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THE ROLE OF AFFERENT ACTIVITY IN THE MAINTENANCE OF PRIMATE NEOCORTICAL FUNCTION

BY E. G. JONES

Department of Anatomy and Neurobiology, University of California at Irvine, Irvine, CA 92717, USA

Summary

The major neuronal populations of the primate cerebral cortex can be classified immunocytochemically according to their transmitters and in terms of the differential expression of certain other molecules such as neuropeptides, calcium-binding proteins and protein kinases. We have been able to chart the time course of developmental expression of these molecules and to show that gene expression for many of them is regulated in adult and infant animals by afferent activity entering the cortex.

In the visual cortex of adult monkeys, levels of immunocytochemically detectable gamma aminobutyric acid (GABA), of its synthesizing enzyme glutamic acid decarboxylase (GAD) and of the tachykinins are greatly reduced in deprived ocular dominance columns within 24 h of blocking impulse activity in the optic nerve by intraocular injection of tetrodotoxin (TTX). Conversely, levels of immunocytochemically detectable calcium-calmodulin-dependent protein kinase (CAM II kinase) are increased in deprived eye dominance columns. These effects are quickly reversible on restoration of binocular vision, and experiments involving *in situ* hybridization and S_1 nuclease protection assays show that the changes are associated with parallel changes in mRNA levels for preprotachykinin and CAM II kinase, but not for GAD, which appears to be regulated by post-transcriptional mechanisms. Experiments in the primate somatic sensory cortex suggest comparable activity-dependent effects on gene expression there also.

It is proposed that effects of this type underlie the establishment of cortical maps during development and their activity-dependent mutability in adulthood.

Introduction

Neural activity, i.e. the initiation and conduction of action potentials and the induction of membrane conductance and polarization changes in postsynaptic cells, plays an important role in the development and maturation of the nervous system, particularly during the phases of stabilization and maintenance of the synaptic connections upon which the normal function of the adult nervous system depends. Although the physiological phenomena that accompany activity-dependent aspects of neural maturation in the higher mammalian nervous system are coming to be understood, especially from work on the visual cortex, the

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mechanisms whereby these phenomena are induced are poorly understood. It is also widely believed that the adult nervous system shows relatively little capacity for activity-dependent plasticity beyond the critical developmental period. However, it has become clear in recent years that even the higher levels of the nervous system in adult mammals show a considerable amount of plasticity, which is dependent upon the nature of their afferent inputs.

Recent work in a variety of fields is beginning to show that neural activity can play an important role in the regulation of gene expression for many neuroactive molecules, including transmitters and those that influence structural proteins. In these observations there appear to be clues for understanding how the development of the nervous system is regulated and how similar regulatory phenomena may govern activity-dependent plasticity of the adult nervous system. In the following paragraphs, the primary focus will be on the cerebral cortex, particularly that of the primate, and an attempt will be made to draw together data from many areas that may help in the understanding of the activity-dependent plasticity that the adult cerebral cortex exhibits.

Afferent activity in the development of the nervous system

Neural activity exercises a powerful influence over the development of the nervous system. Even in the earliest stages of embryogenesis, transmembrane ionic currents and slow changes in membrane potentials may play a significant role in induction and neurulation, while later, with the onset of synaptogenesis and the appearance of action potentials and transmitter release, major effects may be exerted on differentiation, on the induction of pre- and postsynaptic membrane properties of transmitter phenotype, on elimination and stabilization and on histogenetic cell death (reviewed in Patterson, 1979; Harris, 1981). From the point of view of the present paper, which is primarily devoted to the role of neural activity in plasticity of the adult primate cerebral cortex, some of the most revealing studies are those that indicate the role of activity in the establishment of sensory maps and in the induction of neurotransmitter or neuropeptide expression during development. In these there are clues for the understanding of the role of afferent activity in regulating cortical function in the adult.

Among studies that indicate the role of afferent activity in the establishment of topographic maps of a peripheral surface are those on the regenerating retinotectal system of fish and amphibians. In the former, if the sodium channel blocker tetrodotoxin (TTX) is injected into the eyes after transection or crushing of the optic nerves, the regenerating fibers from the eyes reach the optic tectum, but their terminal ramifications fail to segregate as they would normally and the usual alternation of left and right eye dominance domains and a fine grain retinotopy fail to develop until TTX infusion is stopped (Meyer, 1982, 1983; Boss and Schmidt, 1984; Schmidt, 1985). This is probably associated with a failure of inappropriately widespread branches of the terminal arborizations of the axons to withdraw (Schmidt and Edwards, 1983; Kageyama and Meyer, 1988). In frogs, in which the

grafting of a third eye causes the terminations of the regenerated retinal ganglion cell axons of that eye to segregate in the tectum from those of the other two eyes, forming ocular dominance stripes, the blockade of action potentials in the three optic nerves by TTX prevents this segregation (Reh and Constantin-Paton, 1985). The blockade of *N*-methyl-D-aspartate (NMDA) receptors in the tectum by the selective antagonist, aminophosphonovaleric acid (APV) appears to exert a similar preventative effect on the formation of ocular dominance stripes (Cline *et al.* 1987) and the sharpening of retinotopic mapping (Schmidt, 1990). Moreover, the regenerating system shows a greater capacity for the induction of long-term potentiation (LTP) than the established system and the LTP depends upon activation of NMDA receptors (Schmidt, 1990).

As in the mammalian visual cortex (see below), it is probably the coincident activity of groups of neighboring retinal ganglion cells whose axons impinge on the same tectal cells and their induction of correlated postsynaptic effects in the tectal cells that provides for the stabilization of appropriate synapses. Asynchronous activity of retinal ganglion cells in relation to adjacent groups of ganglion cells is probably responsible for the sharpening of the retinotopic map. The NMDA receptor blockade is supportive of earlier theories regarding mechanisms of synaptic stabilization (e.g. Willshaw and von der Malsburg, 1976; Mastronade, 1989). The importance of asynchronous activity seems to be indicated by the failure of sharpening of segregation of the regenerated retinotectal axon terminations and of the retinotopic map when activity of all retinal ganglion cell inputs is synchronized, e.g. by stroboscopic illumination (Schmidt and Eisele, 1985; Cook and Rankin, 1986).

These studies suggest that the coordinated activity of afferent fibers and their capacity to exert postsynaptic effects upon the cells that receive them are major determinants of topographic map formation. The implications for the development of the mammalian cerebral cortex, in which topographic mapping is one of the hallmarks, are obvious. However, as we shall see, these results have implications for the maintenance of topographic maps in the adult cortex as well.

The other area in which fundamental studies in neural development have implications for the development and maintenance of neocortical organization and function is that of the induction of neurotransmitter function. This has been especially well studied in the autonomic nervous system (reviewed in Patterson, 1978; Black, 1978). Here, critical interactions with somatic mesoderm and adjacent tissues during neural crest migration can determine the transmitter phenotype of ganglion cells; sympathetic ganglion cells committed to catecholamine synthesis can be induced, in dissociated culture, to develop cholinergic traits by the presence of nonneuronal cells or conditioned media derived from the culture of nonneuronal cells. The importance of neural activity in the stabilization of the commitment to a particular transmitter phenotype is shown by the observation that preganglionic afferent activity and the activation of nicotinic cholinergic receptors on the cells is necessary for the normal development of adrenergic traits *in vivo*. Moreover, depolarization and the entry of calcium ions

will prevent conditioned-medium-induced cholinergic traits in neurons committed to a catecholamine-producing state (Walicke *et al.* 1977). Hence, patterns of afferent activity operating at critical periods of ganglion cell development may help to determine the commitment of the cells to the cholinergic or to the noradrenergic population.

Afferent activity in the maturation of the visual cortex

The development and maturation of neocortical function has come to be recognized as an activity-dependent process largely as the result of studies on the visual cortex of cats and monkeys. For some years the most overt manifestations of the dependence of cortical development upon afferent activity have been the well-known anatomical and physiological changes induced by monocular sensory deprivation occurring during a critical period of early postnatal development (reviewed in Hubel and Wiesel, 1970; Buisseret and Imbert, 1976; Hubel *et al.* 1977; Blakemore *et al.* 1978; Le Vay *et al.* 1980; Movshon and Van Sluyters, 1981; Sherman and Spear, 1982; Fregnac and Imbert, 1984; Mitchell and Timney, 1984). Under these conditions, alternating regions of visual cortex, that would normally have been innervated respectively by thalamocortical fibers bearing inputs from the left and right eyes, come to receive a preponderant innervation by fibers related to the eye with unperturbed visual experience. Cells that would normally have been binocularly influenced become dominated by inputs from the undeprived eye or exhibit alterations in receptive field structure indicative of a perturbed input from the compromised eye (see Hubel and Wiesel, 1965, 1977; Blakemore and Van Sluyters, 1975).

One of the principal and most overt of these changes, the shift in ocular dominance of cortical neurons in favor of the normal eye, has come to be seen as a manifestation of competition for synaptic space between left eye and right eye inputs. In the course of normal visual cortical development, the asynchronous arrival of activity from coordinated firing of groups of ganglion cells in the left and right eyes is held to be essential for setting up the conditions that determine the boundaries between ocular dominance domains in the visual cortex (Wilson *et al.* 1977). In cats and monkeys, rearing in the dark, or bilateral eyelid suture, conditions in which retinal ganglion cells are still active, results in a considerable degree of anatomical and physiological ocular dominance segregation (Wiesel and Hubel, 1974; Stryker and Harris, 1986). The spontaneous activity of the retinae *in utero* (Mastronade, 1989) is probably responsible for the beginning of segregation of geniculocortical afferents into ocular dominance columns which begins in the last few weeks of fetal life in monkeys (Rakic, 1976). The dependence upon the timing of influences from each eye is shown in experiments in which a surgically imposed strabismus in kittens, although leading to a loss of cortical responses to stimulation of one eye, leads to ocular dominance columns with unusually sharp borders (Shatz *et al.* 1977; Kalil, 1982). The importance of afferent activity is revealed by experiments showing that the anatomical and physiological plasticity

normally induced by monocular sensory deprivation in the critical period in kittens does not occur in the absence of impulse activity in the optic nerve (Stryker and Harris, 1986). Thus, bilateral blockade of retinal ganglion cell activity by tetrodotoxin injections in normal kittens results in a failure of geniculocortical fibers to segregate into ocular dominance columns and cells recorded in layer IV remain largely binocular. The importance of asynchrony of inputs in determining the plasticity of ocular dominance effects is revealed by experiments of Stryker and Strickland (1984) in which asynchronous electrical stimulation of the optic nerves during monocular deprivation of kittens in the critical period can offset the expected effects on ocular dominance in the visual cortex.

The studies quoted above show the general importance of afferent activity in regulating the maturation of visual cortical function. Other studies indicate that, in the course of normal development, the induction of changes in the postsynaptic cells by the afferent inputs are probably critical. Further, it is probably essential for afferent activity entering the cortex to be synchronized with changes in the postsynaptic cells. This will presumably lead to the stabilization of the affected synapses, with a corresponding induction of receptive-field specificity on the part of the cell. In this connection, it is important to note that, if diffuse, temporally modulated illumination of one eye, which does not excite cortical neurons, is imposed in the critical period of kittens, it does not result in an ocular dominance shift (Wilson *et al.* 1977; Singer *et al.* 1977). If visual cortical activity is disrupted by chronic infusion of the excitatory agent glutamate in monocularly deprived kittens during the critical period, ocular dominance shifts and changes in other physiological parameters are also prevented (Shaw and Cynader, 1984). In this case, the enhancement of cortical neuronal discharges by glutamate, being unaccompanied by a concomitant increase in coordinated geniculocortical afferent discharge, appears to be ineffectual in producing an ocular dominance shift. The involvement of postsynaptic cells in the phenomenon of visual cortical plasticity is shown by the observation that, after recovery from chronic intracortical infusion of the γ-aminobutyric acid (GABA) agonist muscimol during monocular deprivation in the critical period, cells in the visual cortex of kittens prefer the formerly deprived eye (Reiter and Stryker, 1988). In this case, less active inputs have been retained and more active inputs have failed to consolidate, presumably because conductance or polarization changes induced by afferent impulses are normally required.

The plasticity of the maturing visual cortex appears to result from the activity-dependent weakening and strengthening of synaptic inputs to cortical neurons. The failure of geniculocortical afferent fibers to segregate completely into ocular dominance domains and the concomitant changes in cellular responsivity in monocularly deprived animals are the most overt demonstrations of this. However, at the single-cell level, reduced inward currents occur when EPSPs are evoked in the cells of the monocularly deprived cortex (Mitzdorf and Singer, 1980), and in dark-reared animals there is a reduction in cyclic-AMP-dependent phosphorylation of a structural protein, MAP2 (Aoki and Siekevitz, 1985). These findings suggest that deprived synapses have become less effective and that

structural modifications have been induced in the synapses and possibly in other membrane or cellular components. The only evidence, however, is that the terminals of deprived geniculocortical terminals in cats are smaller than normal and end on dendritic spines of smaller than usual size (Tieman, 1988).

It is likely that activity-dependent modulations of transmitter function will also be operational during the critical phases of cortical development. In the visual cortex of neonatal kittens, GABA-mediated inhibition is present early (Tsumoto and Sata, 1985; Wolf *et al.* 1986; Albus and Wolf, 1984). A certain percentage of the visual cortical cells that become driven only by the nondeprived eye after monocular deprivation in the critical period for experience-dependent plasticity can be shown to retain a suppressed, deprived-eye input when subjected to ionophoretic application of the GABA antagonist bicuculline later in life (Sillito *et al.* 1981). Chronic infusion or ionophoretic application of bicuculline to the kitten visual cortex during the critical period leads to reductions in orientation selectivity, unusually large receptive fields, a loss of directional selectivity and to certain other changes in the receptive fields of the visual cortex neurons (Ramoa *et al.* 1988; Wolf *et al.* 1986). Hence, GABA-mediated inhibition may play an important role in the setting up of normal patterns of visual cortex maturation and in the physiological plasticity that follows monocular deprivation in the critical period. Gene transcription for glutamic acid decarboxylase (GAD) is increased in kittens during the critical period (Neve and Bear, 1989), but monocular deprivation in this period is unaccompanied by significant changes in the number of cells immunoreactive for GAD (Bear *et al.* 1985). Hence, the changes in receptive field structure revealed by the bicuculline studies may imply that a rearrangement of GABA synapses has occurred. In rats, chronic whisker trimming from birth leads to enhanced responsiveness of somatosensory cortical neurons, accompanied by reductions in their selectivity for direction of movement or angular displacement (Simons and Land, 1987). These experiments also seem to indicate a rearrangement or reduced efficacy of inhibitory synapses in the maturing cortex under the influence of altered afferent activity.

Excitatory transmitter systems may also be involved in the phase of experience-dependent synaptic stabilization in cortical maturation. Infusion of the NMDA-receptor antagonist APV into the visual cortex of kittens in the critical period has been shown to prevent the shift in ocular dominance that can normally be induced by monocular deprivation in that period (Kleinschmidt *et al.* 1987), but also tends to affect the development of normal orientation selectivity (Bear *et al.* 1990). APV infusion in kittens also partially prevents the reversal of the ocular dominance shift that can be induced in the critical period by opening a deprived eye and closing the formerly nondeprived eye (Gu *et al.* 1989). Because the number of APV-sensitive glutamate binding sites increases in the kitten visual cortex during the critical period and falls as it ends (Bode-Greuel and Singer, 1989), it would appear that activity-dependent effects normally involve an excitatory amino acid transmitter. These observations, which seem to have parallels in the NMDA-dependent effects on the development of topography in the regenerating retinotectal system of

certain other vertebrates, suggest that many different neurotransmitter systems in the maturing cortex may be in operation during the period of synaptic stabilization in the critical period. Since afferent activity regulates a number of neurotransmitter and neuropeptide systems in the adult cortex (see below), it is likely that neurotransmitter and receptor modulations may also effect synaptic plasticity in the adult cortex as well.

The data reviewed in this section appear to indicate that the activity-dependent mutability of cortical maps in adults may have its basis in thalamocortical anatomy and in activity-dependent neurotransmitter plasticity. They also imply that similar effects are likely to play a major role in the maturation of the cortex. It may be conjectured that comparable mechanisms will be in operation during the early development of the cortex and that, as neuronal function in the cortex is brought under the influence of peripheral inputs, activity-dependent effects will be major determinants of connectional stabilization and the development of the normal cortical representational maps.

Afferent activity in the maintenance of cortical topographic representation

The maintenance of cortical topographic organization throughout life appears to depend on the balance of sensory activity projected to the cortex. Multi-unit mapping of the somatic sensory and auditory areas and mapping by microstimulation of the motor area in a number of species, particularly in primates, have revealed that, even in adult animals, the maps of the periphery are mutable under activity-dependent conditions (Rasmusson, 1982; Kaas *et al.* 1983; Merzenich *et al.* 1983*a*,*b*, 1984, 1988; Kelahan and Doetsch, 1984; McKinley and Kruger, 1988; Calford and Tweedle, 1988; Sanes *et al.* 1988; Robertson and Irvine, 1989). After removal of a portion of the periphery, for example a finger or part of the cochlea, or after cutting a peripheral nerve, previously silent representations of parts of adjacent fingers or other best frequencies are revealed within the part of the map that would have been expected to be silenced. These effects occur so rapidly, often in a matter of hours, that it is unlikely that they depend upon axon sprouting and the formation of new synapses. Instead, they seem to imply that synaptic connections, previously suppressed under the conditions of the experiments, have been uncovered or strengthened.

Even in the absence of peripheral sensory perturbations, mutability of somatic sensory and other cortical maps may be a normal feature of cortical organization. Mapping a particular part of the peripheral representation in the somatic sensory cortex in the same animal over time (Merzenich *et al.* 1988) has shown that the representation of this part can change dramatically in both size and position. These plastic changes appear to depend upon the patterns of neural activity emanating from the periphery, as revealed in experiments of Clark *et al.* (1988), in which correlated activity of sensory receptors in the skin of two adjacent fingers was obtained by a surgically imposed syndactyly. Under these circumstances, the map of the somatic sensory area showed a reorganization such that the normal

dysjunctive mapping of receptive fields across the two fingers became continuous. One may imagine that, as a young primate commences using its fingers for sensory exploration in a coordinated and goal-directed manner, activity-dependent influences could play a powerful role in shaping cortical maps of both the sensory and the motor periphery. Similar influences in adult animals could ensure that these maps remain mutable and adaptive to new sensory experiences.

The rapidity with which these activity-dependent changes in cortical maps occur, even in adult primates, makes it unlikely that they depend upon axon sprouting and the making and breaking of connections. Instead, one would imagine that existing connections, which were previously suppressed, at least under the experimental conditions, are revealed depending upon the balance of afferent activity entering the cortex. Two possible bases for this would seem to lie in the presence of widely distributed afferent connections whose far-flung nature is normally unrevealed by conventional extracellular mapping (Snow *et al.* 1988) and in the sensitive, activity-dependent regulation of levels of the inhibitory transmitter GABA in cortical neurons (Hendry and Jones, 1986, 1988).

The presence of widely dispersed afferent fibers, whose extent seems out of context with the fine-grain topography normally revealed in conventional single-unit mapping studies, has recently been revealed in adult monkeys. Single thalamocortical axons entering area 3b of the somatic sensory cortex have been shown to terminate in three or more focal zones, each approximately 500 μm in extent but widely separated from one another by terminal free zones and collectively spreading over a cortical territory of 5–10 mm (E. G. Jones, in preparation; Rausell and Jones, 1990). Indirect evidence, from retrograde labeling studies, indicates that a 500 μm focal zone in area 3b can receive axon terminations arising from several score thalamic neurons with the same place and modality properties (Jones and Friedman, 1982; Jones *et al.* 1982). Thus, any cortical focus receives inputs from multiple thalamic axons and, because the axon of a single thalamic cell can have such far-flung focal terminations, the opportunities for multiple, duplicated and overlapping representations of the same small body part are considerable. Suppression or enhancement of certain axons as a result of reduced or heightened stimulation of that body part could, therefore, determine what is revealed by a microelectrode introduced into a cortical focus.

Snow *et al.* (1988) showed that certain terminal bundles of the thalamocortical axons may not reveal themselves. In mapping studies on the somatic sensory thalamocortical system of the cat, thalamic neurons whose receptive fields had first been identified were shown (by antidromic activation from the previously mapped cortex) to project to a wide area of cortex. This was greater than the area containing neurons having receptive fields on the same part of the body as the identified thalamic neurons. Outside the representation of that body part, as mapped at the time of the experiment, certain thalamic inputs are unrevealed and, therefore, presumably suppressed.

The powerful influence of GABA on cortical neurons of adult animals has been demonstrated in both the somatic sensory and visual areas (Sillito, 1975, 1979;

Duffy *et al.* 1976; Tsumoto *et al.* 1979; Sillito *et al.* 1980; Dykes *et al.* 1984; Alloway and Burton, 1986). When GABA receptors in these areas are blocked by bicuculline, elements of receptive field structure indicative of inputs from other, normally suppressed sources, including inputs from other regions of the receptive periphery, are revealed. Activity emanating from the periphery clearly regulates the production of the enzyme involved in GABA synthesis, glutamic acid decarboxylase (GAD), in the cerebral cortex of normal adult monkeys (Hendry and Jones, 1988) and in the barrel cortex of rodents (Welker *et al.* 1989). In monkeys, when action potentials in the optic nerve are blocked by injection of tetrodotoxin into an eye, there is a 50% reduction in the number of neurons immunoreactive for GAD and GABA in the deprived eye dominance columns of the visual cortex within 2–4 days. This effect does not involve cell death and is reversible upon restoration of binocular vision (Hendry and Jones, 1986, 1988). The comparable effect of whisker removal in rodents implies that similar activity-dependent regulation of GAD, and thus of GABA, will occur in other areas of the primate cortex and that this should have a profound influence upon the receptive fields of single neurons and upon sensory and motor maps recorded over time and under conditions in which sensory and motor performance is modified.

Afferent activity as a regulator of gene expression in the nervous system

In the foregoing paragraphs, the role of afferent activity in regulating the anatomical and physiological maturation of the cerebral cortex and its apparent role in the physiological plasticity exhibited by the mature cortex have been reviewed. The task remains to attempt an explanation of the mechanisms whereby activity can affect transmitter and receptor function and possibly even the synaptic morphology of the cerebral cortex.

It is an established fact that neural activity regulates gene expression for a number of neuroactive molecules in the peripheral nervous system. Initially, it was demonstrated that chronic depolarization of cells in the superior sympathetic ganglion by electrical stimulation of preganglionic fibers or treatment of the explanted ganglion with drugs that enhanced synaptic transmission led to marked increases in tyrosine hydroxylase activity and immunoreactivity, i.e. in a major enzyme involved in the synthesis of catecholamine neurotransmitters (Otten and Thoenen, 1976; Zigmond and Ben-Ari, 1977; Zigmond and Chalazonitis, 1979; Zigmond *et al.* 1980). The blockade of nicotinic cholinergic receptors prevents this activity-dependent induction. In studies carried out on explanted autonomic ganglia and on the adrenal medulla, primarily by Black and his associates, depolarization-dependent events have been shown to regulate levels of mRNAs for enzymes involved in catecholamine synthesis and for the precursors of certain neuropeptides. Thus, reserpine-induced increases in tyrosine hydroxylase (TH) activity are preceded by increases in TH mRNA levels, an effect that is prevented by section of preganglionic fibers (Black *et al.* 1985). By contrast, section of the preganglionic nerves and growth of the ganglion in explant culture leads to marked

increases in mRNA levels for preprotachykinin and in tachykinin immunoreactivity in the ganglion cells. The opposite effects seem to occur on TH mRNA levels and on TH immunoreactivity. Blockade of nicotinic acetylcholine receptors also increases tachykinin immunoreactivity and mRNA levels for preprotachykinin, while reflex enhancement of sympathetic activity by drug action causes a drop in immunoreactive tachykinin, and depolarization of the ganglion cells by veratridine blocks the increase in TH mRNA levels. The veratridine effect can itself be blocked by TTX, which prevents action potential discharge and depolarization of the cells (Kessler and Black, 1982; Black *et al.* 1984; Roach *et al.* 1987). These results speak strongly in favor of a differential regulation of TH and tachykinin gene expression by preganglionic activity-induced depolarization of the ganglion cells.

In the adrenal medulla of rats, denervation, nicotinic receptor block in the intact animal or explantation of the ganglion are reported to lead to increases in enkephalin gene transcription, manifested by increases in preproenkephalin mRNA, proenkephalin A and leu-enkephalin, while leaving markers of catecholamine gene expression unchanged (La Gamma *et al.* 1984; La Gamma and Black, 1989). These effects are blocked by depolarizing conditions in the explanted medulla, and the depolarization-induced block is itself antagonized by TTX. Hence, it can be considered that depolarization of the medullary cells induced by afferent activity will normally tend to reduce gene expression for enkephalin. By contrast, it is reported that reflex splanchnic nerve stimulation resulting from 2 h of insulin-induced hypoglycemia in rats actually increases adrenal medullary levels of preproenkephalin mRNA as well as of immunoreactive metenkephalin and metenkephalin fragments cleaved from proenkephalin (Kanamatsu *et al.* 1986*b*). This effect is blocked by administration of ganglionic and muscarinic blocking agents. These results would suggest that the effect of afferent activity on the adrenal medullary cells is to increase enkephalin gene transcription. Whatever the resolution of this discrepancy, it seems obvious that activity-dependent events in the autonomic nervous system are accompanied by changes in gene expression for transmitter-related enzymes and neuropeptides.

At higher levels of the central nervous system, two types of studies reveal activity-dependent effects upon gene expression in neurons. Recurrent epileptiform activity, induced by a number of experimental methods, has been seen to affect the abundance of mRNAs for certain neuropeptides and of mRNAs that encode for known, or putative, transcription factors. In the hippocampal formation of the rat, for example, large selective changes in gene expression can be detected when recurrent epileptiform activity has been induced. Thus, a single injection of kainic acid into the striatum leads to motor seizures recurring over 3–6 h. During this time, levels of immunoreactive metenkephalin and dynorphin fall in the hippocampus, but they return to control levels in 24 h and by 48 h have greatly increased. Mossy fibers of the dentate gyrus granule cells show a similar reduction followed by increased immunoreactive staining for metenkephalin, leu-enkephalin and dynorphin. Preproenkephalin mRNA levels show a 400%

increase in the hippocampal formation and entorhinal cortex after 6 h, and indications of metenkephalin synthesis show parallel increases by 24 h (Kanamatsu *et al.* 1986*a*).

In studies in which recurrent seizures have been induced in rats by unilateral lesions at the hilus of the dentate gyrus, differential regulatory effects have been described (White and Gall, 1987; White *et al.* 1987). Under these conditions, the hippocampal mossy fiber system shows bilateral increases in immunoreactive enkephalin but reduced or no changes in immunoreactive cholecystokinin or neuropeptide Y. These changes are accompanied by increases in preproenkephalin mRNA, slight increases in preproneuropeptide Y mRNA and no changes in preprocholecystokinin mRNA. Furthermore, a large and rapid rise in mRNA levels for the proto-oncogene protein c-*fos* precedes the increase in preproenkephalin mRNA, while mRNAs for the other proto-oncogene proteins, c-*Hras* and c-*myc*, are unchanged.

These results indicate not only that neural activity may differentially regulate neuropeptide gene expression in the central nervous system but also that the selective induction of immediate genes early may be one of the intermediate steps in this induction. Other examples of convulsant-induced or stimulation-induced production of proto-oncogene transcription factor mRNAs have now been reported in the rat central nervous system (Morgan *et al.* 1987; Saffen *et al.* 1988; Hunt and Goedert, 1988).

Afferent activity and gene expression in the monkey visual cortex

A very dramatic effect of afferent activity upon gene expression is exhibited in the visual cortex of adult monkeys subjected to deprivation of vision in one eye. Even in animals more than 20 years old, removal of an eye or blockade of action potentials in the optic nerve by intraocular injections of TTX leads, within 2 days, to large reductions in immunocytochemically detectable levels of GABA, GAD and the tachykinin neuropeptides in GABAergic neurons of the deprived ocular dominance columns in the primary visual cortex. Visual deprivation by monocular eyelid suture produced similar changes but required a longer period: 4–6 weeks (Hendry and Jones, 1986, 1988; Hendry *et al.* 1988; S. H. C. Hendry, E. G. Jones and N. Burstein, unpublished observations). The effect is most readily seen on neurons that lie in the layers that are the main recipients of thalamic afferents. They include neurons that express GAD (and therefore GABA) alone and those that express both GAD and the tachykinins. Neurons most affected appear to be those receiving larger numbers of thalamic axon terminals on or close to their somata than others (Hendry and Jones, 1988). However, the reduction in GABA, GAD and peptide levels can also be shown to extend into other layers, apparently following the lines of connections that pass vertically out of the layers of major thalamic terminations (Hendry and Jones, 1986; Hendry *et al.* 1988).

This rapidly occurring reduction in transmitter protein and peptide levels affects

at least 50 % of the GABA cells in the deprived ocular dominance columns of layer IVC but is not dependent on the death of cells (Hendry and Jones, 1988). All cells remain and can be shown to recover their normal immunocytochemically detectable levels of GABA, GAD and tachykinins within a few days of cessation of TTX injections or of reopening a previously lid-sutured eye (Hendry and Jones, 1988; S. H. C. Hendry and E. G. Jones, unpublished results). The recovery, particularly that related to the recovery of ganglion cell activity after exposure to TTX, seems to be a clear indication that the deprivation effect depends upon afferent activity in the optic nerve.

Accompanying the reduction in immunocytochemically detectable levels of GAD, GABA and tachykinins, there is a parallel reduction in $GABA_A$ receptor density in the deprived eye columns of layer IVC, as determined by immunocytochemistry and by [^{3}H]muscimol or [^{3}H]flunitrazepam binding (Hendry *et al.* 1990). This is a somewhat surprising result since an upregulation of the receptor might have been anticipated, similar to that which follows denervation in other systems and which leads to denervation supersensitivity (e.g. Creese *et al.* 1977; Biggio *et al.* 1981; Drew *et al.* 1987). It is not clear, however, if both the pre- and postsynaptic $GABA_A$ receptors are affected by monocular deprivation. Similarly, it is unclear whether the immunocytochemical and receptor binding studies imply an actual reduction in receptor numbers or a configurational change affecting both immunoreactivity and ligand binding. There will be postsynaptic receptors on other GABA neurons and on the pyramidal cells they innervate (Hendry *et al.* 1983). Presynaptic receptors will be on the axon terminals of the GABA cells. Overall, therefore, the apparent reduction in $GABA_A$ receptors could be largely due to a reduction in synthesis of the receptor by the GABA cells. Such possibilities need to be addressed in attempting to assess the functional implications of the activity-dependent effect.

The reduction in GABA, GAD, $GABA_A$ receptor and tachykinin levels in deprived ocular dominance columns of the visual cortex is accompanied by an *increase* in immunocytochemically detectable levels of a phosphoprotein, type II calcium-calmodulin-dependent protein kinase (CAM II kinase) (Hendry and Kennedy, 1986; S.H.C. Hendry and E.G. Jones, unpublished observations). The alpha-subunit of CAM II kinase is one of the most abundant proteins of the mammalian forebrain (Erondu and Kennedy, 1985) and is localized, among other sites, in association with the postsynaptic densities of forebrain synapses (Kennedy *et al.* 1983; Kelly *et al.* 1984; Miller and Kennedy, 1985). It can phosphorylate a number of substrates, including the enzymes tyrosine hydroxylase and tryptophane hydroxylase, the synaptic-vesicle-associated protein synapsin I and the microtubule-associated protein MAP2 (Yamauchi and Fujisawa, 1983; Bennett *et al.* 1983; Vulliet *et al.* 1984; Schulman, 1984). It also enhances synaptic vesicle mobility (McGuiness *et al.* 1989) and transmitter release from the squid giant synapse, probably by increasing vesicle movement in association with synapsin I (Llinás *et al.* 1985). One particular feature of this protein is its capacity to pass from a calcium-dependent to a calcium-independent state when certain amino

acids of the alpha- and beta-subunits are phosphorylated (Schworer *et al.* 1988; Thiel *et al.* 1988; Miller *et al.* 1988; Fong *et al.* 1989).

In the monkey visual cortex, CAM II kinase is mainly localized in the non-GABA pyramidal neurons (Hendry and Kennedy, 1986; S. H. C. Hendry and E. G. Jones, unpublished observations) and significant amounts of the immunocytochemically detectable protein are found in the dendrites as well as the somata. It is not clear whether the increase in CAM II kinase immunoreactivity represents an increase in protein levels in the pyramidal cells or the appearance of detectable levels in non-pyramidal cells that do not normally express the protein. In either case, the inference is that reduced afferent activity leads to an increase in gene expression for a second-messenger-related protein that can affect synaptic function and exert potentially longer-term changes on structural and other neuronal proteins in the visual cortex.

The introduction of the techniques of molecular biology to the study of activity-dependent effects on the adult primate visual cortex has enabled these effects to be explored at the level of direct gene expression. Species-specific cDNA clones have been generated and used to make antisense cRNA probes that can identify the mRNAs for GAD, preprotachykinin and CAM II kinase-alpha (Benson *et al.* 1989, 1990; D. L. Benson, P. J. Isackson, S. H. C. Hendry and E. G. Jones, unpublished observations). *In situ* hybridization using these ^{35}S-labeled probes in the normal monkey visual cortex revealed that the numbers and distributions of cells expressing the genes for these proteins are within the ranges established by counts in immunocytochemically stained material. The distribution of hybridized CAM II kinase-alpha cRNA, however, is not solely localized to cell somata; significant quantities are found in the large dendrites of the pyramidal cells. It is considered that this reflects the known presence of large numbers of free and attached ribosomes in the large apical and basal dendrites where they are concentrated at the necks of dendritic spines. The presence of significant quantities of CAM II kinase-alpha mRNA in the dendrites suggests that the message is being translated at the spine necks and protein is being synthesized at these sites for immediate insertion into the synaptic regions of the spine heads. A similar distribution of CAM II kinase-alpha mRNA in hippocampal pyramidal cells (D. L. Benson and E. G. Jones, unpublished observations), the association of CAM II kinase with the induction of long-term potentiation in the hippocampal formation (Malinow *et al.* 1989; Malenka *et al.* 1989), its association with the postsynaptic membrane and the evidence for alterations in spine neck polyribosomes accompanying dendritic spine plasticity during reinnervation of the rat hippocampus (Steward, 1983) all favor a mechanism of this type.

The effects of monocular visual deprivation are clearly revealed in preparations of visual cortex involving *in situ* hybridization of the CAM II kinase-alpha probes. There is enhanced hybridization in the deprived eye dominance columns of layer IV. This is readily determined by matching the autoradiographs to adjacent sections stained histochemically for cytochrome oxidase (CO) activity. In deprived eye columns, CO activity is greatly reduced. The enhancement of the hybridiz-

ation signal in the deprived eye columns has been measured at approximately 16 % above that in the normal eye columns (Benson *et al.* 1990). The autoradiographs also reveal that hybridization and, thus, mRNA levels for CAM II kinase-alpha are also increased in regions of layers II–III and V–VI lying immediately above and below the centers of the deprived eye dominance columns of layer IV. These results, therefore, indicate that not only are the effects of reduced afferent activity entering the deprived eye dominance columns of layer IV on CAM II kinase-alpha protein levels in that layer probably mediated by an activity-dependent regulation of gene transcription but also that these effects extend to other layers of the cortex along the established lines of vertical connectivity.

Preliminary results using *in situ* hybridization of complementary cDNA and cRNA probes for preprotachykinin mRNA suggest that the reduction in levels of immunoreactive tachykinin seen in deprived eye dominance columns depends upon an activity-dependent decrease in mRNA levels and thus, probably, a decrease in gene transcription. Paradoxically, however, when *in situ* hybridization of cRNA GAD probes is applied to monocularly deprived visual cortex, no reduction in GAD mRNA levels could be detected in the deprived ocular dominance columns (Benson *et al.* 1990). This suggests that the activity-dependent effect upon GAD protein, and thus upon GABA transmitter levels, is mediated at a post-transcriptional and possibly post-translational level. The cRNA GAD probes used in these studies are known to recognize the transcript of the GAD gene that codes for M_r 65×10^3 GAD (Kaufman *et al.* 1989; D. L. Benson, P. J. Isackson and E. G. Jones, unpublished observations) and this protein is recognized by at least one of the antisera used to detect the effect on GAD protein levels (Hendry and Jones, 1986, 1988). Therefore, it is unlikely that the lack of activity-dependent effects on GAD mRNA levels stems from a failure to detect the transcript of the other known GAD gene which codes for an M_r 68×10^3 protein (Kaufman *et al.* 1989; D. L. Benson, P. J. Isackson and E. G. Jones, unpublished observations) or the transcript of a third, hitherto unidentified, GAD gene.

Among the potential mechanisms that could cause GAD to fall to levels undetectable by immunocytochemistry in the face of maintained GAD gene transcription are those that would operate to affect the structure of GAD post-translationally, and those that would intervene between DNA transcription and mRNA translation or post-translational processing. In the former case, limited proteolysis, deamidation or methylation could not only render GAD enzymatically inactive and cause GABA levels to fall but also cause GAD to become undetectable by immunocytochemistry (see Benyon, 1980, for a review). In this case, we would expect that reduced afferent activity arriving at the GABA cells would lead to the induction of other enzymes that cause such changes.

In the second case, the most likely possibility is that translation of mature, functional GAD mRNA is prevented. There is evidence that levels of certain proteins in animal cells are regulated at this relatively late stage of gene expression (Klausner and Harford, 1989). In this case also, there would be the necessity for the activity-dependent induction of some agent that blocked mRNA translation.

The effects of the activity-dependent regulation of at least one transmitter-related enzyme, one major receptor type, one peptide (or peptides) and one second-messenger-related protein have not yet begun to be explored. There have been very few reports of the physiological effects of monocular visual deprivation on the adult monkey cortex (LeVay *et al.* 1980) and the received dogma is that this is unaccompanied by major changes in ocular dominance or in other receptive field parameters. However, no studies have been made bearing in mind the results described here. The observation that GABA receptor blockade by ionophoretically applied bicuculline can cause significant breakdown in the receptive field specificity of neurons in the adult cat visual cortex argues strongly that similar manifestations could be identified in the monocularly deprived monkey cortex in which GABA levels and $GABA_A$ receptors are down-regulated. Such changes, by comparison with the bicuculline-induced effects in cats, could include a loss of direction selectivity, orientation selectivity and, obviously, of binocularity (Sillito, 1975, 1979; Sillito *et al.* 1980, 1981). These are effects that await study in the monocularly deprived adult monkey.

The effects of a down-regulation of peptide expression can only be guessed at, for the role of the neuropeptides in neocortical function, especially that of the tachykinins, is virtually unknown. There is a similar lack of information about the range of functions of CAM II kinase in the nervous system. It seems to be involved in vesicle movement towards synaptic release sites and thus in the efficacy of synaptic transmission (Llinas *et al.* 1985). There is also growing evidence for its restriction to certain, transmitter-defined classes of cells (D. L. Benson, P. J. Isackson and E. G. Jones, in preparation), which could imply its association with particular types of synapse. Hence, the increased production of CAM II kinase under conditions of monocular deprivation may represent an attempt by cells to maintain particular synapses in the face of altered synaptic drive. However, CAM II kinase is capable of phosphorylating a range of substrates, including those associated with the structural proteins of the nerve cell, and its increased production may reflect the induction of actual structural changes in cortical neurons in response to perturbed activity. In these conjectures there are many possibilities for future experimental studies.

Conclusions

Neural activity appears to be a major determinant of the development and maturation of the cerebral cortex. It seems to express itself in a number of ways, some clearly evident, others conjectured from studies of the development of less complex nervous systems and lower levels of the mammalian nervous system. The stabilization of connections that determine ocular dominance and other selective aspects of the receptive field structure of visual cortical cells during early postnatal development are clearly evident as processes dependent upon the consolidation of synapses by coordinated afferent activity and postsynaptic responsivity. It seems likely, however, that the topographic maps of peripheral receptor sheets that form

in the cortex are also based upon activity-dependent mechanisms. The onset of afferent activity may also prove to play a role in the stabilization or selection of the transmitter phenotype of certain cortical cells, as seems to occur in the autonomic nervous system, although this has not been pursued in the review (see Huntley *et al.* 1988).

Studies of the activity-dependent plasticity of somatotopic maps in the somatic sensory cortex and the activity-dependent regulation of transmitter, receptor, neuropeptide and second-messenger-related protein expression in the visual cortex of adult primates indicate that the adult cortex is by no means immutable. The mechanisms behind these effects in the two sensory areas are likely to be similar. In further studies of these mechanisms the principles that determine the activity-dependent regulation of gene expression in both the developing and the adult cortex are likely to be revealed.

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