THE ROLE OF BASAL FOREBRAIN IN RAT SOMATOSENSORY CORTEX: IMPACT ON CHOLINERGIC INNERVATION, SENSORY INFORMATION PROCESSING AND TACTILE DISCRIMINATION

1993

JACOBS

UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES
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APPROVAL SHEET

GRADUATE EDUCATION

TEACHING HOSPITALS
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Title of Dissertation: "The Role of Basal Forebrain in Rat Somatosensory Cortex: Impact on Cholinergic Innervation, Sensory Information Processing and Tactile Discrimination"

Name of Candidate: Essie Jacobs
Doctor of Philosophy Degree
May 28, 1993

Dissertation and Abstract Approved:

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Abstract

Title of Dissertation: The Role of the Basal Forebrain in Rat Somatosensory Cortex: Impact on Cholinergic Innervation, Sensory Information Processing, and Tactile Discrimination

Stella Essie Jacobs, Doctor of Philosophy, 1993

Dissertation directed by: Sharon L. Juliano, Ph.D.
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The basal forebrain (BF)-neocortical pathway, and lesions disrupting it, have been used to study states of cortical arousal, neuronal responses to receptive-field stimulation, and learning and memory. Cholinergic neurons are a principal constituent of the BF and provide a major source of cortical acetylcholine (ACh). ACh is recognized as an important element in normal cortical function since it enhances neural activity. The mechanisms of ACh-induced cortical enhancement are reviewed in Chapter 1. In Chapter 2 I present findings that stimulus-evoked metabolic activity, assessed by the 2-deoxyglucose (2-DG) technique, is reduced in ACh-depleted somatosensory cortex of rats. Background levels of 2-DG uptake are unaffected. In an effort to restore cortical ACh innervation and ameliorate the functional 2-DG deficit, transplants of ACh-rich embryonic BF were implanted into ACh-depleted rat cortex. Control grafts of embryonic neocortical tissue were placed into cortex of BF-lesioned animals. I report in Chapter 3 evidence that the implants established functional corrections. Regions of cortex within 2 mm from BF transplants contained (a) density of acetylcholinesterase (AChE)-stained fibers comparable to the

opposite normal hemisphere, and (b) 2-DG uptake similar to that found in matched activated barrels in the contralateral hemisphere. Farther than 2 mm from the BF transplant, as well as throughout hemispheres with control neocortical grafts, (a) density of AChE staining is reduced, and (b) 2-DG uptake in barrels within these ACh-depleted territories is diminished from contralateral counterparts. Chapter 4 examines the effect of cortical ACh depletion on a previously learned task of whisker sensory discrimination. Sham-lesioned rats demonstrated no disruption of performance; animals with excitotoxic lesions of the BF exhibited transient impairments. The length of time to return to criteria positively correlated with amount of cortical ACh depletion. Despite their behavioral recovery, the group of animals with excitotoxic lesions continued to demonstrate diminished whisker-evoked 2-DG uptake, suggesting a sustained reduction in cortical sensory processing. A general discussion of the role of the BF in arousal, attention, and Alzheimer's disease is the focus of Chapter 5. Consideration of other factors contributing to transplant-derived improvements in evoked somatosensory cortical processing is also presented.

The Role of Basal Forebrain in Rat Somatosensory Cortex: Impact on Cholinergic Innervation, Sensory Information Processing, and Tactile Discrimination

by

Stella Essie Jacobs

Dissertation submitted to the Faculty of the Department of Anatomy and Cell Biology Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of Doctor of Philosophy 1993

To my mother, Rose, for proving daily that time can stand still so we might as well cram just a little more into life.

To the memory of my father, Alan, whose love of knowledge has been a precious gift.

To Chuan, with love and gratitude for our shared lives.

Acknowledgements

For providing me with guidance, a stimulating area of research to explore, and a model of personal conviction and professional integrity, I am sincerely grateful to my thesis advisor, Sharon Juliano.

I am very appreciative to the members of my committee for their individual efforts towards my reaching this goal. Linda Porter and Asaf Keller have been particularly helpful with thoughtful comments regarding the dissertation. Jim Barrett gave valuable guidance on conducting behavioral research, and made invaluable suggestions during the course of behavioral testing. I also want to thank Katy Lynch for serving on my committee, and for the hours she devoted to our shared discussions of the literature. Although she was unable to attend the defense, I consider her a fully participating member of the committee.

Don Eslin, to whom I have turned so often for technical advice and who has so freely given of himself to teach me many of the histologic, photographic and autoradiographic methods, cannot be thanked enough. His genunine interest in this research project made his suggestions extremely helpful.

I am indebted to Wu Ma for teaching me many of the technical skills I needed to conduct this research, to Becky Code for her systematic approach towards solving a methodological problem which was critical for this work to effectively progress, and to Marina Volkov for guiding me through the statistical analysis of the data and helping me present much of it graphically.

I feel quite fortunate to have been a student in the Department of Anatomy and Cell Biology, where I have been actively encouraged and supported by so many people during these past 6 years. I would like to particularly thank Drs. Rosie Borke, Joe McCabe, Don Newman, and Mark Rollag for generously giving their time to answer my questions. Dr. Michael Sheridan had the gift of offering me motivational words when they were most needed, for which he is warmly acknowledged. Mary Thomson is also gratefully recognized for providing me with both tangible and intangible nurturing.

To the staff in LAM, who provided me with testing space and willingly helped cover weekend feeding schedules for the animals with special diets, I wish to express my appreciation. I also wish to acknowledge the efforts of my friend, Steve Shafer, who made the testing apparatus that served as the cornerstone of the behavioral experiments.

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Chapter 1. Introduction

Acetylcholine in the Neocortex

Acetylcholine (ACh) serves as a potent neuromodulator in widespread regions of the cerebral cortex. The actions of ACh have been the focus of intense investigation for the past two decades. Much of the work over these years has centered on studying and clarifying the modulatory actions of ACh. Although numerous effects have been identified with ACh and both excitation and inhibition observed, in the cerebral cortex cholinergic application appears to primarily enhance neural activity (for review see McCormick, 1992). The excitatory response is slow in onset as well as long-lasting (Krnjević, 1974). An early onset, brief, inhibitory response can be observed that precedes the onset of the slow excitation (Krnjević, 1974). This cholinergic inhibitory response was later found to be mediated by local gamma-aminobutyric (GABA)-containing inhibitory interneurons, which also exhibit an excitatory response to ACh iontophoresis (McCormick and Prince, 1986). Finally, neuronal responses to depolarizing inputs are enhanced in the presence of ACh, while spontaneous activity remains unaffected (e.g., Donoghue and Carroll, 1987; Sato et al., 1987; Sillito and Murphy, 1987).

Excitatory Role of ACh in the Cerebral Cortex

Acetylcholine acts on both nicotinic and muscarinic receptors, however the excitatory effect of ACh in the cerebral cortex is predominantly mediated by muscarinic receptors. Intracellular recording studies conducted by Krnjević et al. (1971) demonstrated that iontophoretic application of ACh in cerebral cortex produced a prolonged and marked excitation that was associated with a slow depolarization and an apparent decrease in K^+ conductance. This was later confirmed by others (Halliwell and Adams, 1982; Brown, 1983; McCormick and Prince, 1986). Cells with little spontaneous firing, or hyperpolarized cells, show minimal changes in responsiveness with iontophoresis of ACh. This characteristic, along with the slow depolarization, makes ACh different from a classical excitatory neurotransmitter, such as glutamate, which results in a fast onset of action and has global effects on cortical activity, both spontaneous and evoked (for review see McCormick, 1992).

It is now known that in the neocortex, several different potassium currents can be reduced by muscarinic (M) receptor activation. One of these M receptors blocks a voltage-dependent potassium current (I_M), which when activated allows K^+ ions to leave the cell, thereby repolarizing the membrane back towards the resting potential

and subsequently slowing the firing rate of the neuron. Therefore, blocking this current leads to a long-lasting increase in neural excitability (Brown and Adams, 1980; Madison and Nicoll, 1984; McCormick and Prince, 1987). ACh acting on muscarinic receptors also inactivates a Ca^+-activated potassium channel, which is nonvoltage-dependent. This current is responsible for the slow afterhyperpolarization (I_{AHP}) that appears during and after the generation of a train of action potentials. Its activation, like that of I_M, reduces the neuronal discharge rate that occurs as a result of a constant depolarizing current and, when blocked, increases the ability of the neuron to generate action potentials during a constant depolarization of excitatory postsynaptic potentials (McCormick and Williamson, 1989; for review see McCormick, 1992). Additional contributions of a Na^+-activated K^+ current block and a slow afterdepolarization of unknown origin have also been implicated as potential mechanisms of ACh action in the neocortex (McCormick and Prince, 1986; Schwindt et al., 1989).

The are 5 subtypes of muscarinic receptors (m_1-m_5) identified to date according to their amino acid sequence; m_1, m_2, and m_4 have been localized immunohistochemically in the cortex (Levey et al., 1990, 1991). Activation of these muscarinic receptors, which appear to be linked to G-proteins, results in various second messenger responses that

mediate the post-synaptic effects of ACh. Correlation of receptor subtype with ionic response and second messenger system has yet to be determined.

Inhibitory Actions of ACh in the Cerebral Cortex

McCormick and Prince (1985, 1986), using an *in vitro* slice preparation, demonstrated that when cortical neurons are depolarized to near firing threshold, application of ACh results in a short latency inhibition followed by slow excitation, identical to that reported *in vivo* by Krnjević (1974). McCormick and Prince (1986) proposed that the initial inhibitory response resulted from the excitation of intrinsic GABAergic inhibitory interneurons. Based on responses to application of atropine and scopolamine, specific muscarinic receptor blockers, the cholinergic excitation of local GABAergic neurons was also found to be mediated by muscarinic receptors (Krnjević et al., 1974; McCormick and Prince, 1985). The mechanism by which ACh acts on inhibitory interneurons is not yet known, however the rapid onset of response has been proposed to be the result of a decrease in input resistance; this contrasts markedly from the increased resistance to K^+ conductance that characterizes the action of ACh on cortical neurons.

The indication that the short-latency inhibition that follows application of ACh may be due to excitation of local GABAergic neurons supports the role of ACh as primarily an excitatory agent in the cerebral cortex. The ability of ACh to depolarize GABAergic interneurons and exert an excitatory effect on cortical neurons makes it potentially a very powerful mechanism for influencing the processing of information in the central nervous system (for review see McCormick, 1990, 1992).

ACh and Cortical Processing of Sensory Stimuli

Studies in sensory cortical information processing have established that ACh enhances the responsiveness of a cortical neuron to relevant stimuli, while spontaneous activity remains relatively unaffected (Krnjević et al., 1971; Sillito and Kemp, 1983; McKenna et al., 1988; Rasmusson and Dykes, 1988). Application of the GABAergic antagonist, bicuculline, enhances a cell's response to sensory information, however, unlike ACh, it also increases the resting discharge of the cell. These effects of bicuculline are the result of the release of the cell from the inhibitory influences of GABA, or disinhibition (Sillito and Kemp, 1983; Lamour et al., 1988). The application of glutamate or other excitatory amino acid neurotransmitters produces an enhanced response as does ACh, but glutamate,

like bicuculline, increases the level of spontaneous activity in the cell (Sillito and Kemp, 1983; Lamour et al., 1988; Metherate et al., 1988a; Tremblay et al., 1990a). A further difference between ACh and these other agents (i.e., GABA and glutamate) is that the increased response to optimal stimulation elicited from ACh iontophoresis occurred without any decrease in response selectivity (Sillito and Kemp, 1983; Murphy and Sillito, 1991). The excitatory post-synaptic actions of ACh (e.g., due to a decrease in the membrane K^+ conductance) provide a basis for the enhanced responsiveness without a reduction in the response selectivity (Murphy and Sillito, 1991).

In the presence of ACh, previously "hidden" receptive fields are uncovered in approximately one-third of the responsive cells; the major effect on receptive field properties is that a lower level of sensory input is capable of generating a neuronal response (Lamour et al., 1988; Metherate et al., 1988a, Tremblay et al., 1990a,b). This is abolished with application of atropine, indicating that the response is mediated by muscarinic receptors.

When application of ACh is paired with stimuli that activate the neurons being studied, the response is greatly enhanced (Donoghue and Carroll, 1987; Sato et al., 1987; Sillito and Murphy, 1987; Lamour et al., 1988; Metherate et al., 1988a). This combination of ACh application and appropriate stimulation leads to resulting neuronal activity

that often substantially outlasts the presentation of the stimulus (Rasmusson and Dykes, 1988; Metherate et al., 1987, 1988a,b; Tremblay et al., 1990b).

Cholinergic Innervation of the Neocortex

Anatomy

For decades it has been appreciated that the mammalian cerebral cortex receives a substantial cholinergic innervation. Early efforts to assess cholinergic projection pathways relied on the use of histologic staining for acetylcholinesterase (AChE), the degradative enzyme associated with ACh (Shute and Lewis, 1967). Subsequent studies, however, demonstrated the presence of AChE in non-cholinergic neuronal regions (Butcher et al., 1975; Lehmann and Fibiger, 1978). It was not until the development of antibodies against the biosynthetic enzyme for ACh, choline acetyltransferase (ChAT), that cholinergic structures could be identified in the CNS with certainty (Sofroniew et al., 1982; Mesulam et al., 1983; Houser et al., 1983; Eckenstein and Sofroniew, 1983; Levey et al., 1984). Studies identifying both AChE and ChAT in the rat indicate that, for most cortical areas, the two staining patterns of the

distribution of fibers are nearly identical (for review see Wainer and Mesulam, 1990).

The majority of the cortical cholinergic innervation is extrinsic, arising from the basal forebrain via cholinergic cells in the ventral pallidum, including the ventromedial globus pallidus, the nucleus basalis magnocellularis and the substantia innominata (e.g., Fibiger, 1982; Mesulam et al., 1983; Rye et al., 1984; Saper, 1984). I will refer to the region of the basal forebrain that supplies cholinergic innervation to the somatosensory cortex as the nucleus basalis magnocellularis, or nbm, even though it encompasses the additional, above-named regions.

A topographical organization has been observed for cholinergic projection patterns, with more rostral and medial basal forebrain cell groups supplying rostral and medial neocortex, while more posterior and lateral cell groups supply lateral regions of the neocortex (Lehmann et al., 1980; Bigl et al., 1982; McKinney et al., 1983; Rye et al., 1984; Saper, 1984).

In addition to the cholinergic innervation from the basal forebrain, the rodent cerebral cortex has a substantial intrinsic cholinergic innervation, which has not been described in other species and whose significance remains to be defined (Levey et al., 1984; Houser et al., 1985; Eckenstein and Baughman, 1987). These intrinsic cholinergic neurons are bipolar, make ChAT-positive synaptic

contacts that are predominantly symmetric onto the distal dendrites, and are also immunoreactive for vasoactive intestinal polypeptide (VIP) (Eckenstein and Baughman, 1984) and GABA (Hallanger et al., 1986).

Basal Forebrain Lesion-Induced Reductions in Cortical ACh

One model used to study cholinergic function in cerebral cortex is that of the basal forebrain lesion, which depletes ACh in the neocortex. Lesions of the basal forebrain made with an excitotoxin, which does not affect fibers of passage, as well as electrolytic lesions and cortical undercutting, reduce cortical levels of both AChE and ChAT (Johnston et al., 1979, 1981; Lehmann et al., 1980; Wenk et al., 1980; Whitehouse et al., 1981; Bigl et al., 1982; Eckenstein and Sofroniew, 1983; Houser et al., 1983; Saper, 1984). Each different lesion yields similar decreases in cortical cholinergic markers, assessed biochemically. For example, when the cortex is undercut or the nbm is lesioned, the total ACh content is depleted by about 70% (Wenk et al., 1980; Fibiger and Lehmann, 1981; Johnston et al., 1981). The approximately 30% remaining cholinergic innervation is derived from intrinsic cholinergic neurons in the cerebral cortex of rats although, as indicated above, intrinsic neurons are not found in other species.

Currently a neurotoxin selective for cholinergic neurons does not exist; the validity of this lesion model has been heavily debated since populations of non-cholinergic neurons within the basal forebrain are also damaged by neurotoxic lesions. Still, basal forebrain lesions appear to be a well accepted means to study the significance of ACh in neocortex. The degree of depletion achieved after such lesions can add substantially to our understanding of the role of cholinergic contributions to cortical function (for reviews see Olton and Wenk, 1987; Dekker et al., 1991).

The Whisker-Barrel Pathway

Anatomy and Physiology

The primary somatosensory cortex (SmI) of several rodent species has an unusual cellular organization in the region of the face representation. The morphology of these aggregations of cells in layer lV, as described by Woolsey and Van der Loos (1970), suggests a 3-dimensional character which led to their being called "barrels".

Woolsey and Van der Loos (1970) report that barrels are discrete, multicellular cytoarchitectonic units made up of a cell-dense ring, which represents the side of the

barrel, surrounding a less cellular area, the hollow. A nearly acellular area, called the septum (containing less cells than the hollow), separates adjacent barrels. The posterior part of the barrel field of SmI was singled out as possessing distinct characteristics that include large barrel size and clearly delineated boundaries, and has subsequently become known as the posteromedial barrel subfield (PMBSF). The 35-40 barrels within this region are arranged in a distinct pattern of 5 rows (referred to as A to E) of 4 to 7 barrels in each row, which mirrors the arrangement and number of large facial whiskers, or vibrissae.

Microelectrode recording experiments have demonstrated that each whisker corresponds in a one-to-one fashion with a barrel-shaped collection of cells in the contralateral somatosensory cortex (Welker, 1976; Simons, 1978). Jensen and Killackey (1987a) reported that thalamocortical afferents terminate in discrete clusters that correspond to the whisker representation in rat SmI. Subsequent studies of the rat barrel cortex have modified the one-whisker-one-barrel hypothesis to suggest a greater divergence of input to the barrel cortex neurons. These multi-whisker receptive fields are probably generated by intrinsic connections of infra- and supragranular cell projections (Simons, 1978; Armstrong-James and Fox, 1987; Armstrong-James et al., 1992). These intrinsic cortical projections have been studied in visual cortex, and only

recently are they the focus of research in somatosensory cortex.

Simons and Woolsey (1979), using electrophysiological recording techniques, proposed that the barrel can be considered to be a cytoarchitectural correlate of a functional cortical column and that cells at all cortical depths in the column are associated with the appropriate whisker.

Experiments using 2-deoxyglucose (2-DG) were conducted by several researchers as a way of visualizing functionally active brain regions anatomically. Since this method allows visualization of glucose utilization during a defined period of time (Sokoloff et al., 1977; for review see Sokoloff, 1981), active or passive stimulation of a whisker during a 2-DG study produces increased 2-DG uptake in a discrete region of cortex associated with that whisker (Durham and Woolsey, 1977; Hand, 1982; Kossut et al., 1988).

A number of studies using the 2-DG technique in normal infant and adult mice and rats have provided visual evidence that barrels belong to a cortical columnar system. For example, in rats, levels of activity within an activated barrel extend throughout all cortical layers, are widest in layer 4 (where 2-DG uptake is most dense), and taper in both the supra- and infragranular layers (Durham and Woolsey, 1977; Hand, 1982; Chmielowska et al., 1986; Kossut et al., 1988).

Barrel Cortex as a Model for Analysis of Cortical Plasticity

The precise anatomical organization and strong peripheral-central correlations in the rat somatosensory system provide an opportunity to study barrel field structural alterations that occur with changes in input. Changes in barrel activity can also be studied using either electrophysiological recording techniques or markers of metabolic activity, as a tight coupling between glucose metabolism and neuronal activity has been shown to exist (Wong-Riley and Welt, 1980).

Van der Loos and Woolsey (1973) demonstrated severe disruption of the normal cytoarchitecture of barrels in mice as a consequence of removal of whiskers early in life, either by nerve cut or cauterization of whisker follicles. Barrels corresponding to the injured periphery were shrunken in appearance and neighboring barrels expanded into the deafferented territory. This phenomenon was also shown in rat somatosensory cortex (Killackey et al., 1976). Such structural alterations in cortical barrels occur when whiskers are removed before postnatal day 5; after this critical period, changes in barrel structure are not observed (Killackey, 1982; Van der Loos and Woolsey, 1973; Weller and Johnson, 1975; Jeanmonod et al., 1981).

Investigators using markers for metabolic activity have assessed alterations in cortical function following neonatal cauterization of selected whiskers. Wong-Riley and

Welt (1980) reported that cautery of a single row of whiskers in mice shortly after birth led to alterations in barrel structure, as seen with cytochrome oxidase (CO) staining, a marker for mitochondrial enzymatic activity. These changes were similar to the previously observed cytoarchitectural changes. Hand (1982), using the 2-DG technique, demonstrated that neonatal denervation of all but one whisker resulted 3 months later in a dramatic expansion of the associated spared whisker representation into cortical territory previously driven by the adjacent denervated whiskers.

It has been suggested that these changes may be related to a change in thalamocortical afferent terminations or changes in intracortical activity. Jensen and Killackey (1987b) found widespread arborization in the thalamocortical projections to barrel cortex in adult rats with early whisker deafferentation, unlike the discrete clustering typical of normal terminal arbors. This has been viewed by some as a failure of the normal segregation of thalamocortical afferents due to disruption of the peripheral input (Erzurumlu and Jhaveri, 1990), and by others as the expansion of thalamocortical afferents associated with intact barrels into the denervated cortical territory (see review by Wall, 1988). Others propose that the changes are due to events intrinsic to the cortex, as a result of horizontal transfer of information in supra- and infragranular layers of a barrel-associated cortical column

(Armstrong-James and Fox, 1987; Armstrong-James et al., 1992). The expanded zone of the spared whisker-associated cortical column, as seen with 2-DG and CO activity, might therefore be the functional consequence of synaptic modifications of such connections. Although the exact mechanism is not clear, this possibility seems more probable than the occurrence of proliferation of terminals to form new synapses since nerve regeneration has been shown to restore the normal pattern (for review see Wall, 1988). The shrunken appearance of the deprived barrels may, in contrast, be due to the loss of synapses that allowed cortical neurons to activate adjacent barrels via intrinsic connections (Kossut, 1992; Fox, 1992).

Functional plasticity following sensory receptor lesions or nerve transections in adult animals also develops, but without gross morphological changes. For example, Wong-Riley and Welt (1980) conducted the same procedure mentioned above in adult mice and found no changes in appearance of barrel structure as determined by Nissl staining, but CO staining was markedly reduced. Similar results were demonstrated by Land and Simons (1985) in somatosensory cortex of adult rats, suggesting that metabolic activity in barrel cortex is regulated by peripheral input.

Evidence is also accumulating that alterations in neonatal and adult cortical connectivity occur as a consequence of more subtle sensory experiences. Studies of

experience-dependent plasticity, in which whiskers were either plucked or cut periodically, resulted in changes in cortical organization as evidenced by 2-DG autoradiography, CO staining, and electrophysiological recording procedures (Hand, 1982; Simons and Land, 1987; Fox, 1992; Kossut, 1992). Changing the balance of input from the periphery along the pathway from the whiskers to the cortex appears to be sufficient for inducing functional changes in the somatosensory system.

ACh Depletion and Barrel Cortex Plasticity

In the following chapter I report that adult rats with unilateral basal forebrain lesions demonstrate reduced 2-DG uptake in response to whisker stimulation in the ACh-depleted hemisphere compared to the activity evoked in the opposite hemisphere. These findings confirm earlier results from nbm-lesioned mice (Ma et al., 1989). The reductions in 2-DG uptake following basal forebrain lesions appear specific to ACh depletion. Although other classes of neurotransmitters and neuromodulators are found in the basal forebrain, dramatic cortical reductions in substances other than ACh have not been reported following nbm lesions (Johnston et al., 1981; McKinney et al., 1982; Dubois et al., 1985; Fine et al., 1987; Höhmann et al., 1987). In addition, lesions destroying other nuclei that transmit

specific neurotransmitters or neuromodulators to the cerebral cortex cause alterations in 2-DG uptake that are distinct from those that occur after ACh depletion. For example, depletion of norepinephrine (NE) from barrel cortex through lesions of the locus coeruleus was found to result in an expansion of stimulus-evoked activity in barrels, in contrast to the shrinkage observed with ACh depletion (Craik et al., 1987).

The following series of experiments were designed to assess (a) the impact of depletion of cortical ACh resulting from basal forebrain lesions on whisker-evoked 2-DG activity in rat somatosensory cortex, (b) the effect of embryonic cholinergic basal forebrain transplanted into ACh-depleted cortex on stimulus-evoked metabolic activity, and (c) the consequences of basal forebrain lesions on the ability of a rat to respond to a previously learned task of whisker sensory discrimination.

CHAPTER 2: Basal Forebrain Lesions Alter Stimulus-Evoked Metabolic Activity in Rat Somatosensory Cortex

Introduction

An earlier study that evaluated the role of acetylcholine in stimulus-evoked activity in the somatosensory cortex of mice revealed that hemispheres depleted of ACh showed a reduction in density and dimension of 2-DG activity elicited by whisker stimulation (Ma et al., 1989). The authors concluded that ACh appears to contribute to the cortical processing of discrete sensory stimuli. The aim of the present study was to determine if ACh plays a similar role in the somatosensory cortex of rats.

Materials and Methods

Female Sprague-Dawley rats (250-350 g) were anesthetized with 7% chloral hydrate (40 mg/100 g) and received unilateral lesions of the basal forebrain. An opening was made in the skull 4 mm anterior to bregma and 2.3 mm lateral from midline. A Hamilton syringe was positioned 35° from vertical and angled 12° laterally to allow for a medial-to-lateral progression as the syringe

advanced from rostral to caudal. This approach permitted preservation of the frontoparietal cortex. The syringe was advanced to a depth of 8.0 mm from the dura, where 0.5 μl of ibotenic acid (10 μg/μl) was pressure injected. Two minutes later, a second injection (0.5 μl) was made at a depth of 7.0 mm. After a one-to-nine week survival, a terminal 2-DG experiment was performed. After inserting a catheter into the jugular vein under halothane anesthesia, the animal was loosely restrained on a block and all but the C3 vibrissae were trimmed bilaterally. Approximately 2 hours after the halothane was discontinued, bilateral mechanical stimulation of the remaining vibrissae was initiated. The 2-deoxy-D-[1-^{14}C]glucose (10μCi/100g) was injected through the jugular catheter 5 minutes after beginning stimulation. The stimulation continued for 45 minutes at which time an overdose of sodium pentobarbital was given and the animal was perfused through the heart with a solution of 4% sucrose in 4.0% buffered paraformaldehyde. The brain was quickly removed and frozen in Freon 22. At a later time, 30 um thick sections were cut with a cryostat (-16°C) in the coronal plane with alternate sections saved for 2DG autoradiography and acetylcholinesterase (AChE) and cytochrome oxidase (CO) histochemistry (Hardy et al., 1976; Jacobowitz et al., 1983; Wong-Riley, 1979).

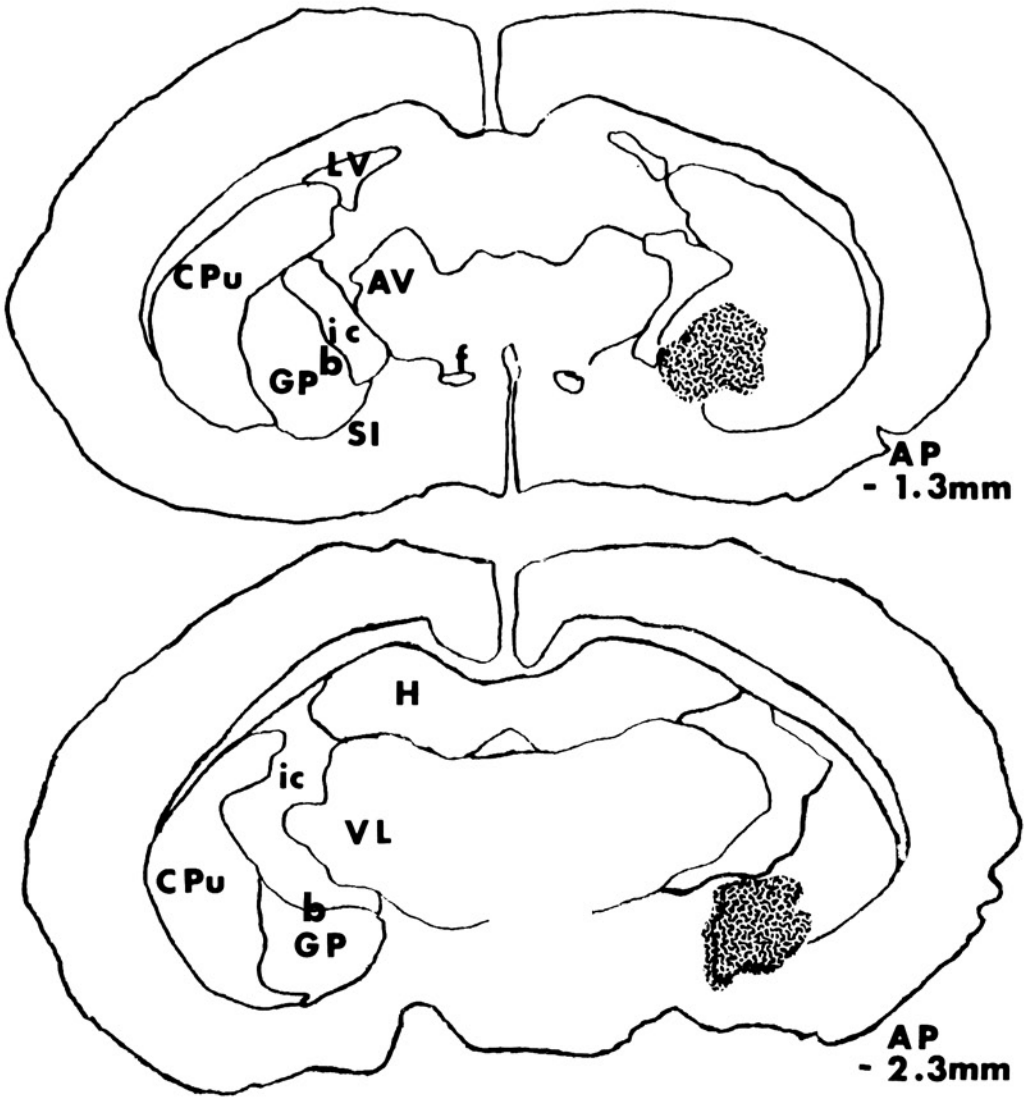
The sections saved for 2DG, along with ^{14}C standards, were processed according to standard autoradiographic

procedures (Ma et al., 1989). Using a video-based image analysis system with a PDP 11/23+ host computer, reconstructions of the frontoparietal cortex on each side of the brain were made from a series of autoradiographic sections (Ma et al., 1989; Tommerdahl et al., 1985). The sections were aligned along the cingulate gyrus and unfolded to visualize the pattern of ^{14}C label throughout the somatosensory cortex. Additionally, autoradiographic images of individual sections were digitized and analyzed, using features of the image analysis system (Tommerdahl et al., 1985).

Results

Unilateral injections of ibotenic acid resulted in basal forebrain lesions that included the ventromedial globus pallidus and the substantia innominata (Fig. 1). The extent of the lesion was confirmed in sections stained for CO histochemistry, an indicator of mitochondrial enzymatic activity, which has previously been demonstrated to be an effective method of determining lesion placement (Ma et al., 1989; Juliano et al., 1990). CO staining within the lesion site is noticeably pale. Nissl staining of adjacent sections confirmed a loss of cell bodies within the lesioned region. A marked reduction of AChE-positive fibers was

Figure 1. Reconstruction of a typical basal forebrain lesion. Drawing to indicate placement of the lesion as viewed in coronal section. Regions affected by injections of ibotenic acid (shading) include the nucleus basalis magnocellularis (b), the substantia innominata (SI), and the ventromedial globus pallidus (GP). The indicated anteroposterior levels (AP) are from Paxinos and Watson (1982). Abbreviations: ac, anterior commissure; AV, anteroventral nucleus of the thalamus; CPu, caudate-putamen; f, fornix; H, hippocampus; ic, internal capsule; lv, lateral ventricle; VL, ventrolateral nucleus of the thalamus.


LV
CPu
AV
ic
b
GP
f
SI
AP
- 1.3mm
H
ic
VL
CPu
b
GP
AP
- 2.3mm

found in the cortex ipsilateral to the basal forebrain lesion compared to the density of AChE staining in the hemisphere contralateral to the lesion (Fig. 2).

Individual autoradiographic sections were analyzed for metabolic activity levels within several sites: (a) the activated barrel, (b) a region of the barrel cortex surrounding the activated barrel but not itself stimulated, and (c) white matter, which represented background activity. By expressing the optical density values found in the barrels as a percent above white matter values, label corresponding to stimulus-evoked activity was calculated to be at least 40% above white matter (Fig. 3). As indicated in Table 1, significant decreases are evident in the intensity of the stimulus-evoked 2-DG uptake in barrels ipsilateral to the lesion compared with corresponding barrels in the contralateral hemisphere ($P < 0.05$). Cortical locations that were surrounding, but not in the activated region were also measured to assess background metabolic levels. These regions did not display differences in activity between the hemispheres.

The areal dimension of the 2-DG label in the cortical barrels was also assessed using the autoradiographic section (Fig. 3). The width was measured along an axis tangential to the pial surface and the height along the extent of the column perpendicular to the pial surface. Table 1 demonstrates that the dimension of the

TABLE 1. Effect of Basal Forbrain Lesion on Dimension and Density of 2-DG Label

	DIMENSION		DENSITY[1]	
	width (um)	height (um)	barrel	surround[2]
NBM 31:				
normal	446.8	348.0	72.6%	35.9%
lesion	431.6	287.6	62.4%*	31.2%
NBM 37:				
normal	362.1	284.2	62.1%	28.8%
lesion	322.8	277.2	56.4%*	26.6%
NBM 38:				
normal	427.4	322.1	47.4%	22.5%
lesion	338.9	253.3	40.9%*	20.2%

[1] mean optical density values expressed as percent above background; background is white matter.

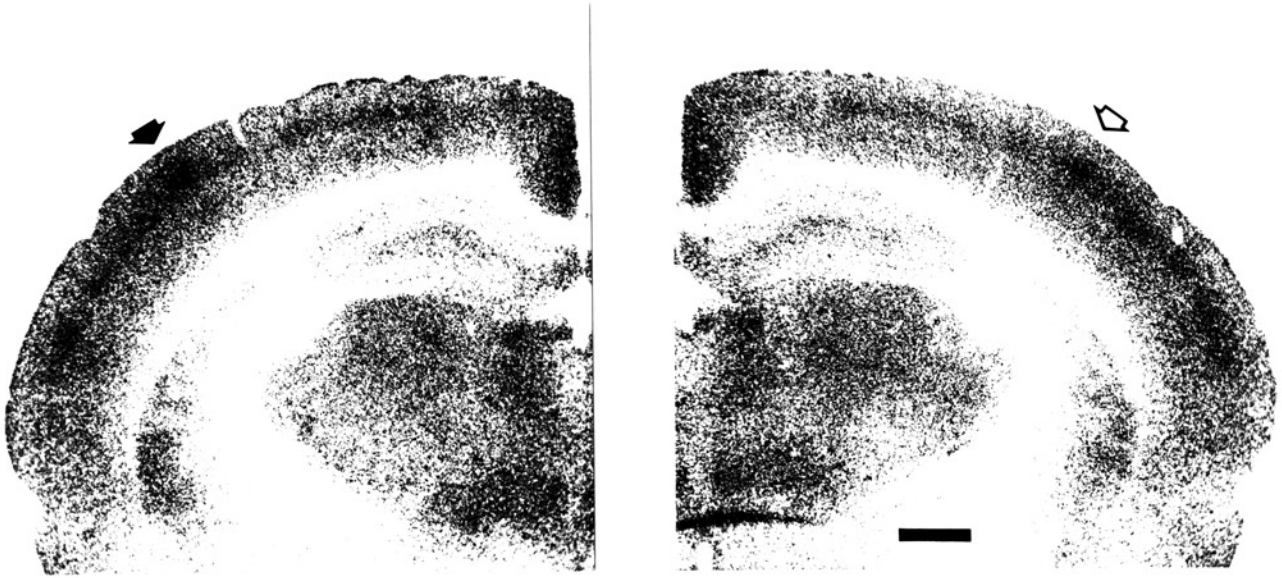
[2] regions of somatosensory cortex within the barrel field that were not specifically stimulated.

* significantly different from normal; Student's t-test, $P < 0.05$.

Figure 2. Pattern of cortical AChE staining from a rat with a unilateral basal forebrain lesion. The normal distribution of AChE+ fibers in rat somatosensory cortex, as demonstrated by AChE histochemistry, is shown on the left. On the right is a photomicrograph of AChE+ fibers from the same tissue section, but in the hemisphere ipsilateral to the basal forebrain lesion. Scale bar, 200μm.



Figure 3. 2-DG autoradiographs from a rat that received a unilateral basal forebrain lesion. Two 2-DG autoradiographs (photographed directly from the film) of coronal sections through a rat brain that received a unilateral lesion of the basal forebrain. Open arrow points to activity evoked in the ACh-depleted hemisphere ipsilateral to the lesion; the solid arrow indicates a spot of label in the opposite, unlesioned hemisphere. The barrel-associated label in the lesioned hemisphere is reduced in density and dimension compared to the normal hemisphere. Scale bar = 1 mm.



activated cortical barrel ipsilateral to the basal forebrain lesion is less than that of the corresponding barrel in the opposite hemisphere. While differences in dimension did not reach statistically significant levels, this is probably a consequence of the small sample size.

Unilateral basal forebrain lesions also alter the spread of stimulus-evoked activity across the ipsilateral somatosensory cortex. Reconstructed maps of 2-DG activity in the somatosensory cortex indicate that the area of stimulus-evoked activity in the C3 cortical barrel ipsilateral to the basal forebrain lesion is reduced compared with that in the contralateral hemisphere (Fig. 4). In a normal animal, the pattern of 2-DG activity appears similar between the hemispheres (Fig. 5).

To additionally assess the impact that basal forebrain lesions might have on cortical activity levels, CO histochemistry was carried out on all animals to evaluate baseline metabolic state. No difference in levels of CO activity is evident between the hemispheres, either in the barrel region or in cortical sites surrounding the barrels. This again suggests that underlying metabolic activity is not affected by depletion of cortical ACh.

Discussion

Figure 4. Digitized maps of stimulus-evoked metabolic activity from a rat that received a unilateral basal forebrain lesion. The hemisphere ipsilateral to the lesion is below the contralateral normal hemisphere. The maps were generated from a series of coronal sections using software that partitions a designated region of cortex into vertical and laminar arrays of high resolution. The sections are then aligned and "unfolded" to produce two 2-dimensional tangential maps. Both hemispheres are digitized at the same time to ensure that both sides of the brain are treated equally. Arrows indicate spots of increased 2-DG uptake evoked by bilateral stimulation of the C3 whisker. The activity in the ACh-depleted hemisphere is reduced compared to activity elicited in the normal contralateral hemisphere. Medial is to the left, rostral is up. Scale bar = 1 mm.

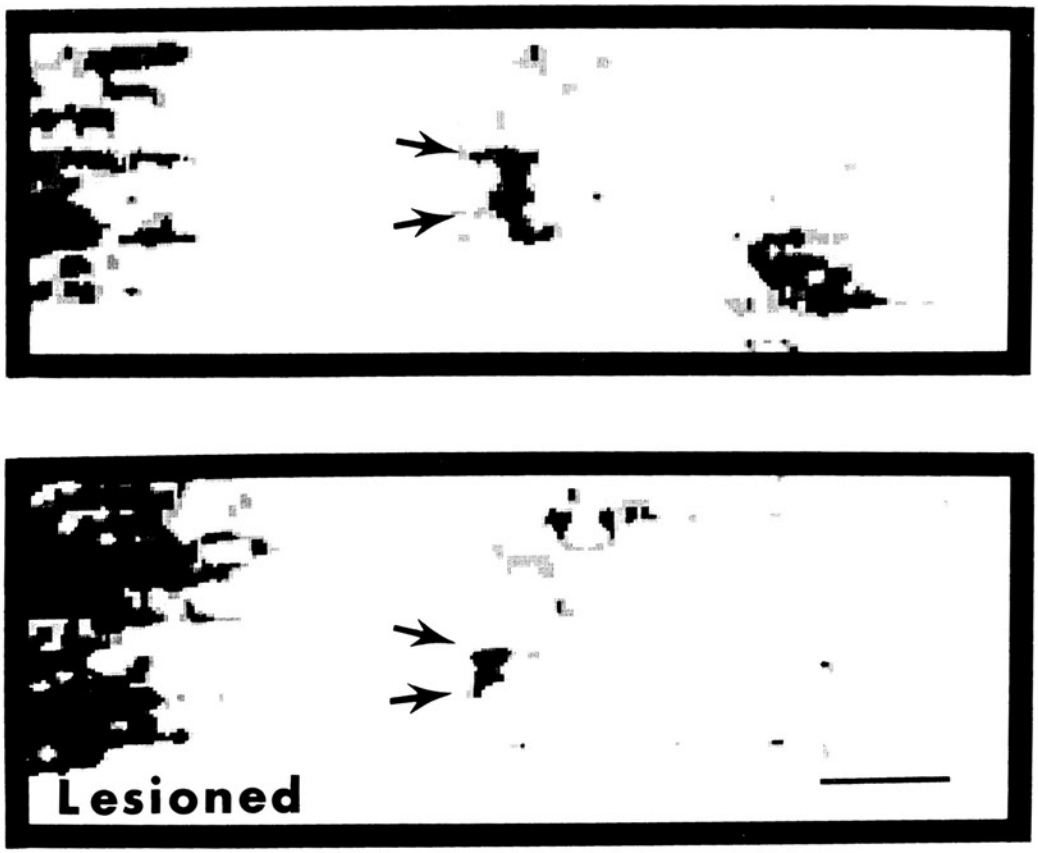
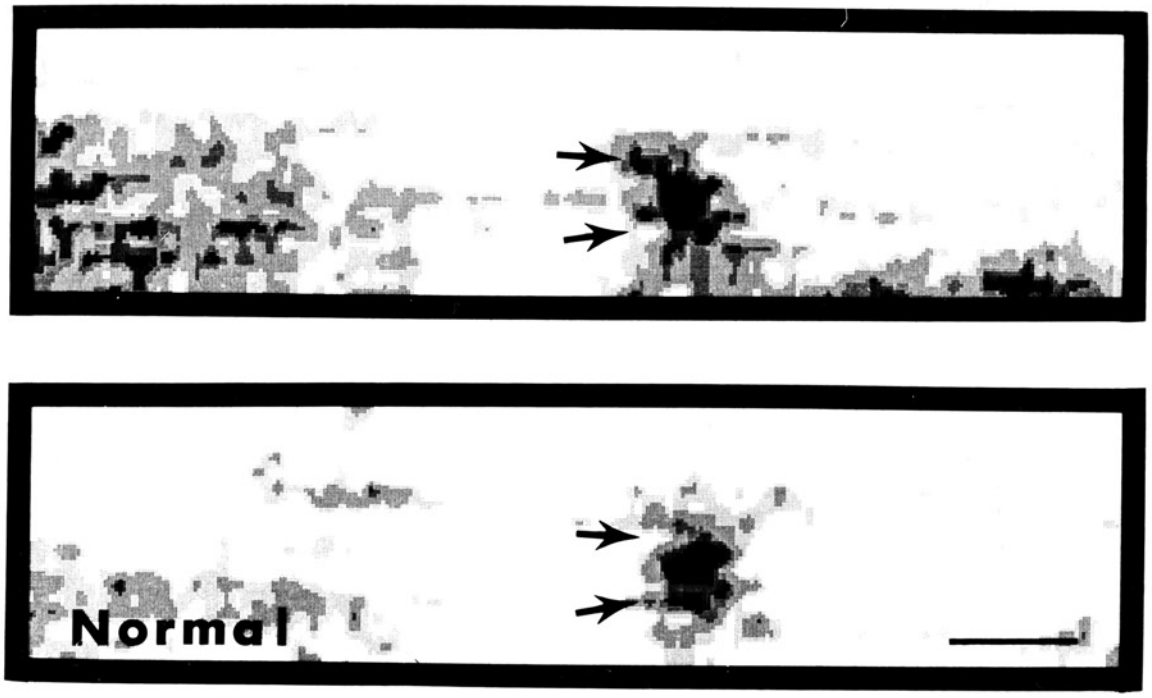

Lesioned

Figure 5. Digitized maps of 2-DG activity generated from a normal rat. Arrows indicate whisker-evoked spots of 2-DG uptake in the C3 barrel and is roughly similar between the two hemispheres. See Figure 4 for conventions. Medial is to the left, rostral is up. Scale bar, 1 mm.


Normal

Depletion of cortical ACh in rats alters the stimulus-evoked metabolic activity pattern in the ipsilateral somatosensory cortex. Reduction of cortical ACh reduces stimulus-evoked 2-DG activity, while background activity appears unaffected. The observation that background activity levels are not altered by basal forebrain lesions is supported by two pieces of evidence: (a) cortical areas surrounding the stimulated area, that are themselves not specifically stimulated, do not demonstrate differences in uptake of 2-DG label between hemispheres; (b) the pattern of CO staining, another indicator of baseline metabolic activity, shows no differences from hemisphere to hemisphere. Similar findings have been reported in mice (Ma et al., 1989).

Evidence is accumulating that ACh is critical in sensory cortical processing. ACh appears to particularly enhance neural responses coupled with specific sensory input, as opposed to affecting responsivity in a general way (Sillito and Kemp, 1983; Metherate et al., 1988a; Juliano et al., 1990). In the present study, lesion-induced ACh depletion reduces the stimulated vibrissal representation in the ipsilateral somatosensory cortex but does not influence background levels of metabolic activity. Thus, ACh appears to be a crucial component of cortical sensory processing. ACh may participate in raising the level of electrical activity in sensory cortical regions in order to allow responses to stimulation that would be subthreshold in the

absence of cholinergic input. The reduced metabolic map seen here in the ACh-depleted cortex may represent an activity picture in a hemisphere without the normal enhancing influence of ACh.

Chapter 3: Cholinergic Basal Forebrain Transplants Restore Diminished Metabolic Activity in the Somatosensory Cortex of Rats with Acetylcholine Depletion

INTRODUCTION

It is well established that basal forebrain lesions lead to cortical depletion of acetylcholine (e.g., Johnston et al., 1979; Wenk et al., 1980). In Chapter 2 I reported that unilateral basal forebrain lesions resulted in decreased cortical metabolic uptake in response to stimulation, similar to earlier findings by others (Ma et al., 1989; Juliano et al., 1990). Using the 2-deoxyglucose technique, activity evoked by whisker stimulation in somatosensory cortex of rats and mice was shown to be reduced in dimension and intensity after unilateral basal forebrain lesions. In these studies, the 2-DG uptake was compared to similarly evoked activity in the opposite normal hemisphere and comparable results were found after unilateral basal forebrain lesions in other species (Juliano et al., 1990). In all previous investigations, background levels of metabolic activity remained unchanged and the 2-DG reductions were particularly associated with evoked activity. These and many other recent studies emphasize the importance of ACh in normal cortical function, particularly

in relation to processing of stimuli (Metherate et al., 1987, 1988a; Lamour et al., 1988; Rasmusson and Dykes, 1988). Although ACh has long been known to play a role in neocortical neuronal activity (Krnjević et al., 1971; Brown, 1983; McCormick and Prince, 1985), more recent experiments emphasize the significance of cholinergic involvement in enhancing neuronal responses to stimulation (Sillito and Kemp, 1983; Donoghue and Carroll, 1987; Sato et al., 1987; Sillito and Murphy, 1987; Lamour et al., 1988; McKenna et al., 1989; Metherate et al., 1988b; Metherate and Weinberger, 1989, 1990).

More recent advances in the technique of transplantation suggest that implants of cholinergic tissue restore reductions in electrophysiological and metabolic activity in ACh-depleted hippocampus (Low et al., 1982; Kelly et al., 1985; Buzsáki et al., 1987; Vanderwolf et al., 1990). Studies by Fine et al. (1985a,b) and Dunnett et al. (1985) revealed the capacity of cholinergic transplants to survive and reinnervate previously ACh-depleted frontoparietal cortex. Furthermore, ACh-rich basal forebrain transplants placed into the neocortex lead to improved performance in learning and memory tasks. For example, basal forebrain-lesioned rats demonstrated improvement in tasks of response retention and spatial learning, (such as passive avoidance and water maze learning), with transplants of cholinergic-rich basal forebrain tissue, but not with non-cholinergic hippocampal

tissue (Fine et al., 1985a,b; Dunnett et al., 1985). These results have subsequently been confirmed by others (Arendt et al., 1989; Hodges et al., 1990). More recently, these results have been extended to primates, demonstrating that cholinergic basal forebrain tissue, but not control hippocampal tissue grafted to the hippocampus of marmoset monkeys can completely reverse profound and specific learning disabilities resulting from fornix transection, which disrupts the cholinergic innervation from the medial septal area of the basal forebrain to the hippocampus (Ridley et al., 1991, 1992). The appropriate placement of fetal tissue grafts as well as their source appear to dramatically increase their potential to restore function. Transplantation of embryonic cholinergic basal forebrain tissue into cholinergically-depleted target sites (i.e., cortex or hippocampus) results in functional recovery, while similar transplants placed into non-target sites are without behavioral effect (Hodges et al., 1990).

As a result of the encouraging findings regarding the possibility of neural transplants to thrive and grow in new environments, as well as to induce functional improvements, I conducted a study that made use of these experimental advances. The experiments described here explore the capacity of cholinergic basal forebrain grafts placed into neocortex to ameliorate the deficits in stimulus-evoked metabolic activity observed after unilateral basal forebrain lesions.

Materials and Methods

The study presented here involved a sequence of experiments. I tested the ability of fetal ACh-rich basal forebrain transplants to restore functional activity in the somatosensory cortex of adult rats with previous basal forebrain lesions. The specific manipulations included: (a) excitotoxic lesions of the basal forebrain that depleted the ipsilateral somatosensory cortex of ACh, (b) transplantation of embryonic basal forebrain or neocortical tissue into the ACh-depleted cortex, (c) 2-DG experiments, during which the rat received whisker stimulation, and (d) tissue processing that included 2-DG autoradiography, staining for cytochrome oxidase (CO) activity and acetylcholinesterase (AChE) histochemistry. Only experiments successful in all phases of this study were used for the final analysis (n = 14). Criteria for success are presented in the results section for each of the manipulations.

Basal Forebrain Lesions

One week prior to transplantation of embryonic basal forebrain, 14 Sprague-Dawley rats, weighing 175-250g, received unilateral injections of ibotenic acid into the basal forebrain. Two sets of coordinates were used, both

aimed at the major source of cholinergic innervation to the somatosensory cortex: the nucleus basalis magnocellularis (nbm), substantia innominata, and ventromedial globus pallidus (Johnston et al., 1981; Mesulam et al., 1983; Rye et al., 1984; Saper et al., 1984). For one set of injections, an opening was made 4 mm anterior to bregma and 2.3 mm lateral from the midline as described in Chapter 2. A Hamilton syringe was angled 35° from vertical and 12° laterally and advanced 8.0 mm deep to the dura where 0.5 μl of ibotenic acid (10 μg/μl) was injected over 2 minutes. The needle was then retracted to a depth of 7.0 mm and an additional 0.5 μl of ibotenic acid injected. The 2nd set of coordinates, previously described by Fine et al. (1985a), required two openings in the skull. One was 1.0 mm anterior to bregma and 2.6 mm lateral from midline. A Hamilton syringe was advanced 7.3 mm deep and 0.5 μl of ibotenic acid was injected. The other injection of 0.5 μl of ibotenic acid was made 7.0 mm deep to an opening 0.2 mm anterior to bregma and 3.4 mm lateral from midline. For this set of injections, the incisor bar was set 5.0 mm above the interaural line.

Transplantation

One week after the basal forebrain lesion, 14 adult host rats received a cell suspension transplant of either cholinergic-rich embryonic basal forebrain (n = 7) from 16 day gestational age rat fetuses, or neocortical control tissue (n = 7) from 14 - 15 day gestational age rat fetuses, into the ACh-depleted, ipsilateral frontoparietal cortex. The cell suspension transplants were prepared as described previously (Björklund et al., 1983; Fine et al., 1985a). Briefly, using aseptic techniques, fetuses were removed by cesarian section from the mother, their brains removed and placed in a sterile, ice-cold solution of 0.6% D-glucose in saline. Either embryonic basal forebrain or pieces of the developing neocortex were dissected and then incubated for 20 min at 37°C in 10 ml of the D-glucose solution with 2.5 mg trypsin (Sigma Type XIII) added. The tissue was then rinsed in D-glucose solution containing DNase (Sigma DNase I) (1 mg/10 ml). The brain pieces were transferred to a vial in a final volume of 10 μl of the DNase/D-glucose solution per brain, and gently dissociated using fire-polished Pasteur pipettes. Viability of the dissociated cells was evaluated using ethidium bromide exclusion before and after transplantation. For all the cell suspensions prepared, at least a greater than 80% viability was found before and after the transplantation session.

Two μl of cell suspension were injected into two sites in the frontoparietal cortex ipsilateral to the basal

forebrain lesion. These sites were in close proximity to, or within, the portion of somatosensory cortex receiving input from the whiskers. One site was 0.3 mm posterior to bregma, 3.2 mm lateral to midline and 2.5 mm deep. The second site was 2.3 mm posterior to bregma, 3.2 mm lateral to midline and 2.5 mm deep. For all injections, the incisor bar was set at -3.3 mm. The animals survived for 6-12 months. One week prior to a terminal 2-DG experiment, the rats received a repeat basal forebrain lesion as described above to reduce the possibility that cholinergic innervation of the cortex might result from remaining or restored connections from the basal forebrain.

2-DG Experiment

Rats were anesthetized with halothane (1-2%) and a jugular vein was catheterized. Following this procedure, each rat was loosely restrained on a block where all but two to four whiskers were trimmed bilaterally and symmetrically, and a long-lasting topical anesthetic placed on the catheter site. The halothane was then removed and the rat allowed to recover from the anesthesia for 1.5 hr prior to a pulse IV injection of 2-DG (10 μCi/100 g).

The selection of the number and the location of specific whiskers for stimulation during the 2-DG experiment was based on several factors. (1) A number of whiskers were

chosen (usually three) in order to increase the possibility of activating barrels in different proximity to the transplant. In each animal, I therefore attempted to activate barrels both close to and far from the transplanted tissue. (2) Whiskers were selected that were not adjacent to one another, but that could easily be stimulated simultaneously. (3) Matching whiskers on each side were always chosen.

Mechanical manual stimulation of the whiskers began 5 min before the animal received the 2-DG injection and continued for 45 min. The animal then received an overdose of sodium pentobarbital (50 mg/kg) and was perfused intracardially with saline, followed by 0.1M phosphate-buffered-paraformaldehyde (4%) with 4% sucrose. The brain was quickly removed, frozen in Freon 22, and stored in a freezer at -70°C until cut.

Tissue Processing

Using a cryostat at -16°C, 30 μm thick sections were cut in the coronal plane. Adjacent sections were saved for 2-DG autoradiography, and AChE and CO histochemistry. Sections for 2-DG autoradiography were collected on slides coated with 2% gelatin and placed on a warming tray (60°C) for rapid dehydration. The slides, along with ^{14}C

methylacrylate standards, were exposed to X-ray film (SB-5, Kodak) in cassettes for approximately 7 days. Sections for AChE histochemistry were also saved on gel-coated slides and processed using the method of Koelle (1955) as adapted by Jacobowitz and Creed (1983). Sections for CO histochemistry were processed according to Wong-Riley (1979).

Data Analysis

Computer analysis of autoradiographic images. Using a video-based image-processing system with a PDP 11/23+ host computer, the autoradiographs were displayed, digitized and calibrated with reference to the ^{14}C standards. By converting the optical density values into either color or grey scales through the use of look-up tables, I was able to quantify variability in the 2-DG label within individual sections.

Measurements of metabolic activity associated with the activated barrels were obtained from the individual autoradiographs. Regions of cortex surrounding the activated barrels, but not receiving specific stimulation, were also measured. These optical density values are expressed as a percent above background (background is considered to be the optical density of white matter).

Two-dimensional maps of the frontoparietal cortex were generated from autoradiographs every 100 μm in the coronal plane, using software that partitions a designated area of cortex into vertical and tangential arrays of high resolution. The individual sections were then aligned and displayed as digitized maps of activity of flattened somatosensory cortex (Tommerdahl et al., 1985). Both sides of the brain were digitized together, under the same conditions, to ensure that both hemispheres would be treated equally.

Specific loci of high activity emerged within the posteromedial barrel subfield (PMBSF), reflecting 2-DG uptake in the barrels. To determine the area of increased activity associated with the barrels, I obtained the average pixel intensity for the entire 2-dimensional map of each hemisphere and measured areas whose pixel value was 2 standard deviations (SD) or more above the average intensity. The area included in the high activity barrel-associated regions was measured using a digitizing tablet and custom designed software. The areal dimensions of these regions allowed the comparison of corresponding barrels in the normal and transplanted hemispheres, since matching whiskers were always stimulated during the 2-DG experiment (see Figs. 15, 16 and 19). 2-DG uptake in the barrels at various distances from the transplants was determined.

Computer analysis of AChE innervation. Optical density measurements were also obtained in the AChE-stained

sections, using an image analysis system. These measurements allowed quantification of the amount of AChE innervation following basal forebrain lesions, as well as an objective measurement of reinnervation following transplants of basal forebrain tissue. Measurements of the optical density of AChE-stained fibers were taken through several trajectories perpendicular to the pial surface. One trajectory was measured in the normal hemisphere that traversed a path through one of the activated barrels. A number of different sites were selected in the transplanted hemisphere that included the transplant itself, each region of cortex that contained a barrel activated during the 2-DG experiment (as identified on adjacent 2-DG autoradiographs), and a path through a cortical site relatively distant from the transplant that had not been specifically activated during the 2-DG experiment. The numerical values of the optical densities in the path of each trajectory allowed us to generate histograms reflecting AChE density (see Figures 9 and 11). The optical density values in the AChE sections also allowed us to determine the amount of depletion (expressed as a percent of levels in the identical region of the opposite, unlesioned hemisphere) at various distances from the transplant, following the basal forebrain lesion.

Results

Basal Forebrain Lesion and Consequent ACh Depletion

The primary tool used to assess the placement of each basal forebrain lesion was the pattern of staining for cytochrome oxidase activity, which has previously been shown to be a reliable marker for excitotoxic lesion sites (Ma et al., 1989; Juliano et al., 1990). Figure 6 is a drawing of a typical lesion site encompassing the ventromedial globus pallidus, the nucleus basalis magnocellularis (nbm), and the substantia innominata. Figure 7 is a section stained for CO activity, which also indicates the site of the lesion. Each lesion was carefully assessed to exclude cases from further analysis where the lesion damaged the thalamus, although I included two cases that had slight damage to rostral portions of the thalamic reticular nucleus. The extent of cortical cholinergic depletion was evident as a marked decrease in the density of AChE-stained fibers in the somatosensory cortex ipsilateral to the lesion, compared to the contralateral normal hemisphere. Experiments considered successful for this phase of the study included animals with lesions that (a) were within the designated boundaries, and (b) resulted in AChE-positive fiber staining in the experimental hemisphere that was no more than 40% of that in the opposite hemisphere, i.e., a 60% depletion. Quantification of the AChE-positive fibers following the lesions is presented below.

Figure 6. Drawings of a typical lesion in the basal forebrain, reconstructed from the brain of a rat that received an injection of ibotenic acid. The sections are in the coronal plane at representative rostrocaudal levels, illustrating the site of a lesion (dark shading). The lesions generally included the nucleus basalis magnocellularis (b), and the ventromedial globus pallidus (GP). The location of the transplant is indicated with hatched lines. The anteroposterior (AP) levels are taken from Paxinos and Watson (1982). Abbreviations: ac, anterior commissure; AV, anteroventral nucleus of the thalamus; CPU, caudate-putamen; f, fornix; fi, fimbria; H, hippocampus; ic, internal capsule; LV, lateral ventricle; SI, substantia innominata; VL, ventrolateral nucleus of the thalamus; VPL, ventroposterolateral nucleus of the thalamus.

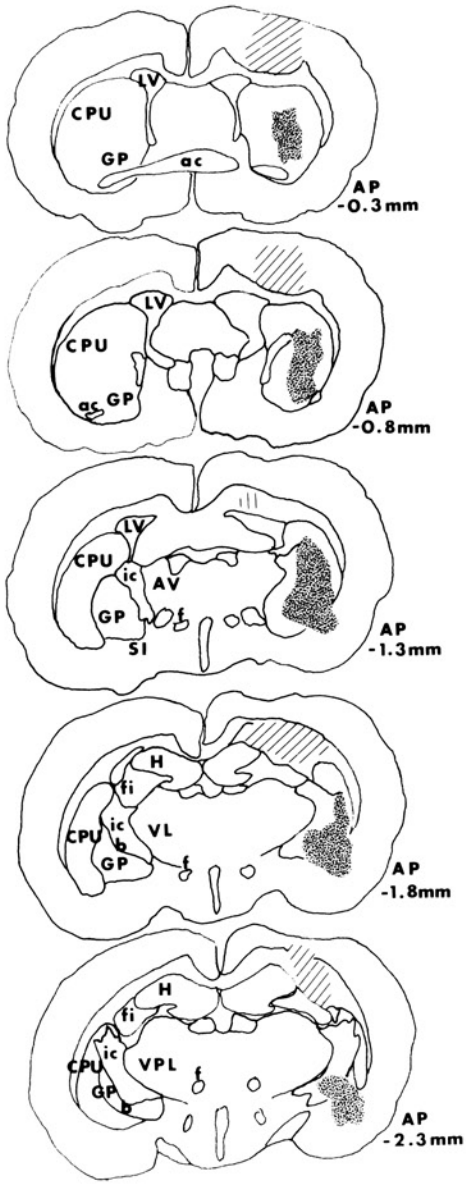

LV
CPU
GP
ac
AP
-0.3mm
LV
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ac
GP
AP
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ic
AV
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SI
AP
-1.3mm
H
fi
ic
VL
CPU
b
GP
f
AP
-1.8mm
H
fi
ic
VPL
CPU
f
GP
b
AP
-2.3mm

Figure 7. A section stained for cytochrome oxidase activity demonstrating reduced staining at the site of the lesion (asterisk).



Histologic Assessment of the Transplants

Basal forebrain transplants. Figure 8A demonstrates the appearance of CO staining in a rat that received a basal forebrain transplant. Within the transplant, regions of increased staining intensity, which correspond to cell-dense clusters, can be detected. Relative to the overall staining pattern in the cerebral cortex however, the intensity of CO staining within the transplant is not grossly different from the surrounding cortex. While there are subtle variations in the appearance of the CO-stained transplants in different animals, the relative similarity of staining between cortex and transplant is a consistent finding.

AChE staining in the section adjacent to the CO-stained section seen in Figure 8A is shown in Figure 8B. Basal forebrain cell-suspension transplants are easily recognizable due to their intense AChE staining. The AChE staining also exhibits fluctuations in density that correspond to cell-dense regions, identified on adjacent Nissl-stained sections. The density of AChE-positive fibers in cortex immediately adjacent to the transplant is increased compared to cortical regions within the same ACh-depleted hemisphere but lying farther from the transplant. An example is shown in Figure 8C, a higher power photomicrograph of the cerebral cortex adjacent to the basal forebrain transplant shown in Figure 8B. A dense network of cholinergic fibers emanates from the transplant; in some

Figure 8. CO and AChE staining of a cholinergic-rich transplant. A) Photomicrograph of a CO-stained coronal section through a rat brain that received a transplant of embryonic basal forebrain tissue. The arrowheads outline the lateral border of the transplant. The intensity of CO staining of the transplant is similar to that of the surrounding cortex. B) Photomicrograph of a section adjacent to 'A' stained for AChE activity. Intense staining can be seen throughout the transplant. Close to the transplant the AChE staining intensity is relatively high; more laterally, density of the staining diminishes, due to a prior lesion of the basal forebrain. The arrows bound the region shown at higher power in Figure 8C (see page 57). The staining in the hemisphere on the left in both A and B represents a normal pattern. Scale bar = 1 mm.

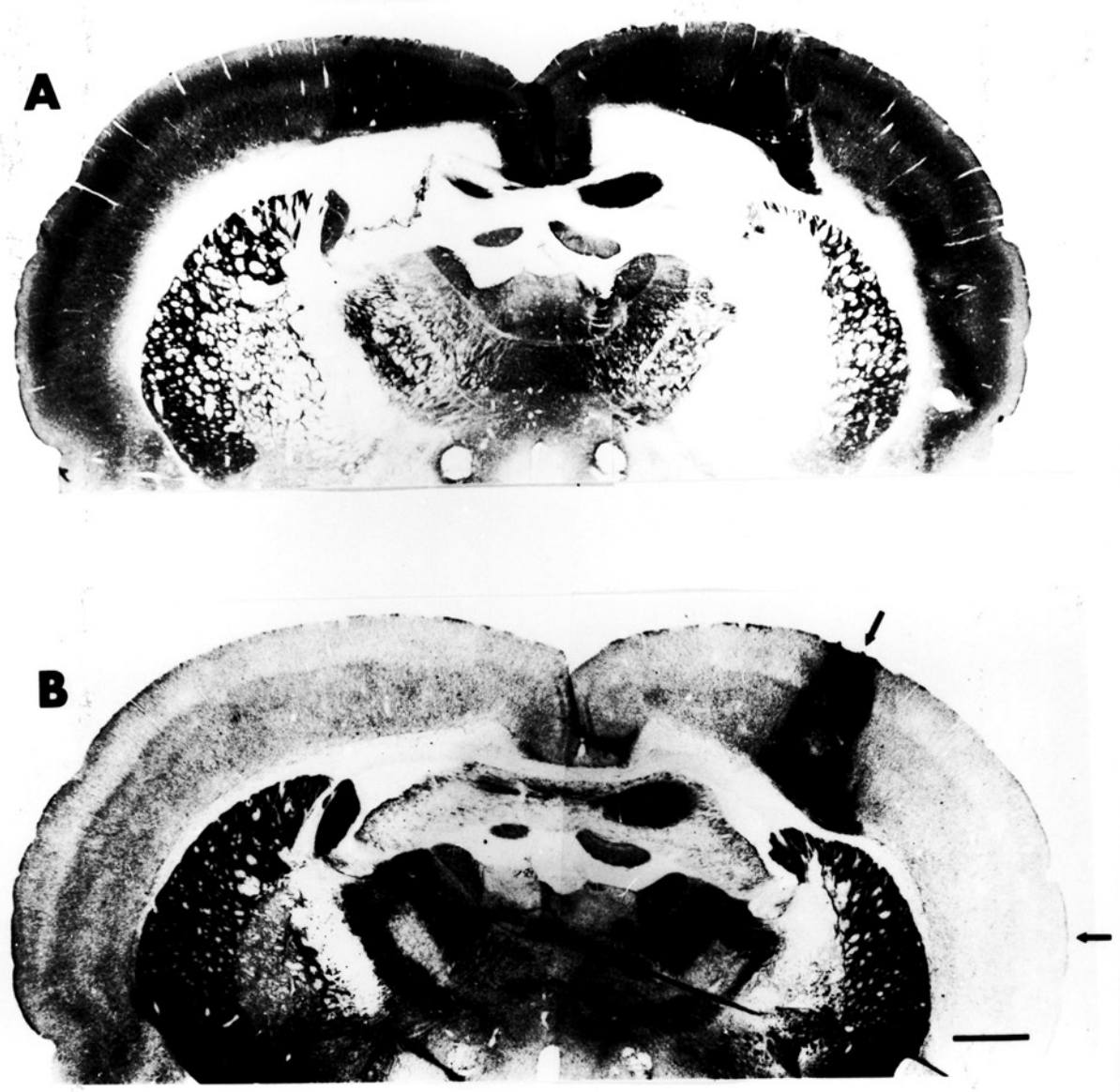

A
B

Figure 8. C) A higher power view of area bounded by arrows in Figure 8B indicating that the density of AChE-stained fibers decreases with distance from the transplant. Scale bar = 1 mm.

C

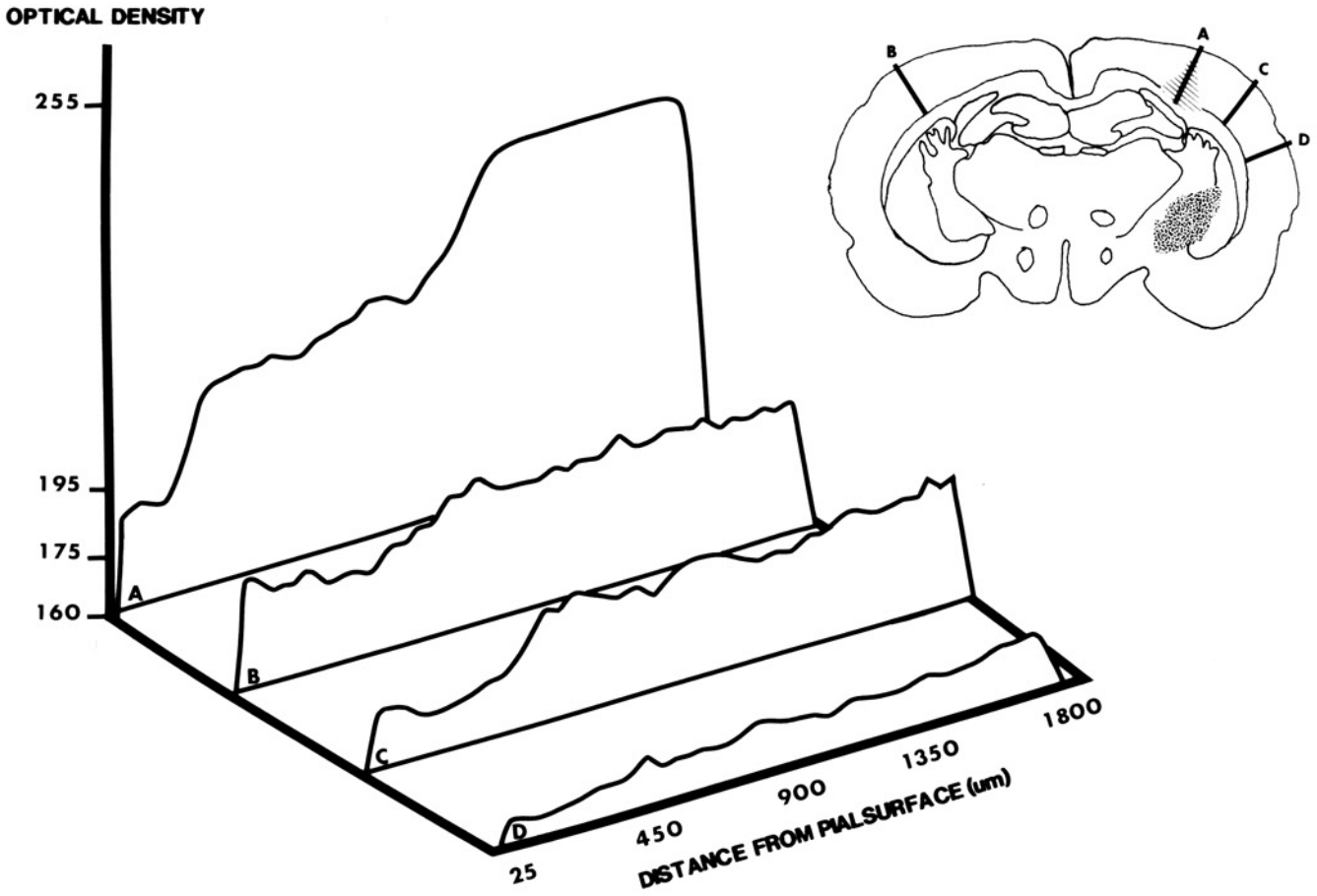


cases, denser than the innervation in the contralateral, normally-innervated hemisphere. Regions farther from the transplant are substantially reduced in AChE staining intensity. Fibers that emerge from the transplant into the deep cortical layers tend to run a horizontal course before turning toward the pial surface; those entering directly into supragranular layers tend to course diagonally as they ascend to the pial surface. In many instances, individual fibers in the surrounding cortex can be traced directly into the transplant. Although it is possible that these fibers do not originate directly from the cell suspension, the pattern of AChE staining surrounding the transplant suggests that the increase is most likely the result of innervation from the transplant. Similar patterns have been described previously (Fine et al., 1985a).

Using an image analysis system, I compared the density of the AChE staining at specific sites in the transplanted hemisphere with the AChE density in the opposite untreated hemisphere (see Methods). Figure 9 contains optical density histograms generated at specified sites in an AChE-stained section.

The optical density of AChE staining within 1.5 mm from the basal forebrain transplants (C in Fig. 9) was at least 85% that of the normal hemisphere, with dense AChE-positive fibers extending from the basal forebrain transplant. The extent of the apparent transplant-derived fiber outgrowth was independent of the size of the

Figure 9. Histograms representing the density of AChE staining in an animal with a unilateral basal forebrain lesion that received an embryonic basal forebrain transplant. See text for details. Each curve in the histogram represents density values taken through a path in the cortex at location A, B, C, or D. 'A' is taken through the transplant itself (hatched region), and thus relatively high in intensity; 'B' is taken through the normal hemisphere, 'C' represents a path through a sector within 1.5 mm of the transplant, i.e., within the territory containing AChE density at least 85% of normal; 'D' represents a path farther from the transplant, with decreased intensity of staining. For purposes of this histogram, the optical density values are represented on a scale from 0-255, with 0 the least dense, and 255 the most dense. The distance of each trajectory through the cortex from the pial surface is indicated on the x-axis. The location of the lesion in this animal is indicated with shading.

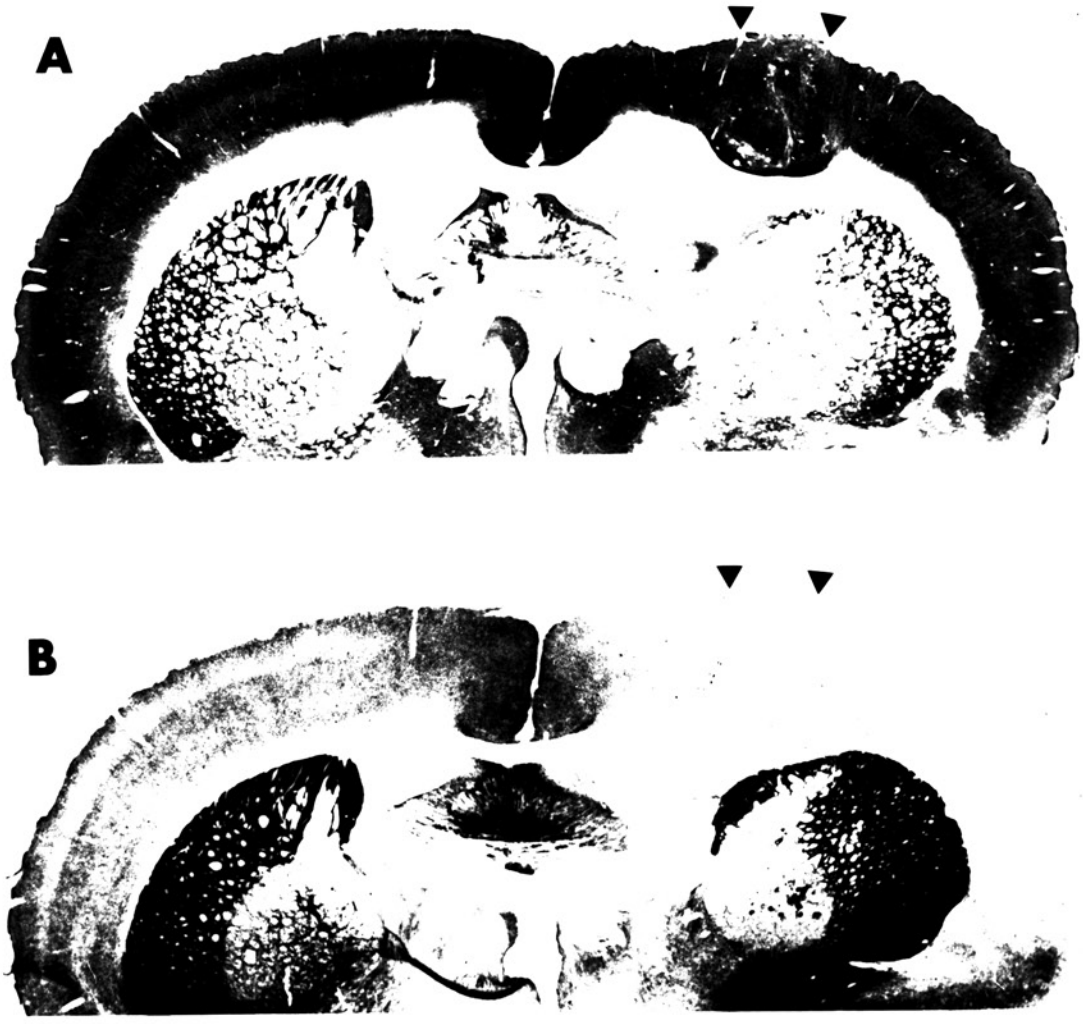

OPTICAL DENSITY
255
195
175
160
A
B
C
D
25
450
900
1350
1800
DISTANCE FROM PIALSURFACE (um)
A
B
C
D

transplant. Between 1.5 - 2.0 mm from the transplant, optical density values vary considerably, from 35-92% of the contralateral hemisphere. Farther than 2 mm from the transplant (D in Fig. 9), the AChE fiber density was 30-40% of normal.

Acceptable criteria for successful transplantation of both basal forebrain and neocortical tissue included visualization of apparently healthy cells as revealed by Nissl staining and demonstration of robust staining for CO activity. In experiments involving basal forebrain transplants, intense staining for AChE was also a required component. I had very few transplants that were not well positioned, but a number of animals that received control cortical cell suspensions were rejected from considerationin this study because the transplants grew into the striatum, and in one instance, into the thalamus.

Neocortical (control) transplants. The appearance of a typical cell-suspension "control" transplant of embryonic neocortical tissue is shown in Figure 10. Sections stained for CO activity were used to confirm both the placement and robustness of the neocortical transplant (Fig. 10A). These transplants stain poorly for AChE (Fig. 10B), confirming the paucity of cholinergic neurons. The distribution of CO activity was usually homogeneous and generally similar in appearance to the surrounding cortex, although in some animals areas of cell-dense clusters accounted for isolated patches of increased staining. After

Figure 10. CO and AChE staining of a neocortical (control) transplant. Adjacent sections in the coronal plane taken from a rat with a prior unilateral basal forebrain lesion that received a transplant of embryonic neocortex. Arrowheads bound the transplant. A) Photomicrograph of a CO-stained coronal section through a rat brain that received a control transplant of embryonic neocortex. In this section, fluctuations in the density of CO-staining are evident. B) Adjacent section reacted for AChE activity. Unilateral AChE depletion is evident on the right. The transplant is barely visible using this type of stain. The site of the ibotenic acid lesion can be seen in the globus pallidus (asterisk). Scale bar = 1 mm.

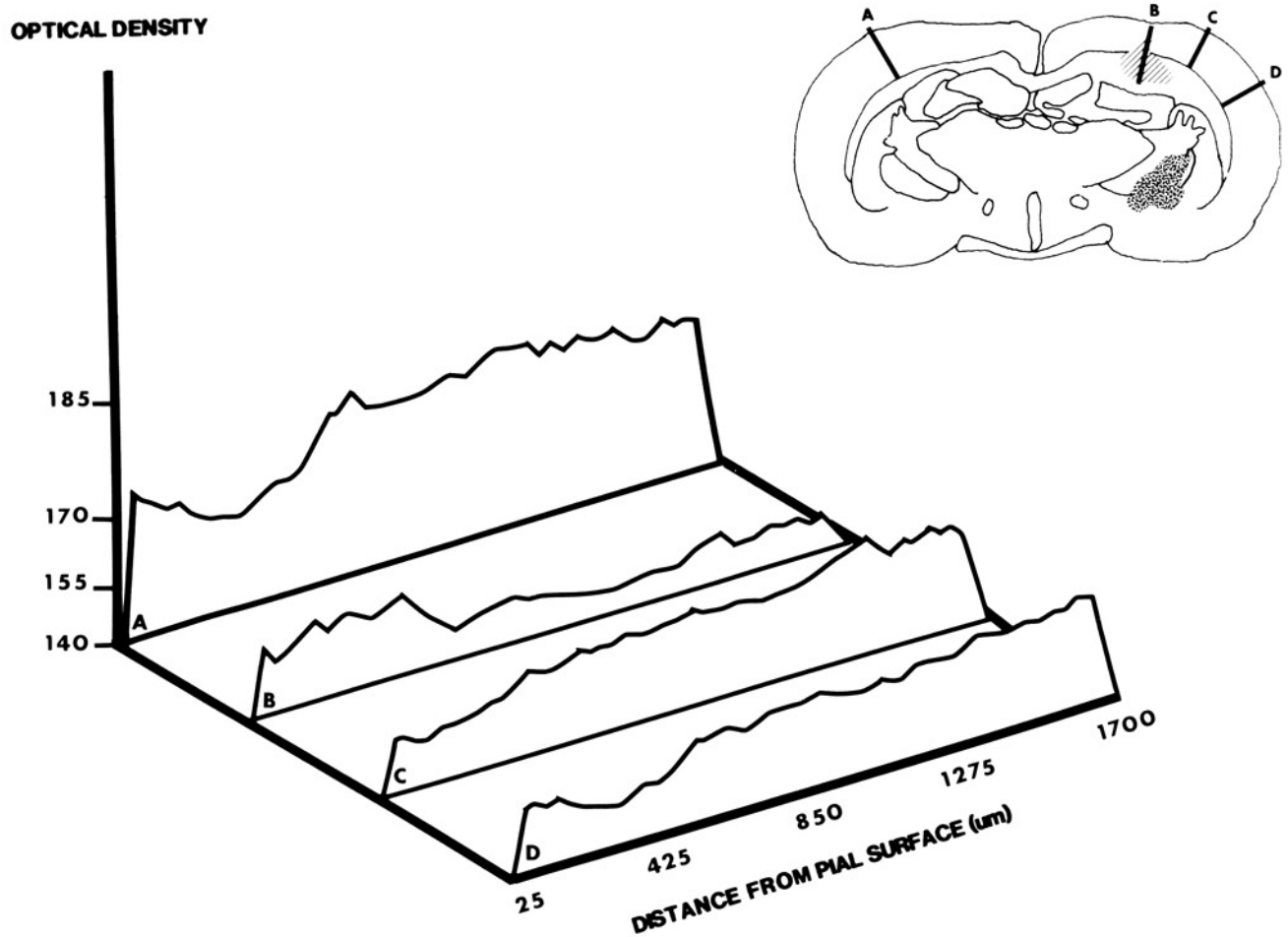

A
B

determining the borders of the cortical transplants, the AChE-stained sections were examined to determine whether staining varied systematically with distance from the transplant. A typical densitometric analysis of AChE-stained fibers in the normal hemisphere and in the hemisphere containing the control graft is shown in Figure 11 (see Methods). There is no difference between the density of AChE staining observed adjacent to the transplant (C in Fig. 11) and that observed within the same hemisphere but farther from the transplant (D in Fig. 11). The AChE staining pattern throughout the transplanted hemisphere is similar to that in animals with AChE depletion subsequent to basal forebrain lesions (Fine et al., 1985a; Ma et al.,1989; also see Chapter 2). The AChE histochemistry in the opposite hemisphere (A in Fig. 11) appears normal. The mean optical density values of AChE staining for all cortical sites ipsilateral to the neocortical transplants were 25-40% of normal, both within the transplant itself and at varying distances from it.

2-DG Uptake

Animals receiving cholinergic basal forebrain transplants. The 2-DG uptake elicited by whisker stimulation was significantly decreased in AChE-depleted cortical territory, similar to results of previous studies

Figure 11. Histogram of the density of AChE staining in an animal that received a unilateral basal forebrain lesion (shading) and a transplant of embryonic cerebral cortex (hatched region). Each curve in the histogram represents AChE density values of trajectories that traversed the normal hemisphere (A), the transplant (B), a path within 1.5 mm of the transplant (C), and a path relatively far from the transplant (D). All the curves through the experimental hemisphere (B, C, and D) are reduced in density from the normal side (A). See Figure 9 and the text for details.


OPTICAL DENSITY
185
170
155
140
A
B
C
D
25
425
850
1275
1700
DISTANCE FROM PIAL SURFACE (um)
A
B
C
D

(Ma et al., 1989; see Chapter 2). The average optical density of barrel-associated label relatively far from the transplant, and therefore in AChE-depleted cortex, was significantly different compared to matched counterparts in the opposite hemisphere (paired *t*-test, $P < 0.05$). In contrast, 2-DG uptake in activated barrels close to the basal forebrain transplants, and within AChE-positive territory, was not significantly different from the opposite unlesioned hemisphere (Table 2).

The 2-dimensional maps of metabolic activity allowed the visualization of 2-DG uptake evoked by whisker stimulation throughout somatosensory cortex. In Figures 12 and 13, maps of the normal (N) hemisphere and the hemisphere containing the transplant (T) are shown; the boundaries of the transplant are indicated with heavy black lines. In both figures corresponding barrels in each hemisphere are indicated (a is matched with a^1, b matched with b^1, and so on). The barrels in cortical territory close to basal forebrain transplants (i.e. within 1.5 mm), with levels of AChE staining at least 85% of the opposite unlesioned side, were not significantly different in areal dimension or optical density of metabolic label from their matched counterparts in the opposite hemisphere (Figs. 12, 13; Table 2). The barrels farther from the transplant, in cortical territory with AChE staining density less than 40% of the unlesioned side, are significantly different in area and

Table 2. Optical Density Levels of 2-DG Activity Associated with Activated Barrels in Normal and Transplanted Hemispheres[1]

BF & AChE +		**BF & AChE -**		**CORTEX & AChE -**	
N	T	N	T*	N	T
71.86	74.05	71.15	69.24	56.12	60.69
78.82	76.00	66.19	67.49	58.88	59.20
82.92	82.02	63.25	60.25	62.91	58.09
74.06	76.26	75.98	72.66	63.74	61.93
80.70	82.57	66.02	66.42	65.74	59.47
76.65	74.88	62.35	59.68	73.52	68.09
70.61	67.15	66.33	58.95	75.45	72.74
		54.77	52.46	73.16	72.22

[1] The mean optical density value for a given activated barrel in a transplanted hemisphere was matched with its equivalent in the opposite hemisphere.

Mean optical density values of stimulus-evoked 2-DG uptake expressed as percent above background; background is white matter. Optical density values were obtained from the autoradiographs.

* Activated barrels in basal forebrain transplanted hemispheres that lay outside the region of cortical AChE reinnervation (BF & AChE -) demonstrate significantly reduced levels of 2-DG activity compared to their paired counterparts in the contralateral hemisphere (paired t-test, $P < 0.05$).

BF & AChE + refers to cortical territory in basal forebrain-transplanted hemispheres where AChE-positive staining is at least 85% of the contralateral normal hemisphere.

BF & AChE - refers to basal forebrain-transplanted hemisphere regions with 30-40% AChE-positive staining compared to normal.

Cortex & AChE - refers to neocortical-transplanted hemispheres (control) where AChE-positive staining is 25-40% of normal.

N, normal hemisphere; **T**, lesioned and transplanted hemisphere.

Figure 12. Two-dimensional maps of 2-DG activity through the somatosensory cortex of a rat that received a transplant of embryonic basal forebrain. Both hemispheres are shown, including the normal side (N) and the experimental side (T), which received a prior basal forebrain lesion and the transplant (location bounded with heavy black lines). During the 2-DG experiment, 2 whiskers were stimulated bilaterally (C3 and D1), which evoked 2 spots of barrel-associated label in the somatosensory cortex of both hemispheres (indicated on the maps as a/a^1 and b/b^1). Both spots of activity in the hemisphere receiving the transplant (a^1 and b^1) are within 1.5 mm from the transplant, and similar in dimension to the spots of activity elicited in the normal hemisphere. The maps are normalized so that values 45% above the mean value of the entire map are visualized as light grey, values 55-60% above the mean are intermediate grey, and values 70% or greater than the mean are black. Values lower than 45% average are set to white. Medial is to the left, rostral is up. Scale bar = 1 mm.

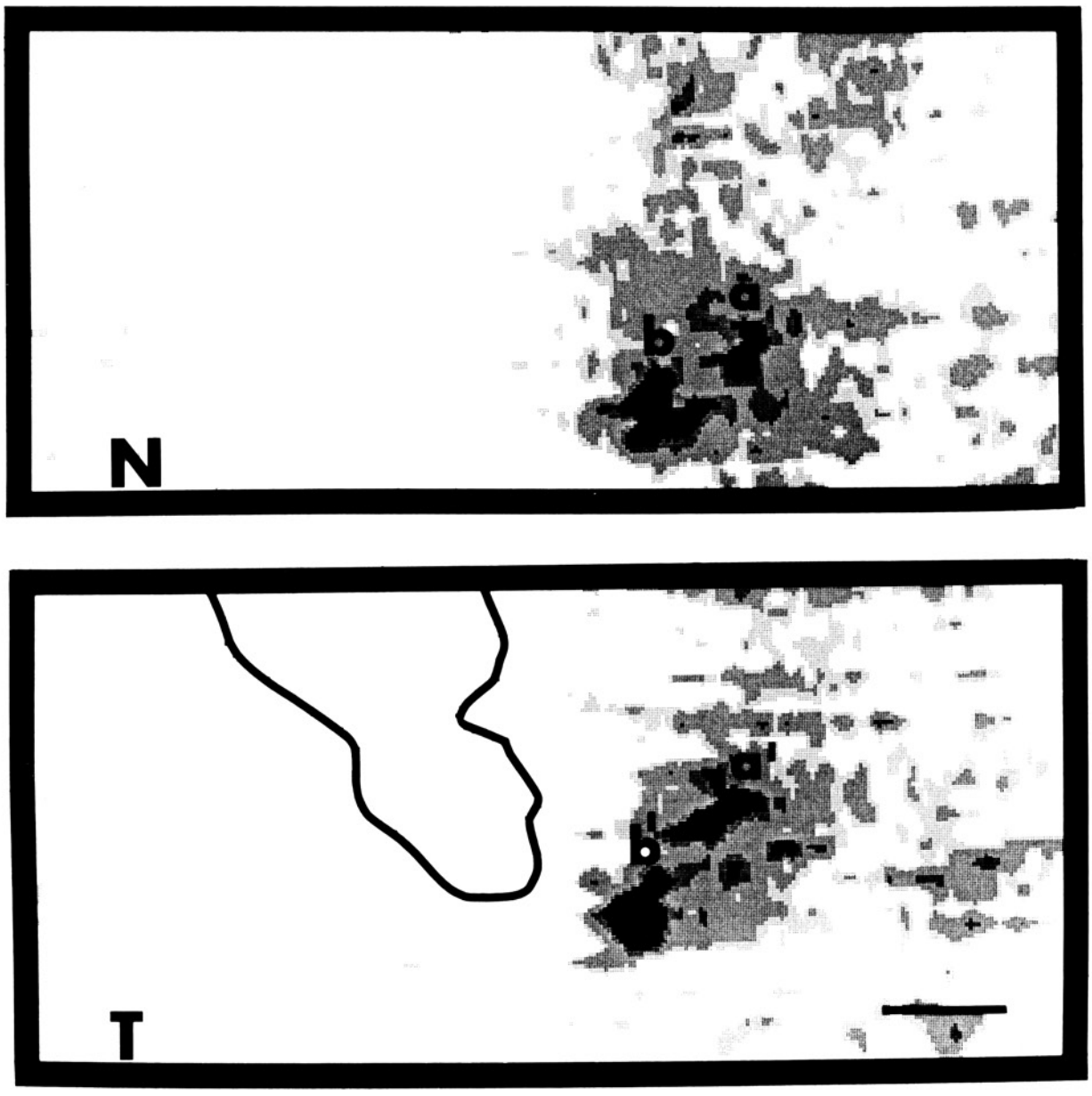
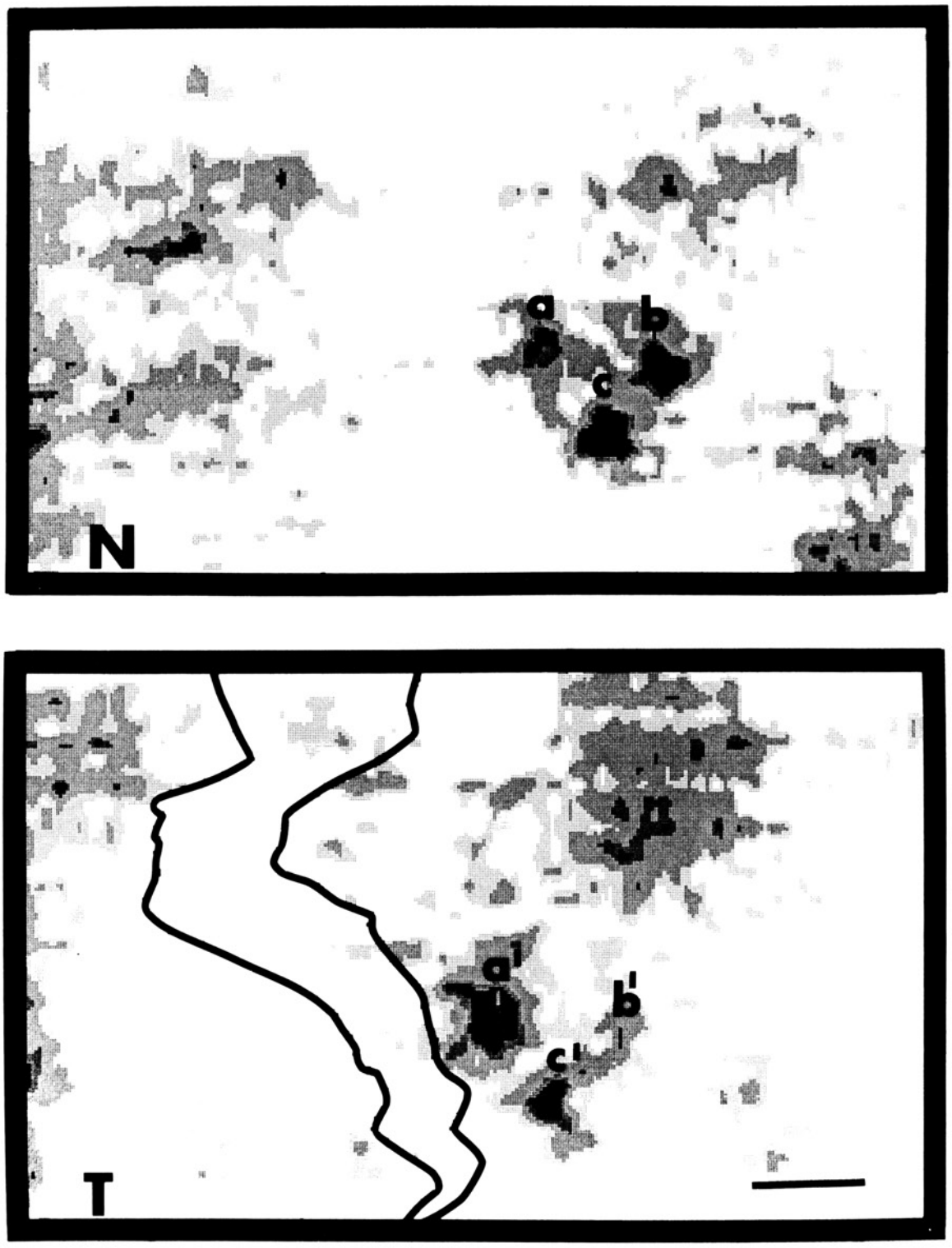
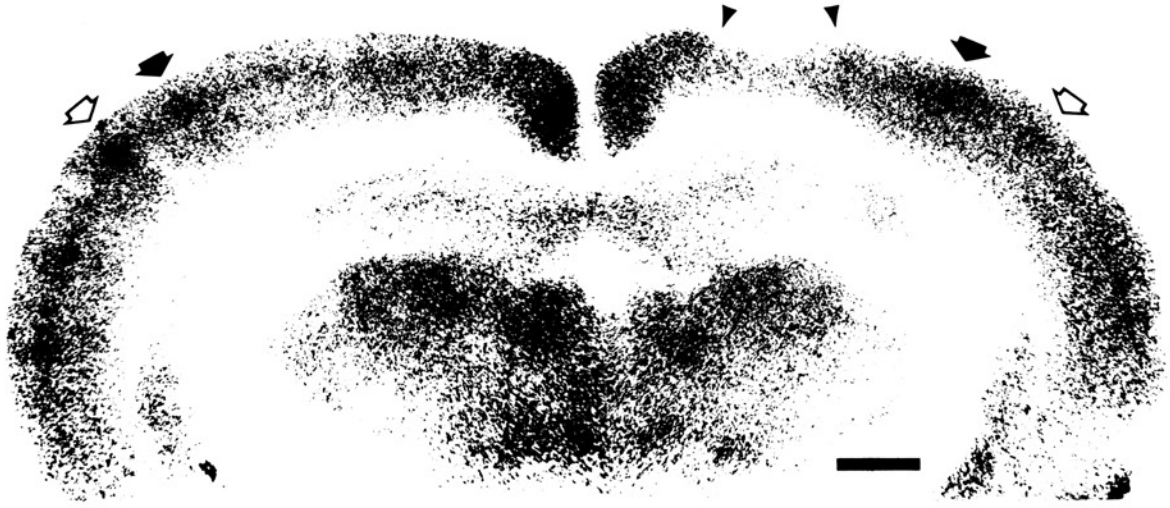

a
b
N
d'
b'
T

Figure 13. Two-dimensional maps of 2-DG activity through the somatosensory cortex of a rat that received a transplant of embryonic basal forebrain. These maps were prepared as described in the text and in Figure 12. During the 2-DG experiment, 3 whiskers were stimulated (B1, B3, and D1), which elicited 3 barrel-associated spots of activity in each hemisphere, indicated as a, b, c in the normal (N) hemisphere and a^1, b^1, and c^1 in the hemisphere containing the transplant (T). In this case, barrels a^1 and c^1 are within 1.5 mm from the transplant and display metabolic activity similar in dimension and intensity to the normal side; barrel b^1 is 1.9 mm from the transplant and reduced in dimension and intensity from b in the normal hemisphere. See Figure 12 for other conventions. Medial is to the left, rostral is up. Scale bar = 1 mm.


a
b
c
N
a'
b'
c'
T

optical density from their counterparts in the opposite hemisphere (Fig. 13; Table 2). In Figure 12, both barrel-associated spots of activity in the experimental hemisphere (T) are within AChE-reinnervated cortical territory; comparison with the normal side indicates that the 2 sets of matched barrels are of comparable size. In Figure 13, barrels a^1 and c^1 of the transplanted hemisphere are also in AChE-positive cortical territory. Barrel a^1 is larger than its contralateral matched barrel while c^1 is comparable in size to its matched counterpart. Barrel b^1, which lies outside AChE-reinnervated cortical territory, is substantially decreased in size compared to its corresponding barrel in the contralateral normal hemisphere. Figure 14 is a 2-DG autoradiograph taken from the animal whose cortical map is represented in Figure 13. The transplant lies adjacent to the PMBSF; the levels of 2-DG uptake in the transplant are slightly decreased in intensity compared to the surrounding cortex. Two barrel-like spots of activity can be seen in each hemisphere. The barrel close to the transplant lies within 1.5 mm from the edge of the transplant (closed arrow) and is of comparable size to its corresponding barrel in the normal contralateral hemisphere. The barrel situated more lateral is 1.9 mm from the transplant (open arrow), where AChE staining density is 40% of normal, and is reduced in dimension relative to its counterpart in the opposite hemisphere.

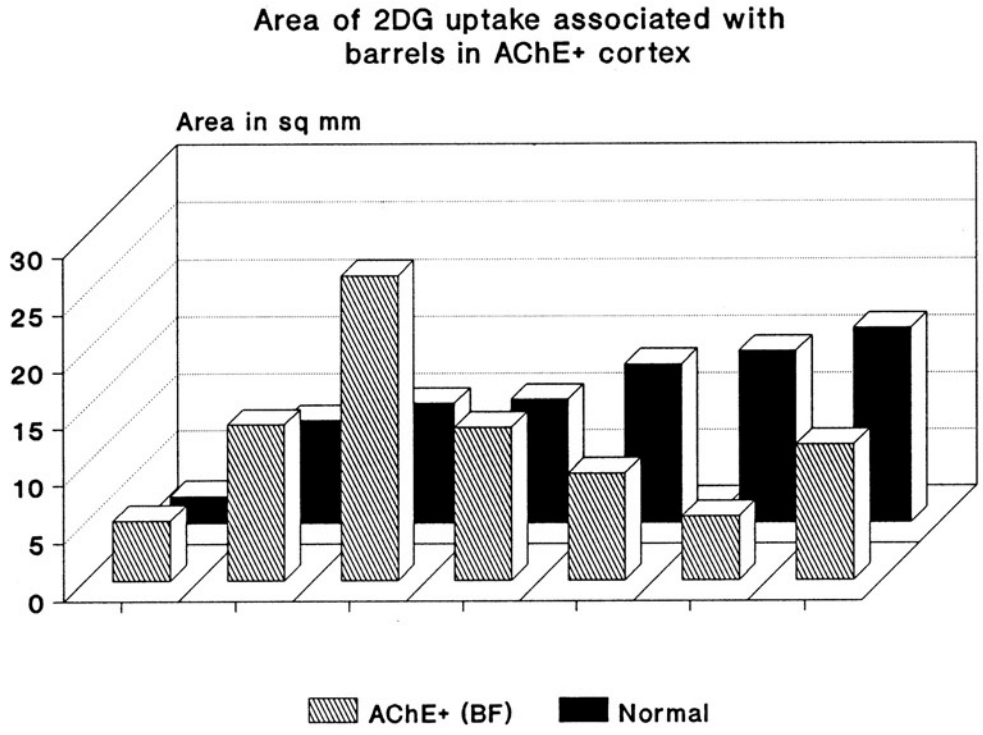
Figure 14. An autoradiograph taken from an animal that received an embryonic basal forebrain transplant indicated by the arrowheads. The autoradiograph was photographed directly from the film and is not digitized. Two regions of barrel-associated increased uptake can be seen in both hemispheres; each pair of arrows (open and solid) indicate matching barrels. The solid arrows point to activity evoked in the normal hemisphere (on the left) and activity evoked in a barrel relatively near to the transplant (on the right). The barrel-associated 2-DG label of these activated barrels is similar in dimension. The open arrows point to a barrel in the normal hemisphere and a barrel 1.9 mm from the transplant. The spot of activity further from the graft is decreased in dimension from its companion on the opposite side. Scale bar = 1 mm.



A paired *t*-test indicates that the areas of the group of barrels within 1.5 mm from the transplant are not significantly different from those in the opposite hemisphere (Fig. 15), while the areas of the barrels outside the AChE-positive territory are significantly different from their matched counterparts in the opposite hemisphere ($P < 0.01$) (Fig. 16).

I also assessed the difference in size between the matched pairs of activated barrels as a group. In order to do this, the percent difference between the areas of activated barrels was determined. For each group ofmatching pairs (Group A: spots of label associated with barrels close to basal forebrain transplants and their matching normal counterparts, Group B: spots of label associated with barrels far from basal forebrain transplants and their counterparts, and Group C: spots of label associated with barrels in hemispheres with neocortical control grafts and their normal counterparts), the area of the activated barrel in the normal hemisphere was considered to be 100%. The size of the activated barrel in the experimental hemisphere was expressed as a percent of its normal counterpart. An analysis of variance (ANOVA) revealed a statistically significant difference between percent size difference of Group A barrels and percent size difference of Group B barrels ($F(1,20) = 8.10$, $P < 0.001$), that is, activated barrels close to the transplant versus those far from the transplant (Fig. 17).

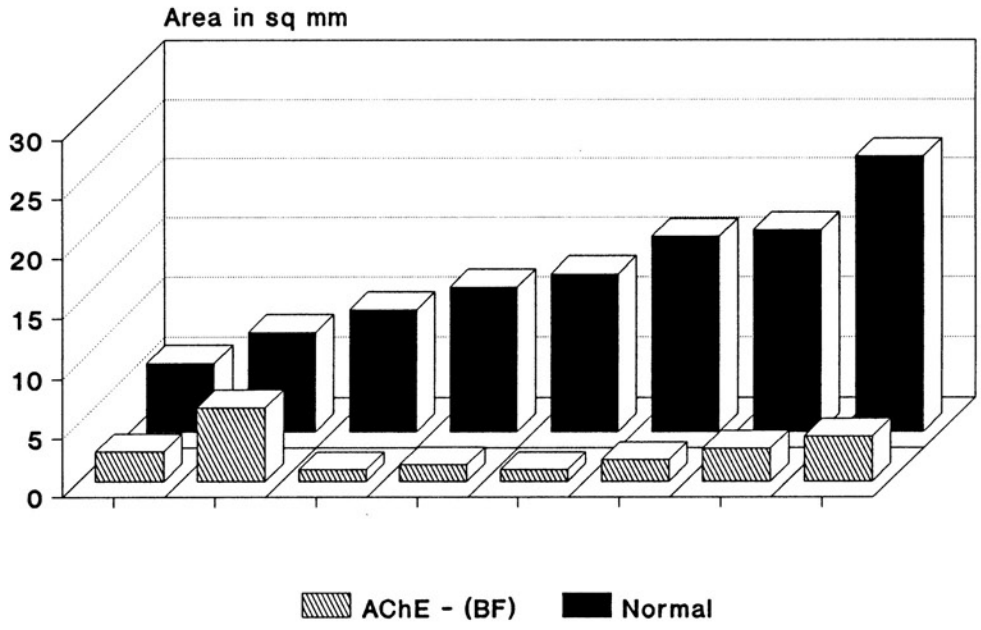
Figure 15. Bar graph illustrating the area (in square μm) of barrel-associated spots of 2-DG uptake, in response to whisker stimulation, taken from both hemispheres of animals that received a basal forebrain transplant. The hatched bars indicate measurements of 2-DG uptake associated with activated barrels in regions of transplanted hemispheres where cortical AChE innervation is at least 85% of normal (AChE +). The solid bars indicate areal measurements of label evoked in the corresponding barrels in the normal hemisphere. The areas of the spots of label were measured on the 2-dimensional maps according to procedures described in the text. The 2 populations of areal measurements were not significantly different (paired *t*-test). BF, basal forebrain.



Animals receiving BF transplant

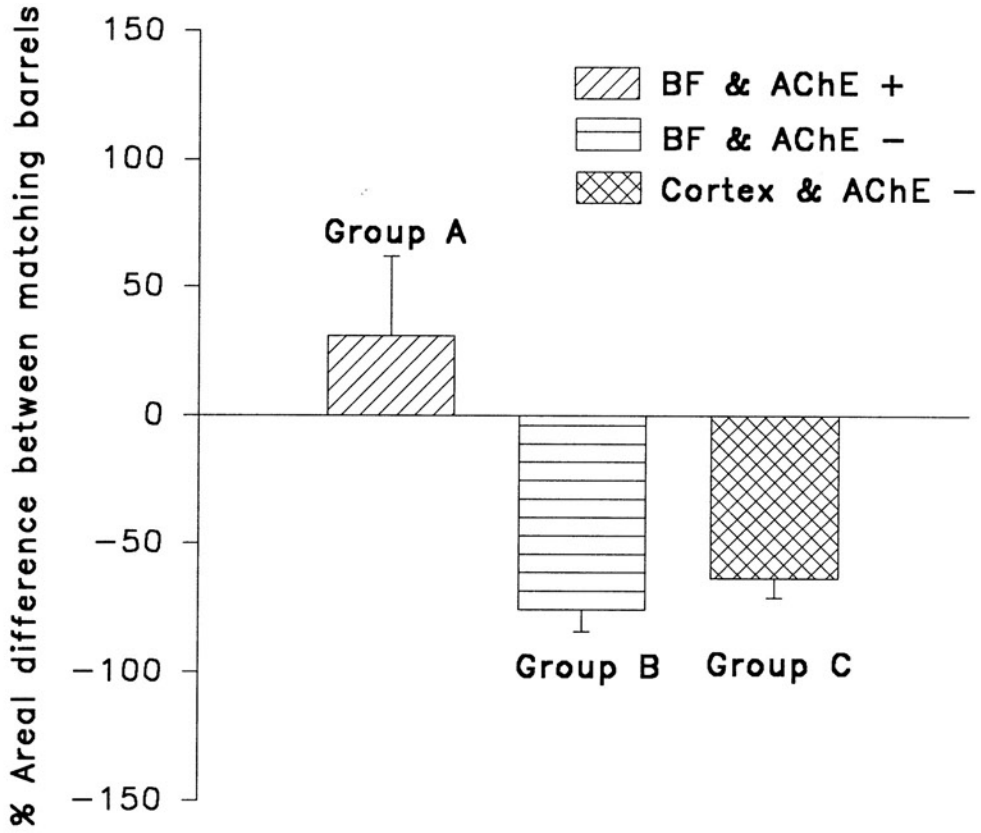
Figure 16. Bar graph illustrating the areal measurements of barrel-associated spots of label in both hemispheres of animals receiving basal forebrain transplants. The measurements taken in the transplanted hemisphere were in cortical territory that was AChE- depleted (i.e., 40% of normal, AChE -), indicated with hatched bars; the measurements in the opposite hemisphere are indicated with solid bars. See Figure 15 and text for details. Significant differences were found between the areas of activated barrels in the AChE-depleted cortical territory in basal forebrain-transplanted hemispheres compared to equivalent barrels in the normal hemispheres (paired *t*-test, $P < 0.01$). BF, basal forebrain.

Area of barrel-associated 2DG uptake
in ACHE- cortex



Animals receiving BF transplant

Figure 17. Bar graph illustrating the percent difference in areal measurements of activated barrels in transplanted hemispheres expressed as a percent of corresponding matched barrels in normal hemispheres. Group A, (BF & AChE +) activated barrels in basal forebrain-transplanted hemispheres within cortical territory containing at least 85% of AChE staining levels of the contralateral unlesioned hemispheres; Group B, (BF & AChE -) activated barrels in basal forebrain-transplanted hemispheres within cortical territory containing 30-40% of AChE staining levels of the contralateral hemispheres; Group C, (Cortex & AChE -) activated barrels in neocortical control-transplanted hemispheres within cortical territory containing 25 - 40% of AChE staining levels of the contralateral hemispheres.
Group A is significantly different from Group B ($P < 0.001$).
Group A is significantly different from Group C ($P < 0.01$).
Groups B and C are not significantly different.


% Areal difference between matching barrels
150
100
50
0
−50
−100
−150
BF & AChE +
BF & AChE −
Cortex & AChE −
Group A
Group B
Group C

Animals receiving neocortical control transplants. Figure 18 is an example of a cortical map generated from an animal that received a basal forebrain lesion and a control transplant of neocortical tissue. Three vibrissae were bilaterally and symmetrically stimulated during the 2-DG experiment. The 3 activated regions in the experimental hemisphere lay within 1.5 mm from the transplant. When the areal dimensions of the 2-DG activity associated with the barrels in the experimental hemisphere were compared to their counterparts in the opposite hemisphere, they were found to be significantly reduced in size (Fig. 19). Although the spots of activity are within close proximity to the transplant, the AChE staining in these sites is 35% of staining density in the opposite non-lesioned hemisphere. The significant decrease in the area of activated barrels in control-transplanted hemispheres is a consistent finding, as demonstrated in Figure 19 (paired t-test, $P < 0.01$).

The percent difference between the areas of matched pairs of activated barrels in control-grafted animals (Group C as defined above) was significantly different from the percent area difference of barrels in AChE-reinnervated territory of basal forebrain-transplanted animals (Group A) ($F(1,20) = 14.00$, $P = 0.01$), but not those outside the region of AChE-reinnervation (Group B) (Fig. 17).

Figure 18. Two-dimensional maps of stimulus-evoked metabolic activity from an animal that received a basal forebrain lesion and a control transplant of embryonic cortical tissue. In the normal (N) and transplanted (T) hemisphere spots of barrel-associated label can be seen (a/a^1, b/b^1, c/c^1). In the map of the hemisphere with the transplant (T), all the barrel-associated spots of activity are reduced in dimension compared with their companions in the normal side (N). All these diminished spots of label were also within 2 mm from the transplant and in cortical territory with less than 35% of AChE-positive fibers in the opposite hemisphere. See the text and Figures 12 and 13 for conventions and details. Medial is to the left and rostral is up. Scale bar = 1 mm.

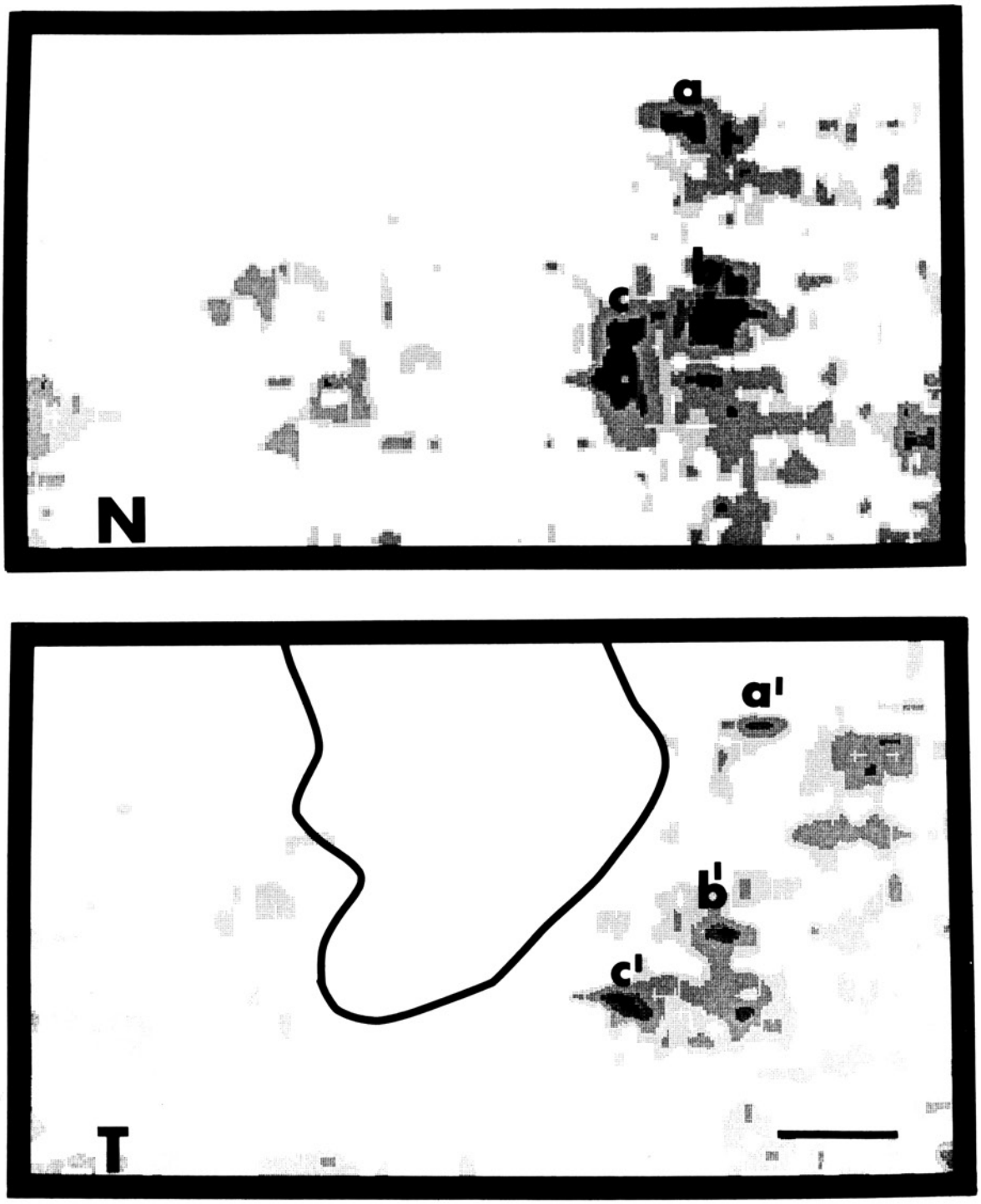
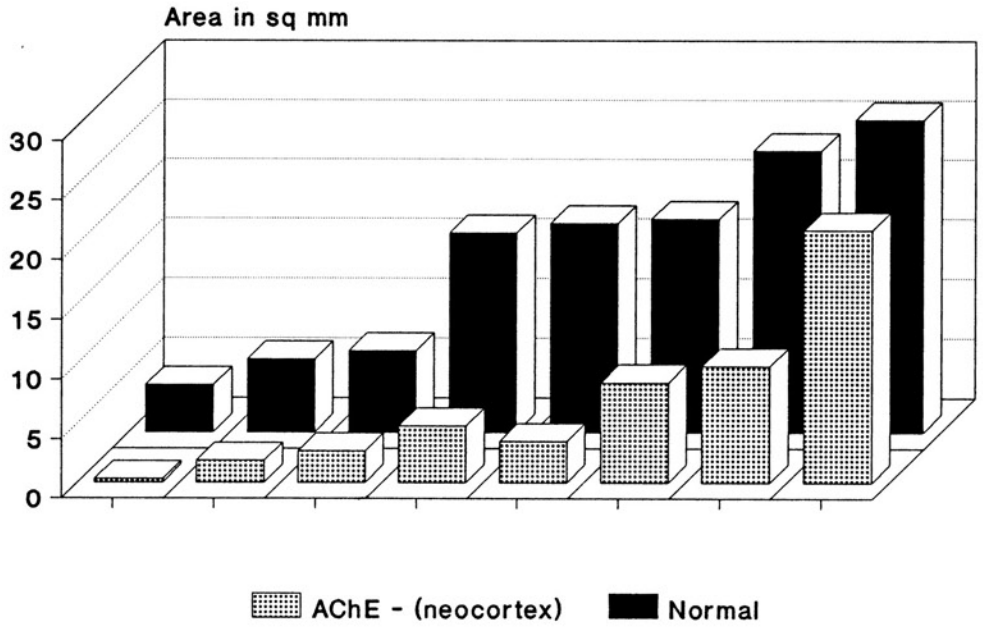

a
b
c
N
a'
b'
c'
T

Figure 19. Bar graph illustrating areas of barrel-associated 2-DG uptake in animals that received neocortical transplants following basal forebrain lesions. The transplanted hemispheres contained 25-40% of AChE staining in the opposite hemisphere. Measurements of the area of stimulus-evoked spots of label in the AChE-depleted hemispheres containing the transplant (AChE -, dotted bars) and their matched counterparts in the normal hemispheres (solid bars) were significantly different (paired *t*-test, $P < 0.01$).

Areas of 2DG uptake associated with barrels in AChE- cortex



Animals receiving neocortical transplant

Optical density measurements were obtained demonstrating that the intensity of metabolic activity in the group of activated barrels in control-transplanted hemispheres was reduced but not significantly different from the corresponding values in the contralateral hemispheres (Table 2).

Discussion

The results of these experiments suggest that cell suspension transplants of embryonic cholinergic basal forebrain tissue (a) increase the density of AChE staining surrounding the transplant and (b) if sufficiently close to activated barrels, the cholinergic transplants can ameliorate deficits in stimulus-evoked metabolic activity resulting from basal forebrain lesions. Control transplants of neocortical tissue did not result in such improvements.

The Effect of Basal Forebrain Lesions on Cortical Function

It is widely accepted that basal forebrain lesions can profoundly impair cortical cholinergic functions, with less clear cut impact on other neurotransmitter systems (for review, see Dekker et al., 1991). The evidence supporting this stems from a number of sources, including previous

investigations by this laboratory of the effect of basal forebrain lesions on metabolic activity. The metabolic studies determined that 2-DG uptake in response to stimulation was decreased in the somatosensory cortex ipsilateral to the lesion (Ma et al., 1989; Juliano et al., 1990; also see Chapter 2). Although basal forebrain lesions almost certainly destroy several classes of neurons, I interpreted the reductions in stimulus-evoked 2-DG activity as most likely due to depletion of cortical ACh. This interpretation was supported in part by the finding that similar reductions in stimulus-evoked activity resulted after topical application of a muscarinic antagonist to the somatosensory cortex (Juliano et al., 1990). In addition, cortical CO staining and baseline cortical 2-DG uptake ipsilateral to the lesion were normal, suggesting that thalamocortical afferent fibers were not disrupted (Ma et al., 1989; Juliano et al., 1990).

Basal forebrain lesions do not appear to cause substantial reductions in cortical levels of other neurotransmitters, such as somatostatin, noradrenaline, dopamine, GABA or serotonin (Johnston et al., 1981; McKinney et al., 1982; Dubois et al., 1985; Fine et al., 1987; Höhmann et al., 1987). Conversely, behavioral impairments induced by basal forebrain lesions are improved by treatments with drugs that enhance or mimic the action of ACh (see Dekker et al., 1991 for review), suggesting that ACh was the functionally relevant substance depleted by the

lesions. In addition, a number of studies emphasize the specific effects of cholinergic depletion on certain cortical processes. For example, research evaluating the role of ACh on the development of cortical morphology in mice determined that only basal forebrain lesions, and not those known to deplete cortical norepinephrine or serotonin, resulted in abnormal cortical cytoarchitecture (Höhmann et al., 1988). In a different study evaluating changes in 2-DG uptake, Levin et al. (1988) determined that noradrenergic-depleting lesions of the locus coeruleus in rats with intact whiskers resulted in expansion of stimulus-evoked 2-DG uptake in individual barrels, in contrast to the reductions caused by cortical ACh depletion. Furthermore, ACh-rich transplants inserted into neocortex, or hippocampus, depleted of ACh lead to substantial improvements in memory and attention, while grafts of tissue containing other neurotransmitters, do not result in behavioral improvement (Fine et al., 1985b; Collier, 1990; Kimble, 1990; Dunnett, 1990, 1991; Ridley et al., 1992).

Basal Forebrain Transplants Restore Activity Toward Normal

For these reasons, I consider it likely that the increases in 2-DG uptake seen after transplantation of embryonic basal forebrain are due to graft-derived cholinergic reinnervation of adjacent neocortex. While it

is likely that cholinergic neurons are only a minority among the constituents of these grafts, many of the other neurotransmitters that might be present (e.g., GABA, glutamate, somatostatin) are common to both the basal forebrain and neocortical embryonic tissue used in this study. In addition, I found a clear correlation between the amount of AChE innervation and the increase of 2-DG uptake in the barrel region. Activated barrels adjacent to the cholinergic transplants, and within AChE-positive regions, exhibited label of comparable dimension and intensity to corresponding barrels in the contralateral normal hemisphere. This was not the case for barrels lying more than 2 mm from the basal forebrain transplant and in ACh-depleted areas. The activity evoked in these areas was typical of that observed in the ACh-depleted hemispheres of the earlier study reported in Chapter 2. This may reflect a "limit" to the distance that axons can extend from these transplants. Previous investigations of embryonic rat cholinergic transplants into ACh-depleted rat neocortex suggest that fiber outgrowth surrounding the graft is restricted to 1-3 mm (Fine et al., 1985a,b; Dunnett et al., 1986). AChE-positive fibers have been reported to extend up to 6 mm from intrahippocampal basal forebrain transplants placed in rats following fimbria-fornix lesions, although the most intense staining occurred within 2 mm of the graft borders (Björklund and Stenevi, 1977; Nilsson et al., 1987; Shapiro et al., 1989). Kelly et al. (1985) found that 2-DG

uptake in the rat hippocampus, reduced by prior lesion of the septohippocampal pathway, was restored to near-normal levels within 3 mm of the intrahippocampal cholinergic basal forebrain transplants, and the density of the AChE staining was also greatest within that region. The mechanism that permits and/or restricts the distance that fibers extend from a transplant is not clear. Dunnett et al. (1986) proposed that since neurogenesis continues into adult life in the hippocampus (Angevine, 1965; Bayer, 1980; Stanfield and Cowen, 1988), a prolonged accessibility to trophic factors may account for the greater distance of AChE-positive fiber outgrowth. It is also possible that the inherent maturity of the transplant may determine the extent of axonal outgrowth; after an implant reaches a mature age, axons may stop growing. The improvements in stimulus-evoked 2-DG uptake following cholinergic transplantation and subsequent reinnervation, as demonstrated by AChE staining, can be understood in light of the significant role that ACh plays in the processing of information in sensory regions of neocortex. Many studies have shown that ACh enhances neural activity through a mechanism depressing potassium permeability (Krnjević et al., 1971; Halliwell and Adams, 1982; Brown, 1983; McCormick and Prince, 1985). In neocortex, this effect is particularly evident in response to specific stimulation (Metherate et al., 1987; Sillito and Murphy, 1987; Metherate et al., 1988a; Dykes, 1990). In addition, ACh appears to facilitate long-term enhancement of

cortical responses and contribute to neural plasticity in both adult and neonatal animals (Bear and Singer, 1986; Rasmusson and Dykes, 1988; Dykes, 1990; Juliano et al., 1991; Webster et al., 1991). As a result, it is not surprising that replacement of ACh in deprived cortex by transplants of ACh-rich tissue, increases the strength of neural responses to stimulation, reflected as increased 2-DG uptake. A recent finding by Nilsson and Björklund (1992) reinforces the notion that heightened metabolic uptake reflects a cholinergic enhancement of activity in response to a functional demand. This study reports that ACh-rich transplants into animals with fimbria-fornix lesions differentially release ACh, as required by the host under distinct behavioral conditions.

These results also reveal that there were no significant reductions in optical density in barrel-associated 2-DG activity near (i.e., within 2 mm) the "control" neocortical transplants but within ACh-depleted territory, compared with significant differences in density values in barrels far (i.e., greater than 2 mm) from basal forebrain transplants, and also in ACh-depleted territory. It is unclear why the density values in control-grafted hemispheres are not notably different from values in the contralateral hemispheres. It is unlikely that ACh played a role, since no evidence for cholinergic innervation was seen in the AChE staining pattern associated with the neocortical tissue transplants. The surrounding cortex had less than

40% of the AChE staining density of the opposite unlesioned hemisphere. It is possible, as has been suggested by others, that unidentified trophic factors participate in functional improvements noted after grafting (e.g., Björklund et al., 1987). If present in this case, these trophic factors may be able to elicit only limited improvements, since despite the slight increases in barrel-associated optical density values near the control transplants, the area of cortex activated by whisker stimulation was still significantly smaller than normal. This suggests that such trophic effects, if present, are not sufficient to restore the normal levels of neural activation in response to stimulation of a given whisker, as cholinergic grafts are able to do.

Clinical Implications

Reduction of cortical ACh is associated with many dementias including Alzheimer's disease, Korsakoff's syndrome, and Parkinson's disease with dementia. It is still not clear that augmentation of neurotransmitters through transplantation is an appropriate therapy for humans with disease processes that may require treatment substantially more complex than replacement of a single substance. Recent results from transplantation of dopaminergic cells into animal models of Parkinson's

disease, and into some humans, have provided optimism for the possibility of improvements in motor impairments associated with this disease (Lindvall et al., 1990; for review see Lindvall, 1991; Freed et al., 1992). In addition, numerous studies in ACh-depleted animals, demonstrating cognitive improvements following basal forebrain transplants (Ridley et al., 1991, 1992), sustain the possibility of human treatment with transplantation in the future. Observations that deficits in somatosensory processing are present in patients with Alzheimer's disease (Freedman and Oscar-Berman, 1987) underscore the significance of the results reported here and emphasize that Alzheimer's disease affects the entire neocortex.

Chapter 4: The Impact of Basal Forebrain Lesions on the Ability of Rats to Perform a Sensory Discrimination Task Using the Whiskers

Introduction

The effects of lesions of the basal forebrain cholinergic system on behavior have been typically interpreted in relation to cognitive processes, even when the task might suggest the involvement of non-cognitive systems (e.g., sensorimotor impairment, increased locomotor activity) (for reviews see Collerton, 1986; Olton and Wenk, 1987; Wenk and Olton, 1987). For example, several studies used sensory discrimination tasks to assess learning or memory (Ridley et al., 1985, 1986; Everitt et al., 1987; Irle and Markowitsch, 1987; Wozniak et al., 1989). While the findings reported by these authors suggest that basal forebrain lesions impair an animal's ability to accurately perform on tasks of visual or somatosensory discrimination, the previous experiments were not specifically designed to be sensitive to deficits in sensory processing. The findings of the earlier studies investigated the ability to acquire a new task (learning), and rate of correct responses with the imposition of a time delay between stimulus and response (memory).

A number of clinical studies with Alzheimer's disease patients have suggested that ACh is involved in sensory-based functions. Alzheimer's disease is characterized by marked cell loss in the nbm (Whitehouse et al., 1981, 1982; Price et al., 1982; Candy et al., 1983) as well as reductions in cortical cholinergic activity (Bowen et al., 1976; Davies and Maloney, 1976; Perry et al., 1977); clinical studies with such patients have routinely related the cholinergic neuropathology to memory decline (for reviews see Bartus et al., 1982; Coyle et al., 1983; Collerton, 1986). Freedman and Oscar-Berman (1987) demonstrated pronounced deficits in tactile discrimination learning in individuals with Alzheimer's disease compared to those with Parkinson's disease. Nissen et al., (1985) found that patients with Alzheimer's disease were unable to visually discriminate between coarse and fine patterns. These findings suggest that a loss in sensitivity occurs in Alzheimer's disease that affects thresholds for sensory discrimination.

Although these and other experiments suggest a relationship between ACh and sensory behavior, very few studies have investigated sensory discrimination performance after ACh depletion. Therefore, the studies presented here test the ability of rats to perceive and respond to a tactile stimulus before and after ACh depletion. Their performance was correlated to 2-DG uptake.

Materials and Methods

Experimental Design

Rats were trained to respond to a stimulation of the whiskers. When a rat reached a pre-set level of performance ability, it was randomly assigned to receive either a saline injection or an ibotenic acid injection into the region of the nucleus basalis magnocellularis in the right hemisphere. Post-lesion behavioral testing continued until the animal returned to pre-lesion criteria at which time a 2-DG experiment was conducted.

Subjects

A total of 13 female Sprague-Dawley rats, weighing 175 - 200 g were used in this study. The rats were individually housed and maintained on a 12/12 hr light/dark cycle. Water was provided ad libitum; food was weighed and offered once daily to maintain the rats at 85% of their free feeding rate. The rats were weighed before each behavioral session to monitor their weight and, later, to ensure maintenance of desired weight.

Apparatus

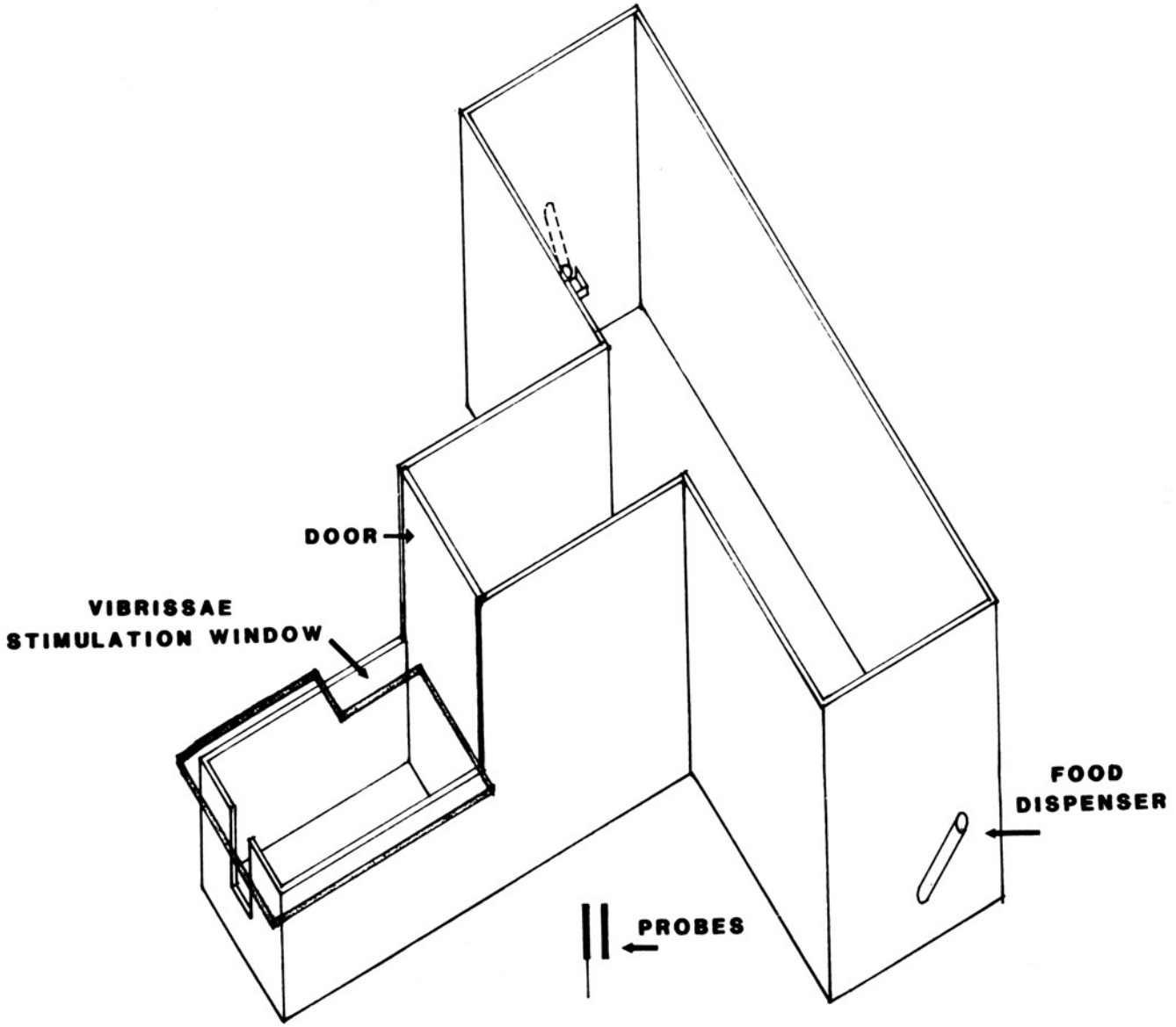
A wooden T-maze painted black with polyurethane coating was used for the behavioral task (Figure 20). As previously described by Hurwitz et al. (1990) a start chamber, separated from the rest of the apparatus by a guillotine door, was covered by a clear plexiglass lid with an opening 6.5 x 1.5 cm adjacent to the door. Stimuli were presented through this opening to the left set of whiskers of each rat. The end of each goal arm contained a food cup into which Noyes dustless pellets (45 mg) were manually dispensed. Throughout the training and testing, the room was illuminated by a red safety light, which hung 80 cm above the floor of the T-maze, oriented in a manner to provide only indirect light. A handheld probe of insulated wire (20 gauge) was used to manually stimulate the whiskers (Figure 20).

Behavioral Procedure

Shaping. During the shaping phase, a rat was placed in the start chamber and the plexiglass lid put in place, after which the door to the main alley was opened. To encourage the rat to explore, food pellets were placed on the floor of the main alley and both goal arms, and in the

Figure 20. The T-maze apparatus used for the whisker sensory discrimination task. While in the start box, the rat received a whisker deflection using the long probe, or a sham deflection using the short probe. The door then opened and the rat received a food pellet reward for turning right when the whiskers were deflected, or for turning left when the whiskers were not deflected.

T-MAZE TASK



food bins. The rat was allowed to eat pellets in the main alley and in whichever goal arm it first entered. It was returned to the start chamber before being allowed to enter the opposite food-baited goal arm. Food pellets were replenished and the procedure repeated for a total of 10 min. When a rat first ate food from the food bin, additional pellets were manually dispensed into it, to accustom the animal to the sound and appearance of food being delivered. Soon after the rat ate out of the food bin, pellets were no longer placed on the floor and were available only in the bins. When a rat reached a point in training when it went directly to a food bin after being placed in the T-maze, the shaping phase was replaced by a training phase.

Training. The rat was placed in the start box facing the guillotine door and the plexiglass lid put in place to cover the space. With the rat in this position, a stimulus was presented through the lid opening that consisted of either (a) a brisk caudal-to-rostral deflection of the whiskers along the left side of the face using a probe 15 cm long, or (b) a sham stimulus using a 10 cm long probe that provided all the stimulus cues but in which no whisker contact was made (Figure 20). Immediately after administration of the stimulus, the door was opened to the main alley. The rat was rewarded with a food pellet for turning right if its whiskers were deflected, and for turning left if the whiskers were not deflected.

Determination of the goal arm was therefore dependent on the ability of the rat to discriminate whether or not its left-side set of whiskers had been displaced. Training was conducted 6 days a week during which time problem behaviors (e.g., side preferences, aversive reaction to handling) were eliminated. When a rat performed at better than chance level on 3 occasions, the training phase ended and a formal testing phase was initiated.

Testing. Testing was conducted 3 times a week with each test session consisting of 80 trials divided equally into 40 whisker deflections and 40 sham deflections. Selection of the stimulus to be administered (actual deflection vs sham deflection) was randomly determined with the restriction that no more than 3 consecutive trials were the same. Pre-lesion criteria was established as 3 consecutive sessions at the 80% correct level. Within 48 hr after achieving this criteria, the animal was prepared for surgery.

Surgery

Basal forebrain lesion. Animals were randomly assigned either to a group receiving an excitotoxic lesion or to a group receiving a sham lesion of the basal forebrain. Each animal was anesthetized with 7% chloral hydrate IP (40 mg/100 g), placed in a stereotaxic apparatus,

and prepared for surgery. A small opening was made in the skull 4.0 mm anterior to bregma and 2.3 mm lateral from midline. A Hamilton syringe containing 1 μl of either sterile saline (sham lesion) or the excitatory amino acid, ibotenic acid, (10 μg/μl) (excitotoxic lesion) was angled 36° from vertical and 12° lateral, and advanced 8 mm from the dura. These coordinates have previously been established as an effective way to approach the area of the basal forebrain without traversing the somatosensory cortex (see Chapter 2). The syringe remained in place for 5 min, after which 0.5 μl was injected over a period of 2 min. The needle remained in place for 2 min and was retracted 1 mm, where the remaining 0.5 μl was similarly injected. The animal then returned to its cage under a warming light for recovery and was closely monitored until it was moving spontaneously. Forty-eight hr after surgery, the post-lesion behavioral phase of the experiment began. The rat participated in testing 3 times/week until its performance again reached the pre-lesion criteria level. At this point a terminal 2-DG experiment was performed.

2-DG procedure. Each rat was anesthetized with halothane (1.5%) and a catheter placed in the jugular vein. A local anesthetic was applied to the skin around the catheter, and the rat loosely restrained on a block. All whiskers were trimmed bilaterally except for C3 or C2. The halothane was then discontinued and the animal allowed to

recover from the effects of the anesthetic for 1.5-2 hr. At this time, using handheld brushes, manual stimulation of the remaining matched pair of whiskers began. Five min after the onset of stimulation, a pulse IV injection of 2-DG (10 μCi/100 g) was administered and the stimulation continued for 45 min, at which time sodium pentobarbital (50 mg/kg) was administered IV and the animal transcardially perfused with saline, followed by a solution of 0.1M phosphate-buffered paraformaldehyde (4%) with 4% sucrose. The brain was quickly removed, frozen in Freon 22, and stored in a freezer at -70°C.

Tissue Processing

Brains were cut in a cryostat at -16°C at a thickness of 30 μm. Adjacent sections were saved for 2-DG autoradiography, acetylcholinesterase (AChE) histochemistry using the method described by Koelle (1955) and adapted by Jacobowitz and Creed (1983), and cytochrome oxidase (CO) histochemistry according to the procedure of Wong-Riley (1979).

Sections saved for 2-DG autoradiography were picked up on 2% gelatin-coated slides and placed on a warming tray at 60°C for rapid dehydration. To prepare the 2-DG autoradiographs, the slides were placed in an X-ray cassette

along with ^{14}C methylacrylate standards and exposed to X-ray film (SB-5, Kodak) for 7 days.

Data Analysis

Quantification of cortical ACh depletion. To assess the effectiveness of both the excitotoxic and sham lesions, the density of AChE-stained fibers in both hemispheres of all rats was quantified. Three brains were evaluated by counting AChE-stained fibers, according to the method described by Stichel and Singer (1987), and by optical density measurements of cortical AChE-staining using a video-based image analysis system with a PDP 11/23+ host computer and custom-designed software. Cortical regions specifically evaluated for AChE density included the regions containing the activated barrels in both hemispheres, as identified by adjacent autoradiographic sections, and a region of cortex within the barrel field but not itself activated. A linear relationship was found between the fiber counts and the optical density measures. By expressing the mean optical density of AChE staining in the experimental hemisphere as a percent of the normal hemisphere, the amount of cortical AChE depletion in the lesioned hemisphere was determined for each animal.

2-DG autoradiographic analysis. Individual autoradiographic sections containing barrel-associated regions of increased activity were measured in specified sites, including the activated barrel, a region of cortex surrounding the activated barrel but not itself stimulated, and the underlying white matter (considered to be background). The optical density values in the region of the activated barrel and in the surrounding cortex were then expressed as a percent above background. The percent difference in the intensity of 2-DG uptake between the hemispheres was determined.

Digitized maps of metabolic activity in the somatosensory cortex were generated from a series of coronal autoradiographic sections. The autoradiographs were displayed and details of the 2-DG label enhanced using look-up tables that transform the ^{14}C values to gray scales. The cortical area of interest was outlined using a digitizing tablet and partitioned into vertical and laminar arrays of high resolution. The individual sections were then aligned and displayed as flattened digitized maps of the somatosensory cortex (Tommerdahl et al., 1985). Both sides of the brain on a single section were digitized and analyzed simultaneously to ensure identical treatment of the two hemispheres.

Tangential maps generated from each hemisphere revealed spots of increased 2-DG uptake corresponding to the

barrels activated by whisker stimulation during the 2-DG procedure. The average pixel intensity and standard deviation (SD) for the entire cortical map of each hemisphere were obtained. The area of activity at least 1.5 SD above the average intensity was displayed and measured using a digitizing tablet allowing for comparison of the areas of the barrel-associated spots between hemispheres.

Statistics. Descriptive statistics, analysis of variance (ANOVA) and correlation coefficients were computed using SYSTAT 5.0 for Windows (SYSTAT, Inc.). A probability (*P*) value of 0.05 was used for all statistical tests of significance. Statistical comparisons between Group (Sham vs Excitotoxic lesioned) and sessions to reach criteria, and between Group and areal dimension of 2-DG label were conducted using a 2 x 2 ANOVA.

Results

Analysis of the Lesion and AChE Depletion

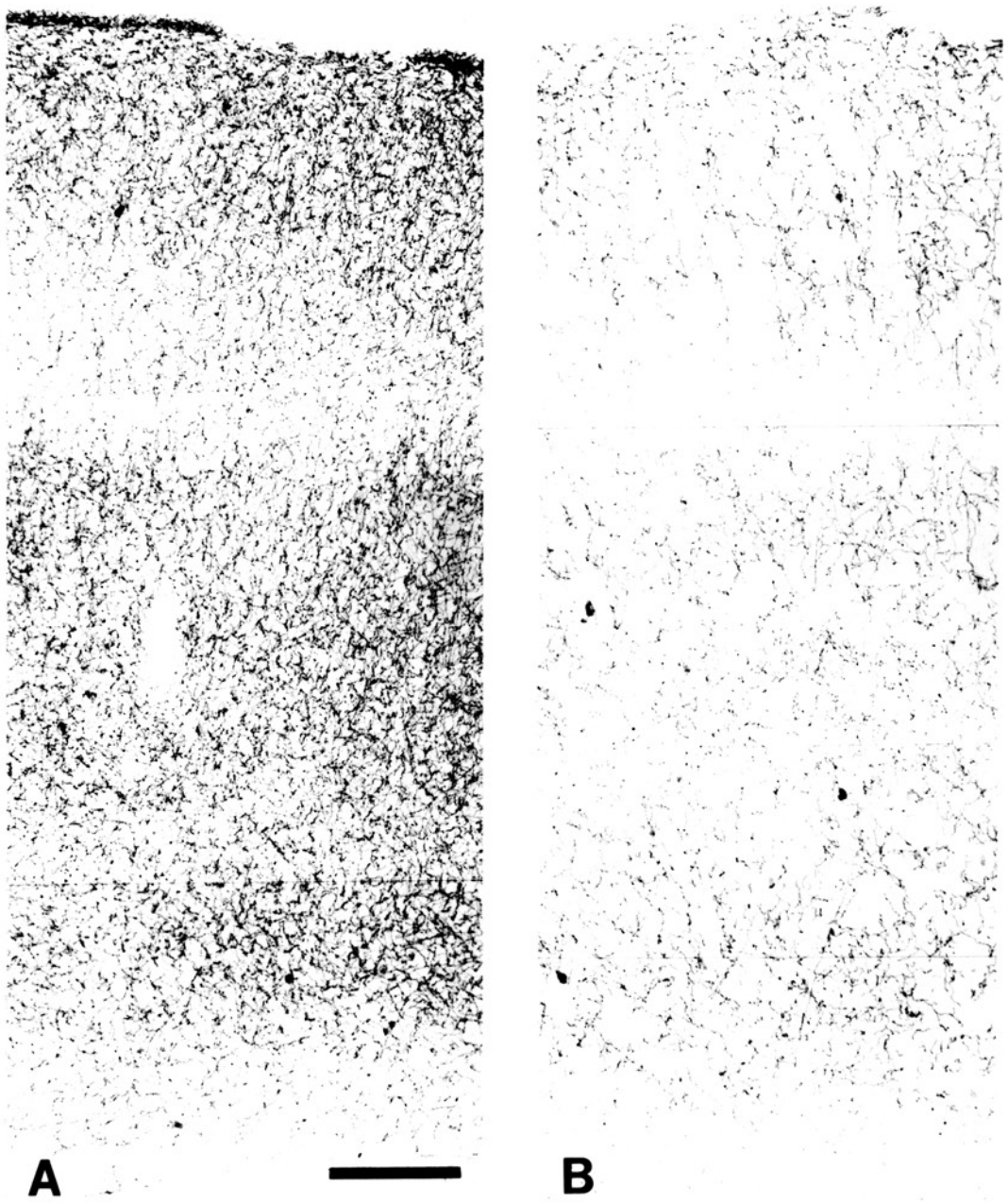
Each lesion was carefully analyzed for placement. A successful lesion was confined to the nucleus basalis magnocellularis (nbm) and the ventromedial globus pallidus, and did not encroach on the thalamus. CO histochemistry was used to define the lesion borders, along with Nissl and AChE

staining (Ma et al., 1989; Juliano et al., 1990). The intensity of AChE staining was reduced in the frontoparietal cortex ipsilateral to the excitotoxic lesion throughout all cortical layers. The amount of AChE depletion was quantified as described in the Methods and from 16-75% below the intensity of the unlesioned hemispheres. An example of cortical AChE depletion is demonstrated in Figure 21, and reflects a decrease of 51.8% in AChE-stained fibers. Sham lesions did not lead to differences in AChE staining density between experimental and control hemispheres; differences between hemispheres ranged from 0-3.5%. The amount of depletion was also correlated with the performance of each rat in the maze task (see below).

Maze Performance

Prior to the lesion procedure, rats were trained to respond appropriately to whisker stimulation. When a rat performed at the 80% level for 3 consecutive sessions, it was randomly assigned to receive either a sham (N = 5) or excitotoxic (N = 8) basal forebrain lesion. During the

Figure 21. Photomicrograph of AChE fiber staining in the normal hemisphere (A) and, from the same section, the hemisphere ipsilateral to a basal forebrain lesion (B). The amount of AChE depletion shown in (B) was quantified using the image analysis system, and reflects 51.8% of the staining in (A). Scale bar = 250 μm.


A
B

post-lesion testing sessions the examiner was blind to which rat was performing at any given time to ensure no bias in the procedure. All sham lesioned rats returned to their pre-lesion performance levels within the first week post-lesion (1-3 sessions). Figure 22 shows performance profiles for 3 sham and 3 excitotoxic lesioned rats. Also indicated in Figure 22 is the amount of AChE depletion in the right hemisphere of each rat, which was determined using the quantification methods described previously. Comparison between the number of sessions required to return to pre-lesion criteria for the sham lesioned rats ($\overline{X}$ = 1.8, SEM = 0.490) vs the excitotoxic lesioned rats ($\overline{X}$ = 11.0, SEM = 2.478) was significantly different ($F_{1,11}$ = 8.21, $P < 0.015$) (Fig. 23). Animals that received excitotoxic lesions required variable periods of post-lesion testing before reaching pre-lesion criteria, ranging from 4-24 sessions. Those with less effective excitotoxic basal forebrain lesions (i.e., 16-47% depleted) returned to criteria within 2-3 weeks, while rats with substantial ACh depletion (51-75%) required 3-8 weeks to demonstrate restored performance ability. There was a strong correlation between the amount of AChE depletion and the number of sessions required to return to pre-lesion criteria ($r = 0.81$, $P < 0.002$) (Fig. 24).

Figure 22. Performance profiles for 3 sham lesioned rats (top row; circles) and 3 rats that received excitotoxic lesions of the basal forebrain (bottom row; triangles). Pre-lesion testing sessions, indicated by open symbols, concluded when the animal performed at the 80% level for 3 consecutive performances (criteria). Closed symbols indicate testing sessions conducted following the lesion procedure until criteria was again achieved. Animals were tested 3 times a week. The amount of cortical AChE depletion, expressed as a percent of staining in the opposite normal hemisphere, is indicated below each profile. All sham lesioned rats returned to criteria within one week. Excitotoxic-lesioned animals required variable lengths of time (3 - 8 weeks) to return to pre-lesion performance levels.

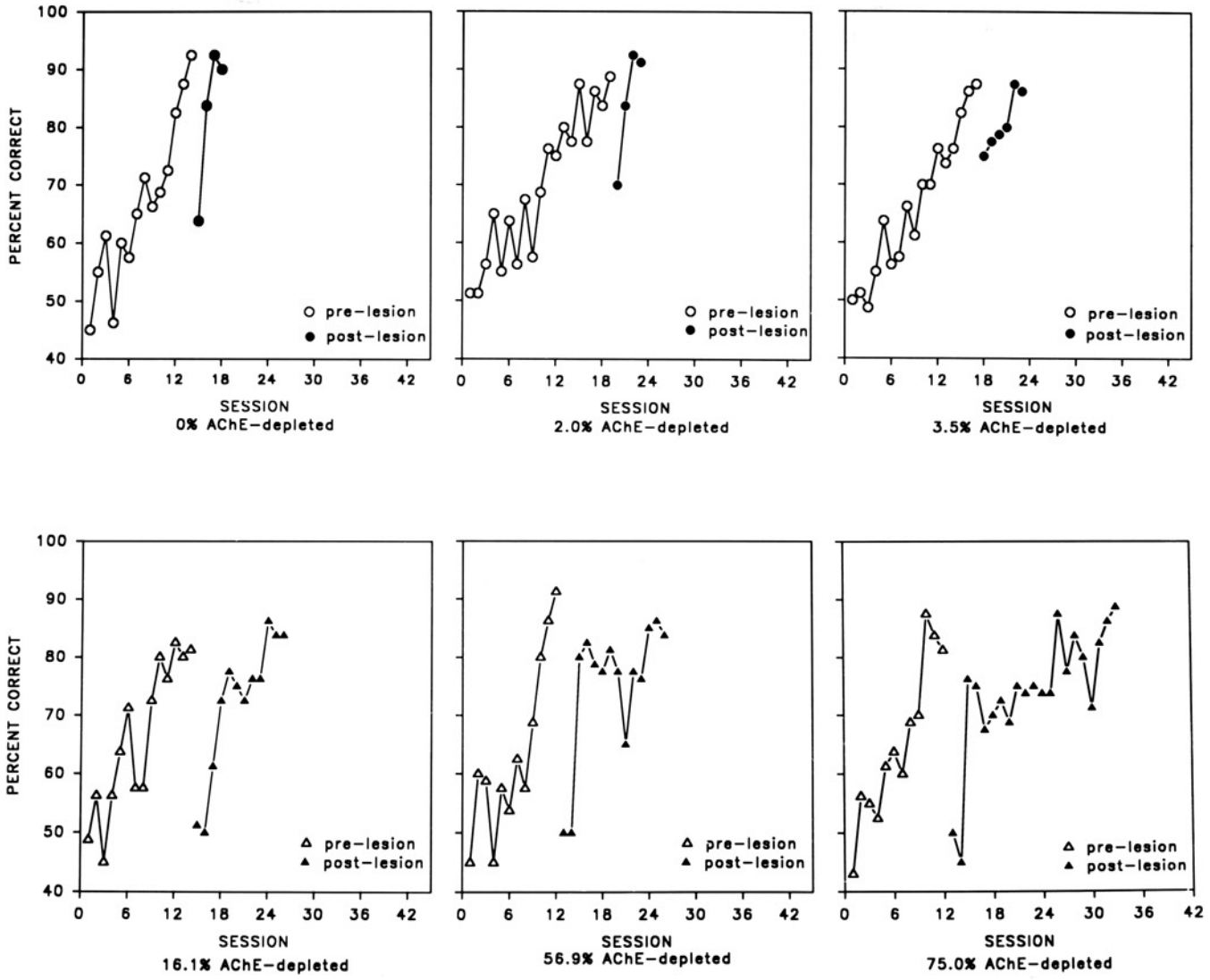

PERCENT CORRECT
100
90
80
70
60
50
40
0 6 12 18 24 30 36 42
SESSION
pre-lesion
post-lesion
0% AChE-depleted
2.0% AChE-depleted
3.5% AChE-depleted
16.1% AChE-depleted
56.9% AChE-depleted
75.0% AChE-depleted

Figure 23. Graph of mean number of post-lesion sessions required by sham lesioned vs excitotoxic lesioned rats. The number of post-lesion testing sessions required by rats in the sham lesioned group (striped bar) was significantly different from the number of sessions required by the excitotoxic lesioned rats (open bar) ($P < 0.015$). The mean number of post-lesion sessions for the sham lesioned animals was 1.8 (SEM = 0.490), while for the excitotoxic lesioned animals the mean was 11.0 (SEM = 2.478).

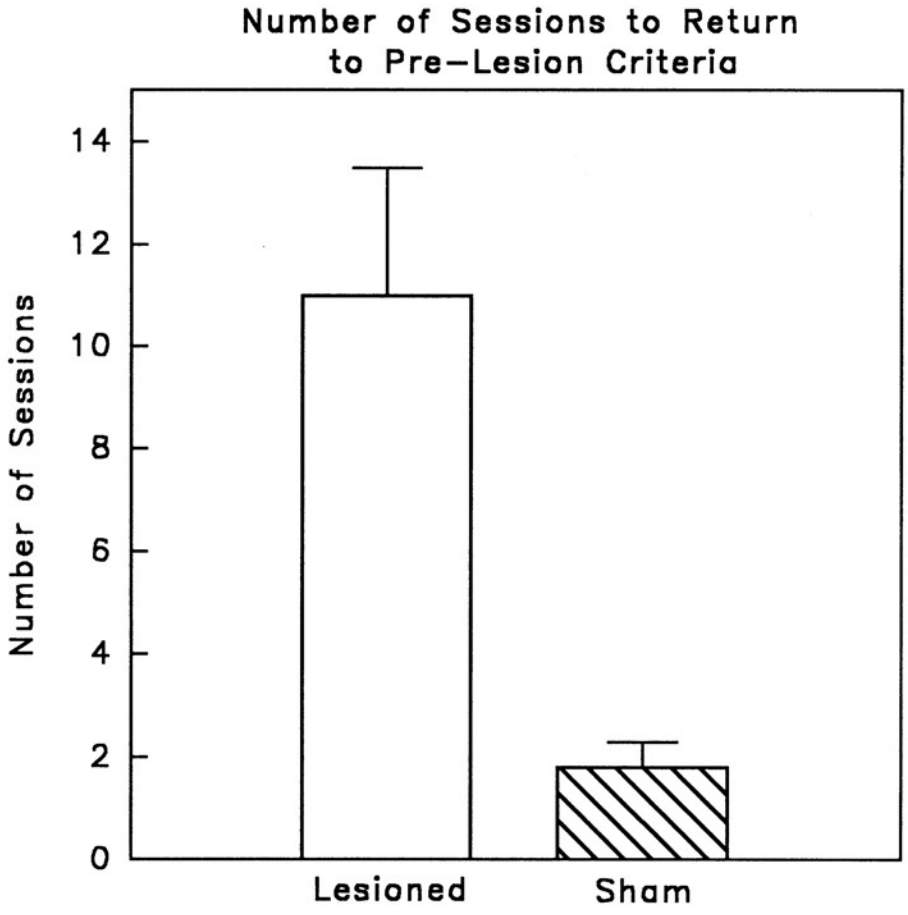
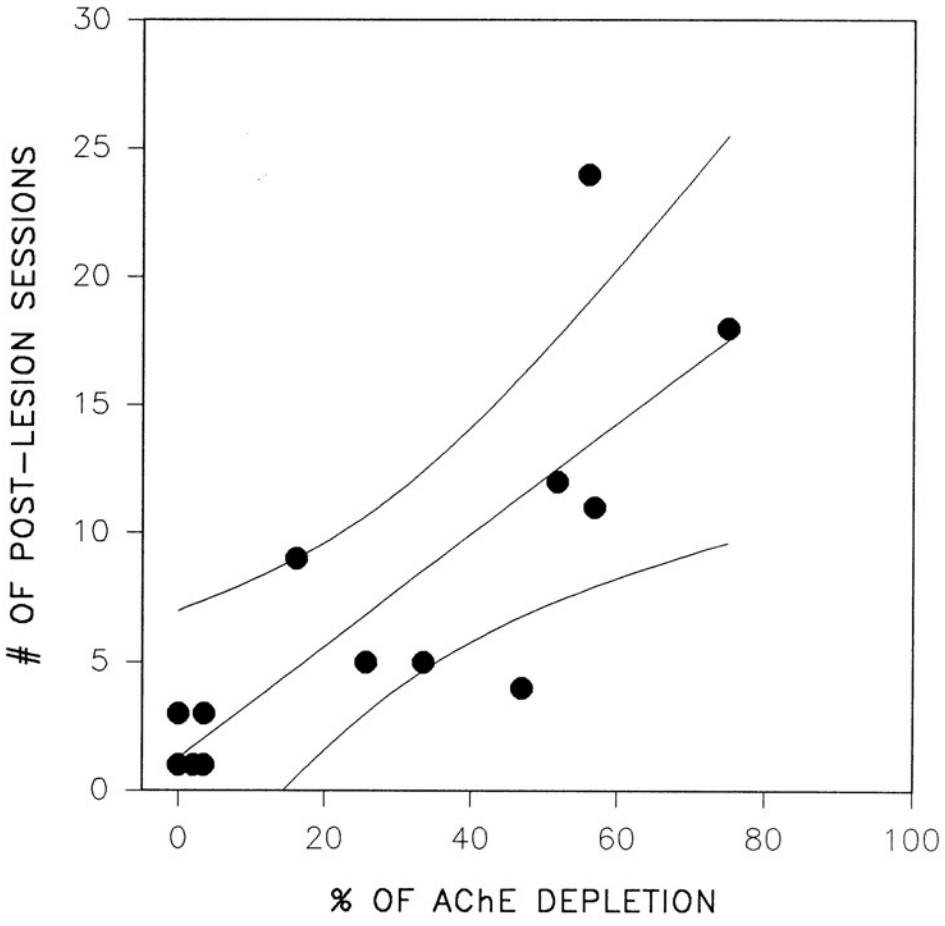

Number of Sessions to Return
to Pre-Lesion Criteria
Number of Sessions
14
12
10
8
6
4
2
0
Lesioned
Sham

Figure 24. Graph indicating a strong correlation between the amount of cortical AChE depletion and the number of post-lesion sessions to return to criteria ($P < 0.002$).


30
25
20
15
10
5
0
OF POST-LESION SESSIONS
0
20
40
60
80
100
% OF AChE DEPLETION

2-DG Uptake

Metabolic activity evoked by single whisker stimulation was evaluated in the individual autoradiographs and in digitized 2-dimensional maps of 2-DG uptake. Column-like spots of label were found in the individual autoradiographs in regions corresponding to the stimulated barrels. Within the spots of label and the surrounding cortical regions, there were no significant differences in mean density of 2-DG label between the normal and lesioned hemispheres. This held for excitotoxic and sham lesioned animals.

The 2-dimensional maps of activity indicate that the area of label evoked by whisker stimulation in the ACh-depleted hemisphere is reduced in comparison to the matched counterpart in the opposite hemisphere. A typical example of this is shown in Figure 25. Stimulus-evoked 2-DG uptake was similar between the hemispheres of rats that received sham lesions of the basal forebrain (Fig. 26).

By expressing the size of the activated barrel in the experimental hemisphere as a percent of the size of the corresponding barrel in the normal hemisphere, the percent size difference in activated barrels within experimental hemispheres was calculated for all animals for which cortical maps had been generated (sham $N = 3$, excitotoxic $N = 4$). While there was a difference between the sham and

Figure 25. Digitized maps of stimulus-evoked metabolic uptake through the barrel cortex of a rat that received behavioral training on a tactile discrimination task and a unilateral excitotoxic lesion of the basal forebrain (nbm). The experimental hemisphere (below) reveals a reduced barrel-like spot of activity compared with activity evoked in the corresponding barrel in the contralateral normal hemisphere (above). Arrows indicate spots of activity. The reduction in activated barrel size persisted despite behavioral recovery. Scale bar = 1 mm.

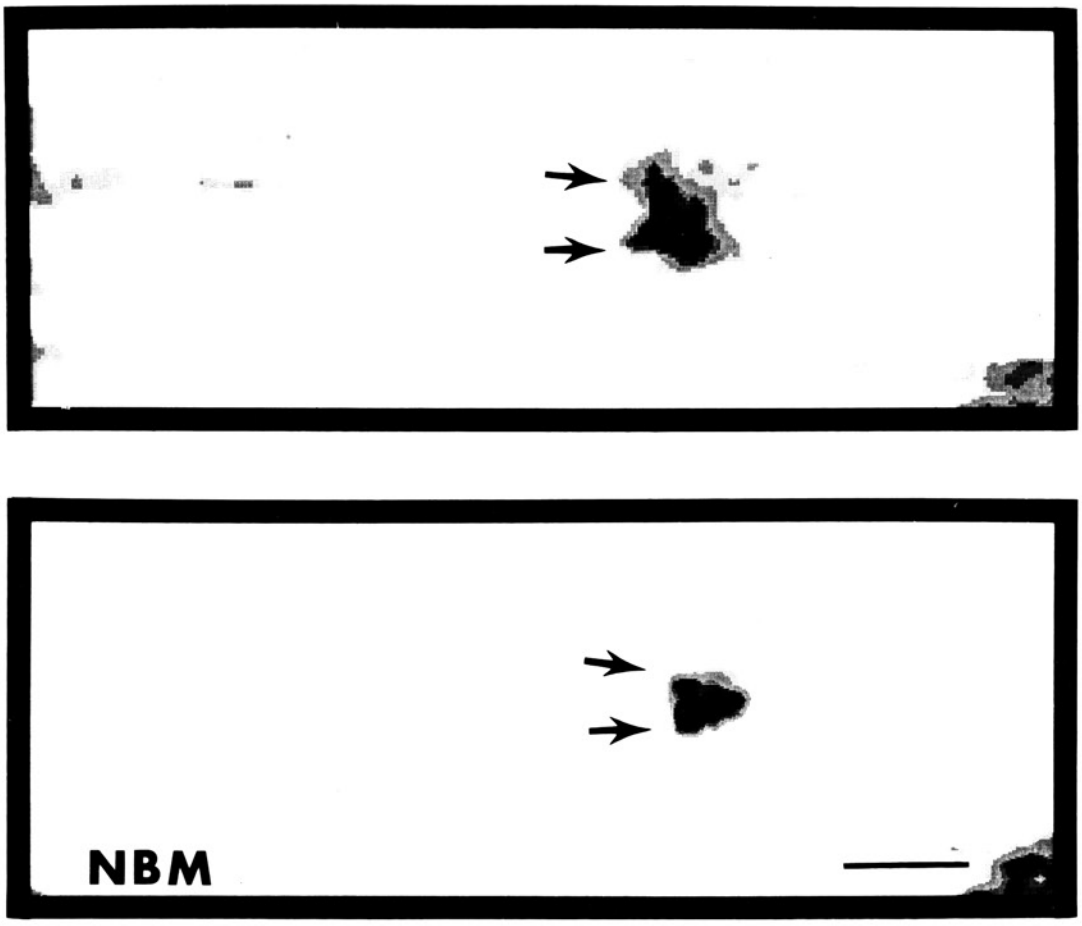
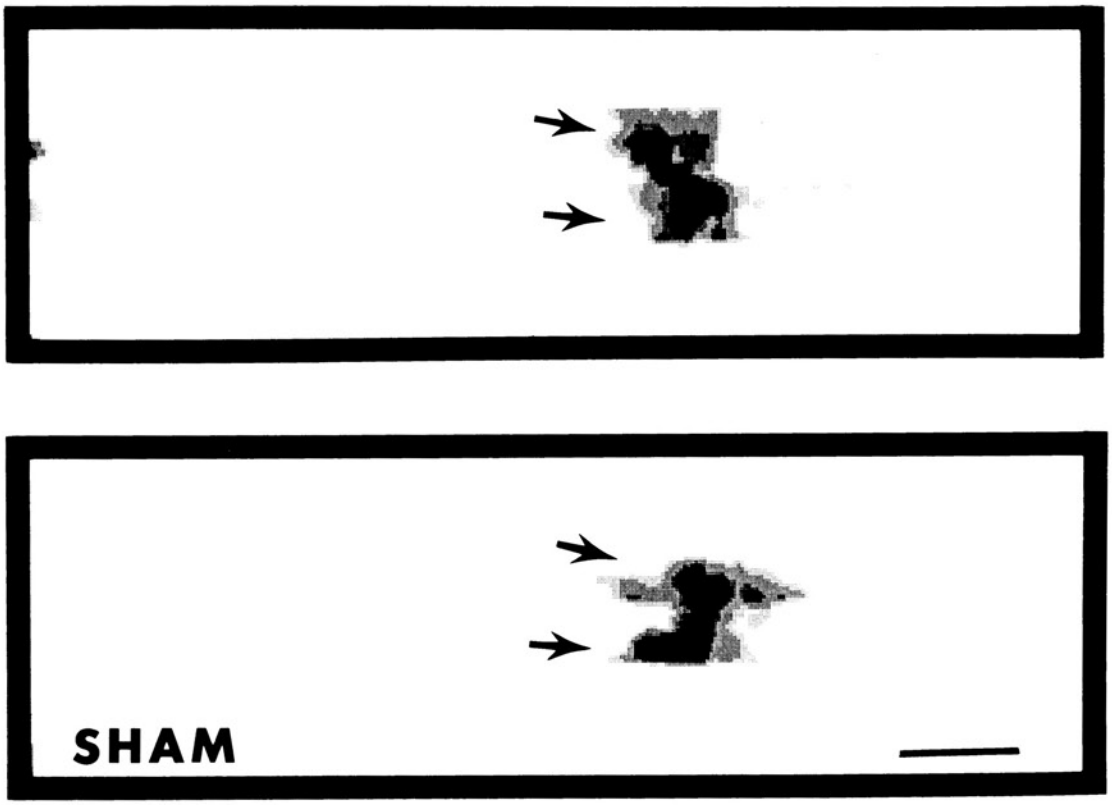

NBM

Figure 26. Digitized maps generated from a rat trained on the tactile discrimination task that received a sham lesion of the basal forebrain (Sham). The stimulus-evoked 2-DG activity associated with bilateral stimulation of the C3 whiskers appears similar between the experimental hemisphere (below) and the normal hemisphere (above). Arrows indicate spots of increased metabolic uptake. Scale bar = 1 mm.


SHAM

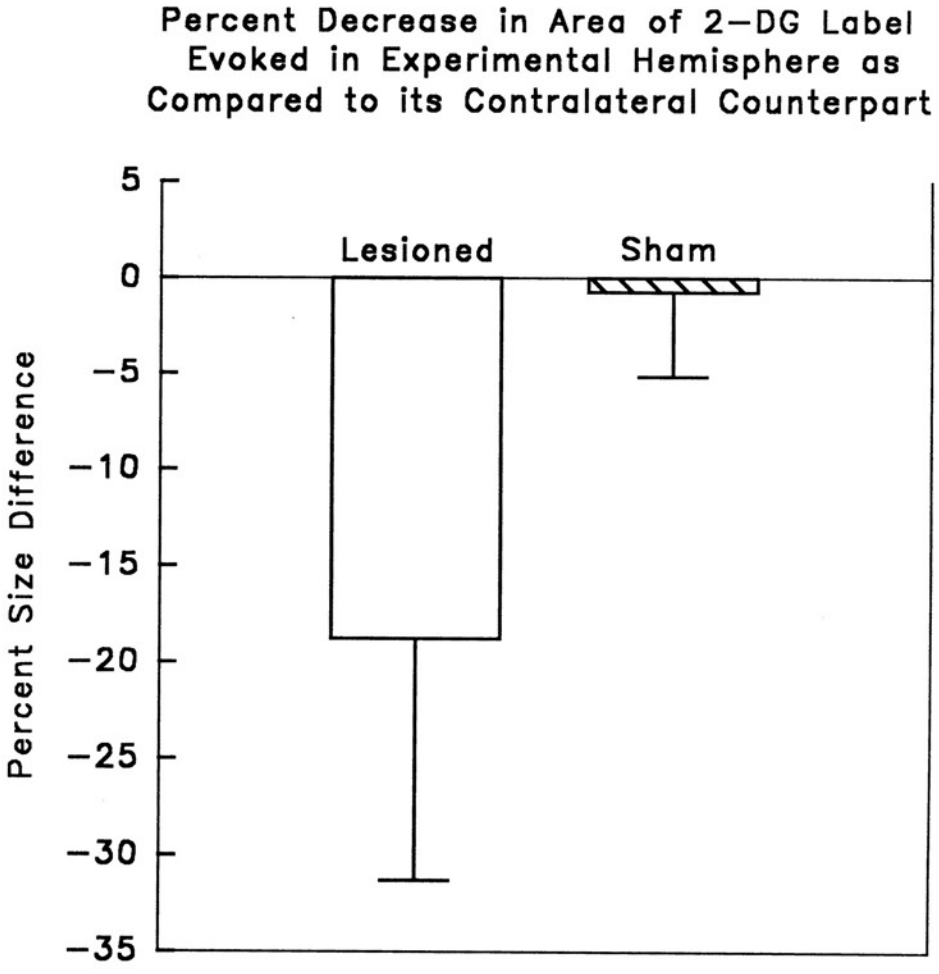
excitotoxic lesioned groups, it was not significant, which may be the result of the small *N*, as well as the amount of variability. (Sham $\overline{X}$ = -0.73, SEM = 4.42; excitotoxic $\overline{X}$ = -18.77, SEM = 12.55) (Fig. 27).

Discussion

Summary

The behavioral consequences of basal forebrain lesion-induced cortical ACh depletion were examined in this study. The performances of ACh-depleted rats on a previously learned task of processing sensory information with the whiskers were initially impaired, but after 2-8 weeks of post-lesion testing, returned to pre-lesion levels of ability. Sham lesioned rats experienced no such post-lesion disruption of task performance. The time it took an animal to return to pre-lesion levels of sensory discrimination was positively correlated with the extent of AChE depletion. The findings suggest that basal forebrain lesions, which disrupt cholinergic innervation to the somatosensory cortex, interfere with the animal's ability to process sensory information passively applied to the whiskers. The specific impairment was transient as the

Figure 27. The areal dimension of activated barrels in experimental hemispheres of 3 sham lesioned rats and 4 excitotoxic lesioned rats expressed as a percent of the corresponding matched barrels in the contralateral normal hemisphere. The percent size difference between the two groups, although not significant, is made evident by this graph.


Percent Decrease in Area of 2—DG Label
Evoked in Experimental Hemisphere as
Compared to its Contralateral Counterpart
Percent Size Difference
5
0
−5
−10
−15
−20
−25
−30
−35
Lesioned
Sham

ACh-depleted animals returned to pre-lesion performance levels.

Despite this behavioral recovery, the areal spread of 2-DG uptake in response to whisker stimulation in the ACh-depleted hemispheres was reduced compared to the activity evoked in the opposite unlesioned hemispheres. This observation is similar to findings in animals that received basal forebrain lesions and subsequent 2-DG experiments without participating in behavioral testing (Ma et al., 1989; also see Chapter 2). No differences in 2-DG uptake were observed between hemispheres in sham lesioned animals. Unlike the earlier findings reported in Chapter 2, the intensity of 2-DG uptake was not significantly different between hemispheres. I noted, however, in Chapter 3 that no significant differences were found in the optical density of activated barrels in ACh-depleted cortex with neocortical (control) grafts compared to matched barrels in the normal contralateral hemisphere. It is unclear why these discrepancies occur and whether they represent a functional change in barrel activity or some methodological\technical inconsistency.

Effect of Cholinergic Depletion on Other Sensory Modalities

Depletion of cortical ACh as a consequence of nbm lesions has been shown to result in significant impairment

of discrimination accuracy for other sensory modalities. Ridley et al. (1986) demonstrated a disruption of the ability to visually discriminate between previously-learned stimuli after bilateral basal forebrain lesions. In the same study, presentation of a new visual discrimination task during administration of a cholinergic agonist improved performance of lesioned animals which was later disrupted by administration of scopolamine, a cholinergic antagonist. These results suggest that visual discrimination performance was impaired as a result of the disruption of the cortical cholinergic input from the basal forebrain.

Everitt et al. (1987) assessed the effects of bilateral nbm lesions in rats pre-trained on a conditional visual discrimination task and found that sham lesioned rats reachieved pre-lesion performance requiring many fewer retraining sessions than the lesioned group. Additionally the greatest amount of ChAT depletion was associated with the greatest behavioral impairment.

In other studies relating the degree of cholinergic depletion (ChAT and/or AChE) to resulting behavioral deficits, there is consensus within the group of studies assessing performance of pre-trained animals on tasks of sensory information processing, that greater depletion results in the animals requiring a longer time to return to baseline levels of performance. Kesner et al., (1987) found a positive correlation between nbm lesion-induced cortical AChE depletion and extent of behavioral impairment on a

previously learned radial maze task of spatial information processing. This may imply that sensory processing is a graded phenomenon in terms of the amount of cholinergic depletion, consistent with the contribution ACh makes in improving the 'signal-to-noise' ratio of neuronal activity (McCormick and Prince, 1986).

Does ACh Depletion Impair Tactile Discrimination?

It has been suggested that simple detection of tactile stimulation to the whiskers does not require barrel cortex, and is a task that can be performed at the level of the thalamus (Hutson and Masterton, 1986). A number of recent studies have shown that the barrel cortex is, in fact, required for the animal to process information with its whiskers (Simons et al., 1975). Guic-Robles et al. (1989) and Carvell and Simons (1990) demonstrated that rats can learn a complex roughness discrimination task using only their whiskers. Following bilateral ablations of the cortical barrel field, the rats demonstrated lack of task retention and failure to return to pre-lesion performance levels. When they were then permitted to use their forepaws to explore the stimuli, rats rapidly reachieved pre-lesion criteria (Guic-Robles et al., 1992). In another experiment, Hurwitz et al. (1990) pretrained rats on the same behavioral task of passive whisker deflection used for the study

reported here. They then photochemically induced a unilateral thrombotic infarction of the barrel field. Sham lesioned rats performed at pre-infarction levels within a week post-surgery. While infarcted rats demonstrated improved performance over time, they never fully achieved pre-infarct levels of ability. These findings suggest that performance on an identical previously-learned tactile discrimination task requires an intact somatosensory barrel cortex.

In this study, excitotoxic lesioned animals were able to eventually perform the sensory discrimination task as well as sham lesioned rats, however the 2-DG pattern continued to demonstrate diminished stimulus-evoked cortical processing. It is possible that the lack of ACh interfered with the ability of the animal to process the relevant stimulus effectively. In terms of the behavioral improvement, since the task was relatively simple, the animal may have been able to compensate with other sensory cues. Efforts to control for some of the factors that often confound the data (e.g., relying on cues from other senses, using related abilities) included the use of sham lesioned controls, within group pre-and post-surgery performance comparisons, and use of a darkened room during the testing sessions. It is possible that a more complex task, involving active discrimination with whiskers, would lead to more enduring behavioral deficits. Since ACh has been shown to enhance cortical neural activity when combined with an

appropriate stimulus, perhaps performance in a more demanding task would require the continued enhancement of neural activity that ACh provides.

While a number of studies suggest that nbm lesion-induced deficits are a consequence of impaired attention or arousal (for review see Richardson and DeLong, 1988), the literature suggests that cholinergic projections from the reticular activating system are responsible for arousal (for review see Steriade et al., 1990; Woolf, 1991), whereas the nbm provides ACh innervation to sensory cortex, which impacts the neuronal activity to incoming stimuli. In the following chapter I discuss attention and arousal further.

Research has consistently demonstrated that ACh is an important component in cortical sensory processing. While memory-related interpretations are frequently applied to explain nbm lesion results, the findings from this study support and expand the view that ACh is an important component in executing a learned response to a sensory discrimination task.

Chapter 5: Discussion

Introduction

Considerable research implicates the basal forebrain cholinergic system in processes of learning and memory, as well as the neuropathology of Alzheimer's disease. Through the series of experiments that I have presented, I suggest a role for acetylcholine in cortical sensory processing and tactile discrimination. In this section I discuss mechanisms of global regulation of cortical ACh and speculate on how this might have influenced the results. I also propose an alternative to the possibility that ACh was responsible for improved functional activity demonstrated in the animals with basal forebrain transplants.

Appetitive Rewards and Cortical ACh Release

Electrophysiological studies indicate that the discharge rates of nbm neurons increase transiently during the acquisition of learned behaviors that are closely tied to the expectation of a food reward (Richardson and DeLong, 1990). This neuronal responsiveness to an appetitive stimulus was one of the earliest reported findings with nbm

cells and has been confirmed in subsequent studies (for review see Richardson and DeLong, 1990). Not only do nbm neurons respond to the reward delivery, but they also are activated by stimuli that consistently precede the reward delivery (Richardson and DeLong, 1986). The associated changes in firing pattern appear more related to the ability of the stimulus to predict the delivery of a significant reward than to specific sensory stimulation (McCormick, 1990).

The task I used with the nbm-lesioned and behaviorally-trained rats coupled whisker stimulation with a food reward. It is therefore important to consider how this coupling might have affected the performance of the ACh-depleted animals. Stimulation of the basal forebrain has been shown to result in an increase in cortical ACh release (Rasmusson and Dykes, 1988). Presenting an appropriate sensory stimulus in the presence of ACh has been shown to increase the effectiveness of the neuronal response to the sensory input (e.g., Donoghue and Carroll, 1987; Metherate et al., 1987). Since association of a stimulus with a food reward activates the basal forebrain, under normal conditions this would optimize the cortical response to a sensory stimulus. This appetitive association works to the animal's advantage as the stimulus "primes" the nbm cells, which results in cortical ACh release and enhanced cortical processing of the sensory stimulation by the ACh-mediated changes detailed in Chapter 1.

The excitotoxic lesioned rats in the behavioral study (see Chapter 4) exhibited different amounts of cortical ACh depletion, reflecting the varying extent of cell damage in the basal forebrain. The remaining cells may release sufficient cortical ACh to increase the signal-to-noise ratio or allow a sensory cortical neuron to reach threshold when presented with an appropriate stimulus. The fact that the less depleted rats returned to criteria faster than those with more extensive depletion may be due to more surviving nbm neurons releasing ACh, allowing more efficient cortical sensory processing during behavioral testing. During the 2-DG experiment where the type and manner of whisker stimulus were not closely tied to a food reward, however, it is possible that nbm neurons were not activated as readily. For this reason, the smaller cortical area activated during the 2-DG experiment in the ACh-depleted hemispheres may reflect sensory cortical processing without the normal complement of basal forebrain cholinergic neurons. What may be a more relevant relationship between the 2-DG and behavioral data is the fact that, within the central region of the activated barrel, the density of 2-DG uptake was comparable between the depleted and normal hemispheres. That this central region of the barrel appears able to function within a normal range of response (as measured by 2-DG) may be the reason why the animal was able to improve behaviorally, even though the shrunken pattern of

metabolic uptake in the depleted hemisphere reflects long-term deficits in cortical functioning.

Arousal and the Basal Forebrain

The role of attention on behavioral performance has been addressed by several studies. Microelectrode recordings and positron emission tomography have further demonstrated that cortical functioning is enhanced when attention is focused on the task, and impaired with distraction (Robbins et al., 1989; Meyer et al., 1991; Sweeney et al., 1992). Some of the findings associated with attentive behavior include improved localization of briefly presented target stimuli, increased neuronal firing rate, and a focal increase in cerebral blood flow in the activated cortical region.

Until recently the brainstem cholinergic system has been viewed as the mediator of brain mechanisms of attention and arousal with stimulation of the midbrain reticular formation resulting in release of ACh and electroencephalogram (EEG) desynchronization, which is associated with arousal (Moruzzi and Magoun, 1949; Kanai and Szerb, 1965). While the ascending reticular activating system plays a role in modulating cortical activation as well as its behavioral correlates of attention and arousal, few direct cholinergic projections have been found from the

brainstem to the cortex (for review see Wainer and Mesulam, 1990). A direct projection from the pontomesencephalic tegmentum does, however, terminate in the nbm (Wainer and Mesulam, 1990). Studies in which the nbm was either stimulated or damaged by excitotoxic lesions show a corresponding increase or decrease in cortical ACh release and EEG activation patterns associated with arousal (Stewart et al., 1984). These findings have led to the current consideration of the cholinergic nbm-neocortical pathway as a major site of cortical activation (Détári and Vanderwolf, 1987; Steriade and Buzsáki, 1990; Metherate et al., 1992).

If the nbm does mediate the state of cortical activation, as reflected in the EEG, then damage to these cells would likely impair ability to attend during sensory stimulation. It is possible that the disruption I noted in tactile discrimination performance of the nbm lesioned rats was the result of impaired attentional processes. The fact that the animals were pre-trained on the task may have served as a basis for the improvement in performance following a transient impairment. That is, the responsiveness of specific cells could have been "conditioned" during the training period, and, since this was not a task of new learning, this conditioning may have allowed the animal to ultimately perform the task regardless of the reduced cortical activity reflected in the 2-DG data. Another interpretation, one that also considers the 2-DG profiles of the ACh-depleted animals, is that the loss of

nbm neurons and the reduction in arousal, reflected by a relatively more synchronized EEG indicative of a low level of cortical activation, may have rendered a previously adequate sensory stimulus no longer sufficient for the cortical neuron to reach threshold, and lowered the ability of a cell to maintain neuronal responses, thereby impairing activation of adjacent neurons. The animals may have compensated behaviorally using other intact systems, but functional cortical activity, as assessed by the 2-DG technique, continued to demonstrate reduced responsiveness.

It is also important to consider the role of the thalamus as a mediator of cortical processing. In many respects, responses in the neocortex depend on state-related changes in the thalamus (for review see Steriade and Llinás, 1988). There is a direct projection from the cholinergic cells of the midbrain reticular formation to the thalamic nuclei (for review see Steriade and Buzsáki, 1990). During sleep, at which time these cells demonstrate a relative lack of activity, thalamic relay neurons are under the influence of local GABAergic interneurons and the ability of information to be transferred to the cortex is diminished. In this way, the thalamus blocks the transfer of information (McCormick, 1990). During arousal, which is associated with increased activity in brainstem cholinergic neurons, thalamic relay neurons become depolarized and GABAergic inhibition is moderated via an increase in K^+ conductance.

In general, both of these alterations in cell activity result in an enhanced flow of information through the thalamus (for review see Steriade and Llinás, 1988). Consequently, increases in arousal are thought to be associated with a greatly increased ability of thalamocortical systems to receive and process information (McCormick, 1990).

Non-cholinergic brainstem nuclei that project diffusely to neocortex have also been implicated in the forebrain processing state, as revealed by the EEG (Vanderwolf, 1988). Ascending noradrenergic fibers from the locus coeruleus (LC) send direct projections to the thalamus as well as to neocortex (Foote and Morrison, 1987). In a manner similar to cholinergic neurons, LC noradrenergic neurons show a slow, steady rate of discharge activity during sleep which becomes faster as the animal becomes more alert. Iontophoresis of norepinephrine on thalamic and neocortical neurons generates responses similar to that of ACh, including slow depolarization due to a decrease in K^+ conductance (McCormick and Prince, 1988; McCormick and Williamson, 1989). Norepinephrine has been proposed to exert neuromodulatory effects similar to those of ACh, including an increase in signal-to-noise ratio of neurons and increased neuronal response to stimulation (McCormick, 1989; McCormick and Williamson, 1989). Unlike ACh, application of NE alters spontaneous activity in cortical

neurons; the specific change is a decreased rate of firing (McCormick, 1989).

The transition from sleep to arousal is also marked by an alteration in slow-wave, synchronized rhythmic thalamocortical activity to high-frequency desynchronized activity in serotonergic neurons (for review see Foote and Morrison, 1987). The existence of a serotonergic (5-HT) projection to cortex and thalamus has been demonstrated (Moore et al., 1978), but the impact of serotonin on cortical neuronal activity has been less well studied.

It is evident that changes in the animal's state of alertness, reflected by the EEG, is related to increased firing in cholinergic, noradrenergic, and serotonergic neurons (Foote and Morrison, 1987). In addition to inputs to the thalamus from these above-mentioned modulatory neurotransmitters from the brainstem and basal forebrain, the cholinergic basal forebrain cells appear to receive an afferent supply from dopaminergic, serotonergic, and noradrenergic neurons, as well as from the cholinergic neurons of the brainstem tegmentum (Jones and Cuello, 1989). This suggests that final control over cortical excitability may depend on the interplay of several neural systems, of which nbm neurons form the primary source of direct ACh-mediated cortical actions (Metherate et al., 1992).

Nerve Growth Factor

Within the past decade a number of studies have demonstrated that NGF acts as a neurotrophic factor for central cholinergic neurons and plays a role in the function of these neurons (for review see Hefti et al., 1990). Briefly, the evidence in humans, monkeys, and rats includes (a) identification of NGF and messenger RNA for NGF (NGF mRNA) in the target structures of the basal forebrain neurons (neocortex and hippocampus) (b) NGF receptor immunocytochemical labeling of basal forebrain neurons co-localized with either AChE or ChAT, (c) increased ChAT activity with intraventricular injections of NGF, and, in rats, (d) rescue of cholinergic neurons from lesion-induced degeneration by NGF administration, as well as improved performance on memory-based tasks (for review see Hefti et al., 1990).

The relationship between NGF and the basal forebrain cholinergic system is of particular relevance to the transplantation study reported in Chapter 3. Perhaps the presence of this factor in the graft, and not that of ACh, was responsible for the restored somatosensory-evoked cortical activity. If this were the case, either of the two types of transplanted tissue, basal forebrain or neocortex, should have resulted in improved cortical processing. Clearly, this was not the case for the transplants of fetal neocortical transplants. This does not, however, diminish the importance of NGF in the transplantation paradigm.

Sofroniew et al. (1986) demonstrated that the atrophy of basal forebrain neurons, which typically occurs following excitotoxic lesion of the cerebral cortex, was prevented by transplantation of fetal neocortical tissue into the damaged cortex. Since target-derived trophic factors are important for the maintenance of the basal forebrain neurons (Hefti and Weiner, 1986; Williams et al., 1986; Kromer, 1987), the authors concluded that the trophic support necessary for ensuring the preservation of the cholinergic basal forebrain neurons was supplied by the transplanted cortical cells (Sofroniew et al., 1986). The fact that basal forebrain neurons benefitted from the presence of NGF in cortical transplants of neocortical tissue, but that such transplants were not able to alter the diminished 2-DG uptake discussed in Chapter 3, tends to rule out NGF as the critical component for functional 2-DG recovery.

There have been studies demonstrating that the site of the transplant is also important for optimizing functional improvements. It is possible that the graft survival and functional recovery I report required placement of the fetal basal forebrain cells within the cortex where growth factors are readily available. In one study, fetal grafts of basal forebrain placed into the previously-lesioned basal forebrain region of adult rats did not elicit improvements on a behavioral task of spatial memory; similar tissue placed in target sites (i.e., hippocampus and

neocortex) did achieve beneficial results (Hodges et al., 1990). NGF is synthesized in the cortex and hippocampus; NGF receptors, located on the terminals of ChAT-positive basal forebrain neurons, take up NGF for retrograde transport to the cell bodies (Schwab et al., 1979; Seiler and Schwab, 1984). It is possible that cholinergic cells placed directly into the target tissue may have thrived, while similar cells placed far from the necessary substance were unable to benefit. Transplants may act by providing local availability of the necessary substance; grafting appropriate cells some distance from the site where the substance is required may render the transplant ineffective in achieving a functional advantage. In the study by Hodges et al. (1990) reported above, even though the benefits were demonstrated in the basal forebrain, which is relatively distant from the cortical transplant, the basal forebrain neurons were intact and it is reasonable to presume that the NGF receptors present on the cholinergic terminals of these healthy neurons were apposed to the cortical graft where the NGF required for the maintenance of the cholinergic neurons was present.

None of the above evidence rules out the possibility that ACh was the key element required to restore stimulus-evoked metabolic activity in the transplantation study presented in Chapter 3. On the contrary, one could argue that the functional benefits observed were derived by making ACh locally available to the ACh-depleted cortex using

transplants of cholinergic-rich tissue. Use of embryonic neocortical tissue that, but for ACh, provided many similar substances including growth factors, was unable to improve or restore cortical function.

One final point that seems appropriate to make relates to possible treatment strategies for individuals with Alzheimer's disease, which is associated with a selective loss of cholinergic neurons of the basal forebrain (Bartus et al., 1982; Coyle et al., 1983), and also affects other neuronal systems (Hardy et al., 1985). For example, neuronal loss in the locus coeruleus has been noted with concomitant decreases in cortical levels of norepinephrine in Alzheimer's patients. There is no correlation, however, between this neurotransmitter loss and clinical dementia as with ACh (Perry et al., 1981). Numerous other neurotransmitters are implicated, but the changes are poorly documented and the extent of their contribution to the cognitive deficits are unknown (Hefti and Weiner, 1986).

The loss of cholinergic neurons and cortical cholinergic innervation is highly correlated with the memory loss that is characteristic of Alzheimer's disease (Perry et al., 1978; Hefti and Weiner, 1986). This correlation between the cholinergic deficit and Alzheimer's disease has been the impetus for clinical studies in which exogenous substances were substituted for the missing ACh (for reviews see Bartus et al., 1982; Coyle et al., 1983). Clinical experiments in which interventions have been targeted at

this single neurotransmitter have failed, however, to demonstrate significant improvements (Davis, 1985). Additionally, side effects associated with cholinergic medications have been a problem for a number of patients.

Because NGF prevents cholinergic neuronal degeneration in the adult brain, and because cholinergic degeneration may contribute to symptoms in patients with Alzheimer's disease, it has been speculated that NGF may be useful in the treatment of the disease (Hefti, 1983). Butcher and Woolf (1989) caution that such therapy might promote and exacerbate the neuropathological processes of neuritic plaques and tangles which form as a consequence of accumulations of cytoskeletal components in nerve endings and in the soma. Instead, they propose the administration of agents which block or interfere with the action of neurotrophic factors. Geddes and Cotman (1989) suggest that even if an excess of growth factors contributes to an aberrant sprouting response in Alzheimer's disease, it may still be detrimental to administer growth factor inhibitors since the global impact of such agents could adversely affect healthy neurons.

So far there is no direct evidence supporting a primary role for NGF in the pathogenesis of Alzheimer's disease (Goedert et al., 1986). A more complex picture has emerged suggesting that Alzheimer's disease is a multisystem disorder with the primary insult originating in the cerebral cortex (Mesulam, 1986; Price, 1986). The abundance of

evidence implicating cholinergic neurons in higher cognitive and memory processes (Collerton, 1986) justifies the assumption that the cholinergic deficit, even if only a secondary process and not the probable cause, is responsible for many of the clinical symptoms characteristic of Alzheimer's disease.

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