EXTRATHALAMIC MODULATION OF CORTICAL FUNCTION

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INTRODUCTION

THE DEMONSTRATION OF EXTRATHALAMIC CORTICAL AFFERENTS As recently as 25 years ago, it was commonly assumed that essentially all afferents to the neocortex arose from the thalamus. With the development of methods for visualizing noradrenergic, serotonergic, and dopaminergic neuronal processes, it gradually became clear that axons arising from subthalamic cell groups that contain these putative transmitters travel rostrally through the brainstem and monosynaptically innervate the neocortex. With the more recent demonstration that acetylcholinergic (ACh) neurons in the basal forebrain project monosynaptically into the neocortex, it is now clear that there are at least four substantial extrathalamic projections to the neocortex. Recently, these afferents have been demonstrated to be much more dense and more highly organized than was initially thought.

The existence and nature of these cortical afferents raises major questions about cortical organization and function. Thalamocortical and corticocortical systems appear to be organized to implement both serial and parallel processing of information through modules that are interconnected with precise topography, thus permitting functional segregation.
This well-known columnar, radial organization of the neocortex is apparently violated by the tangentially organized extrathalamic afferents in which single axons may innervate not only different columns within a functional region but different functional areas. What are the functions of these systems? What information do they convey that could not be conveyed as readily and perhaps more precisely by thalamic afferents? How do these afferents alter cortical processing so that the neocortex can better accomplish its functions?

The scope of this article Answers to these questions are not presently available, but recent anatomic and physiologic observations offer tantalizing clues about how they might be experimentally addressed. The purpose of this article is to briefly review some of these recent findings and discuss their implications for future research. The literature review concentrates on primate neocortex, since it is clear that certain aspects of neocortical organization, including the characteristics of its extrathalamic innervation, are qualitatively different in primates and rodents. We review the current knowledge about the four extrathalamic cortical afferent systems that have been well documented: (a) the noradrenergic afferents arising from the pontine nucleus locus coeruleus (LC); (b) the serotonergic afferents arising from the mesencephalic raphe nuclei; (c) the dopaminergic afferents arising from the substantia nigra–ventral tegmental area complex (SN/VTA); and (d) the ACh afferents arising primarily from the nucleus basalis of the substantia innominata. (For descriptions of a less extensively studied system, see Saper 1985 and Saper et al 1986.) After presenting the anatomy and physiology for each system, we briefly discuss the functional implications of these data. In the concluding section, we discuss the possible general impact of these systems on cortical function.

The most recent data available suggest the following principles of organization for extrathalamic afferents to neocortex:

1. Each extrathalamic afferent system exhibits pronounced regional and laminar specialization.
2. These systems differ from each other in terms of which major cortical subdivisions, cytoarchitectonic areas, and cortical laminae constitute their principal termination sites.
3. The extensive phylogenetic enlargement, elaboration, and specialization of primate neocortex is paralleled by analogous development of extrathalamic afferent systems.
4. Regional innervation patterns of an extrathalamic system in neocortex may be matched by its preferential innervation of functionally related thalamic and other subcortical structures.
These principles of organization raise fundamental questions about the processes underlying the ontogenetic development of the systems. We have recently reviewed the available data bearing on the development of extrathalamic systems (Foote & Morrison 1987) and do not therefore deal with these issues in the present article. Also, due to space constraints, we do not review the currently available data about receptor localization for extrathalamic systems in neocortex.

NORADRENERGIC INNERVATION OF NEOCORTEX

Anatomy: Source Cells and Termination Patterns of Neocortical Afferents

The noradrenergic innervation of neocortex arises solely from the nucleus locus coeruleus, which is located in the pontine brainstem (reviewed in Foote et al 1983). This nucleus innervates every major region of the neuraxis, even though it is composed of a relatively small number of neurons: approximately 1600 per hemisphere in the rat, 5000 per hemisphere in the monkey, and 13,000 per hemisphere in the human. Individual LC neurons often innervate widely separated brain regions, thus indicating that these axons must be highly divergent. It has also been demonstrated that individual LC neurons innervate different cortical regions. Thus, after an LC axon has traveled through the brainstem to the frontal regions of cortex, it diverges as it sweeps in a rostrocaudal trajectory through the cortical hemisphere, sending off collaterals to many different cortical regions and to both superficial and deep cortical layers (Morrison et al 1978, 1979, 1981, Loughlin et al 1982). The noradrenergic fibers are primarily distributed in a tangential (i.e. parallel to the cortical surface) fashion, particularly in layer VI, where they are oriented predominantly in the anteroposterior plane, forming a continuous sheet of longitudinal fibers overlying the white matter. In rat, these very fine caliber fibers branch to innervate all six layers of the neocortex, and the pattern of noradrenergic axon distribution possesses a geometric orderliness and distinct laminar pattern that is consistent throughout the lateral neocortex (Morrison et al 1978, 1981), with significant regional specialization present only in cortical regions on the medial surface of the hemisphere (Morrison et al 1979). Layer I possesses a lattice of rostrocaudal and mediolateral tangential fibers. The layer VI axons branch profusely into upper layer V and layer IV; possibly they represent terminal axons. The fibers in layers II and III are predominantly radial in orientation. The layer I fibers do not necessarily all arise from local fibers in deeper layers; some fibers may run long distances within this layer.
In contrast, the noradrenergic innervation of primate cortex exhibits striking regional specialization in both density and laminar pattern of innervation (Morrison et al 1982a,b, 1984, Morrison & Foote 1986, Levitt et al 1984). For example, primary somatosensory and motor regions are densely innervated in all six laminae, whereas temporal cortical regions are very sparsely innervated. In primary visual cortex, the density of innervation is intermediate, but there is a striking absence of fibers in lamina IV. As in the rat, a strong tangential, intracortical trajectory is a dominant feature of the noradrenergic innervation of the much more convoluted primate cortex (Morrison et al 1982b).

The presence of regional specialization in noradrenergic, and other extrathalamic, innervation patterns raises the question of whether there are underlying organizing principles for these regional variations. The presently available rodent and primate data do not support a simple scheme for the distribution of noradrenergic fibers in the neocortex, such as preferential innervation of motor versus sensory structures, one sensory modality in preference to others, or primary sensory versus secondary sensory or association areas. Since a large portion of the primate neocortex is visual in nature, we have utilized immunohistochemical methods to characterize the noradrenergic innervation of several cortical and subcortical visual areas in monkey in order to search for possible organizing principles in these afferent systems (Morrison & Foote 1986). Cortical areas 17 and 18, as well as visual areas in the temporal and parietal lobe, were found to exhibit regional specialization of noradrenergic innervation. Precisely at the border between areas 17 and 18, the laminar innervation patterns characteristic of noradrenergic fibers in area 17 shift such that layer IV of area 18 contains more fibers than layer IV of area 17, and the overall density of fibers in area 18 is higher. The visual region of the inferotemporal cortex was found to be very lightly innervated by noradrenergic fibers, while area 7 of the parietal lobe was much more densely innervated. Visual thalamic nuclei exhibited pronounced regional differences in noradrenergic innervation density. The lateral geniculate was found to be virtually devoid of noradrenergic fibers, while the pulvinar-lateral posterior complex was densely innervated. In the mesencephalon, the superficial layers of the superior colliculus were found to be densely innervated by noradrenergic fibers.

The patterns of innervation indicate that, in these primate species, functionally related visual regions share common and distinguishable densities of noradrenergic innervation. Specifically, tectopulvinar-juxtastriate structures are more densely innervated than geniculostriate and inferotemporal structures. These relationships suggest that, within the visual system, noradrenergic fibers preferentially innervate regions involved in spatial analy-
sis and visuomotor response rather than those involved in feature extraction and pattern analysis. This is especially interesting given the proposed involvement of the LC-noradrenergic system in attentional mechanisms.

**Physiology**

**Activity of Source Neurons** LC neurons in unanesthetized, nonparalyzed rat, cat, and monkey have been shown to be most active during waking, less active during slow-wave sleep, and silent during rapid-eye-movement sleep (reviewed in Foote et al 1983). Within the waking state, the mean discharge rates of LC cells increase when enhanced levels of arousal or attentiveness are exhibited by the animal. In monkey, for example, discharge rates vary from second to second, and anticipate by several hundred milliseconds subsequent electroencephalographic changes indicating increased or decreased levels of alertness (Foote et al 1980).

**Conduction Properties of Afferent Axons** The conduction properties of primate LC axons projecting to neocortex have recently been characterized (Aston-Jones et al 1985b). Such information is obviously crucial in delineating the mechanisms whereby neurons residing at such a great distance from the neocortex, but innervating it so substantially, could influence neocortical activity. Discharge activity was recorded extracellularly from individual, histologically verified LC-noradrenergic neurons in anesthetized squirrel monkeys. These neurons were found to exhibit several properties previously described for LC cells in rat, including slow (0.2–2 Hz) spontaneous discharge rates, distinctive impulse waveforms (notched, entirely positive in unfiltered recordings, and 2–3 msec in duration), antidromic activation from many target areas, a period of suppressed discharge activity following either antidromic or orthodromic driving, and responsiveness to noxious stimuli presented as subcutaneous electrical stimulation of a rear foot. Antidromically driven action potentials (verified by collision tests) were recorded from individual LC neurons in response to electrical stimulation of neocortical (frontal, somatosensory, and occipital sites) and thalamic areas. These cells reliably conducted impulses from various cortical sites, some of which were up to 100 mm from the LC. The very high reliability of collision testing that we observed indicates that orthodromic impulses were also conducted with high reliability and temporal fidelity. Many monkey LC neurons projecting to the thalamus and neocortex were found to exhibit more rapid conduction velocities than previously observed in rat (e.g. approximately 34% were greater than 1 M/sec), resulting in similar conduction latencies to distant target areas for the two species.

These observations indicating that action potentials are conducted from
LC to neocortex with high reliability are significant because they lend additional credence to the hypothesis that noradrenergic release is controlled by the rate of impulse production in LC-noradrenergic neurons, rather than by local factors within the neocortex, as has been postulated by others. The demonstration of faster conduction velocities for long LC axons in the monkey is also important since extrapolation from the slow conduction velocities found in rat to species with larger brains (such as primates) would predict that exceptionally long latencies (i.e. hundreds of milliseconds) would prevail for LC impulse activity to reach distant target areas, such as the neocortex. Such a property would have profound implications for proposed functions of the LC system in vigilance and sensory processing. The data indicate that the conduction times required for LC impulses to reach distant target areas may be conserved across different species and sizes of brains and suggest that these latencies play an important role in the general function of the LC system in brain and behavioral processes.

LC-NORADRENERGIC EFFECTS ON CORTICAL NEURONAL ACTIVITY There have been numerous studies of the possible effects of the LC-noradrenergic projection to the cortex on putative target cells in various neocortical regions. The initial study of noradrenergic effects in the neocortex of unanesthetized monkeys was performed several years ago (Foote et al 1975). Most studies of LC-noradrenergic effects had been performed in anesthetized animals, on target neurons that were not being activated (or inhibited) in a manner relevant to their function. Adequate characterization of the functional properties of the noradrenergic system, however, requires that its impact be considered with regard to other neural systems projecting to the same target neurons. That is, LC-noradrenergic effects on functional activity, rather than spontaneous activity, of the target neuron must be assessed. This requires simultaneous activation of other inputs to target neurons while activating the LC-noradrenergic system. In this experiment, the effects of iontophoretically applied noradrenaline were studied, using as test cells auditory cortex neurons that were activated acoustically by species-specific vocalizations in unanesthetized, unparalyzed squirrel monkeys. Poststimulus-time histograms and raster displays of neuronal responses to the vocalizations were computed before, during, and after iontophoresis. Noradrenaline caused dose-dependent inhibition of spontaneous and vocalization-evoked activity. A given dose of noradrenaline reduced spontaneous activity proportionately more than it reduced activity evoked by acoustic stimuli. During auditory responses, segments with lower discharge rates were reduced proportionately more than segments with higher discharge rates. These observations led the
authors to propose that noradrenaline may act on such neurons to enhance elicited activity relative to spontaneous activity; in engineering terms, it enhances the "signal-to-noise" characteristics of the cells.

Many analogous experiments have been conducted in the past several years, aimed at determining whether LC-noradrenergic system differentially affects various aspects of target cell activity. Waterhouse, Woodward, and their colleagues have intensively investigated the effects of iontophoretic noradrenaline on neocortical neuronal activity in anesthetized rats (Waterhouse et al 1980, 1981, Woodward et al 1979). They have observed a similar reduction of background activity relative to stimulus-elicited activity in the somatosensory cortex. Additionally, in some cells, evoked excitatory responses were enhanced by noradrenaline application. Often, noradrenaline also augmented stimulus-bound inhibition and post-excitatory suppression of activity. Iontophoretic noradrenaline enhances responses to iontophoretic GABA and ACh, and enhanced excitatory sensory responses are produced and blocked by alpha- but not beta-adrenergic agents. The effects of LC electrical stimulation are generally a depression of spontaneous cortical neuronal activity (reviewed in Foote et al 1983; see also Jones & Olpe 1984).

HYPOTHESES OF LC-NORADRENERGIC EFFECTS ON TARGET NEURONAL ACTIVITY Various hypotheses have been advanced to describe the electrophysiological effects produced by the LC system. Some emphasize the enhancement of evoked activity relative to spontaneous activity (Foote et al 1975, Woodward et al 1979). Others describe this process as "enabling" (Bloom 1979), by which coactivity in LC terminals enables other systems converging on the same target neurons to transmit more effectively during the period of simultaneous activity. Yeh et al (1981) and Moises & Woodward (1980) present evidence that such enhancement by noradrenaline may occur in Purkinje cells only with certain transmitters and suggest more specific versions of these hypotheses (see also Moises et al 1983). The term "modulation" has often been used to describe these types of effects (see Dismukes 1979).

From the data, we draw the following conclusions: (a) the noradrenergic effects are too complex to describe as simple excitation or inhibition; (b) several studies find similar effects in different LC-noradrenergic terminal areas; (c) these effects constitute a substantial modification of the operation of the presumed target neurons.

Hypothesis

Taken together, the data concerning LC projections, the activity of source neurons, and the effects of the transmitter on target neurons indicate that
the LC is activated during alerting or arousal and releases noradrenaline onto target neurons in many brain regions, including the neocortex. This transmitter then acts to enhance the selectivity and vigor of responses to subsequent sensory stimuli or other synaptic input to the target neurons. The LC may well also play a role in more tonic behavioral state changes, such as the sleep-wake cycle. It has been proposed that the function of LC could best be described as altering behavioral modes from internally oriented and generated states, such as sleep, grooming, and food consumption, to an externally oriented mode that involves active matching of appropriate behaviors with novel, stressful, or informative stimuli (e.g. Aston-Jones & Bloom 1981a, 1981b, Astron-Jones et al 1984c).

Recent evidence concerning the anatomy and physiology of the LC-noradrenergic cortical projection permits the development of more specific hypotheses dealing with the site, mode, and time-course of action of this system on target neurons (reviewed in Foote et al 1983). First, studies of the activity of LC neurons in behaving animals suggest environmental and behavioral conditions under which this system is active in releasing noradrenaline onto target neurons: Specifically, because LC neurons exhibit phasic responses to certain sensory stimuli and systematically alter their discharge in anticipation of phasic arousal, this should be reflected in response patterns of target cells. Second, light-microscopic studies demonstrate that in primate neocortex, unlike rodent neocortex, terminal arborization patterns of noradrenergic axons are distinctive for each cytoarchitectonic region in such a way as to suggest that within each area specific neuronal classes receive this innervation. Third, other studies (Olschowka et al 1981) have demonstrated that noradrenergic axon terminals form synaptic contacts onto their target neurons, thus suggesting that this system acts on target elements in a temporally and spatially restricted fashion. Fourth, the data just reviewed indicate that noradrenaline, either released from LC-noradrenergic fibers or applied by microiontophoresis, has specific effects on well-defined functional activity of target neurons. These four sets of data suggest a specific interaction of the LC-noradrenergic system with thalamocortical and corticocortical circuits to alter the latter’s functioning via spatially localized and temporally discrete modification of neuronal information processing.

SEROTONERGIC INNERVATION OF NEOCORTEX

Anatomy: Source Cells and Termination Patterns of Neocortical Afferents

The serotonergic innervation of the rat neocortex arises primarily from the dorsal and median raphe nuclei (e.g. O’Hearn & Molliver 1984, Porrino
The serotonergic innervation of adult rat neocortex is more dense than is the noradrenergic innervation (Lidov et al 1980). The entire neocortical mantle is penetrated by these fine, varicose, and highly convoluted fibers, which appear in relatively uniform density across all cortical layers. The density and distribution of these fibers are such that they might innervate every neuron in the neocortex (Lidov et al 1980).

The serotonergic innervation of the monkey neocortex is also very dense, but in the monkey the innervation of different neocortical regions exhibits differences in density and laminar distribution of axons (Morrison et al 1982a, Takeuchi & Sano 1983, Kosofsky et al 1984, Morrison & Foote 1986). The serotonergic innervation of primary visual cortex (area 17) has been characterized in greater detail than that of any other region. The serotonin fibers here are very dense and are distributed in a strictly laminated fashion. Serotonin fibers show a strong preference for layer IV in area 17, a tendency that is also evident in other cortical areas. This tendency is especially interesting since this is the lamina that is the primary recipient of thalamocortical afferents. The morphology of serotonin fibers in the cortex is quite heterogeneous, with a mixture of thick, nonvaricose fibers, large, varicose fibers, and extremely fine, varicose fibers. Since the very thick fibers are most evident in white matter and the deep cortical laminae, and are more evident in young animals, it is highly likely that they are fibers of passage. The fibers are usually tangential in orientation. Differences between primate species are evident in the distribution and density of serotonin fibers in area 17.

In primary visual cortex (area 17) of the squirrel monkey, noradrenergic and serotonergic projections exhibit a high degree of laminar complementarity: Layers V and VI receive a dense noradrenergic projection and a very sparse serotonergic projection, whereas layer IV receives a very dense serotonergic projection and is largely devoid of noradrenergic fibers (Morrison et al 1982a, Kosofsky et al 1984, Foote & Morrison 1984). Also noradrenergic fibers manifest a geometric order that is not readily apparent in the orientation of serotonergic fibers. These patterns of innervation imply that the two transmitter systems affect different stages of cortical information processing. For example, the raphe-serotonergic projection may preferentially innervate the spiny stellate cells of layers IVa and IVc, whereas the LC-noradrenergic projection may innervate pyramidal cells.

We have examined the serotonergic innervation of several cortical regions subserving visual functions (Morrison & Foote 1986). Often a degree of complementarity exists between the density of noradrenergic and serotonergic innervation in these areas. For example, as mentioned above,
the noradrenergic innervation of temporal lobe visual areas is very sparse. Serotonin innervates these same areas very densely.

**Physiology**

**Activity of Source Neurons** The discharge activity of raphe neurons has been studied extensively in unanesthetized, behaving cats (reviewed in Jacobs et al 1984). Presumed serotonin neurons in the dorsal raphe nucleus have been found to exhibit slow, regular discharge patterns during quiet waking and to show increases in firing with phasic arousal. Presentation of a simple auditory or visual stimulus results in a single spike response, followed by a period of reduced activity lasting about 200 msec. Mean discharge rates decrease to about 1 Hz during slow-wave sleep, and the neurons become almost silent during REM sleep. With no external stimulation, these cells show very periodic activity, which various studies indicate is very likely caused by pacemaker activity intrinsic to the neurons themselves. Jacobs and colleagues (1984) have hypothesized that dorsal raphe discharge rates in behaving animals is closely related to activity in central motor systems, not in the sense that raphe neurons are motor or premotor neurons themselves but rather that their discharge rates appear to covary with general levels and intensity of movement.

**Raphe-Serotonergic Effects on Cortical Neuronal Activity** The responses of cortical neurons to microiontophoretically applied serotonin have been much less extensively evaluated than for noradrenaline. Neuronal responses to iontophoretic application of serotonin have been found in virtually every brain region tested. Depending on the region tested, the responses of unidentified neurons can be either excitation or depression. However, in areas known to receive a dense and uniform serotonergic innervation, the most common response to iontophoretically applied serotonin (reviewed in Bloom et al 1972, Nelson et al 1973) or electrical stimulation of the median raphe (e.g. Wang & Aghajanian 1977) is marked inhibition of spontaneous activity. Particularly in cerebral cortex, the variability of serotonergic effects (excitation versus inhibition) has led to much controversy and questioning of methods. However, more recent studies suggest that such discrepancies may be due to the unconventional nature of serotonergic effects. It has been suggested that serotonin, like noradrenaline, may have primarily a “modulatory” role, rather than a classical inhibitory or excitatory action.

The most extensive evidence for a modulatory action of serotonin comes from the work of Aghajanian and co-workers in the facial motor nucleus (reviewed in Aghajanian 1981). Iontophoretically applied serotonin failed to excite motoneurons in the facial nucleus over a large dose range, but
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small amounts of serotonin were shown to markedly facilitate the excitatory effects of both threshold and subthreshold doses of iontophoretically applied glutamate and the excitation evoked by stimulation of trigeminal afferents (McCall & Aghajanian 1979, 1980).

Although substantial interlocking evidence exists to suggest a potent neuromodulatory role for noradrenaline in the neocortex, however, little evidence exists to substantiate or dismiss a similar role for serotonin.

Hypothesis

Strong analogies between the LC-noradrenergic system and the raphe-serotonergic system suggest that the system may perform similar but distinct functions in the neocortex. Single-cell recordings in freely moving animals indicate that the spontaneous firing of both LC-noradrenergic and raphe-serotonergic neurons fluctuates as a function of sleep-wake cycle, level of arousal, and phasic sensory stimulation. However, raphe neurons exhibit activity that is most strongly correlated with behavioral “state,” whereas LC-noradrenergic neurons exhibit more phasic fluctuations, associated with “attentiveness.” Both systems in turn innervate vast areas of neocortex via widely ramifying axonal projections. At the gross morphological level, both systems exhibit parallel trajectories and regions of innervation. However, more detailed analyses of their laminar distributions suggest that noradrenergic and serotonergic fibers may terminate on different cell types, or at least on distinctive portions of the same cell type. Taken together, these observations suggest that the raphe-serotonergic system may serve a similar, yet distinct (and perhaps complementary) role in cortical information processing from that proposed for the LC-noradrenergic system; namely, alteration of cortical neuronal responses to afferent input in response to changes in state. Since serotonergic innervation of the neocortex is even more dense than noradrenergic innervation, serotonin may have even more profound, or more widespread, effects on neocortical neuronal activity than does noradrenaline. A major question yet to be addressed is whether serotonin also plays a modulatory role in the neocortex, since it does play a modulatory role on target neurons in other brain areas.

DOPAMINERGIC INNERVATION OF NEOCORTEX

Anatomy: Source Cells and Termination Patterns of Neocortical Afferents

The first evidence for the existence of a dopaminergic projection to the neocortex was obtained through biochemical studies of dopamine syn-
thesis in the neocortex of rats (Thierry et al 1973). This study was followed by histochemical confirmation of a dopaminergic projection to the cortex (Hökfelt et al 1974, 1977, Lindvall et al 1974) and the demonstration that the projection originated in the mesencephalon (Lindvall et al 1974, 1978, Fuxe et al 1974). The anatomic organization of the dopaminergic projection to the neocortex has been analyzed in great detail in the rat (see Lindvall & Bjorklund 1984 for review). This innervation originates exclusively from the substantia nigra–ventral tegmental (SN/VTA) area cell groups and, until recently (see below), was thought to be restricted to four discrete terminal fields: a prefrontal dopaminergic projection that is subdivided into (a) anteromedial and (b) suprarhinal terminal fields, (c) a supragenual terminal field that is coincident with the anterior cingulate cortex, and (d) a perirhinal terminal field. Specific laminar patterns of termination and topographically restricted cell bodies of origin exist for each terminal field. For example, the terminal fibers of the anteromedial and suprarhinal systems are primarily directed at layers V and VI and originate from the medial and dorsolateral ventral tegmental area (VTA), respectively.

The supragenual dopaminergic system is restricted to the anterior cingulate cortex, where fine terminal fibers are present in layers I–III. Unlike the prefrontal systems described above, the supragenual system of fibers originates largely from substantia nigra neurons, with a possible minor contribution from the lateral VTA (Swanson 1982), which is more likely to be related to the caudal extension of the anteromedial terminal field.

Although it is clear that the SN/VTA complex projects topographically to the four major neocortical terminal fields in rat, certain aspects of the relationship between cell bodies of origin and terminal fields in the ascending dopaminergic systems remain controversial. For example, the degree of collateralization of the dopamine fibers remains a controversial issue. Swanson (1982) maintains that a given dopamine cell has one major target, even though the cell bodies of origin of two discrete projections may be in the same portion of the cell group. However, Fallon & Loughlin (1982) maintain that single neurons in the central portion of the substantia nigra and VTA project to multiple targets, whereas the more peripherally situated neurons tend to project to a single target area. The issues of topographic organization (dopaminergic vs. nondopaminergic cortically projecting cells) and the degree of collateralization will be critically important to a characterization of this system in the primate, where we suspect that the terminal fields in the cortex are far more diverse and widespread than in the four target regions described above. In the monkey, Porrino & Goldman (1982) demonstrated a topographically organized projection from VTA to various regions of the frontal cortex (Porrino & Goldman
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1982); however, the cells bodies of origin of the terminal fields outside of the frontal lobe have not been analyzed, and the Porrino & Goldman study did not differentiate dopaminergic from nondopaminergic projection neurons in VTA. In addition, no data are available on the collateralization of the dopamine neurons in primate VTA.

In addition, recent data suggest that the dopaminergic innervation of rat cortex may be more widespread than the four terminal regions described above. Recent anti-tyrosine-hydroxylase immunohistochemical studies (Berger et al 1985) suggest that additional, less densely innervated, regions exist. Berger and her colleagues demonstrated convincingly that well-defined portions of motor and visual association cortices receive a dopaminergic projection. In addition, evidence exists for a dopaminergic projection to the visual cortex in cat (Tork & Turner 1981).

It is difficult to accurately determine the regions in primate cortex that might be directly homologous to the neocortical regions in the rat that receive a dopaminergic projection. Biochemical studies (Bjorklund et al 1978, Brown & Goldman 1977, Brown et al 1979) demonstrated that dopamine levels as well as dopamine/noradrenaline ratios were elevated in various frontal and temporal regions of the monkey cortex. These careful biochemical studies offered the first evidence that the dopaminergic projection in the primate was likely to involve numerous discrete regions of association cortex within the frontal and temporal lobes, as well as the primary motor cortex, and that the density of innervation was likely to be highly region-specific. Based on endogenous dopamine levels in carefully dissected regions, Brown & Goldman (1977) proposed that within the frontal lobe, the densest dopaminergic innervation was in the prefrontal cortex and a rostrocaudal gradient of decreasing density existed. In fact, the rostrocaudal gradient could be extended into the parietal and occipital lobes, with low levels in parietal lobe relative to frontal, and only trace amounts of dopamine present in occipital cortex. Bjorklund et al (1978) relied more heavily on dopamine/noradrenaline ratios in their interpretation and supported the notion of a rostrocaudal gradient within the frontal lobe. Anatomic analysis of the dopaminergic system in primate neocortex has been hampered by the fact that the fluorescence histochemical method does not distinguish between noradrenaline and dopamine fibers. Immunohistochemical studies using antibodies to tyrosine-hydroxylase (TH) potentially suffer from the same problem. However, there are reports that TH levels are below the levels necessary for immunohistochemical detection in noradrenaline fibers. For example, this appears to be the case for the anti-TH that we have used (Lewis et al 1987) to study primate neocortex.

Levitt et al (1984) have completed an extensive fluorescence histo-
chemical analysis of primate neocortex, in which they differentiated dopamine from noradrenaline fibers on the basis of morphological characteristics. Fibers with "dopamine-like characteristics" were present only in the frontal and temporal lobes, and were most numerous in the prefrontal cortex, precisely the region exhibiting the highest endogenous levels of dopamine. In addition, dopamine-like fibers were present in the motor cortex, thus supporting the earlier prediction of a dopaminergic innervation of motor cortex that was based on biochemical data. In frontal and cingulate cortex, most of the dopamine-like fibers were present in layers II and III; however, no details on the laminar or regional distribution of the dopaminergic innervation of temporal lobe were given, and no dopamine-like fibers were seen in the parietal or occipital lobes. The most important finding of Levitt and co-workers was the anatomic confirmation that in the primate, the dopamine system extended well beyond the medial, prefrontal, and perirhinal areas characterized in the rat, and was likely to innervate vast areas of frontal and temporal association cortices. Our preliminary data using anti-TH support this contention and, in fact, suggest that the dopaminergic innervation extends throughout all four lobes of the neocortex, with dramatic regional variations in density that coincide with cytoarchitectonic and functional boundaries (Lewis et al 1987). We have carefully compared our anti-DBH (dopamine-beta-hydroxylase) staining patterns with anti-TH staining patterns, and have analyzed the anti-TH staining pattern in monkeys in which the ascending noradrenergic projection to cortex has been ablated. Our immunohistochemical findings suggest that the differentiation of dopamine from noradrenaline fibers based on morphologic grounds in the histochemical studies of Levitt et al (1984) must have been misleading in several cases. Our data suggest that extensive regional heterogeneity of the dopaminergic innervation exists within frontal cortical areas, such that the variations in density are more complex than a simple rostrocaudal gradient, and that the primary motor cortex has the densest dopaminergic innervation of any frontal area. Also, there is an extensive dopaminergic innervation of the inferior parietal lobe (area 7), such that the innervation of this area is denser than several prefrontal areas (see Figures 1 and 2). Primary visual, auditory, and somatosensory cortices all exhibit a very sparse dopaminergic innervation. For all three modalities, density is significantly higher in related association areas. The laminar pattern of fibers in a given region is correlated with fiber density. In very sparsely innervated regions, dopamine fibers are limited to layer I, and areas of intermediate density have dopamine fibers in layers I, superficial III, and deep V–VI. The primary motor cortex displays fibers in all laminae. Throughout the neocortex, layer IV has the lowest density of innervation. In summary distribution patterns reveal a
Figure 1  Darkfield photomicrograph of dopamine fibers visualized using TH immunohistochemistry. This image is from a coronal 40 μm section through area 7, the inferior parietal lobule, of a cynomolgus monkey. The numerals at left indicate the cortical laminae. Calibration bar = 200 μm. WM = white matter. From Lewis et al (1987).
Reverse-image photographic reproductions of darkfield photomicrographs of dopamine fibers revealed by TH immunohistochemistry in various cytoarchitectonic areas of cynomolgus monkey cortex. Note the extensive regional heterogeneity in the density and laminar distribution of labeled fibers. A, dorsomedial prefrontal cortex (area 9); B, primary motor cortex (area 4); C, primary somatosensory cortex (area 3); D, posterior parietal cortex (area 7); E, primary visual cortex (area 17); F, rostral superior temporal gyrus; G, rostral inferior temporal gyrus. Calibration bars equal 200 μm. From Lewis et al (1987).
functional specialization of the dopaminergic innervation of primate cortex such that fibers preferentially innervate motor relative to sensory regions, sensory association relative to primary sensory areas, and auditory association relative to visual association areas.

**Physiology**

**Activity of Source Neurons** There have been a limited number of studies of the discharge activity of SN/VTA neurons in unanesthetized, behaving animals. In cat, these neurons discharge more rapidly during active than quiet waking, but do not further decrease their activity during sleep (Trulson et al. 1981, Steinfels et al. 1983, see also Miller et al. 1983). Brief excitatory and inhibitory responses to phasic auditory or visual stimuli are observed during quiet waking. Various stressful and arousing stimuli do not alter the discharge rates of substantia nigra neurons, although phasic responses to neutral auditory or visual stimuli are blocked by such manipulations (Strecker & Jacobs 1985). The most striking change in activity is a prolonged suppression of activity that accompanies orientation toward and fixation of a novel or meaningful stimulus in the environment (Steinfels et al. 1983). In monkeys, substantia nigra dopamine neurons show little relation to the phasic movements or other aspects of an operant paradigm (DeLong et al. 1983). It is not known whether the subset of dopamine neurons projecting to neocortex exhibits any of these properties.

**Effects of Dopaminergic SN/VTA on Cortical Neuronal Activity** In iontophoretic tests, dopamine has been found to be inhibitory on neurons in the frontal and cingulate cortices. Bunney & Aghajanian (1976) observed in rat prefrontal cortex that cells in layers II and III are more sensitive to the inhibitory effects of noradrenaline than those of dopamine on spontaneous activity. The converse was true for cells in layers V and VI. This corresponds to the preferential innervation of superficial layers by noradrenaline fibers and of deep layers by dopamine. Ferron et al. (1984) observed that stimulation in the region of dopamine cells projecting to neocortex in the anesthetized rat blocked the excitatory effect on cortical neurons of thalamic stimulation. In the orbitofrontal cortex of behaving monkeys, Aou et al. (1983) observed that those cells most sensitive to microiontophoretically applied noradrenaline decreased their activity during a food-acquisition behavior whereas those most sensitive to dopamine increased their activity during this behavior.

**Hypothesis**

In both rodent and primate, the dopaminergic innervation of neocortex exhibits a far greater degree of regional heterogeneity than either the
noradrenergic or serotonergic systems, with the highest densities occurring in limbic and association cortices. Laminar and regional patterns of innervation suggest that the dopaminergic system is in a position to influence the activity of corticocortical rather than thalamocortical circuits and higher-order integrative processes rather than the more analytic aspects of sensory processing. In addition, this system is likely to be involved in some aspect of cortical regulation of motor control and associated functions in the frontal lobe. It will be of interest to determine whether cortically projecting SN/VTA neurons respond to environmental and behavioral manipulations differently from dopamine neurons projecting to the basal ganglia. It may be that the mesocortical system exhibits quite different properties that have yet to be described.

CHOLINERGIC INNERVATION OF THE NEOCORTEX

Anatomy: Source and Termination Patterns of Neocortical Afferents

Putative acetylcholinergic (ACh) axons in the neocortex have been visualized by acetylcholinesterase (AChE) histochemistry, AChE immunohistochemistry, and choline acetyltransferase (ChAT) immunohistochemistry (see Wainer et al. 1984a for review). The ACh extrathalamic innervation of rat neocortex appears to be widespread and to arise primarily from the nucleus basalis and portions of the diagonal band, although ACh neurons in other sites contribute a minor portion of this cortical projection (Lehmann et al. 1980, Bigl et al. 1982, Mesulam et al. 1983). ChAT fibers in the motor cortex are distributed with approximately equal density through all cortical layers, while in somatic sensory cortex there is an increased density in layer V and a decreased density in layer IV (Houser et al. 1985). There is general agreement that ChAT neurons are intrinsic to the neocortex. These cells are bipolar and are found in layers II–VI, with a slightly higher density in layers II and III (Eckenstein & Thoenen 1983, Houser et al. 1983, 1985, Levey et al. 1984), but lesion and biochemical data indicate that such intrinsic innervation probably constitutes a small fraction of the ACh innervation of neocortex. Double-labeling studies indicate that cortically projecting ACh neurons exhibit only limited collateralization, and a coarse topographic relationship exists between the locations of cells of origin and the locus of cortical termination (Bigl et al. 1982, McKinney et al. 1983). Perhaps source cells receive input from those cortical regions to which they project (Saper 1984). ChAT synapses have been described as predominantly symmetrical and occurring
on dendritic shafts and spines in the cingulate and entorhinal cortices (Wainer et al. 1984b) as well as in motor and somatosensory cortices (Houser et al. 1985).

In area 17 of the cat (Bear et al. 1985) AChE histochemistry reveals heavily stained pyramidal cells in layer V, as well as a network of fibers with striking laminar variations in density. Experimental manipulations indicate that the reactive fibers arise from the ipsilateral basal forebrain: Undercutting eliminates virtually all AChE fibers; injection of HRP into area 17 yields retrogradely labeled neurons in the basal forebrain; and basal forebrain lesions substantially reduce the density of AChE fibers. The projection appears to be strictly ipsilateral. These observations on innervation patterns (see Figure 3) have been verified with ChAT monoclonal antibodies (Stichel & Singer 1985). No ChAT+ neuronal profiles are observed in area 17, of the cat (Stichel & Singer 1985).

It has not yet proved possible to reliably visualize ChAT-immunoreactive fibers in the monkey cortex. Cortical fibers have been visualized with AChE histochemistry (Mesulam et al. 1984) and AChE immunohistochemistry (Hedreen et al. 1984). With histochemistry, regional specializations are evident in terms of laminar distribution and density of fibers. Primary visual, auditory, and somatosensory cortices contain a distinctive band of fine processes in layer IV. Motor and premotor cortices exhibit prominent, radially oriented fibers in deep layers. Association cortices contain the lowest density of reactive fibers of all cortical areas (Mesulam et al. 1984). With AChE immunohistochemistry (Hedreen et al. 1984), area 17 of the monkey (see Figure 4) exhibits enhanced fiber density in laminae in which lateral geniculate afferents terminate. Some moderately stained neuronal profiles are evident in layer VIB. Regional heterogeneity is also evident in monkeys when regional concentrations of ChAT are determined (Lehmann et al. 1984).

In monkey, cortical ACh fibers appear to arise from the nucleus basalis of Meynert and, to a lesser extent, neurons within the diagonal band of Broca. Different subdivisions of the nucleus basalis project preferentially to major regions of the cortical mantle (Mesulam et al. 1983). The topographic organization of this projection is more readily evident in primate than in rat (Rye et al. 1984). In monkey, cortically projecting ACh neurons appear to receive input from a limited set of cortical and subcortical sites (Mesulam & Mufson 1984, Russchen et al. 1985). Cortical input arises from prepyriform, orbitofrontal, anterior insular, temporal pole, entorhinal, and medial temporal areas, while subcortical afferents arise from septal nuclei, amygdala, nucleus accumbens–ventral pallidum complex, and the hypothalamus.
Figure 3 Camera lucida drawing of ChAT-immunoreactive fibers in a coronal section through area 17 of an adult cat. G = blood vessel. Courtesy of C. C. Stichel and W. Singer.
Figure 4  Drawing of AChE-immunoreactive fibers in an 8 μm section through area 17 of cynomolgus monkey. Laminar boundaries in the Broadmann system are indicated along the left-hand edge. From Hedreen et al (1984). Reprinted with permission.
**Physiology**

**Activity of Source Neurons**  Recordings from nucleus basalis neurons in behaving monkeys have revealed that the neurons are most active in situations involving the sight and taste of food rewards (DeLong 1971, Burton et al 1975, Rolls et al 1979). The desirability of the food reward and the animal's state of hunger both influence the intensity of activity. In the operant paradigms utilized in these studies, the neurons were not activated reliably as a function of simple sensory or motor variables.

**Conduction Properties of Afferent Axons**  Cortically projecting nucleus basalis neurons have been identified during single cell recordings in the nucleus basalis region by antidromic activation from frontal and parietal cortices in rats (Aston-Jones et al 1984a, 1985a) and monkeys (Aston-Jones et al 1984b). Such cells are physiologically heterogeneous, exhibiting a variety of rates and patterns of spontaneous discharge. A wide range of conduction latencies is also observed (1–26 msec for rat frontal cortex). One outstanding characteristic of these neurons in both rat and monkey is the tendency to exhibit multiple, discrete antidromic latencies, as a function of stimulus intensity and stimulation depth within the cortex. These authors interpret such results as evidence for pronounced branching of nucleus basalis axons within local cortical terminal fields. Conversely, there was no evidence of branching from a single neuron to innervate different cortical fields, since no cells were found to be driven from both frontal and parietal cortices. Finally, by examining conduction velocity as a function of depth of cortical stimulation, Aston-Jones et al (1985a) were able to determine that intracortically nucleus basalis fibers conduct impulses at about 0.3–0.8 m/s, whereas subcortically the impulse travels at 1.8–3.4 m/s. Thus, nucleus basalis fibers may be myelinated subcortically, but they lose their myelin sheaths as they approach their targets within cortical gray matter.

The nucleus basalis–ACh system differs from other extrathalamic cortical afferents in that these neurons form a physiologically heterogeneous population, with wide variations in spontaneous discharge, spike waveform, and conduction latencies to cortex. The functional significance of this heterogeneity remains unclear. However, Aston-Jones et al (1985a) note that the physiologically homogeneous noradrenaline-LC neurons appear to have more divergent efferent projections, such that individual neurons are found to project to widely separated brain areas. They speculate that the more restricted terminal fields of nucleus basalis–ACh neurons may correspond to their physiologic heterogeneity, such that more re-
Strict target areas are differentially controlled by individual neurons to a greater extent than in the LC system.

**Nucleus Basalis--ACh Effects on Cortical Neuronal Activity**

ACh has usually been found to have excitatory effects on cortical neurons when iontophoretically applied (e.g. Krnjevic & Phillis 1963, Krnjevic et al 1971, Spehlmann 1969, Spehlmann et al 1971, Foote et al 1975). It is of interest to note that the application of ACh to auditory cortex neurons in the unanesthetized monkey results in a large increase in spontaneous activity without a corresponding increase in stimulus-elicited activity (Foote et al 1975). Thus, the net effect of ACh is opposite that of noradrenaline, that is the signal-to-noise ratio of sensory responses is decreased. However, Inoue et al (1983), recording from monkey dorsolateral prefrontal cortex during bar-press feeding behavior, observed that the continuous iontophoretic application of ACh enhanced phasic neuronal responses to various aspects of the task. Scopolamine diminished task-related activity. Electrical stimulation of the nucleus basalis produced driven activity in cortical neurons, which was abolished by iontophoretic application of atropine.

**Hypothesis**

As noted by Mesulam & Mufson (1984), in monkey the subcortical afferents to nucleus basalis--ACh neurons projecting to neocortex are limbic and paralimbic. The information characteristic of these structures may be transmitted to neocortex by this ACh afferent system. Also, because the cortical regions projecting to the nucleus basalis complex are much more restricted than those regions receiving input from it, the cortical structures afferent to the nucleus basalis can control the ACh input to themselves and to other cortical structures. These authors speculate that this afferent system may be responsible for conveying information concerning relationships between complex environmental events and the internal milieu to many cortical areas involved in many different, specific functions. The possible heterogeneity of function may correspond to the physiological heterogeneity of the neurons described by Aston-Jones et al (1985a).

**Extrathalamic Modulation of Cortical Function**

What are the implications of our knowledge of the cellular anatomy and physiology of these extrathalamic systems for speculations concerning their roles in normal and abnormal brain function?
1. The source neurons for these systems generally reside outside the major sensory and motor pathways of the brain. However, the efferent pathways of each system innervate primary and secondary sensory and motor structures. Thus, each system probably plays a role in influencing such activities, probably by imposing state-dependent effects onto these highly topographic systems. The dopaminergic and ACh systems may well exert these influences with a greater degree of topographic specificity than do the noradrenergic and serotonergic systems.

2. The electrophysiologic effects of these putative transmitters on post-synaptic neurons also indicate a modulatory role. The present data suggest that these effects constitute well-defined alterations in the electrophysiologic properties of target cells that result in specific alterations of their operating characteristics. For example, the noradrenergic system appears to enhance the signal-to-noise characteristics of sensory neurons in many brain regions.

3. Electrophysiologic studies of the activity of extrathalamic source neurons indicate that noradrenaline neurons exert their effects in both a tonic and a phasic fashion; they are more active during waking but also exhibit bursts of activity during episodic increases in attentiveness during waking. Serotonin neurons appear to be more responsive to the sleep-wake cycle per se and might well initiate and maintain more tonic effects of behavioral state on target neuron function. The activity of dopamine and ACh neurons has been less extensively studied in behaving animals. Dopamine neurons appear to be involved in orienting behaviors, while ACh neurons are most active during relatively specific conditions involving motivated, or perhaps emotional, behaviors.

4. Although all three monoamines innervate the neocortex, each of the four clearly has preferred regions and laminae of termination. This suggests that their effects on the neocortex are not generalized excitation or inhibition but rather region-specific enhancement or diminution of activity in limited neuronal ensembles during certain stages of information processing.

5. Finally, because each monoamine innervates diverse functional systems, one would not expect a simple correlation of "one transmitter—one behavior." In both normal and abnormal states, each of these transmitters must influence a variety of behaviors.

The studies aimed at determining the behavioral functions of extrathalamic neocortical afferents have been limited in number (e.g. Brozoski et al 1979, Arnsten & Goldman-Rakic 1985). Such studies must be viewed as initial attempts to approach a very complex problem, but it is of interest that limited lesions and pharmacological manipulations of these systems
can produce profound impairments in carefully controlled behavioral paradigms.

ACKNOWLEDGMENTS

Gary Aston-Jones provided essential assistance in evaluating available data on axonal conduction properties. The work reported here from our laboratories was supported by USPHS Grants MH40008 (S.L.F.), NS21384 (S.L.F.), AA06420 (S.L.F., J.H.M.), AG015131 (J.H.M.), and the MacArthur Foundation (S.L.F., J.H.M.).

Literature Cited


Brown, R. M., Crane, A. M., Goldman, P. S.


Kosofsky, B. E., Molliver, M. E., Morrison,
EXTRATHALAMIC MODULATION OF CORTEX


Morrison, J. H., Foote, S. L., O'Connor, D., Bloom, F. E. 1982b. Laminar, tangential and regional organization of the noradrenergic innervation of monkey cortex: Dopamine-hydroxylase immuno-


Spehlmann, R., Daniels, J. C., Smathers, C. C. 1971. Acetylcholine and the synaptic transmission of specific impulses to the visual cortex. *Brain* 94: 125–38


microscopic immunohistochemical study employing a monoclonal antibody against choline acetyltransferase. *Brain Res.* 308: 69–76


