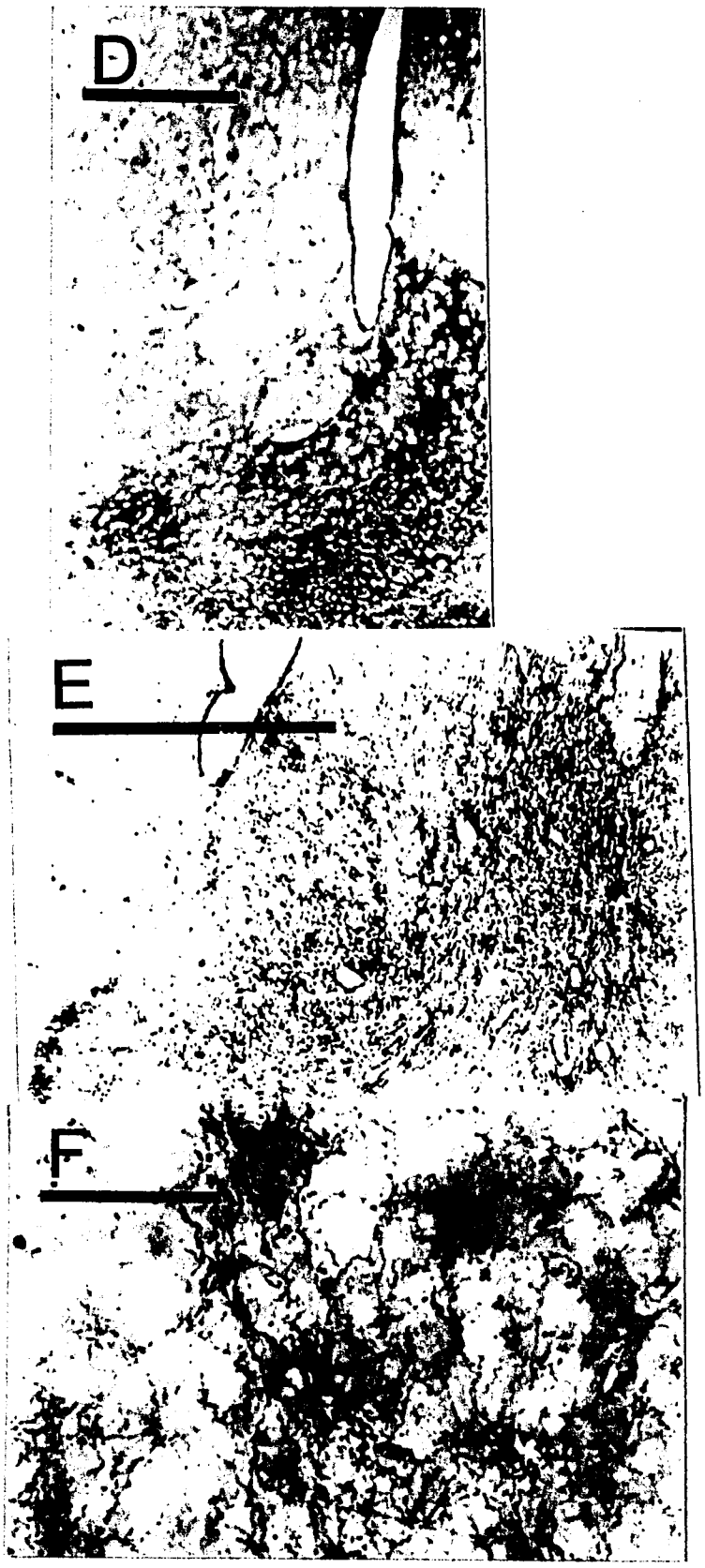

D
E
F

Figure 46. Photomicrograph of 20-day-old coronal section through the NAc stained for 5-HT-like immunoreactivity (G). Bar = 500μm. (H) represents the framed area in (G) at higher magnification. Bar = 200μm. (I) represents the framed area in (G) at higher magnification. Bar = 50μm.

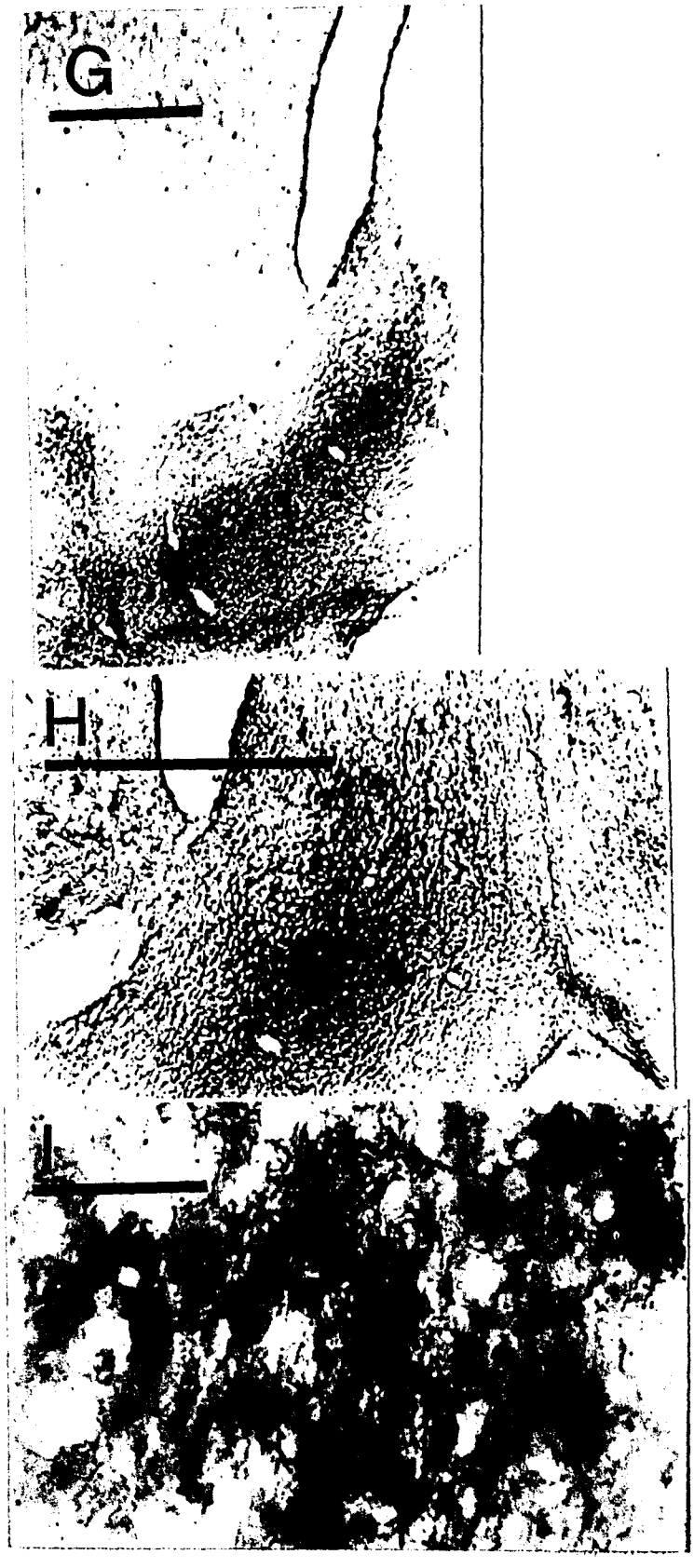

G
H
I

Figure 47. Photomicrographs of 1-day-old coronal section through the NAc assayed for Enk-like immunoreactivity (A). The entire extent of the nucleus is densely stained. Bar = 500μm.

Figure 48. Photomicrographs of Adult coronal section through the NAc taken through rostral (B), medial (C) and caudal (D) levels. These sections are representative samples of tissue processed for Enk-like immunoreactivity. The entire nucleus is densely stained with some patches of more intense staining. Bar = 500μm.

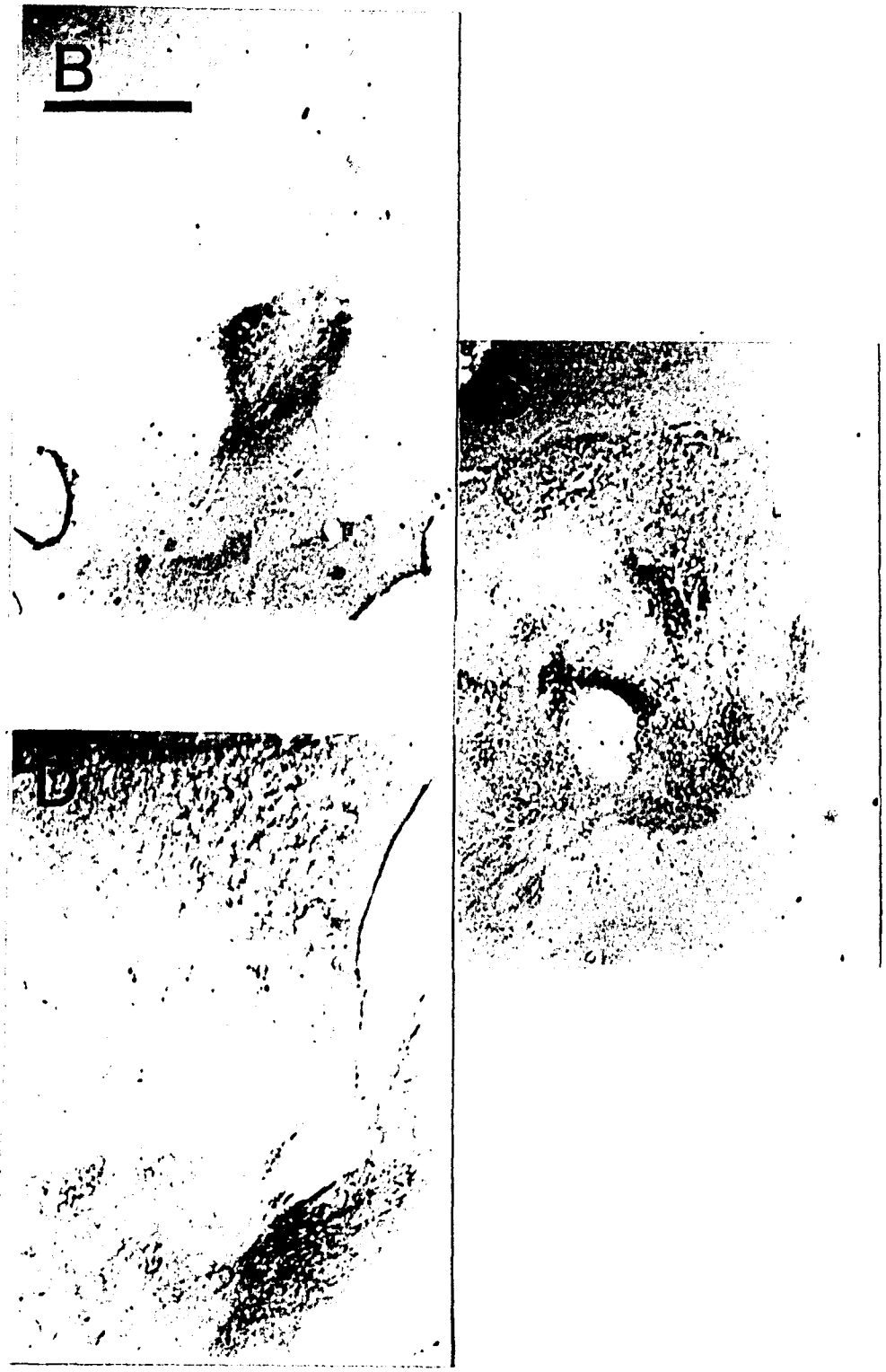

B

Figure 49. Photomicrograph of 1-day-old coronal section through the NAc. This section was processed for GABA-like immunoreactivity. Note the abscence of staining within the nucleus and the prescence of cell body staining in the cortex (A) (B). Bar = 500μm.


A

Figure 50. .Photomicrographs of 3-day-old coronal section through the NAc taken through rostral (C) and caudal (D) levels. GABA-like immunoreactivity is abscent in the NAc, but cell body staining is evident in the same section in the neocartex (E). Bar = 500μm.

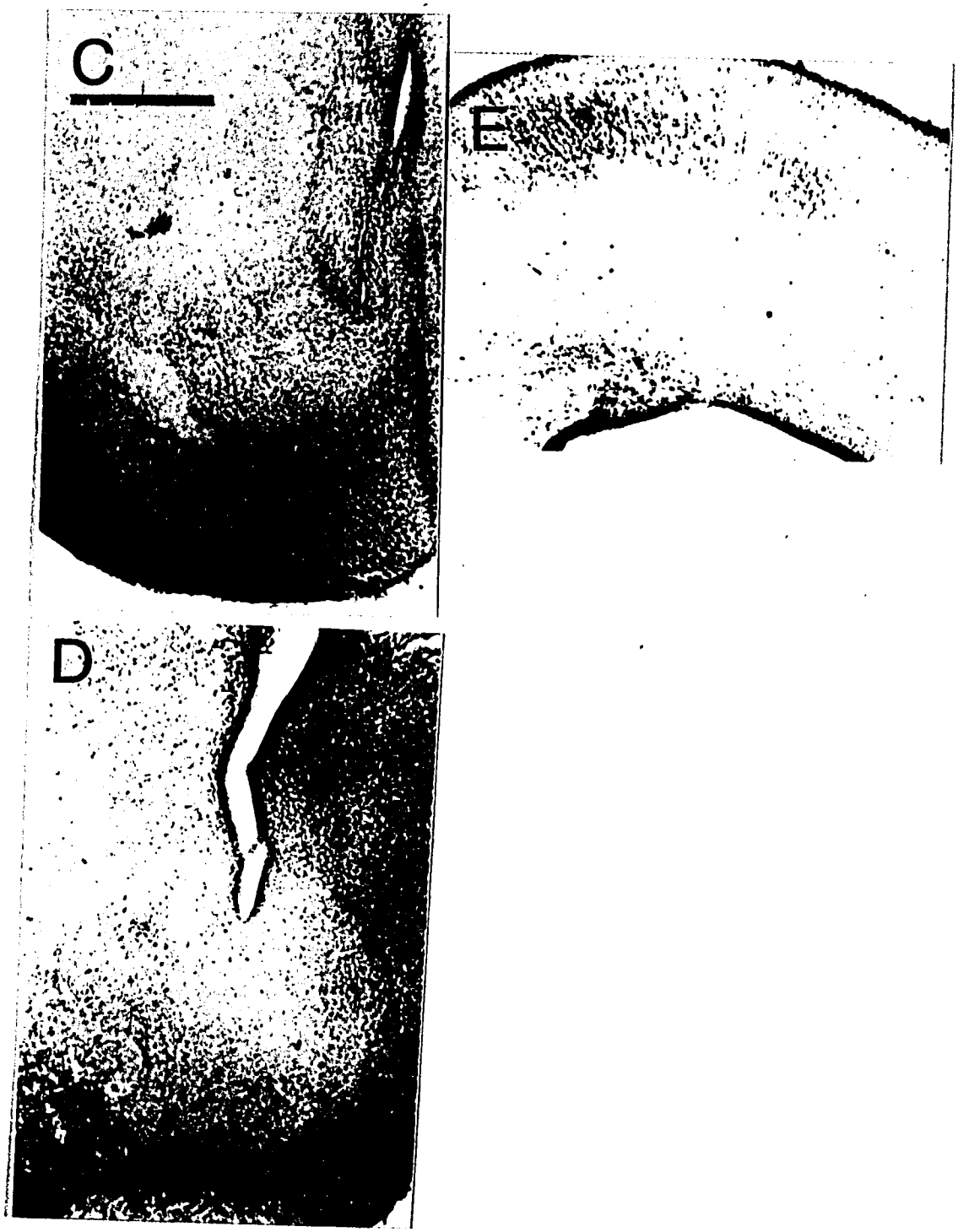

C
E
D

Figure 51. .Photomicrograph of 5-day-old coronal sections through the NAc taken throgh rostral (F) and caudal (G) levels. Tissuewas processed for GABA-like immunoreactivity. A conspicious abscence of staining is revealed in the NAc compared to the cerebral cortex. Bar = 500μm.

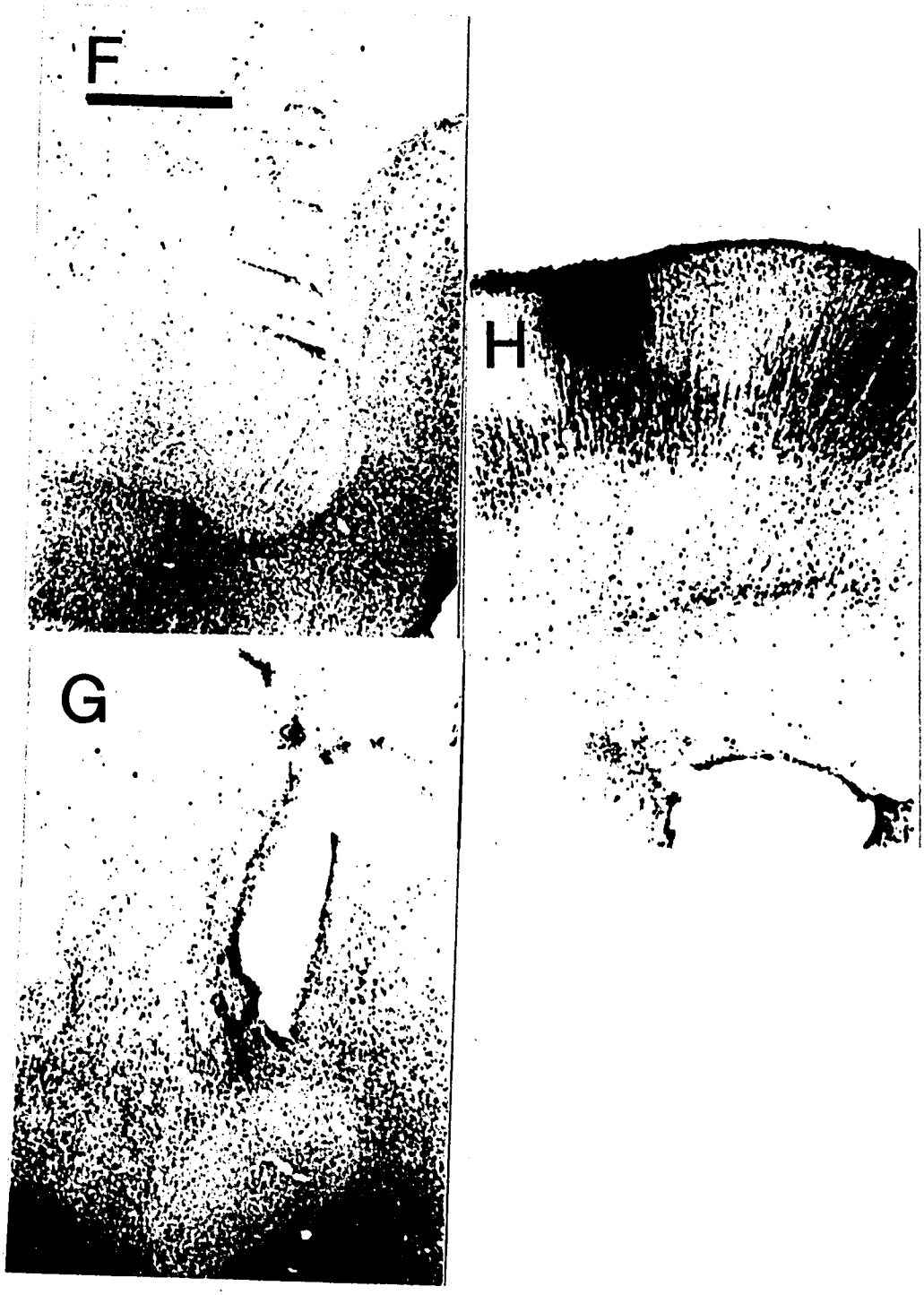
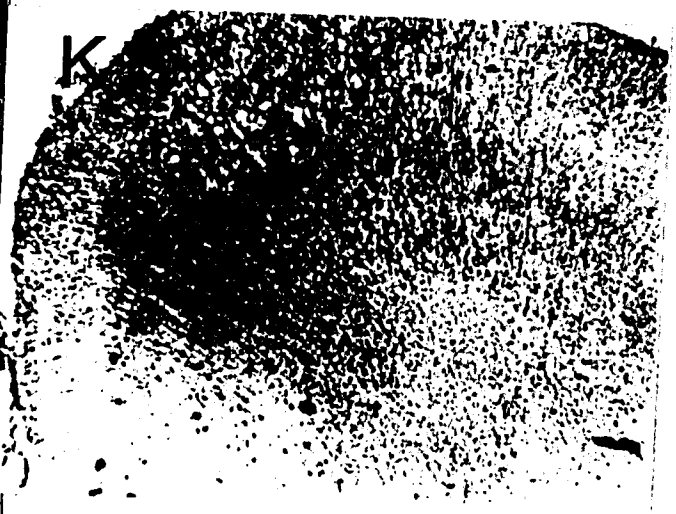

F
G
H

Figure 52. Photomicrographs of 10-day-old coronal sections through the NAc taken through rostral (I) and caudal (J) levels, stained for GABA-like immunoreactivity. Some cell body staining is evident, but not as intense as the cerebral cortex. Bar = 500μm.


I


K


J

Figure 53. Photomicrograph of 14-day-old coronal section thrugh the NAc taken at caudal level (L), processed for GABA-like immunoreactivity. A moderate amount of staining is revealed with few cell bodies stained. Cortical cells are heavily labeled (M). Bar = 500μm.

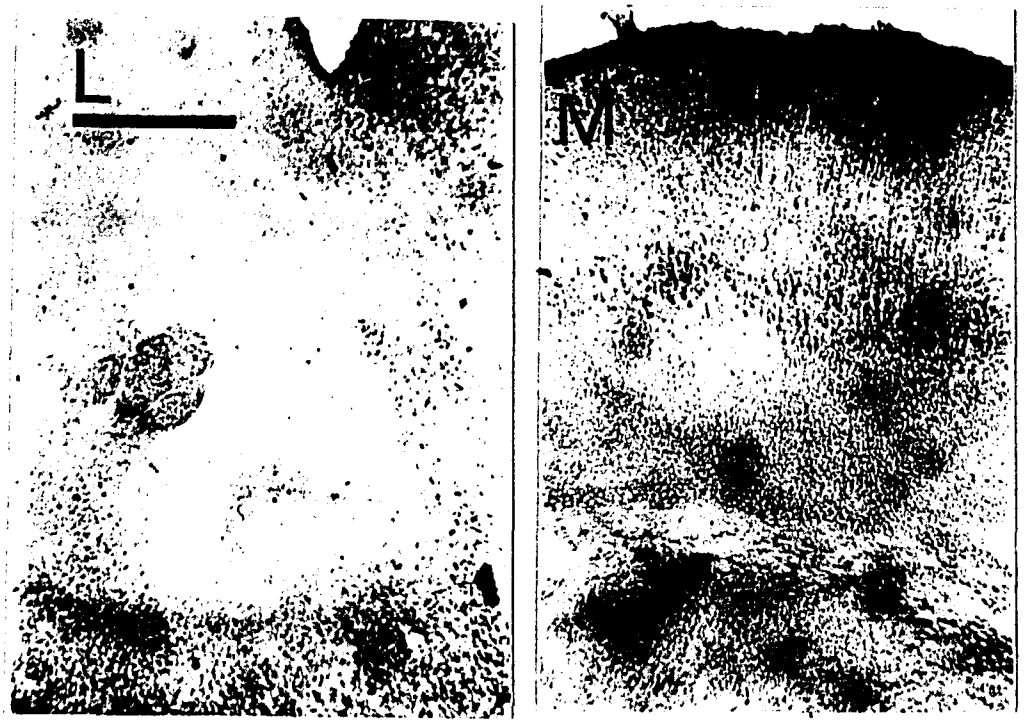
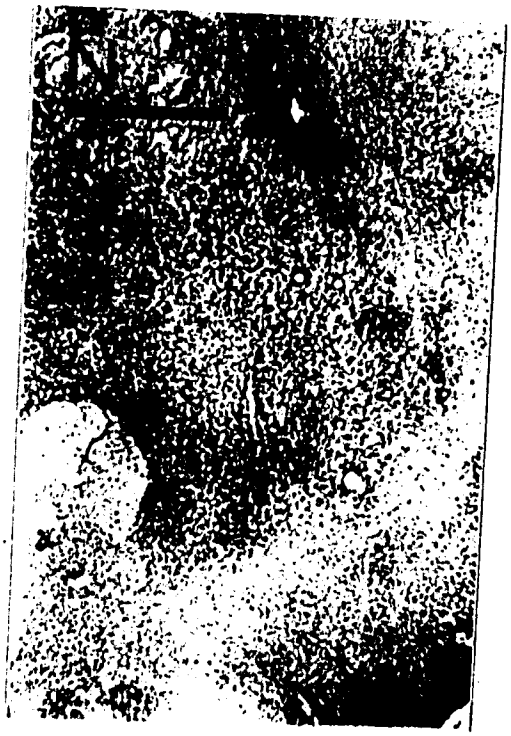

L
M

Figure 54. (N)is a representative photomicrograph of 20-day-old coronal section through the NAc at rostral level.s. Intense GABA-like immunoreactivity is present. Bar = 500μm.



DISCUSSION

Behavioral Activation

Periodic trains of electrical brain stimulation directed toward the NAc elicited reliable and dramatic changes in the behaviors of preweanling rat pups. These behavioral patterns observed following stimulation have been used to define the state known as behavioral activation. The subset of behavioral components elicited by stimulation of the NAc were mouthing, licking, pawing, probing, and locomotion. These behaviors were elicited repeatedly. These behaviors did not appear in all the pups tested and varied in both magnitude and frequency with maturation. The level of activity during these tests was particularly impressive because the infant rat usually exhibits few spontaneous behaviors (Hall, 1979 a). Brain stimulation initiated and maintained this set of behavioral responses in the absence of any relevant goal objects. These findings demonstrate that portions of the neural circuitry for behaviors such as feeding are present at birth in the rat.

Stimulation of the NAc in pups produced behaviors that were most related to ingestive activity (ie. suckling and feeding). Mouthing was the most robust behavior for all animals, at all ages, while probing was the least represented of the behaviors elicited. These findings, however, cannot be generalized to 14-day-old rat pups. These stimulation parameters were not effective in eliciting behavioral activation at this age.

The behavioral profile elicited from animals 3 to 14 days of age as a result of electrical stimulation directed towards the NAc must be interpretted in the context of the methodology employed within this study. An important methodological issue is that all variables were held constant without regard to age. Not only are the results attributed to neuroanatomical and neurochemical changes, but also behavioral changes. Animals 14 days of age undoubtly respond differently from 3 day olds in response to contextual variables. Animals were deprived of contact with the dam and littermates for at least 24 hrs before testing. Pups at different developmental stages handle these insults differently and may be contributary to the dramatic changes seen in the younger animals behavior. The temperature of the testing chamber was

consistently maintained at 32 ^{0} C. Pups level of activation may also be related to ambient temperature. Young pups are primed for high levels of activity at nest temperature, whereas, older pups might find this temperature extreme, and thus engage in hightened levels of activity for different reasons which are under differnt physiological control. Motoric differences cannot be ruledd out as a contributaory factor controlling the behaviors seen at differnt ages. And finally, regardless of age, each pup was tested utilizing the same stimulation parameters. These factors must play an important role in determining whether activity is expressed as either mouthing or rolling, for example.

Additionally, studies of electrical brain stimulation bring to the experimental setting a number of methodological concerns. First, to augment experimental reliability, a constant current stimulator was used A repetive stimulation schedule was used, which is used to evoke a behavioral response. Third, the volume of stimulated brain area is a consistently dynamic feature of the experimental subjects within ages and across age groups. And, hence, caution was used when suggestions were made about the observed behavioral output resulting from the functional integrity of the inputs and ouputs of the NAc. This feature is critical when examininng the neural elements that are influenced by electrical stimulation. Changes in membrane permeability are occuring at a number of sites, such as , at the cell body, throughout the dendritic arborizations, and along the exent of the axons (influencing both presynaptic input, intrinsic neural elements and postsynaptic outputs that participate in the final expression of the behavior). In a physiologically intact system, however, electrical stimulation of the NAc initiates changes in only the intrinsic neural elements as well as on the efferents of the system. Therefore to suggest that the later emerging 5-HT immunoreactivity as accountable for the dissolution of behavioral activation is presumptive. To suggest a GABAergic contribution is easier. There is no doubt that 5-HT influences this brain locus, however its direct function is not directly revealed here. Further analysis of the other intrinsic and efferent neurotransmitter systems are necessary to find those systems responsible for behavioral activation and its eventual dissolution.

These data are consistent with those obtained by Hall (1979 a and b, 1983). The pups made mouthing movements and they also made movements that resembled feeding of the adult. Head probing and rolling over were the dominant behaviors. These behaviours are consistent in their appearance and function in the motivated behaviors of food getting and nipple search.

These data are also similar to those obtained by Moran et al., (1983). In that study, the termination locus of the stimulating electrode was the MFB at the level of the lateral hypothalamus, and the ages used were similar to those used in the present study. Pups emitted the full constellation of behaviors that define behavioral activation. These included mouthing, licking, pawing, gaping, probing, and stretch and lordosis responses. Within this constellation of behaviors, one sees the potential forerunners to many later adult motivated behaviors. Although these behaviors are different and serve different purposes for the pup they come to be organized together. Finally, the present data are similar to those obtained by Lee (1988). In this study the locus of electrical stimulation was the NAc but only 3-day-olds were used. The predominant behavioral responses that were elicited were mouthing, licking, pawing, and locomotion.

The results from the present study differ from those obtained by Moran, et al., in the following ways: First, there was no progressive recruitment of behaviors with prolonged stimulation. The sequence in which behaviors occurred for individual animals was rank ordered so that the first behavior that was elicited received a score of 1, the second a score of 2, and so on. No particular response pattern emerged in this study within age groups or across age groups. It was observed by Moran et al., (1983) that once mouthing behaviors were elicited, further stimulation resulted in the elicitation of more integrated behaviors (ie. lordotic responses, and ear wiggling). Behaviors were elicited in a particular sequence. Mouthing and licking preceded gaping and stretch responses at all of the ages. Behaviors appeared to be clustered around particular organized "end behaviors" or perhaps "motivational systems". Second, pups never engaged in the variety of end behaviors that were elicited from MFB stimulation. Stretch responses, ear wiggling or lordotic behaviors were never observed from NAc stimulated pups.

The expression of the phenomenon that Hall (1979 a and b) identified and termed behavioral activation in neonatal rats appears to be both stimulus- and age-specific and dependent. These results are consistent with other findings (Camp & Rudy, 1987) that suggest that the behaviors that define behavioral activation are mediated through different systems (mouthing- serotonin or rolling-dopamine) Thus, different stimulation types determines whether activity is channeled into the behavioral profile across varying ages. Behavioral activation may best be viewed as many motor patterns that are part of more general neural systems under similiar neurochemical control. These precocious abilities may serve no immediate function for the pup but may simply reflect the manner in which the neural systems develop. In either case, these adult-like behaviors that infant pups display should be extremely useful as measures of the development of funciton of various neuroanatomical and neurochemical systems important for the display of adult motivatied behaviors.

These differences in results may be attributed to the complex neurochemical anatomy of the MFB. The MFB is a bundle of both ascending and descending axons that travel within the rostral-caudal axis from the midbrain to the rostral basal forebrain. This bundle contains short axons that connect adjacent areas as well. Stimulation directed towards the MFB elicits a variety of species-typical appetitive responses. These behaviors may represent recruited outputs from a variety of behavioral systems all traveling within this fiber system.

The fact that the results depend upon the locus of stimulation is relevant due to the nature of both the afferent and efferent fiber system within the NAc. The NAc receives input from limbic structures and sends efferent fibers to the globus pallidus, an important component of the motor system. Perhaps these connections enable the cells of the NAc to modulate the activity of the globus pallidus, and hence the rats observed behavior. The nucleus accumbens may be a response initiator, and/or "gate" all motivated behaviors, and only let through those that are age appropriate. The circuitry involved in the other behaviors may bypass the NAc completely. This would explain the greater variety of behaviors observed by Moran, et al. Stimulating the MFB may have elicited both NAc gated behavior and NAc independent behavior.

It has been suggested (Fentress, 1976) that "boundaries" exist between motivational systems and these boundaries become inflexible as development continues. As a result of increasing neural complexity at the level of the NAc, behavior no longer switches from one mode to another at the height of activation. This may represent a progressive specificity and directedness in those behaviors that are more crucial to the immature pup. Electrical stimulation at a particular site in rat pups might elicit behaviors associated with only one motivational system (eg. feeding) at first. But prolonged stimulation may lead to behaviors associated with other motivational systems (eg. sex) as well. As the rat develops and the "boundaries" become established this type of progressive recruitment of behaviors may become less likely. The motor system is organized in a hierarchial manner with increasing complexity of intergration along the neuroaxis (Bernstein, 1967; Allen and Tsukahara, 1974). The lowest end of the neuraxis corresponds to the lowest level of the hierarchy of neural organization which controls activities which represent a relatively limited level of integrative function. Higher CNS structures, represent a higher level of integration in the hierarchy, contributing to motor responses that are more complex and precise. This may account for the progressive recruitment of behavior seen by Moran et al., (stimulating the MFB, a more caudal structure) and the lack of such progression seen in the present study in which the NAc, a more rostral structure, was stimulated.

As behavioral activation disappears from the rats behavioral repertoire, what happens to the behavioral patterns and the underlying neural components? Are the neural substrates inhibited or suppressed? Do these behavioral fragments become incorporated as a whole or inpart into other adult behavioral systems? Does this behavioral system provide any adaptive advantage for the altricial animal?

The behaviors that are elicited from the NAc as a result of electrical stimulation represent behavioral fragments that are recruited outputs from the pups' repertoire. The neural circuitry for the exicution of these behaviors is present and organized at an early age, long before these complex behaviors normally occur. Such activity reflects more than a general hyperactive state because strong tactile stimulation does not produce similiar behaviors or activation (Hall, 1979 a). These behavioral components may represent potential forerunners of food getting and social

and/or sexual behaviors.

The eventual disposition of the circuity underlying behavioral activation illustrates the complexity of and the current vagueness of the notion of developmental continuities. New behaviors do not seem to be added to the animals' repertoire during ontogeny, but rather become organized differently and come under different neurological controls and can only be elicited by specific stimuli (ie. goal objects- food, water, conspecifics).

Hence, electrical stimulation of the NAc elicits a constellation of behaviors called behavioral activation in the preweanling pup. After day 14, stimulation is ineffectual in eliciting these behaviors. The behaviors that initially define behavioral activation become incorporated into different complexes of behaviors and now these components come to define such behaviors as feeding, grooming, and sexual behavior.

The loss of involuntary reflexes as development progresses is a common process evidenced in young mammals. In human infants, for example, the rooting and sucking reflexes of the mouth, the grasping reflex of both hand and foot, and the coordinated stepping pattern all become increasingly difficult to elicit as development progresses (Cratty, 1970). Instances of neurologic damage or senility provide examples from the clinical setting of the reappearence of many of the repressed reflexes of infancy. Their neural substrates have not been lost, but are merely inhibited by overlying neural controls. This situation has been experimentally reproduced in the laboratory (Moran et al.,1986). In an attempt to identify the contribution of higher inhibitory cortical centers on the dissolution of electrical stimulation induced behavioral activation, rat mothers were treated with the anitmitotic agent methylazoxymethanol (MAM) during pregnancy. It was found that the MAM pups continued to respond to electircal stimulation well into the second postnatal week. These results suggest a role for the normal cortical development in behavioral organization. The circuitry for infantile forms of behavior are continually present but are under higher cortical control. Studies of behavioral activation illustrate the general difficulty in discerning the relationship between adaptive

behaviors in infancy and functionally similar representations in adult.

Neuroanatomy and Neurochemistry

Several cytoarchitectonic features of the NAc were constant throughout ontogeny. First, at all ages the boundary between the caudate and the NAc was difficult to discern. There appears to be a blending between these two structures. Second, the vesicles of the internal capsule appear to pierce only the caudate. And finally, the zonula limitans of Johnston, a cell poor region along the medial border, was evident throughout ontogeny. This border is perhaps one of the most dramatic in the CNS (Chronister, et al., 1981).

The cells of the NAc were smaller and more densely packed than those of the caudate. At more caudal levels, the most dramatic feature of the NAc appeared. This structure was the large Islets of Calleja. This dense cluster of small cells and associated larger cells made its first appearance in tissue section of 3-day-olds and was a constant feature throughout the development of the NAc, and was evident at all subsequent ages. The identification of Bodian stained fibers corresponds to embryonic work carried out by Bayer (1981). Axon fibers did not appear until after the second week.

The results of the present study demonstrated the heterogeneity of neurotransmitter development in the NAc. There were clear differences in the ontogenesis of Enk-, 5-HT-, and GABA-like immunocytochemisrty. The topographic and developmental differences in the appearance of the immunoreactive products represent differences in the development of properties of each neurotransmitter system.

Enk-like immunoreactivity was detectable as early as day one. These data are in agreement with anatomical and biochemical studies whch reveal the early presence of opioid receptors and peptides. In general, opioid peptides appear late in embryogenesis (day 16 and 17) in rat brain. Ontogenesis of receptor binding correlates well with the presence of endogenous opioids in the brain, being present at E14 with increasing density until adulthood. (Bayon et al., 1979; Spain et al., 1985).

However, serotonergic synapses are mainly acquired between the time of birth and the third postnatal week (Liu et al., 1987). These developing features of the serotonergic phenotype, including the appearance of stores of 5-HT reflect serotonergic synaptogenesis that occurs during the postnatal perid and is reflected in the present findings.

GABAergic staining developed later than either 5-HT or Enk. It was virtually absent at birth, appearing in a mature form at about the second postnatal week. Portions of the GABAergic neurotransmitter system, particularly in caudal brain regions, exhibit substantial levels at birth. For instance, brain stem levels of this predominately inhibitory neurotransmitter system approaches adult levels at parturition (Coyle and Enna, 1976; Hedner et al., 1984) GABA uptake is likewise near adult levels at birth, although binding in all regions is only approximately 25% of adult levels during the first postnatal weeks. These findings are consistent with the present results obtained when neonatal tissue was immunohistochemically reacted.

It is important to realize, however, that the presence of neurotransmitter-like immunoreactivity does not necessarily imply an integrated, functional neurotransmitter system. For example, Enk-like immunoreactivity is present as early as day 1, but it is uncertain whether this neurotransmitter system is fully functional at birth. Immunocytochemical localization of neurotransmitter systems indicates only the presence of the ligand. That this neurotransmitter's synapses are fully functional in conventional neurotransmission proir to birth, (ie. before all of the properties of the synapses are present) is suspect. Certainly, it is unlikely that a great deal of neurotransmission will occur until the terminals have acquired their full compliment of precursors and significant stores of the neurotransmitter itself.

The differential maturational patterns of these neurotransmitter systems within the NAc may be correlated with the differential maturational patterns of CO activity observed in the present study. The reciprocal distribution of Enk and CO staining suggest that Enk neurons do not drive oxidative metabolism in the NAc. Further, the early appearance and distribution of Enk in the NAc suggests that the

axons stained with Bodian silver stain are not enkephalinergic. In contrast to Enk, 5-HT and GABA development is predominantly postnatal.

The NAc is known to control locomotor activity in familiar environments; pharmacological studies suggest a critical dependence on levels of neurotransmitter in the NAc which are able to alter spontaneously occuring or drug-induced hyperactivity (Pijnenburg et al., 1973; Anden and Johnell, 1977; Costall et al., 1980). Reviews of studies in which various opioids were injected into the NAc suggest that locomotor depression and catelepsy may be mediated by opiate receptors (μ type), whereas locomotor stimulation may be due to an activation of the δ type receptor (Havemannn and Kuschinsky, 1985).

The early appearing Enk-like immunoreactivity may demonstrate the contribution of this system to the locomotor component of behavioral activation. Similarly, the later appearing GABA- and 5-HTergic inhibitory components may be critical for the initial elicitaiton of mouthing, licking and other ingestive behviors so necessary for the attachment of the neonate to the dam (Enters and Spear, 1985) and then contribute to their dissappearance, as well (Mogenson and Nielson, 1984).

The results presented in this thesis strongly suggest that maturational changes in both the anatomy and neurochemistry of the NAc may be constituents participating in the changing temporal and topographic patterns of behavioral activation seen in the neonate. However, an important issue is the generalization of such data to globally explain complex behavioral changes that occur during ontogeny. While these findings characterize neural mechanisms in the preweanling, this work invites further studies that would manipulate these systems within the NAc during maturation. Studies employing specific ligands would clarify the functional roles of particular neural systems mediating behavioral activation. Thus, if behavioral activation is thought to be mediated by particular neurotransmitter systems, then the pharmacology of these systems would similarly change with development.

Initial studies incorporating such a strategy have been done in our laboratory (Lee, 1988). Administration of both typical and atypical neuroleptics, haloperidpol

and clozapine (dopamine antagonists), were observed to interfere with elicitation of NAc-stimulation-induced behavioral activation. These findings suggested to Lee, that behavioral activation at least in part, is mediated by early maturing (mesolimbic) dopamine systems. Thus, if behavioral activation is thought to be mediated by a particular neurotransmitter system, then drug effects (from either agonists or antagonists) should not be evidenced until that neurotransmitter system is fully developed.

The notion of convergent systems arises from anatomical observations of the NAc. The NAc receives prolific and diverse sets of inputs. This area is important for the assimilation of information or the association of diverse communication. It provides a crucial area for information processing and its modulation. Pharmacological analysis reveals that the NAc contains at least 16 different neuroactive substances including DA, NE, 5-HT, amino acids, and peptides (Nierwenhuys, 1986). Moreover each of the pharmacologic agents tested is known to interact with processes directing the activity of 2 or more of these neuroactive substances making it extremely extremely difficult to obtain insight into the precise mechanism of action of this brain locus.

There are additional concerns when interpreting mechanisms of action. Developmently, postsynaptic receptors may precede presynaptic release of the endogenous ligand. Thus, receptor agonists may be effective while drugs that act at the presynaptic terminal may not and the neural system may not be physiologically functional although some components are and some drug actions are active as well. It is necessary to examine the pre- and post-synaptic elements of the system as well as the inputs and outputs (Barr, in press).

Conceptually, the major difficulty in generating interpretations of these data is due to the interrelated nature of neural systems as well as particular features common to developing systems. Such contributory factors as myelinization, modification of connections as a result of sprouting, preening and cell death and refinement in the establishment of favorable connections, all contribute to the postnatal remodeling of the CNS and, ultimately, difficulties in generating interpretations.

The neural changes that have been examined within this study comprise a small fraction of potential systems contributing to the final expression of such a complex behavior as behavioral activation. Neural changes within the NAc are not the sole factors for the changes in behavioral activation, but are contributary. Yet the goal of such studies is the elucidation of portions of the neural circuitry of complex behaviors. These and companion studies open the possibility for exploring the characteristics of complex behaviors like behavioral activation, during development and the possible biological circumstances that engage these mechanisms.

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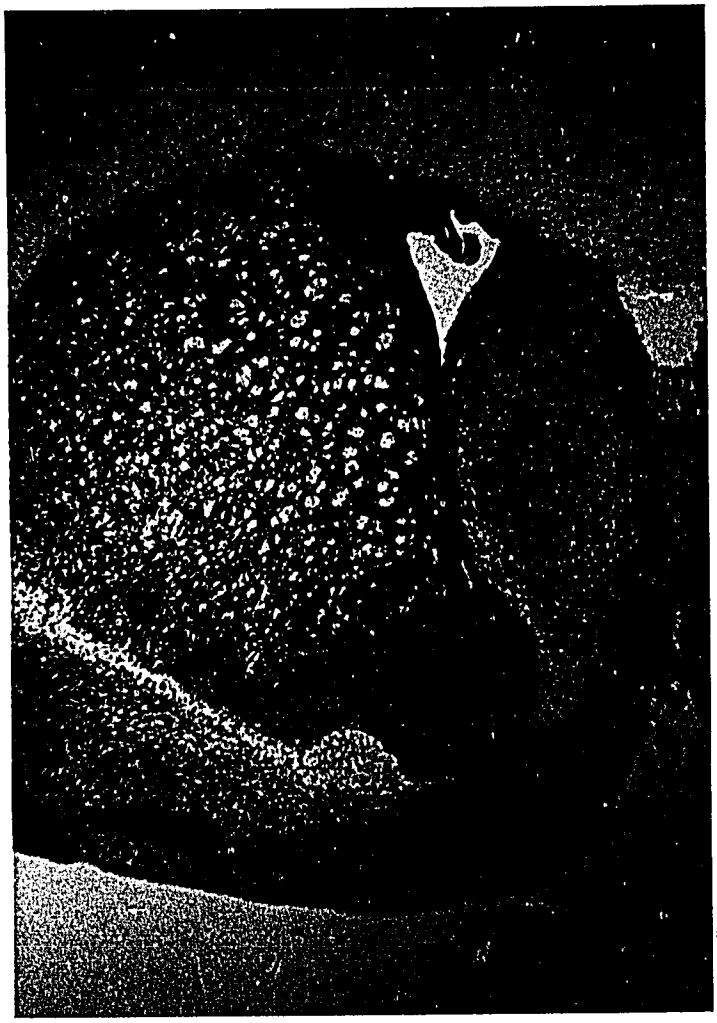

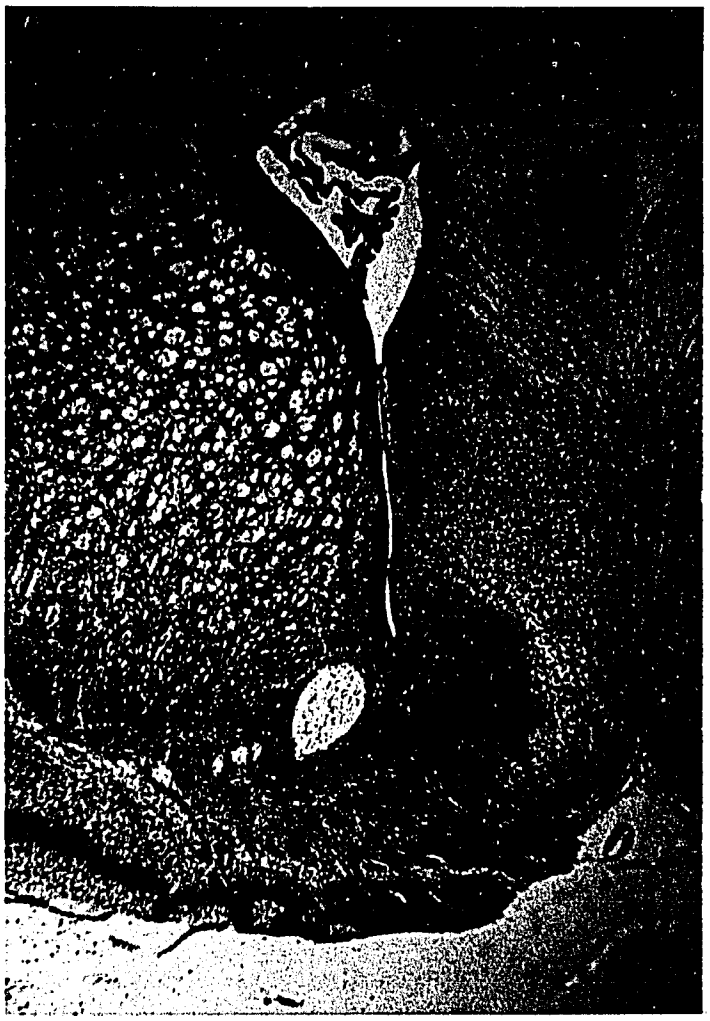

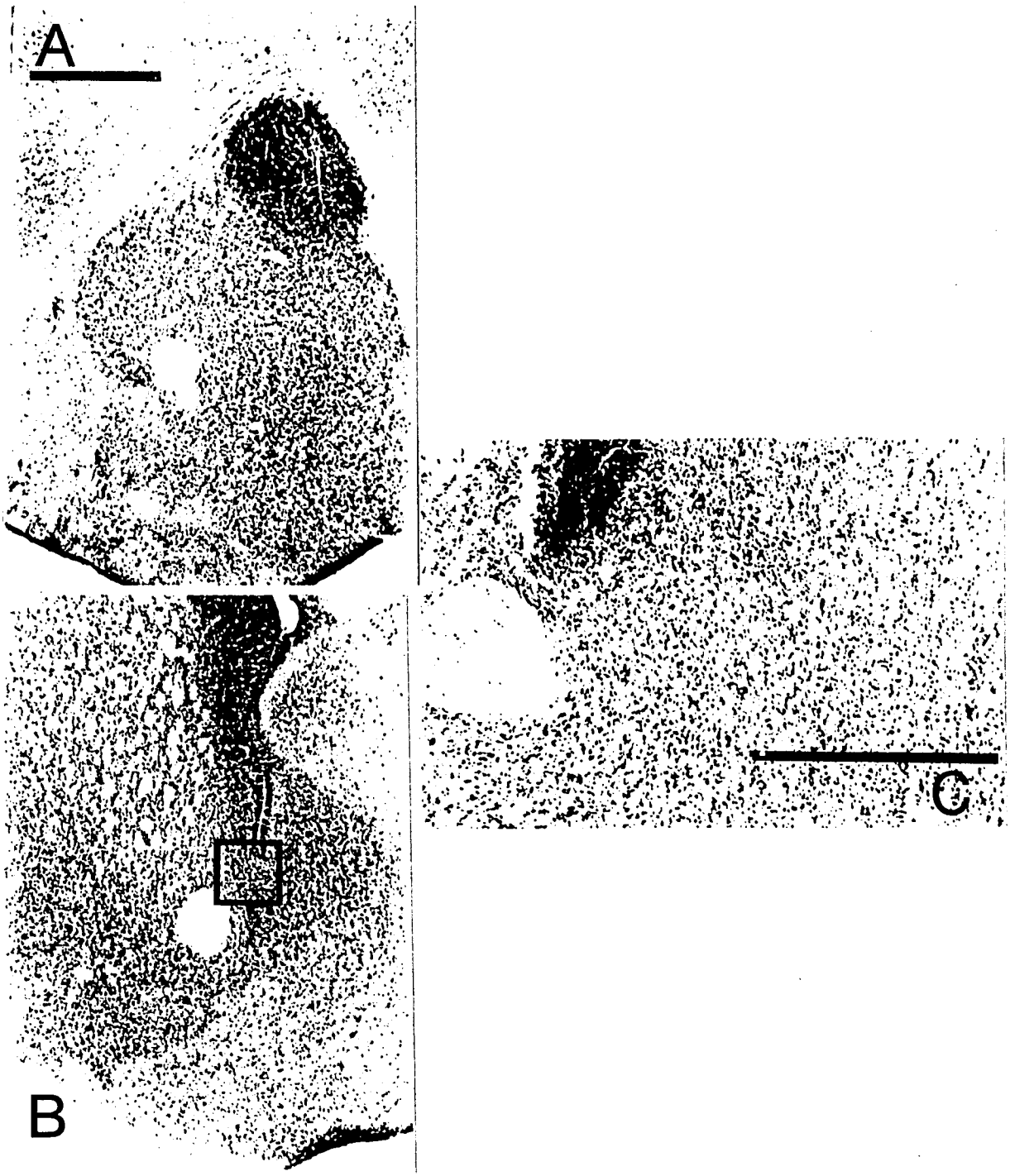
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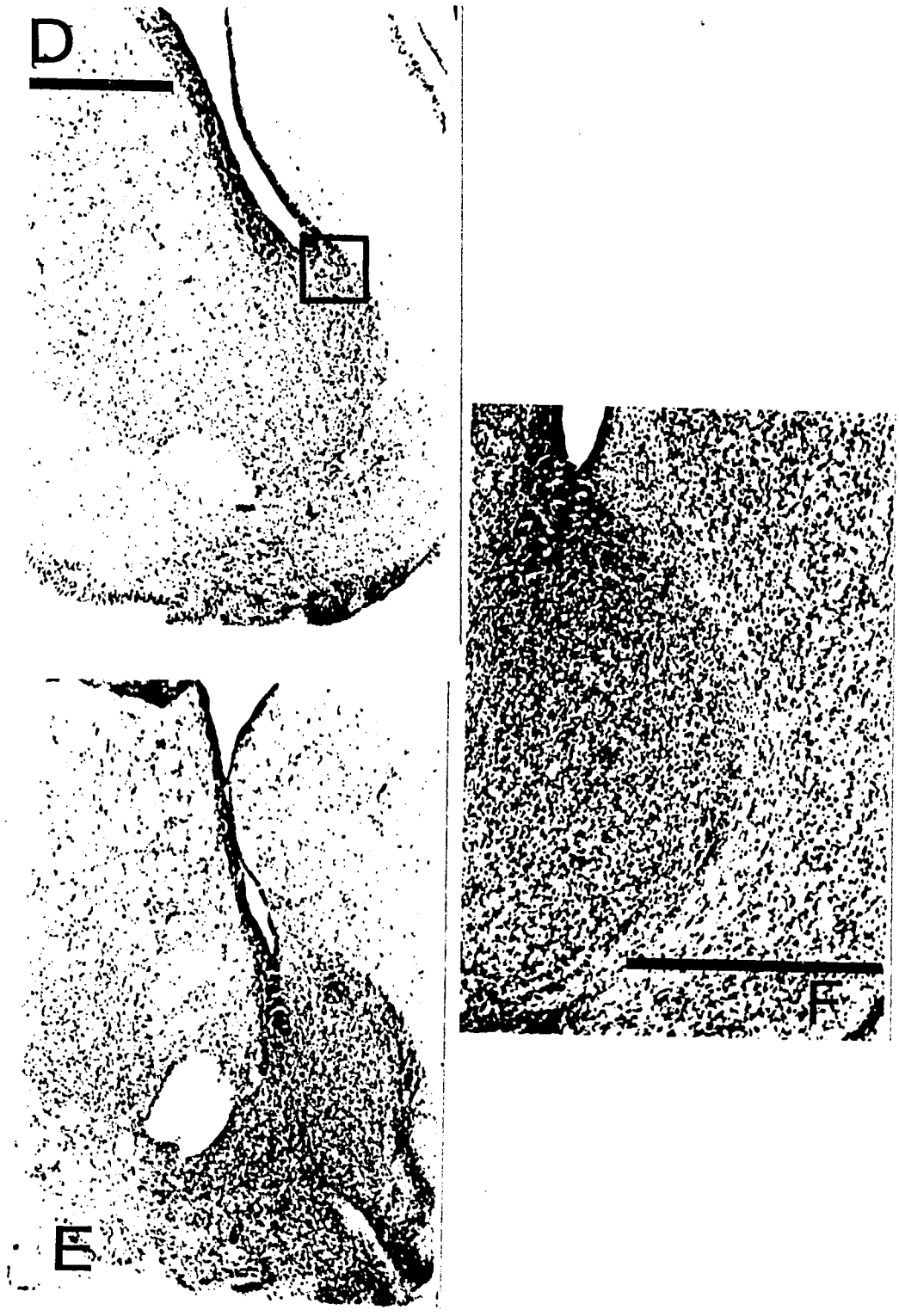
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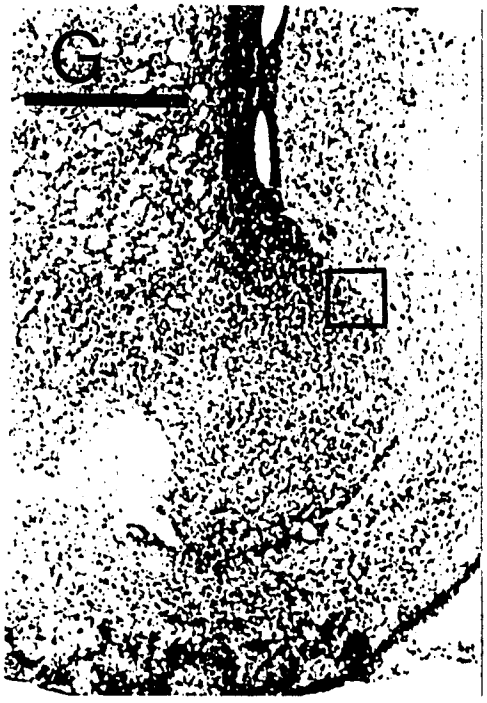
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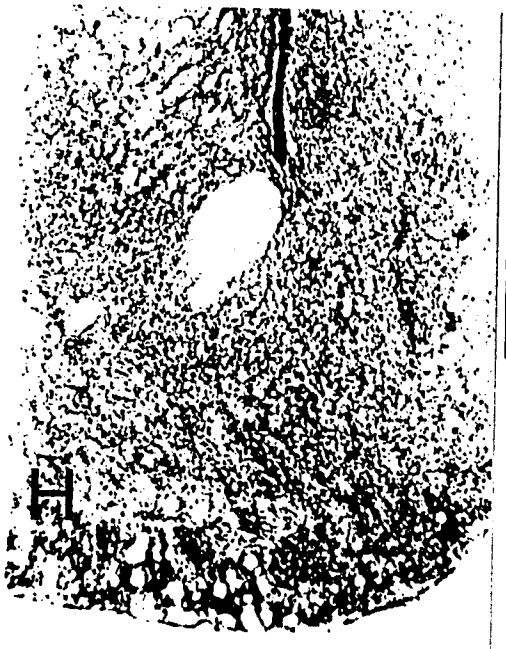
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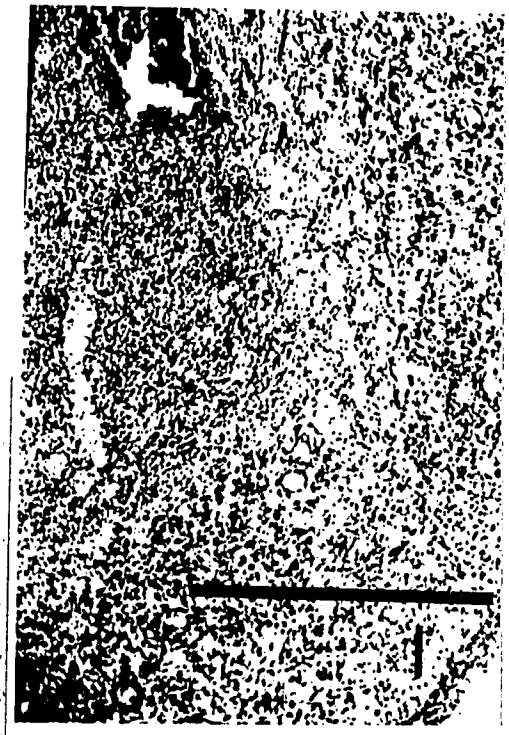
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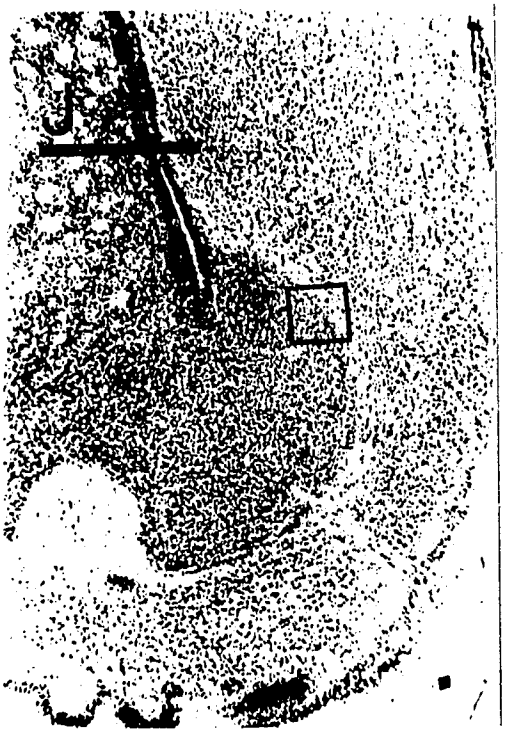
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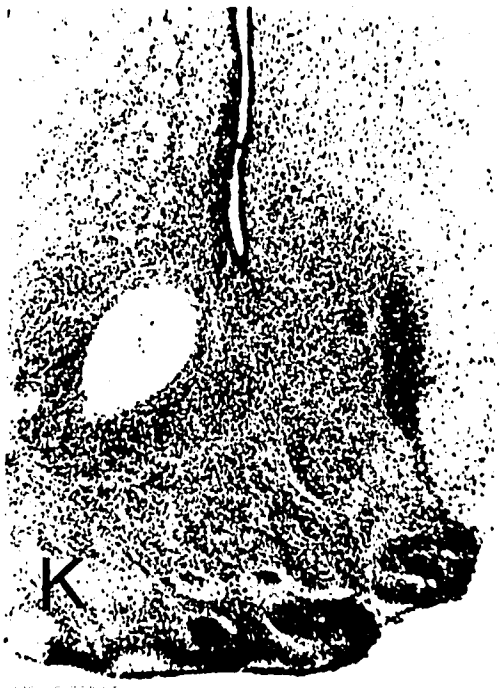
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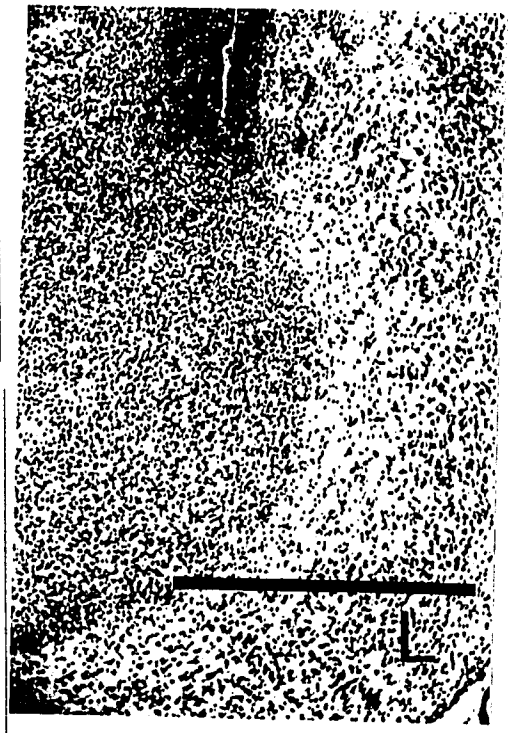
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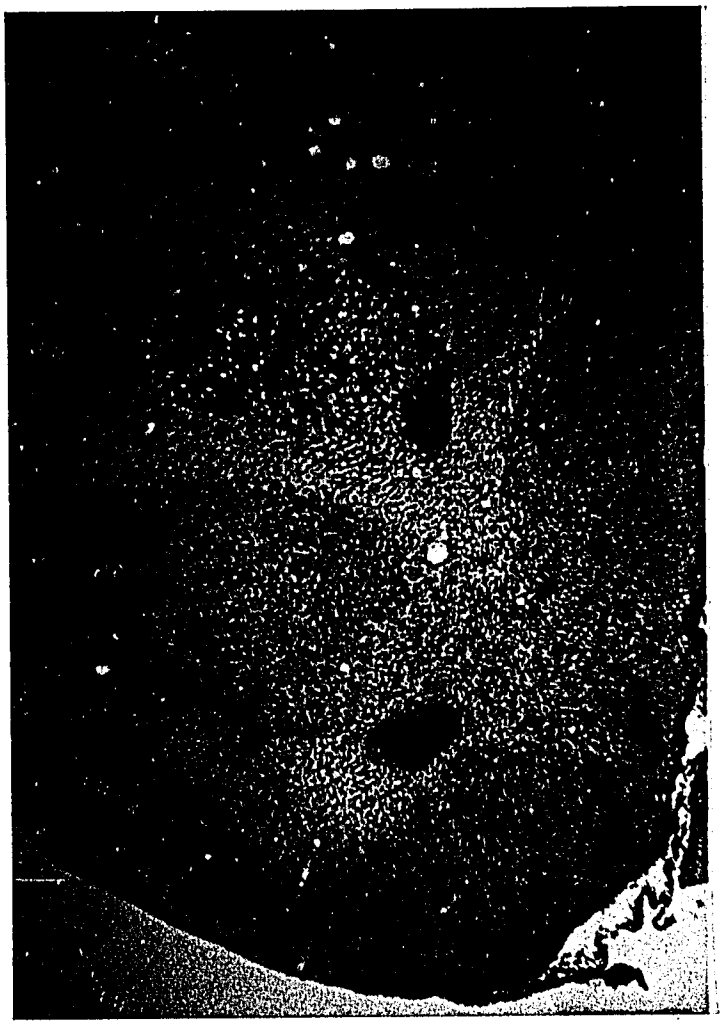

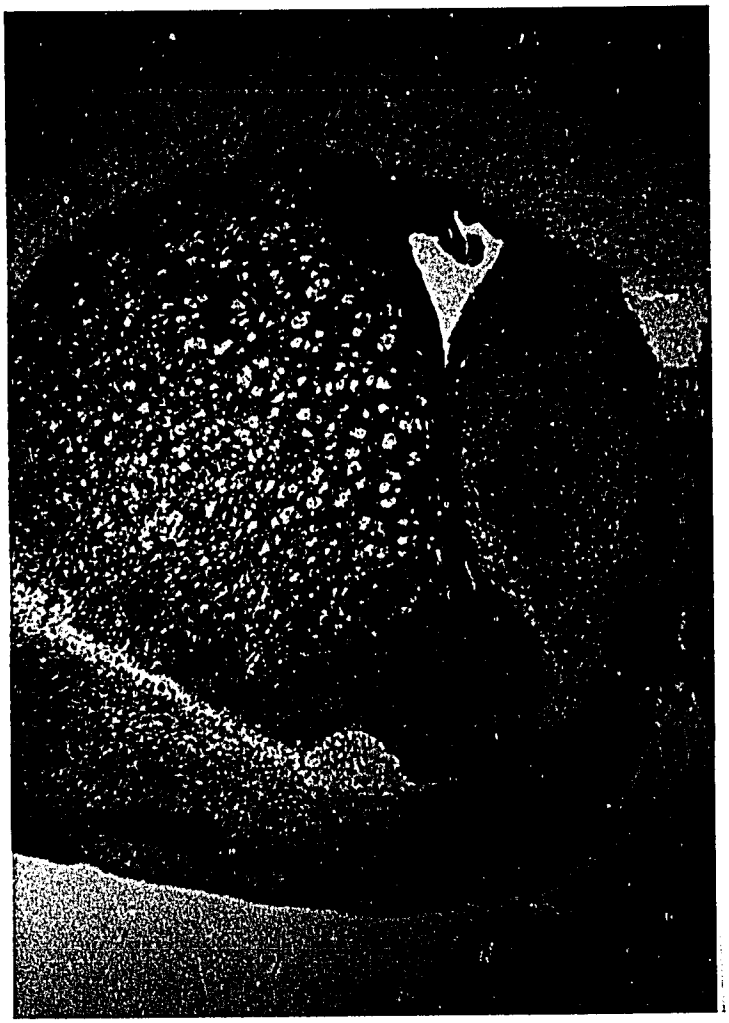

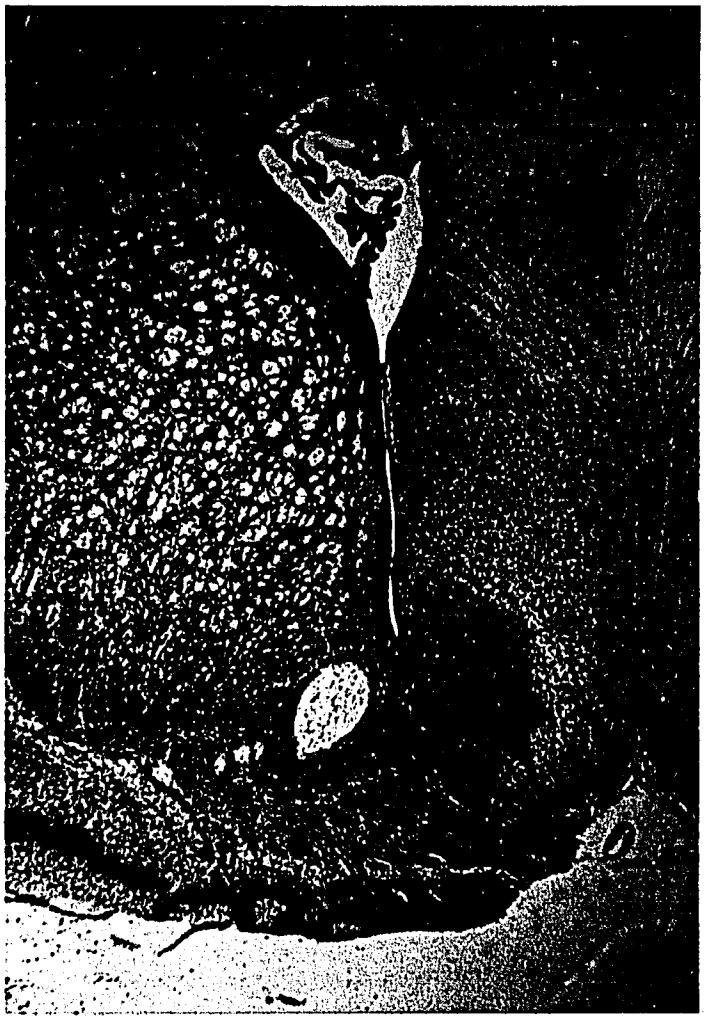

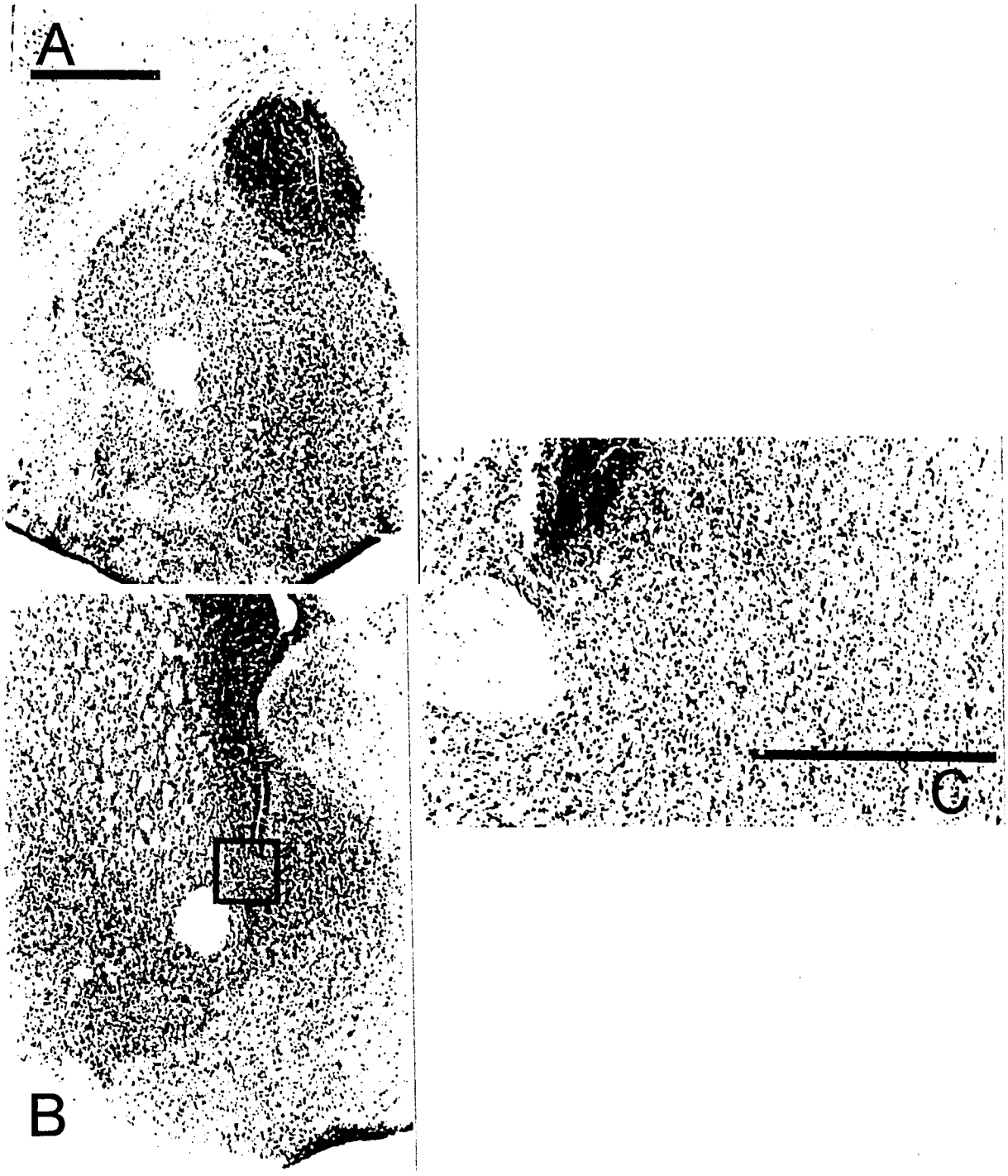
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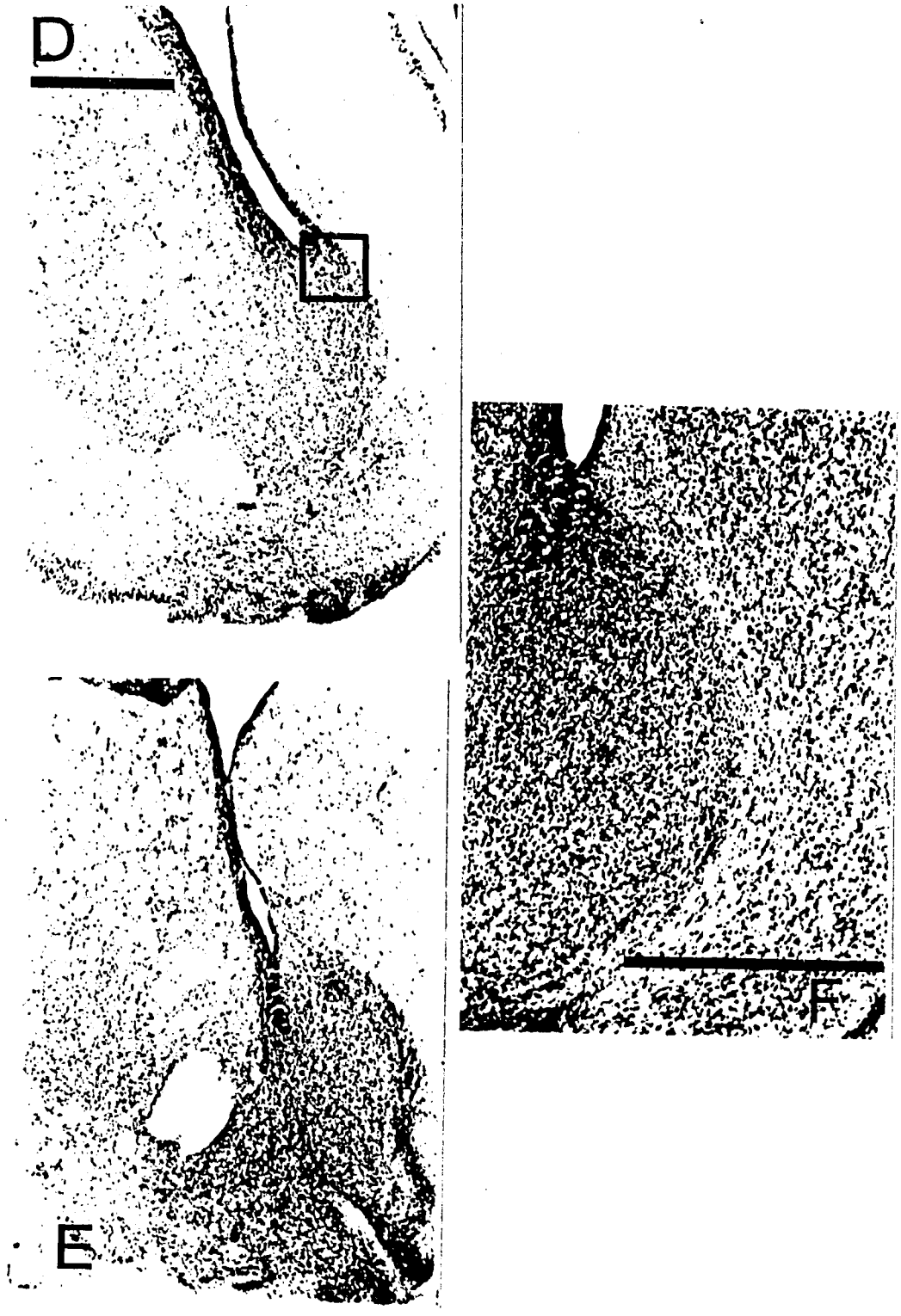
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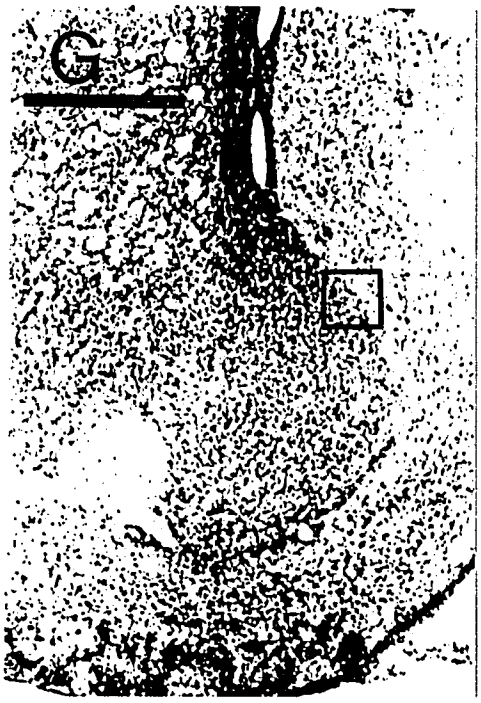
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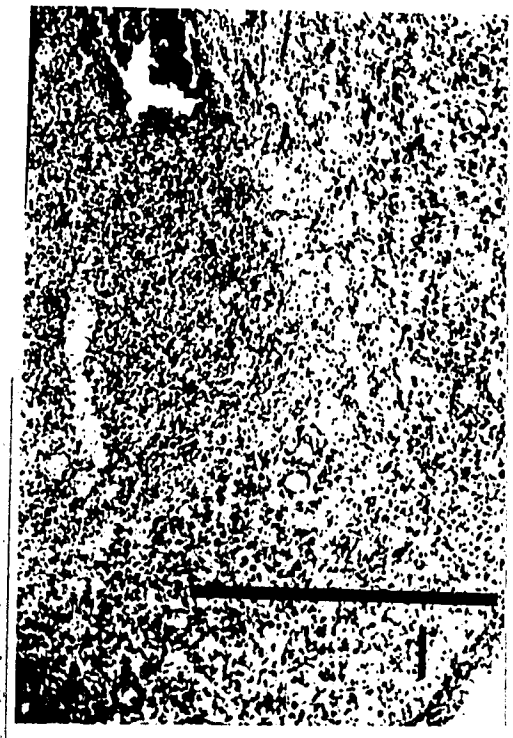
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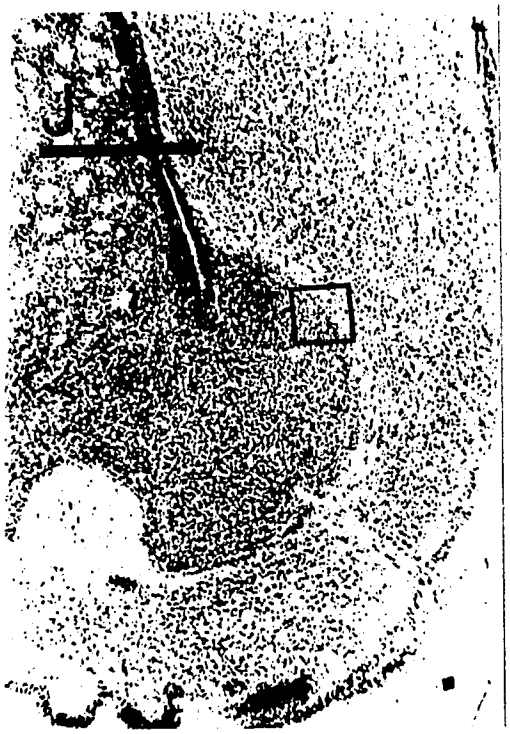
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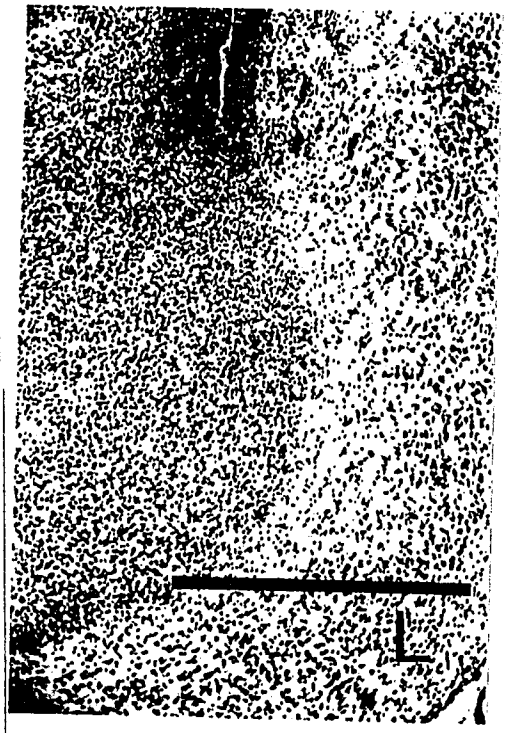
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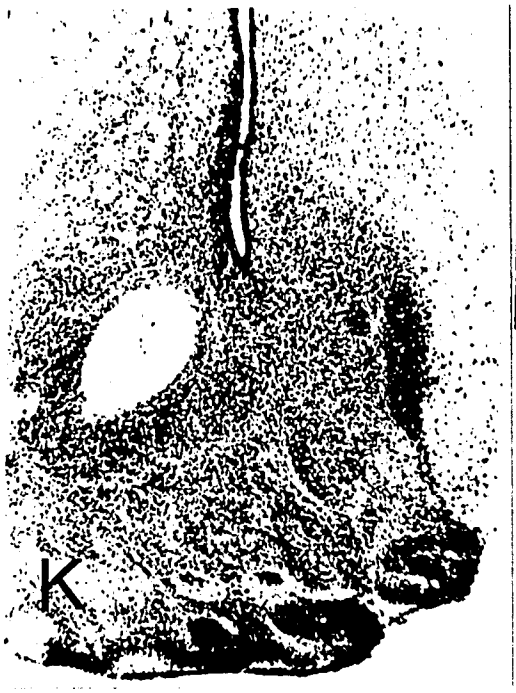
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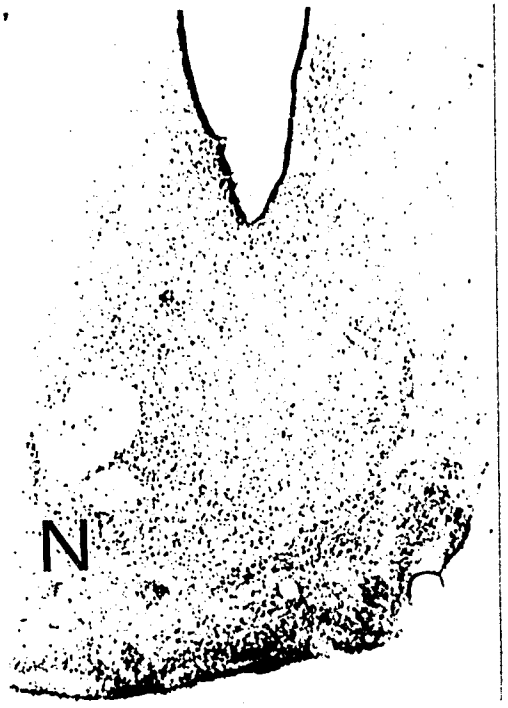
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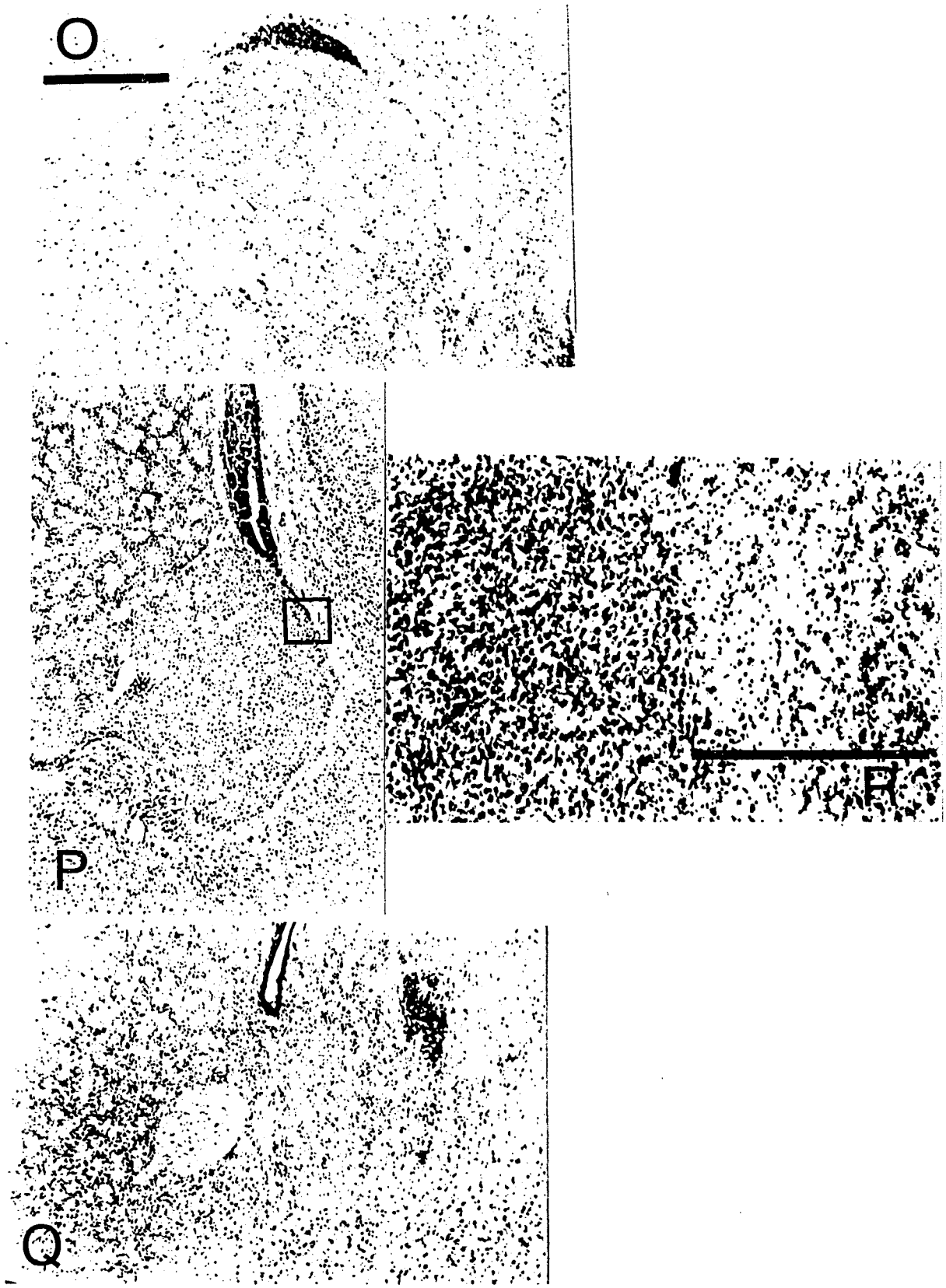
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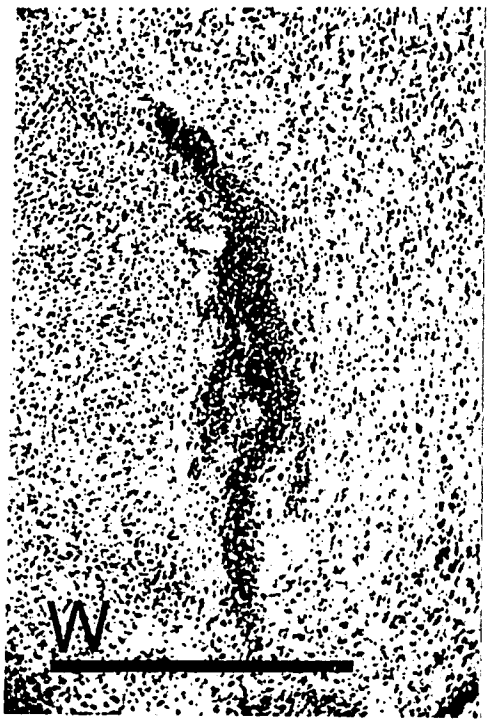
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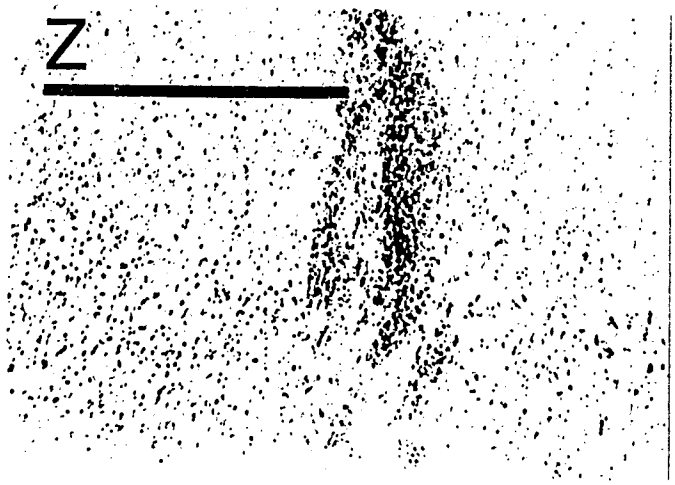
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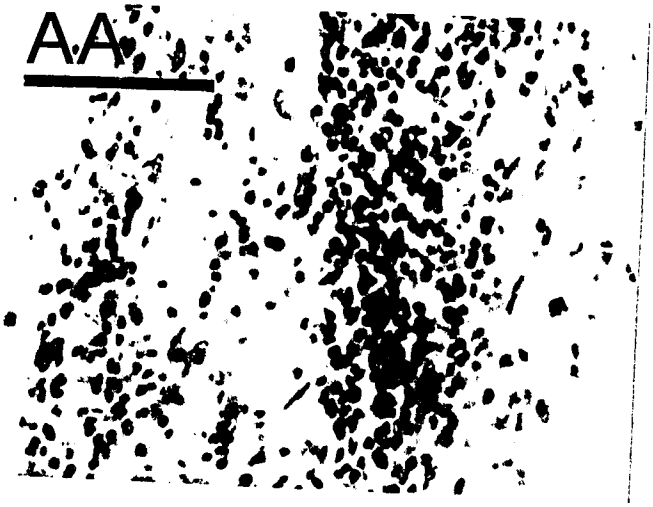
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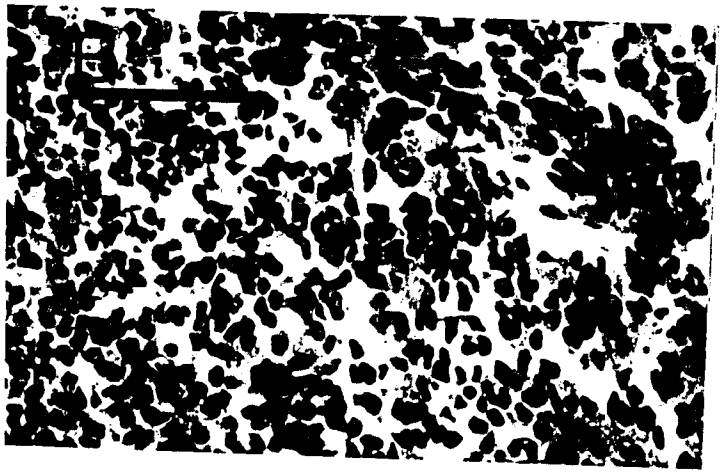

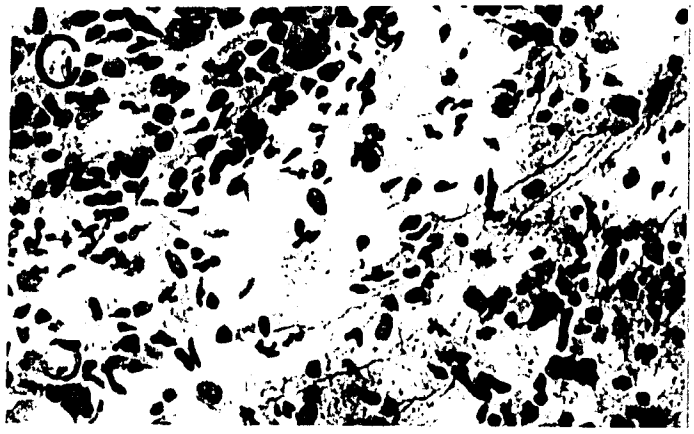

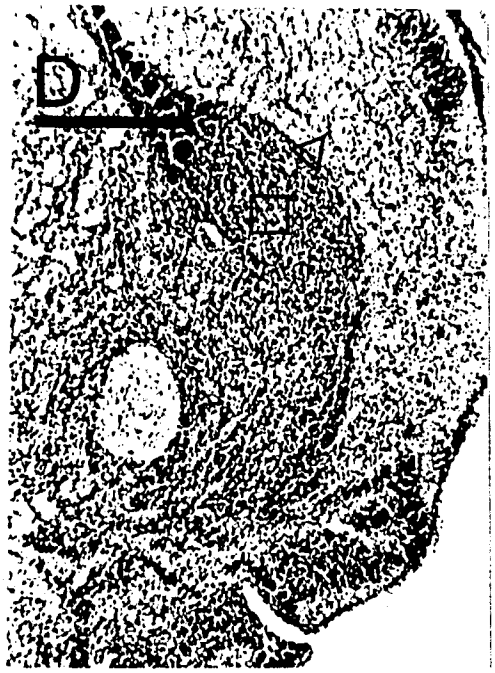
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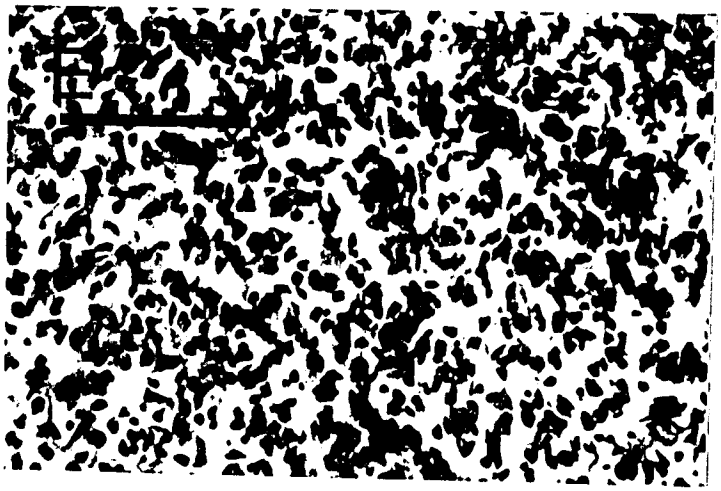

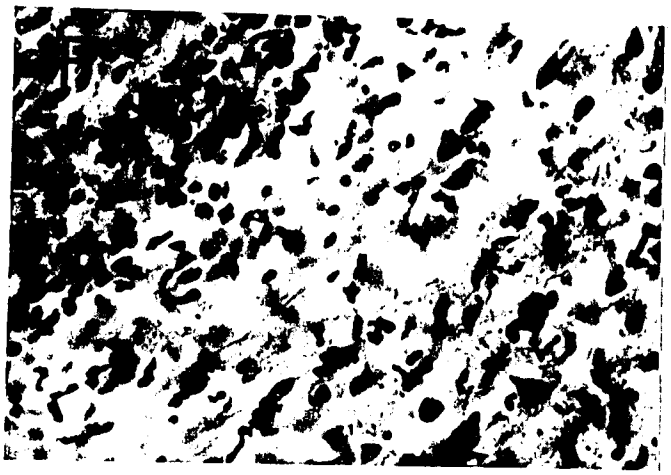


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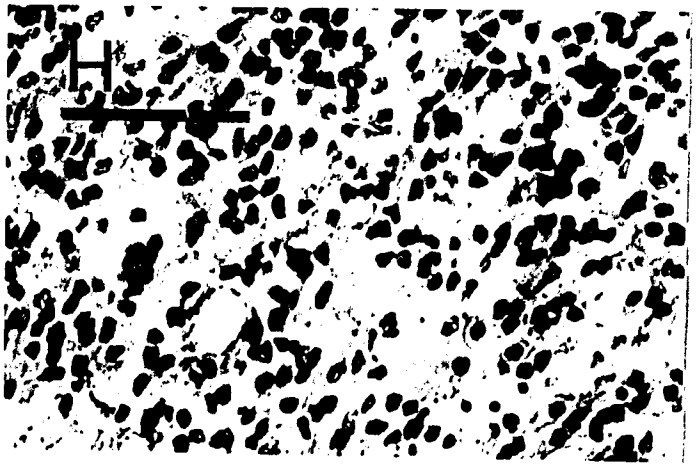

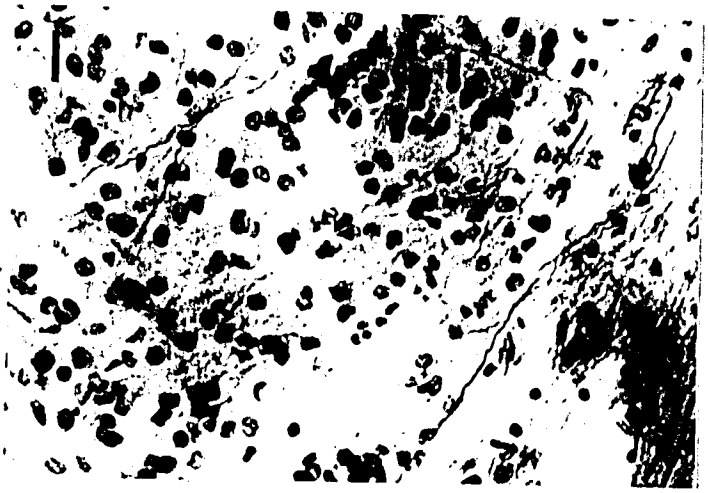


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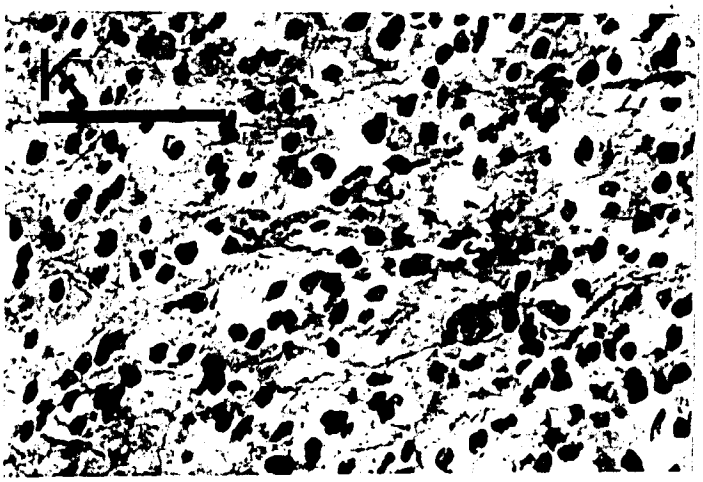

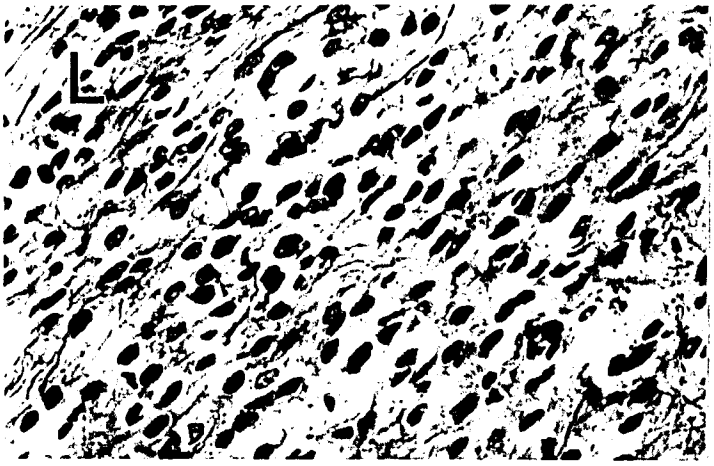


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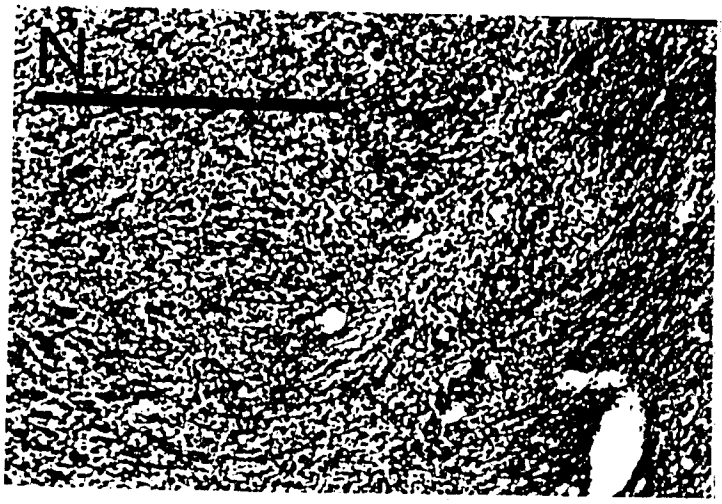
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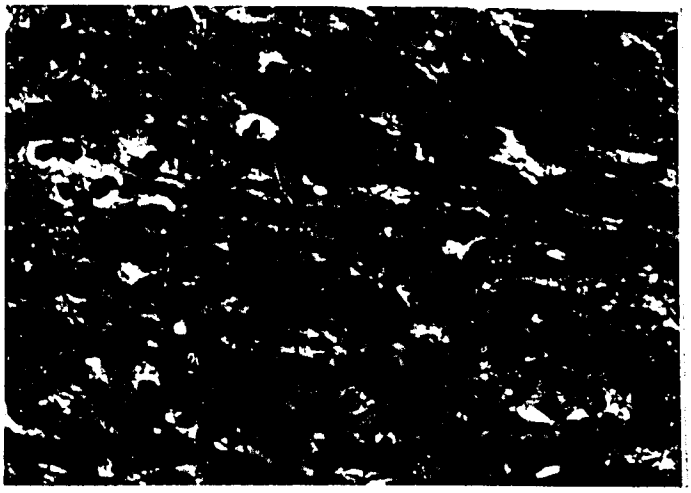

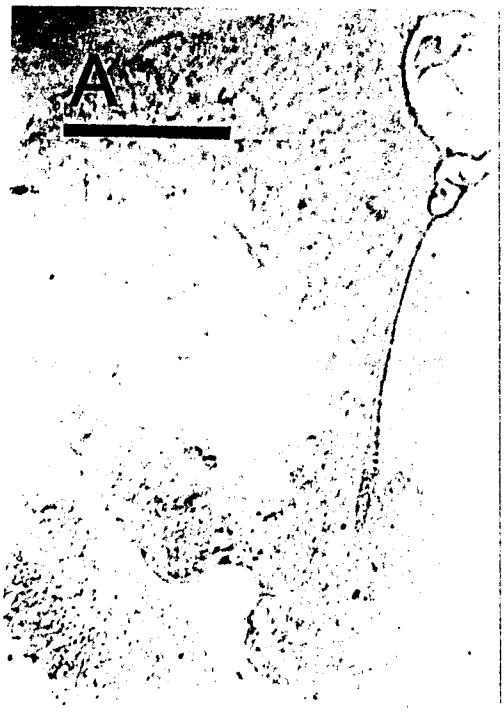
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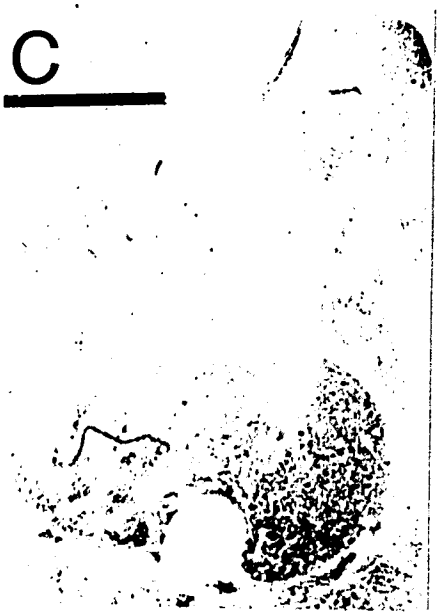
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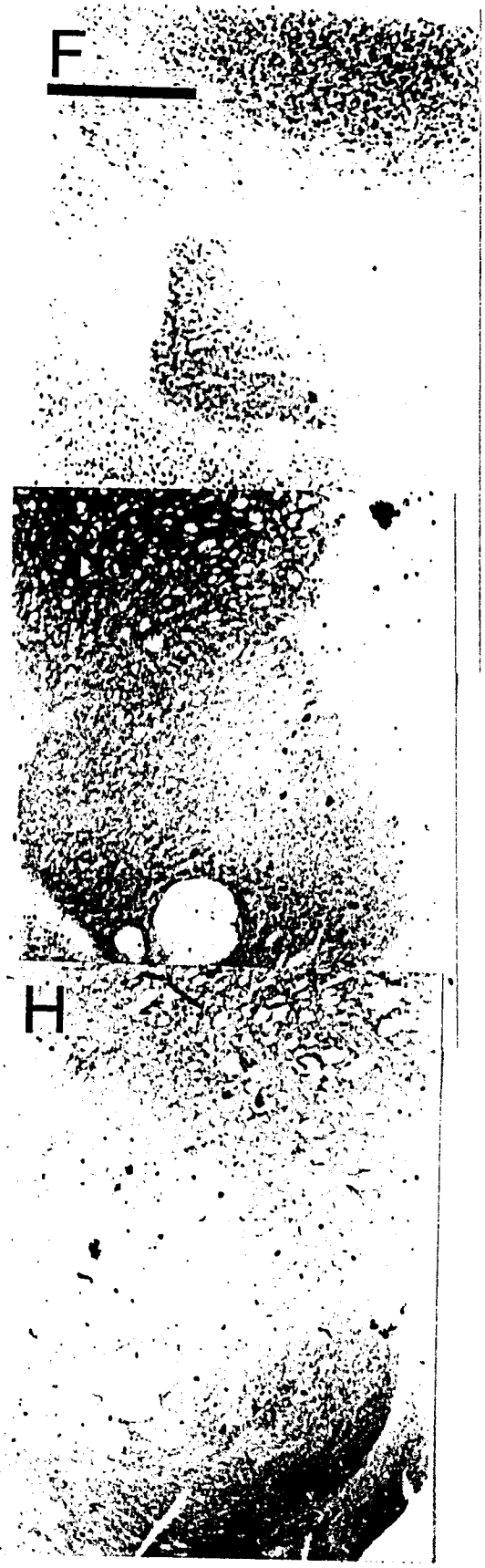
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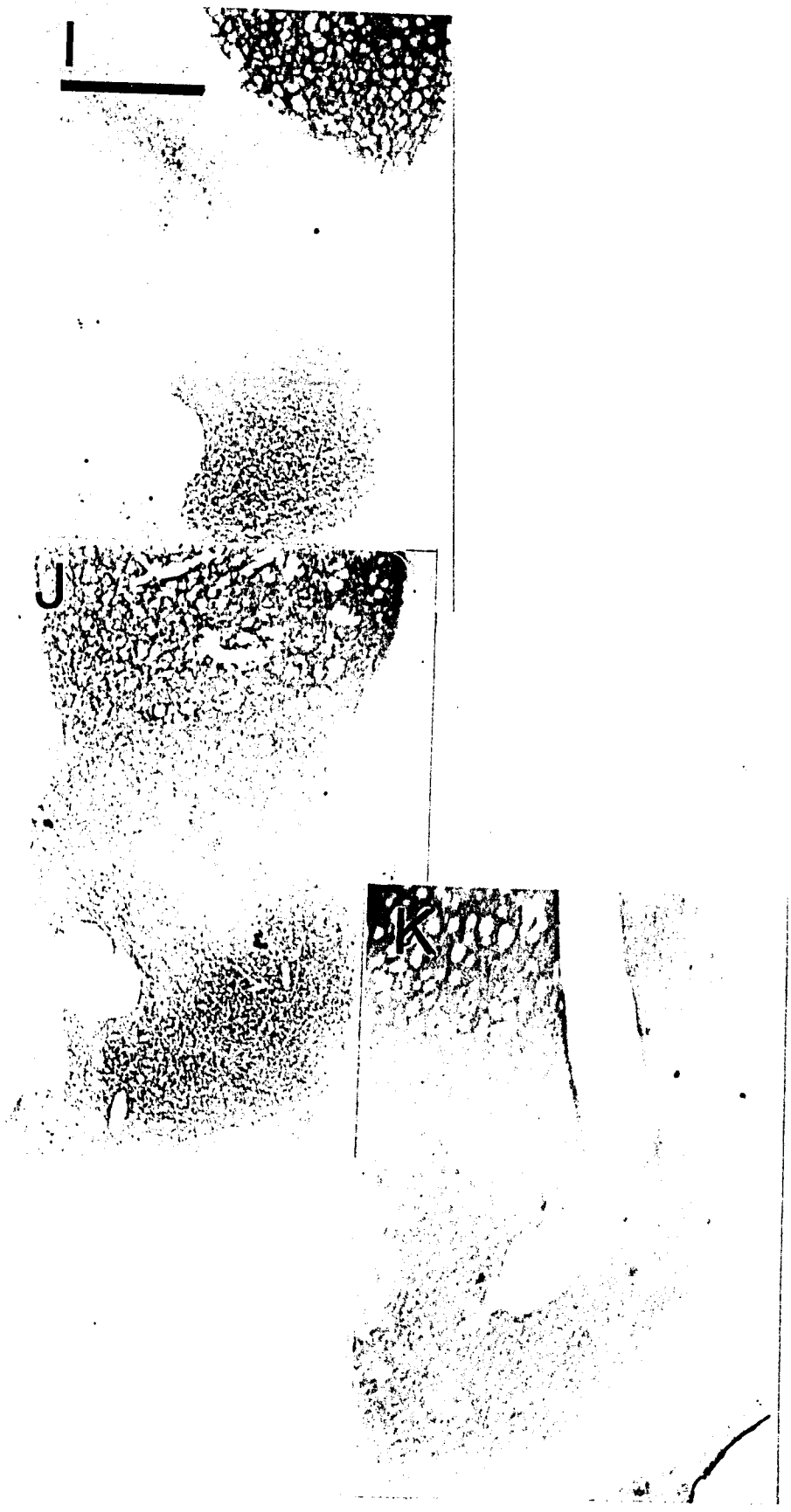
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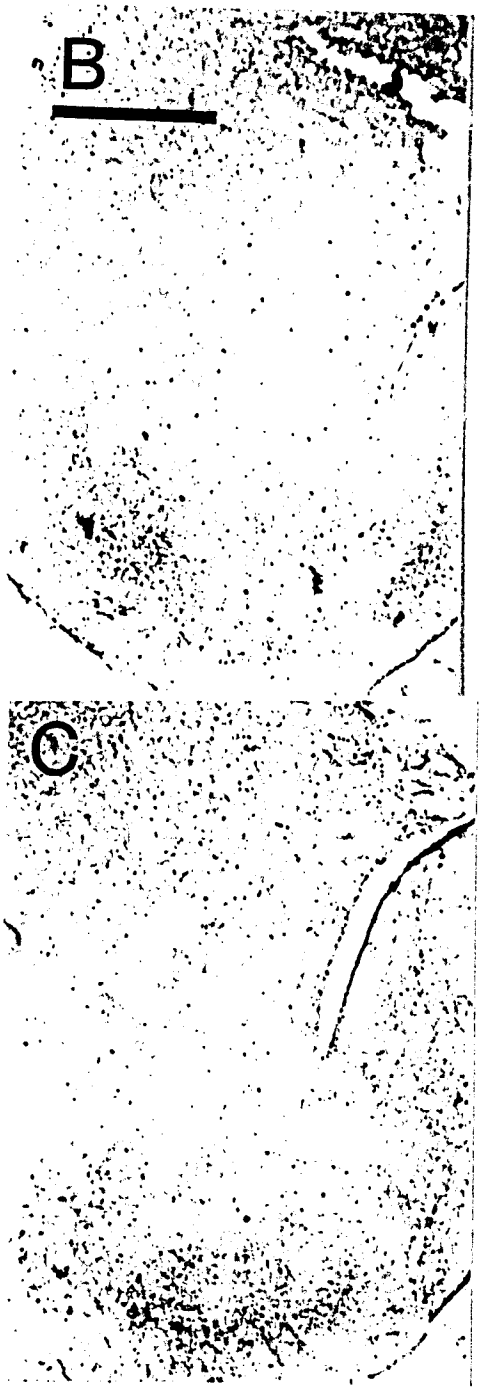
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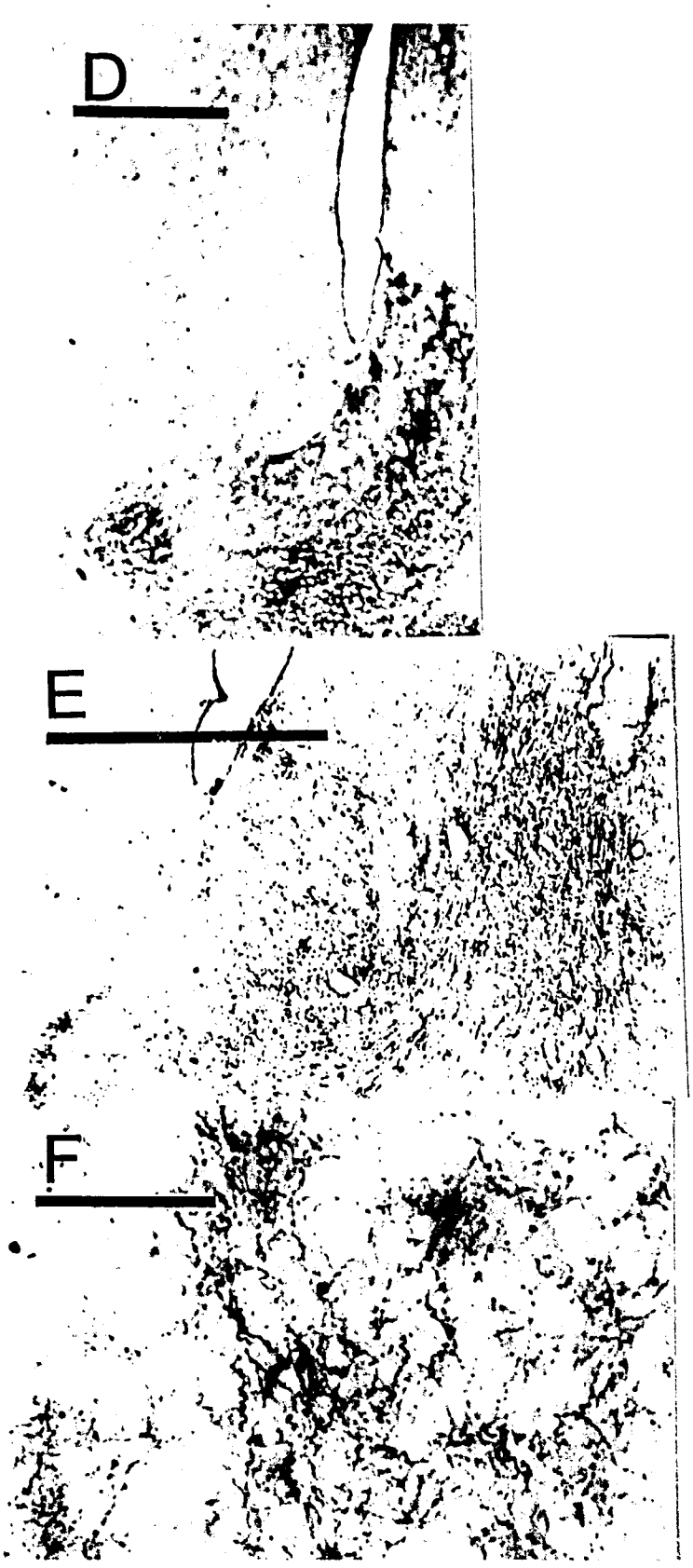
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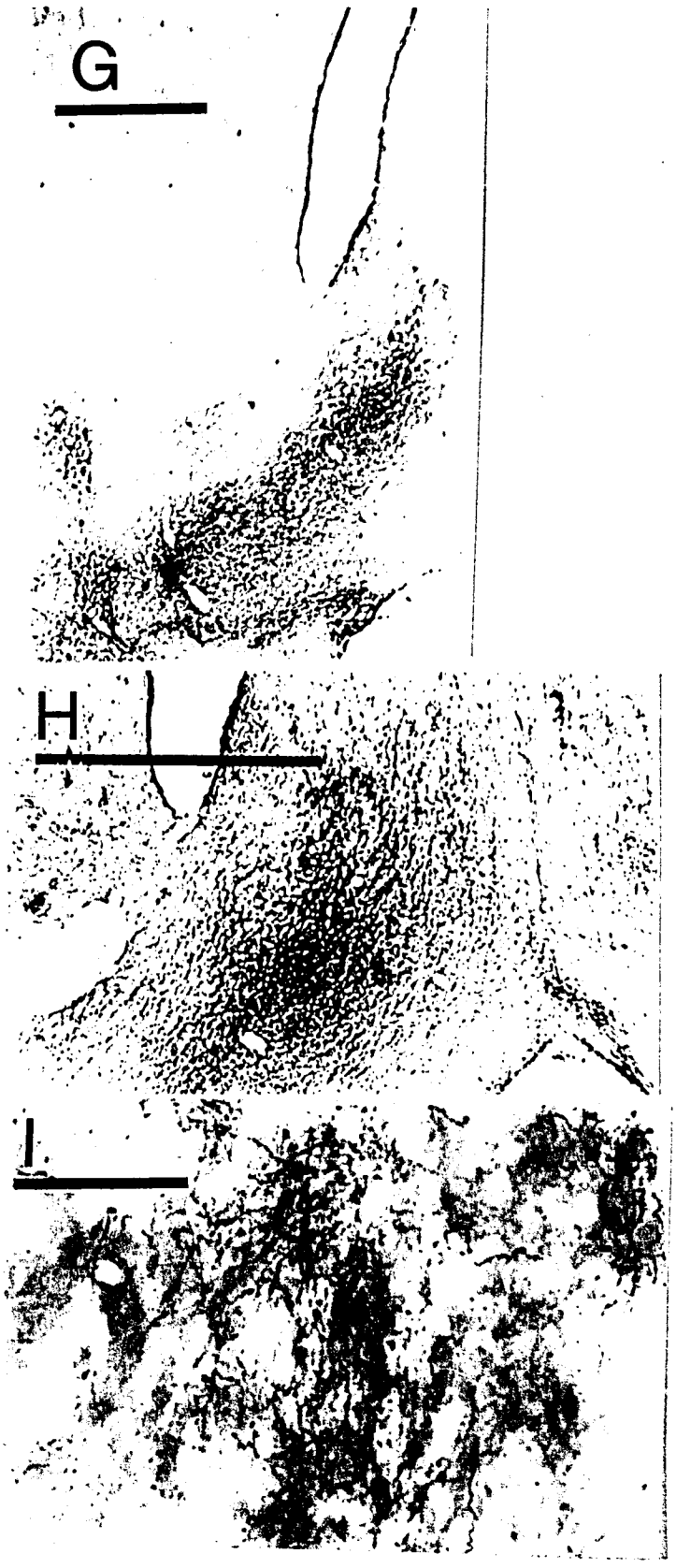
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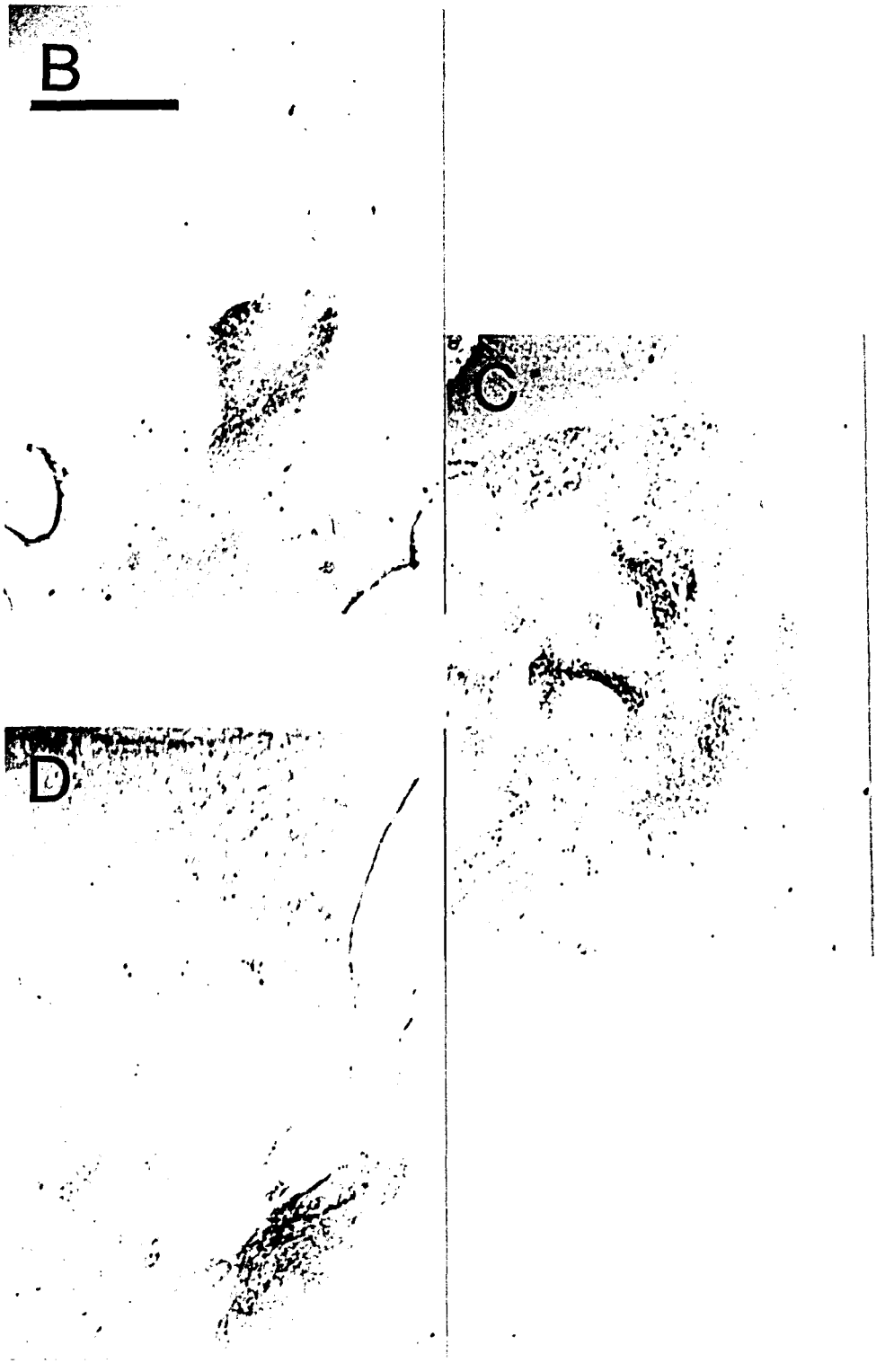
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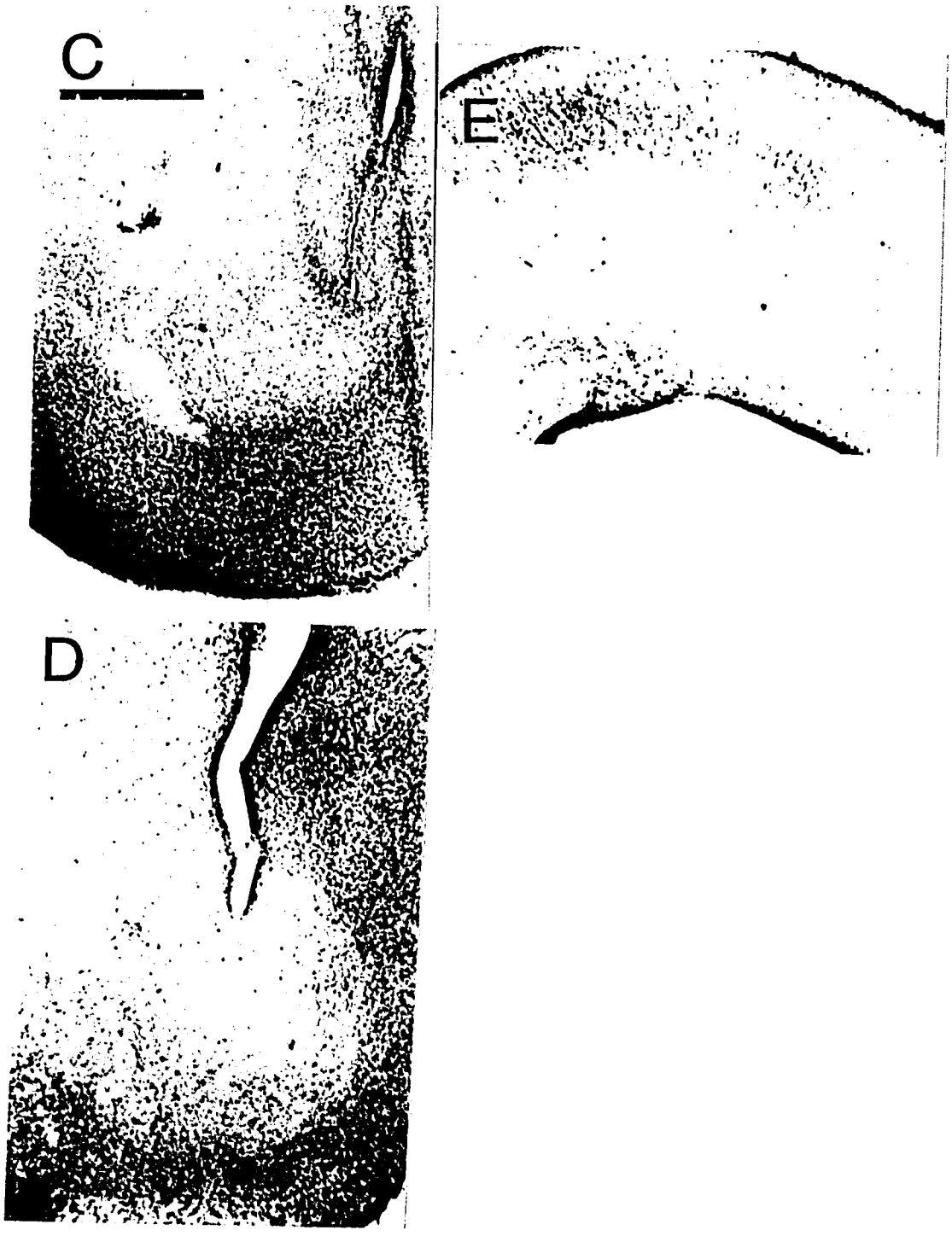
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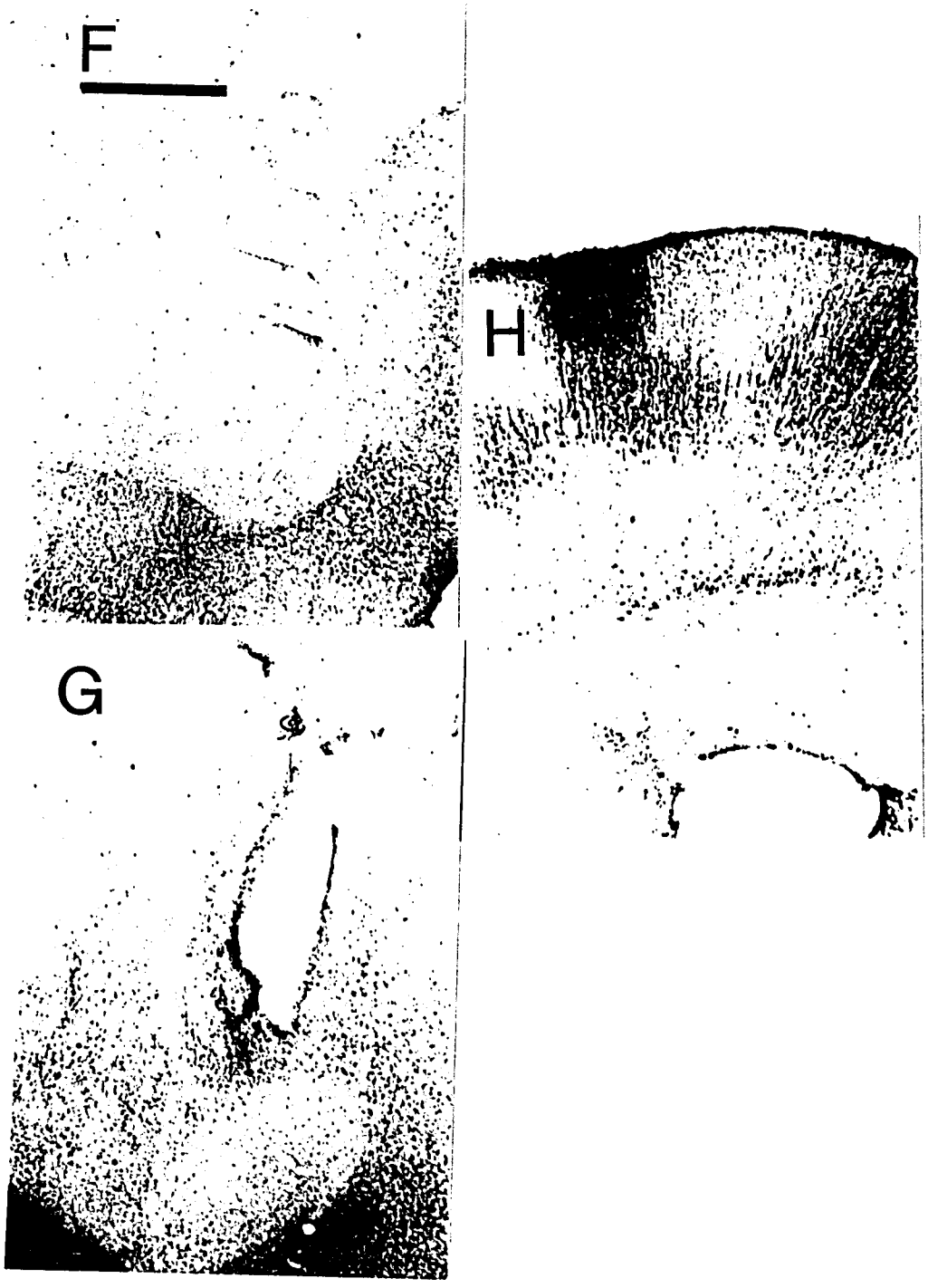
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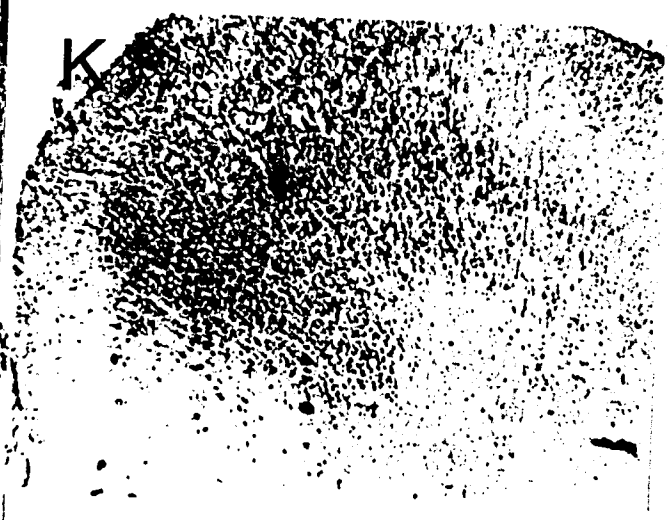
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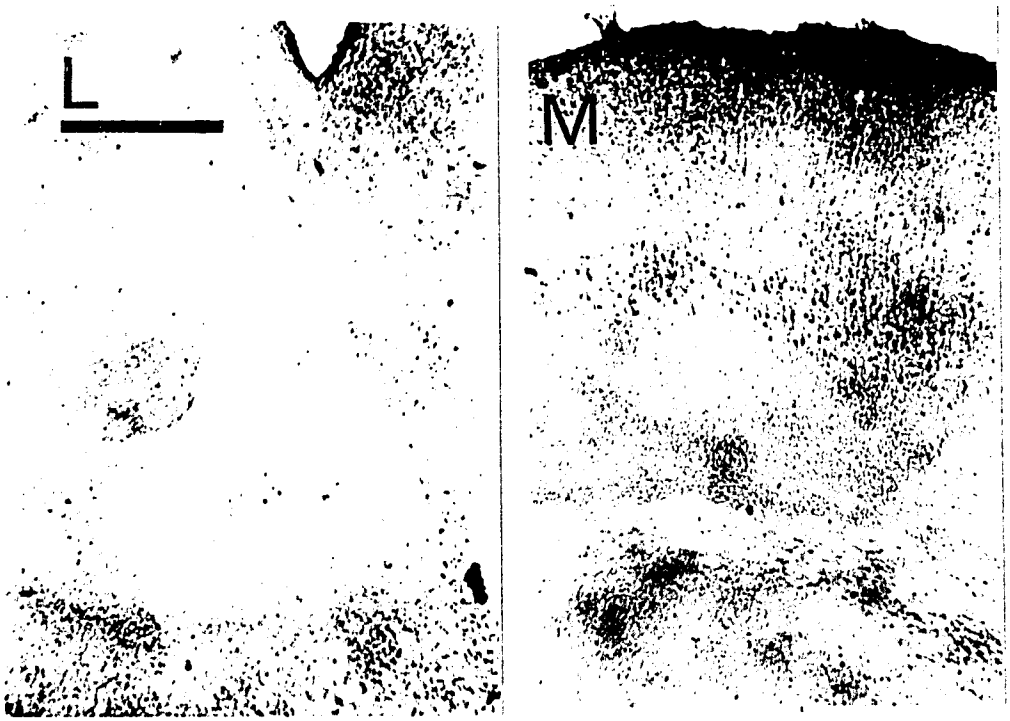
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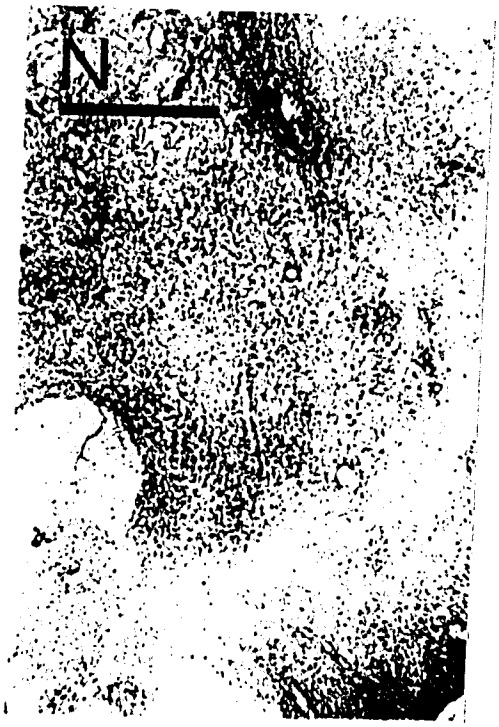
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