Nucleus Locus Ceruleus: New Evidence of Anatomical and Physiological Specificity

STEPHEN L. FOOTE, FLOYD E. BLOOM, AND GARY ASTON-JONES

Arthur Vining Davis Center for Behavioral Neurobiology,
The Salk Institute, La Jolla, California

I. Introduction ......................................................... 844
II. Locus Ceruleus Anatomy .............................................. 845
   A. Nucleus: location, cytoarchitecture, and cell number .......... 845
   B. Nucleus: histochemistry and phylogeny ......................... 846
   C. Afferents to locus ceruleus ..................................... 848
   D. Intrinsic organization ........................................... 851
   E. Locus ceruleus efferents: pathways and termination patterns 852
   F. Ontogeny of locus ceruleus and its efferents .................. 863
III. Locus Ceruleus Physiology .......................................... 867
   A. Spontaneous and sensory-evoked activity ...................... 867
   B. Antidromic activation and axonal conduction properties .... 874
   C. Locus ceruleus pharmacology .................................... 876
   D. Summary and conclusions ........................................ 880
IV. Synaptic Structure and Function of Locus Ceruleus System .... 883
   A. Ultrastructure of locus ceruleus terminals .................... 883
   B. Postsynaptic effects of locus ceruleus efferents ............. 886
   C. Molecular mechanisms of central norepinephrine synapses .... 889
   D. Locus ceruleus–norepinephrine effects on target neuron functional activity .......................... 894
V. Conclusions ......................................................... 897
   A. What is specificity? ............................................... 897
   B. Why is specificity important? ................................... 898
   C. Toward testable hypotheses of locus ceruleus function ....... 899

I. INTRODUCTION

In the 5 years since Amaral and Sinnamon's (12) comprehensive review of the neurobiology of the locus ceruleus (LC), there has been substantial progress in characterizing the anatomy and physiology of these norepinephrine (NE)-containing neurons. Our review summarizes the most important of these developments and synthesizes them, along with previous knowledge, into working hypotheses concerning the physiological functions of these noradrenergic neurons of the LC (denoted in this review by LC-NE). We have selected data relevant to four basic characteristics of the LC system for detailed review and interpretation: 1) electrophysiological data indicating...
that LC neurons in behaving animals exhibit phasic responses to sensory stimuli and also systematically alter their discharge in anticipation of phasic changes in arousal; 2) light-microscopic data demonstrating that terminal arborization patterns of LC efferents are differentiated for each terminal field, suggesting that within each area specific classes of neurons serve as targets for this innervation; 3) ultrastructural data demonstrating that the majority of LC-NE terminals form specialized synaptic contacts onto neuronal elements; and 4) data suggesting that NE, either released from LC-NE fibers or applied by microiontophoresis, has specific effects on well-defined functional activity of target neurons. We have chosen these areas because substantial progress has been made in them, and the results obtained have been unexpected in many cases and have caused the most dramatic shifts in current perception of the LC and its function. The data are mutually reinforcing and suggest a specific interaction of the LC-NE system with other major brain systems to alter the functioning of these other systems via spatially localized and temporally discrete modification of neuronal information processing.

The scope of this review is limited. First, work described in Amaral and Sinnamon's (12) review is included only when relevant to current developments. Second, the subject matter has been limited to studies on the cellular physiology and anatomy of the LC-NE system. Presynaptic control of NE release and NE-mediated neurotransmission are discussed only as they directly relate to the functioning of the LC system (352, 447, 469, 481). The behavioral effects of lesioning LC or its ascending projections have been previously reviewed (286, 292) and generally offer only indirect evidence about the cellular physiology of the LC system. Finally, pharmacological studies of LC are described only when they offer evidence concerning the mechanisms underlying LC function in undrugged animals (for review see 154).

II. LOCUS CERULEUS ANATOMY

A. Nucleus: Location, Cytoarchitectonics, and Cell Number

The LC is a distinct cluster of neurons located near the wall of the fourth ventricle at the level of the pons. The location of the LC, and the general appearance of its constituent neurons, is relatively stable across mammalian species, although some distinctive differences exist. The nucleus has been most intensively studied in the rat, and studies of Nissl, Golgi, and immunohistochemical material from this species remain the most comprehensive light-microscopic analyses of LC cytoarchitectonics and cell types (459, 462). Swanson's description of the nucleus as containing some 1,600 neurons and being divisible into dorsal and ventral components based on cell types and cell densities has not been challenged, although more recent descriptions of
Golgi and immunohistochemical studies have been more complete. In summary, descriptive anatomy alone of the LC has yielded no new perspective on functional issues in the last 5 years, and the basic description of the nucleus has changed little. Previous reviews (267, 303) describe this work in detail, and only new findings are presented here.

B. Nucleus: Histochemistry and Phylogeny

1. Norepinephrine cell types and their distributions in rat

The LC became the object of intensive investigation when it was demonstrated that it is the largest group of NE-containing neurons in the brain (85). These neurons have been studied in detail with Nissl, Golgi, catecholamine fluorescence, and immunohistochemical methods. In the rat, recent developments in the light-microscopic study of these neurons have primarily involved the use of antibodies to dopamine β-hydroxylase (DBH), the final synthetic enzyme for NE (171). Grzanna et al. (159-162) found that antisera to rat DBH have an affinity for rat DBH several thousandfold greater than that of a similarly produced antiserum directed against cow DBH. This enhanced affinity yields improved sensitivity and selectivity in the immunohistochemical demonstration of DBH-containing neurons and processes (162). These antisera have also been used to visualize DBH-reactive neurons in 100-μm sections, which permits visualization of the cell processes, much like a Golgi impregnation but with the transmitter specificity of the antisera (162). In addition, if a horseradish peroxidase (HRP) method is utilized, permanent sections are created that can be viewed with bright-field microscopy. Grzanna and Molliver (159, 160) have used this anti-DBH method with sections of various thicknesses to study the morphology and distribution of LC neurons in detail. They observed four contiguous but cytotologically distinct sets of neurons composing the rat LC: 1) the LC proper, identical with Swanson's (459) dorsal division of LC; 2) the A4 cell group, described by Dahlstrom and Fuxe (85); 3) the ventral division, similar again to Swanson's (459); and 4) a group of cells not previously identified that are DBH reactive and extend anteriorly from the LC. These are the largest NE-containing neurons in the brain, with cell bodies 35-45 μm in diameter. They are scattered throughout the lateral portion of the central gray and extend into the periaqueductal gray of the caudal midbrain.

Within the LC proper, Grzanna and Molliver (159, 160) had difficulty determining the structure of individual neurons due to the extremely dense packing of DBH-immunoreactive processes. In the ventral LC, highly ramified dendritic processes from these predominantly multipolar neurons could be observed. Other recent studies have detailed the Golgi morphology of LC neurons in the rat (347) and demonstrated that these neurons contain tyrosine hydroxylase (TH) (353, 354).
Thus in the past 5 years new immunohistochemical procedures have revealed a new NE cell group contiguous with the LC proper (rostral cell group), the basic findings of previous studies were confirmed, and greater detail was revealed about the dendritic processes of these neurons.

2. Phylogenetic variation of locus ceruleus

The cell types and numbers of neurons composing LC have been studied in detail in a large number of species, and a trend toward more detailed study in primates, including humans, is evident. At the other end of the phylogenetic spectrum, a recent study described the presence of an LC analogue in the chicken (96), complementing earlier studies of these neurons in another bird (472) and in the frog (474).

In the rabbit, LC neurons are more loosely arranged and the subceruleus is more extensive than in the rat (33). The rabbit LC is not, however, as diffuse as previously described for the cat (70, 205, 278), and the subceruleus, though possibly more extensive, does not appear to contain a large number of neurons. A recent study proposes that the cat LC is made up of four classes of neurons (256): 1) medium-sized catecholamine (CA) neurons, 2) small, nonmonoamine interneurons, 3) intermediate-sized CA neurons, and 4) intermediate-sized indolamine neurons. The cat LC region has been reported to contain about 9,150 CA cells unilaterally; about 5,300 of these are within the LC proper (497). In the opossum, as in the cat, NE-containing neurons are interdigitated with non-NE neurons (82).

Before 1977, studies revealed that the nucleus of the nonhuman primate is similarly located to that of the rat LC and is also composed of NE-containing neurons (24, 89, 118, 127, 139, 143, 192, 195). Garver and Sladek (139) had observed CA neurons in the central gray, anterior to the LC proper. Hwang et al. (193), studying Macaca mulatta, found 7,316 ± 930 (SEM) cells in the LC proper and 749 ± 113 cells in A4. Recent studies of the monkey LC have been consistent with previous reports concerning general distribution and morphology of LC neurons (403, 404).

Also prior to 1977, monoamine histofluorescence studies in human fetal brains indicated that the human LC was very similar to that of nonhuman primates (320, 332). Monoamine fluorescence yielded poor results in adult postmortem brain (324), but more recent immunohistochemical studies with a TH antibody revealed an LC similar to that of the monkey in the fetal (358) and adult (341, 342) human. Also recently, Bogerts (49) mapped melanin-containing neurons throughout the adult human brain stem. The LC was found to be ~1 cm long with cells extending away from the main nucleus into A4 (see also 52), the subceruleus, and rostrally into the mesencephalon in analogy to the rat group A7. Both putative NE and dopamine (DA) cells contained melanin, preventing differentiation of the two cell classes (see also 311). Bogerts (49) argues that melanin is an adequate marker for CA neurons.
because 1) previous studies have indicated that melanin is generated from polymerization of oxidized CA products and 2) the distribution of the melanin-containing neurons in human brain is analogous to that observed for CA neurons in other species.

Bondareff et al. (50) counted LC neurons in adult human brains and found that cell counts in control brains varied between 9,551 and 16,427 per LC with a mean of 12,534 and a standard deviation of 2,336 (see also 475). Vijayashankar and Brody (482) reported a higher mean of 16,840 cells.

3. Other histochemical characteristics of locus ceruleus

Iijima (195) demonstrated that the squirrel monkey LC exhibits hexokinase, monoamine oxidase, L-gulonolactone oxidase, and nicotinamide adenine dinucleotide–linked xylitol dehydrogenase activity, consistent with its catecholaminergic content. The LC neurons have also been found to exhibit acetylcholinesterase (AChE) activity (9, 227, 262). Some observations of indolamine neurons have been made in cat LC (256, 257). Indolamine neurons within or adjacent to LC have been observed in monkey with formaldehyde-induced fluorescence techniques (403, 404, 441, 443). Neurotensin-containing cell bodies have also been reported in the rat LC (478).

C. Afferents to Locus Ceruleus

Information on afferent inputs to LC has come from five types of studies: 1) HRP injections into LC with determination of the distribution of retrogradely labeled neurons; 2) injection of tritiated amino acids into other nuclei with subsequent observations of orthograde terminal labeling in LC; 3) the localization of various types of receptors on LC neurons, implying a chemically appropriate afferent to these neurons; 4) the observation of transmitter-specific fibers and/or terminals in LC; and 5) the demonstration of degradative enzymes for particular transmitters in LC neurons. Of course any particular pathway should ultimately yield positive results on most of these tests. To date, however, there is very little convergence of these various types of information to permit adequate demonstration of any particular system as both a chemically and anatomically specific input to LC. In section III we present related data on the chemical sensitivity of LC neurons as demonstrated by microiontophoresis and the sensitivity of LC neurons to orthodromic electrical stimulation.

1. Horseradish peroxidase injections into locus ceruleus

The major study of this type is the one performed by Cedarbaum and Aghajanian (62). They found a complex array of retrogradely labeled neurons spread throughout the brain and spinal cord.
Forebrain structures containing labeled neurons included the insular cortex, the central nucleus of the amygdala, the medial, lateral and magnocellular preoptic areas, the bed nucleus of the stria terminalis, and the dorsomedial, paraventricular and lateral hypothalamic areas. In the brainstem reactive neurons were observed in the central grey substance, the reticular formation, the raphe, vestibular, solitary tract and lateral reticular nuclei. In particular, the areas of catecholamine cell groups A1 [for more recent information see 3941, A2 and A3 appeared to contain many reactive cells. Labeled neurons were also observed in the fastigial nuclei and in the marginal zones of the dorsal horns of the spinal cord.

Most of these findings have been confirmed by Clavier (72) with the same technique. In this later study, however, labeled cells were not observed in dorsomedial or paraventricular hypothalamic nuclei or in the medial preoptic area. Labeled cells were observed in the dorsal raphe, ventrolateral periaqueductal gray, pontine reticular formation, pontine and medullary NE and epinephrine (E) groups, lateral hypothalamic area, contralateral LC, deep cerebellar nuclei, ventrolateral and parafascicular thalamic nuclei, and the parabrachial regions of the pons and midbrain. An analogous experiment has been performed in the cat (381), but such an experiment is extremely difficult to interpret given the interdigitation of NE and non-NE neurons in the cat LC.

2. Orthograde labeling of terminals in locus ceruleus

Tritiated leucine and proline injections into the ventral tegmental area produce labeled fibers entering the LC, whereas injections into pars compacta of the substantia nigra do not (27). Projections from median and dorsal raphe to LC have been demonstrated with similar methods (76, 360). The nucleus cuneiformis of the midbrain reticular formation has been shown to project into the LC region of the cat (101). Projections from the ventromedial and lateral hypothalamic areas of the rat to the pontine central gray medial to LC, and in some cases more weakly to LC itself, have been demonstrated by orthograde transport (240, 383, 384). Labeled terminals in the cat LC have been observed after injection of labeled leucine into the central nucleus of the amygdala (188). However, other investigators have failed to find labeling over LC after tritiated amino acid injections into central and other amygdala nuclei in the rat (239, 363). In the monkey, Price and Amaral (364) observed no label over the dorsal LC but did observe some over ventral and lateral LC and over pigmented subceruleus cells after injections into the central nucleus of the amygdala.

3. Receptors on locus ceruleus neurons

A high concentration of opiate receptors has been visualized in rat LC with [3H]diprenorphine binding (21). The monkey LC, however, exhibits only
a moderate concentration of these receptors (484). In agreement with physiological results described in section III, a high density of $\alpha_2$-adrenergic receptors has been localized to LC, with a lower density of $\alpha_1$-adreceptors (509). A moderate density of muscarinic acetylcholine (ACh) receptors has been visualized in rat LC (379).

4. Histochemically labeled fibers projecting into locus ceruleus

Fibers displaying $\beta$-endorphin immunoreactivity have been observed originating from cell bodies in the arcuate nucleus and projecting into the anterior pole of LC (44). The monkey LC exhibits sparse to moderate labeling for Met-enkephalin-immunoreactive fibers and terminals (166). Swanson (460) has observed neurophysine-containing neurons in the paraventricular nucleus that project to LC. A network of angiotensin II fibers in LC has also been observed with immunohistochemical methods (136). With an antibody to phenylethanolamine N-methyltransferase (PNMT), Hökfelt et al. (187) observed reactive, presumably adrenergic, terminals in LC. With antibodies to tyrosine and tryptophan hydroxylase, Pickel et al. (355) demonstrated that serotonergic fibers surrounded the perikarya and dendrites of LC catecholaminergic neurons. In these ultrastructural studies, the serotonin varicosities were rarely found to form synaptic contacts with thickened membrane specializations. Nerve terminals exhibiting serotonin uptake have been shown in LC (255). Occasional substance P-positive cells have been observed in LC along with a low to medium density of positive fibers (271). In an ultrastructural immunohistochemistry study, Pickel et al. (356) found substance P and enkephalin axons and axon terminals that were structurally similar to each other and to profiles seen to contact TH-labeled elements. Finally, neurotensin-immunoreactive cell bodies, fibers, and terminals have been observed in LC (478).

5. Acetylcholinesterase in locus ceruleus neurons

To date, only one inactivating mechanism for a putative LC afferent system has been demonstrated. Albanese and Butcher (9), expanding earlier observations (227, 262), have performed a combined CA, Nissl, and ACh histochemistry study demonstrating that the rat LC is composed entirely of CA neurons that are also reactive for ACh. Acetylcholinesterase reactivity was confined to somata and proximal processes.

6. Circulating factors

The close approximation of LC neurons to blood vessels in some species (120, 425) has led to speculation that these neurons may be sensitive to
circulating chemicals (for review see 12). The LC neurons have been shown to concentrate intravenously administered $[^3]H$estradiol and $[^3]H$stanolone (177, 178).

7. Summary

In conclusion, there is no afferent pathway to LC for which the cells of origin, transmitter identity, and postsynaptic effect on LC neurons have been clearly established. The data reviewed above clearly show that LC is not a relay nucleus for primary sensory information and that it is innervated by a large number of brain regions that are presumably functionally diverse. Neurons in these source nuclei have been found to exhibit activity related to internal and external sensory stimuli as well as to affective state. This suggests that the function(s) of LC is (are) integrative and linked to abstract properties of internal and external sensory stimuli. These conclusions are compatible with what is known about LC-NE neuronal activity in behaving animals (see sect. IIIA3).

D. Intrinsic Organization

The data summarized in this section are functionally important and derived from anatomical observations within LC. Recent data concerning the organization of LC dendrites are also summarized and suggest that functional contacts between LC neurons exist.

The study of Grzanna and Molliver (160) demonstrated that there are DBH-positive cells in the roof of the fourth ventricle that extend their dendrites between ependymal cells to the ventricular surface. Thus this subpopulation of LC neurons may be directly in contact with cerebrospinal fluid. In the ventral LC, where neurons are less densely packed, these investigators could visualize LC-NE dendrites and determine that they are extensively ramified, extending well beyond the boundaries of the nucleus. This characteristic was also one of the outstanding features of LC-NE neurons in Golgi studies in the rat. In such material, Swanson (459) found that it was possible to follow dendrites of somata located within LC for up to a few hundred micrometers outside the boundaries of the nucleus. Also using the Golgi method, Shimizu et al. (427) reported that LC dendrites appear to be most extensive in the medioventral direction, extending into the pontine central gray adjacent to LC. This site might constitute a convergence point for LC afferents. Groves and Wilson (157, 158) performed a detailed ultrastructural analysis of rat LC intrinsic organization. In coronal sections, most dendrites and axons appear in cross section, whereas most are cut longitudinally in parasaggital sections. The authors concluded that "locus coeruleus neurons possess disc-shaped dendritic fields parallel to the anterior posterior axis of the brainstem, with predominantly longitudinal axo-den-
dritic synaptic configurations” (157). They also reported that most of the afferents to LC-NE neurons terminate on dendrites 0.5–2.5 μm in diameter or onto spinelike appendages derived from somata and dendrites. Relatively few synaptic endings were found on large proximal dendrites and somata. With glyoxylic acid perfusion and post-fixation in permanganate, Shimizu et al. (426) reported ultrastructural evidence of dendrodendritic and dendrosomatic contacts between LC neurons. Koda et al. (229) saw dendritic profiles containing NE-storage granules in permanganate-fixed sections of the LC. In material from the LC of rats injected with 5-hydroxydopamine (5-OHDA) intraventricularly, Groves and Wilson (158) described three types of labeled presynaptic profiles: 1) putative serotonin-containing, 2) putative CA axons of intrinsic or extrinsic origin, and 3) LC dendrites. There were no differences observed between various categories of either labeled or unlabeled afferent terminals as to their distribution on postsynaptic neuronal targets in LC (157, 158). In light-microscopic studies, DBH has been observed in somata and proximal dendrites of rat LC neurons (71, 160), an observation borne out by ultrastructural immunocytochemical examination (71). Catecholamine fluorescence reveals NE in LC dendrites of primates (115, 442). Thus there is substantial evidence that NE as well as the enzyme required for its manufacture reside in LC dendrites. Physiological evidence that NE is released from LC dendrites as a consequence of impulse activity has also been recently developed (229; see sect. III.B).

There have been reports of special vascularization in the LC of various species. Felten and Crutcher (116) described “direct apposition of blood vessels to perikarya and dendrites” of LC neurons in squirrel and rhesus monkeys. No glia interposition was found between cells and vessel basement membranes. The LC in rhesus monkey was also reported to have a dense capillary bed relative to other brain sites but not relative to the paraventricular or supraoptic nuclei (120). Interestingly, Finley and Cobb argued, based on vascular differences, that the mesencephalic nucleus of V and the LC are functionally distinct units, a clarification that was not finally resolved in some quarters until the advent of CA fluorescence; for example, an atlas of the squirrel monkey brain published in 1963 labels a large part of LC as the mesencephalic nucleus of the trigeminus (103).

E. Locus Ceruleus Efferents: Pathways and Termination Patterns

In this section, light-microscopic data concerning the trajectories and arborization patterns of LC efferents are discussed. This literature is massive, which is not surprising given the extensive distribution of LC efferents. Immunohistochemical methods have provided substantial new insights into the distribution of LC efferents, especially in regions such as the neocortex where these fibers are of very fine caliber. These neocortical termination patterns are described in detail in order to document the specificity of LC innervation in certain brain areas.
The most general descriptions of LC efferent pathways and termination sites have been generated by CA histochemical methods (135, 266, 269, 479) and by injection of tritiated amino acids into LC with subsequent tracing of efferent pathways (51, 206, 357). Lesion and biochemical studies have determined the contribution of different source nuclei to the NE innervation of certain terminal areas and the relative density of innervation of different terminal areas by LC. For example, Jones et al. (204) found that medullary NE neurons provide ~75% of the NE innervation of hypothalamus, 25% of thalamus and midbrain, and none of cortex or hippocampus. Kobayashi et al. (228) demonstrated that unilateral LC lesions produced the magnitudes of NE losses in various terminal areas that would be expected from anatomical studies.

1. Neocortex

Descriptions of the NE innervation of neocortex have progressed in complexity and specificity as a direct result of improvements in techniques for visualizing these fine-caliber fibers. The early freeze-dried CA fluorescence techniques (56, 110, 111) provided the first histological evidence of the existence of NE fibers in cerebral cortex (135) and were used in combination with lesion techniques to suggest that the cell bodies of origin for this projection were contained within brain stem NE-containing cell groups (13).

These fluorescence techniques revealed a very sparse CA innervation throughout several cortical regions with the highest density in limbic cortices. The innervation appeared especially sparse in neocortical regions, and, although it was regionally widespread, it was thought to be largely restricted to the molecular layer (13, 24, 135). In 1968, Fuxe et al. (137) utilized refinements of the original CA fluorescence techniques to provide the first detailed regional and laminar analysis of CA cortical innervation, which they suggested was primarily NE on the basis of its biochemistry. Fibers were observed in many allo- and isocortical areas; however, terminals still appeared to be rare or even absent in some regions of neocortex. In 1971, Ungerstedt (479) used a modified Falck-Hillarp fluorescence technique combined with smear preparation and lesions to provide more convincing evidence that the LC was the source of NE neocortical fibers and to describe the pathways by which LC-NE fibers reached and penetrated various cortical regions in greater detail. Ungerstedt (479) suggested that neocortical NE innervation was achieved by a dorsal bundle of fibers that curved anteriorly through the septum, traveled caudally within the cingulum bundle, and furnished laterally directed branches that innervated the entire neocortex. These observations provided crucial anatomical support for the existence of a unified, direct, monosynaptic NE projection ascending from the brain stem to the cerebral cortex.

In the 11 years since Ungerstedt's study, anterograde transport and more sensitive histochemical procedures have been used to develop a more
detailed description of the trajectory of the cerulocortical projection (51, 206, 266, 279, 357). It now appears that the pathway through the septal region may be only one of three major routes that NE fibers follow as they enter the cerebral cortex. A lateral route through the ventral amygdalofugal pathway and an intermediate trajectory through the ventral caudate and around the rostral portion of the external capsule have also been described (206, 428, 473). The specific contribution of each of these pathways to cortical NE innervation has remained a difficult issue to resolve. The pathway from LC to forebrain termination sites has now been demonstrated in monkey with anterograde transport techniques (51), and its trajectory is very similar to that previously observed in the rat and monkey by other methods (140).

The lack of sensitivity of early histochemical methods in revealing cortical NE fibers became apparent with the introduction of the glyoxylic acid histofluorescence method (43, 266), which revealed that the actual density of the CA innervation of cortex had been greatly underestimated. With the glyoxylic acid method, as well as with anterograde and retrograde transport techniques, Freedman et al. (127) demonstrated that the NE innervation of monkey neocortex originated in LC. This innervation was more dense than that previously described for either the rat or monkey (24) and was distributed over all six layers of neocortex. Subsequently, others made similar histofluorescent observations in the rat (259, 264, 265) and cat (198). It has now been repeatedly demonstrated that LC is the primary (and probably the only) source of NE fibers in a number of neocortical regions in the primate (94, 141, 191, 223, 296, 297).

Immunohistochemical techniques have revealed an even more elaborate and orderly NE innervation of cortex. Most recently, antisera directed against the final NE-synthesizing enzyme DBH have proven to be extremely sensitive and specific in the characterization of the NE projection to cortex. With an antiserum directed against rat DBH and a highly sensitive glyoxylic acid method, Molliver and colleagues (161, 162, 261, 264, 265, 307-310, 331) have performed a detailed analysis of the pattern and organization of the NE innervation of rat cortex. With DBH immunocytochemistry, the NE innervation of cortex appears much denser than previously reported, extending throughout all six cortical layers (307). Also the pattern of NE axon distribution possesses a geometric orderliness and distinct laminar pattern that is consistent throughout the lateral neocortex (307). In contrast to the uniform pattern of innervation present in the lateral cortex, the medial cortex possesses regional variations in pattern and density of NE innervation that adhere strictly to cytoarchitectonic boundaries (261, 309; for an interesting example revealed by CA histofluorescence, see 265). The NE fibers in both medial and lateral cortex are found to be primarily distributed in a tangential 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 (308, 310). In addition, it has been demonstrated that the cingulum bundle is not the major intracortical NE
pathway in rodent, because it furnishes only locally arborizing branches. Rather the dorsolateral neocortex is innervated by a group of NE fibers that continue rostrally from the medial forebrain bundle (MFB) into the frontal pole, turn dorsally over the subcortical white matter, and continue caudally through the deep layers of cortex, supplying the NE innervation throughout their trajectory (308, 310).

Investigation of the NE innervation of primate cortex has been limited (127, 305, 306). Clearly, given the extensive development of neocortex in primates, descriptions of intracortical trajectory and laminar distribution in the rat provide only sketchy predictions concerning the characteristics of these features in the primate. Recently, in collaboration with Morrison (306), we have been using an antiserum directed against human DBH (130, 324) to visualize NE fibers in the cortex of normal and lesioned monkeys to provide initial characterizations of intracortical NE pathways and the laminar distributions of NE fibers in three distinct neocortical regions. Although the NE innervation of primate cortex exhibits a far greater degree of regional variation in density and pattern than is seen in the rat cortex, a strong tangential, intracortical trajectory similar to that observed in the rat (310) is a dominant feature of the NE innervation of the gyrencephalic primate brain. To date, the cortical regions examined in detail have been the dorsolateral prefrontal cortex and the primary somatosensory cortex of the squirrel monkey and the primary visual cortex of the cynomolgus and squirrel monkey. These three functional regions have been found to differ in density of NE innervation, and each has been found to exhibit a unique laminar pattern of NE innervation. Our observations on density of innervation have shown good agreement with previously published biochemical measurements of NE levels (53).

We (305) have also studied the distribution and organization of serotonergic fibers in primary visual cortex. The NE and serotonergic projections in this neocortical region exhibit laminar complementarity; layers V and VI receive a dense noradrenergic projection and an extremely sparse serotonergic projection, whereas layer IV receives a very dense serotonergic projection and is largely devoid of NE fibers. Additionally, the NE fibers manifest a geometric order that is not readily apparent in the distribution of serotonergic fibers. These patterns of innervation imply that the two transmitter systems participate in different stages of cortical information processing; the raphe-cortical serotonergic projection may preferentially innervate the spiny stellate cells of layers IVA and IVC, whereas the cerulocortical NE projection may innervate pyramidal cell dendrites. The suggestion that NE fibers terminate on pyramidal cell dendrites is similar in some respects to one made by Caviness and Korde (58) based on data summarized in section II.F.

These characteristics of the NE innervation of primate neocortex suggest that, coincident with the extensive phylogenetic development and differentiation of neocortex, there is a parallel elaboration and differentiation
of the cerulocortical projection, perhaps reflecting increased functional specialization of this system in the primate (123, 125).

2. Thalamus

There have been few new developments in descriptions of the LC-NE innervation of thalamus beyond the extensive work of Lindvall et al. (266, 268) in the rat. These authors described the ascending fibers from LC that course through the mesencephalon in the dorsal tegmental bundle, through the zona incerta, into the MFB. Branches then leave the MFB to innervate many diencephalic regions, most notably the anterior, ventral, and lateral nuclear complexes, and the medial and lateral geniculate bodies. The rat lateral geniculate nucleus (LGN) receives a bilateral LC innervation that is the sole source of NE innervation for this nucleus (247). The innervation of ventral LGN is moderate, whereas that of dorsal LGN is very dense. Ishikawa and Tanaka (197) found LC-NE fibers throughout most of the thalamus of the rhesus monkey, except for the midline and medial nuclei. The pulvinar was found to be sparsely innervated with LC-NE fibers.

3. Amygdala

Fallon et al. (112) describe the LC-NE innervation of amygdala as arising from fibers entering the posterior basal forebrain via both the stria terminalis and ansa peduncularis-ventral amygdaloid bundle system. These fibers then form a moderately dense innervation of the central and basolateral nuclei of the amygdala and a less dense innervation of other amygdala areas. Fallon and Moore (113) have also studied in some detail the NE innervation of the olfactory bulb, anterior olfactory nuclei, olfactory tubercle, and piriform cortex with biochemical and CA fluorescence techniques. Labeled LC cells are seen after HRP injections into the rat or cat olfactory tubercle (444). Halasz et al. (167) have described NE terminals in the granular and external plexiform layers of the rat olfactory bulb. An LC projection to the anterior amygdaloid area and the central nucleus of the amygdala has been demonstrated in monkey with orthograde transport techniques (51), and labeled LC neurons have been shown after HRP injections into monkey amygdala (294, 321). Similar results have been obtained in the rat and cat (336).

4. Hippocampus and septum

The NE innervation of hippocampus was first described comprehensively by Blackstad et al. (32) by use of Falck-Hillarp techniques. The NE terminals were observed throughout the hippocampal region, most densely in the hilus...
of the area dentata. In the cortical areas approaching hippocampus there was no clear differentiation of regions on the basis of NE innervation. However, Collier and Routtenberg (73) have observed fibers of a morphological type suggesting an LC origin innervating only a restricted posterior portion of entorhinal cortex, an area where HRP injections have been shown to produce labeled cells in LC (26). A subsequent description by Swanson and Hartman (462) of hippocampal fibers visualized by DBH immunohistochemistry differed slightly from the CA fluorescence study, possibly because of the difficulty of resolving DA from NE fibers with CA fluorescence. More recently, Loy et al. (275) have demonstrated that LC is the sole source of hippocampal NE in the rat and that the projection is 75-90% ipsilateral. They describe the pattern of innervation as being similar at all septotemporal levels, with the densest terminal and preterminal elements localized to the infragranular hilus of the area dentata, stratum lucidum of CA3, and the molecular layer of the subiculum (see also 420). In the monkey, HRP injections at various rostrocaudal levels of hippocampus have been shown to label cells in ipsilateral and contralateral (~10% of ipsilateral) LC. Labeled cells were observed throughout the length of the LC in both dorsal and ventral divisions, with a few cells subjacent to LC (11). These findings are similar to those of an earlier study in the rat (420).

Use of HRP injections has also shown that LC projects to the septum in rat (421) and monkey (238). With CA fluorescence histochemistry, Moore (302) and Lindvall and Stenevi (269) have described this septal innervation as moderately dense and including the anterior hippocampus, the medial septal nucleus, the nucleus of the diagonal band, and the interstitial nucleus of the stria terminalis, as well as a sparse innervation in the lateral septal nucleus and the septofimbrial nucleus. Moore (302) found that hemisections posterior to LC produce a 47% decrease in septal NE, and LC lesions cause a similar depletion. This is interpreted to indicate that half of the septal NE innervation arises from LC and half from more posterior NE cell groups.

5. Hypothalamus

The NE innervation of hypothalamus is extremely complex in that various hypothalamic nuclei receive different densities of NE innervation from one or more of three different NE source nuclei. For example, Sawchenko, Swanson, and colleagues (394, 395, 463) recently studied the organization of NE pathways from the brain stem to the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus in the rat. They have found that injections of the retrograde tracer “true blue” into either the PVN or SON label NE cells in A1, A2, and LC (394). Thus immunohistochemical studies of NE fibers within PVN may, may not, or may partially be studies of LC terminal fibers. Furthermore, after either injection, LC neurons constitute only a minority of retrogradely labeled NE neurons. In addition to
these observations from retrograde transport studies, Sawchenko and Swanson (395) have also injected anterograde tracers into the NE source nuclei. Each source nucleus innervates distinct subnuclear regions within PVN and SON. The LC appears to innervate only the most medial part of the parvocellular division of PVN. Because cells in this subnucleus project to the median eminence, Sawchenko and Swanson suggest that LC may selectively participate in neuroendocrine functions rather than in the autonomic functions suggested for other PVN subnuclei. Sawchenko and Swanson (395) did not see detectable labeling in SON after tritiated amino acid injection into LC. They suggest that LC may not project to SON and that the retrograde LC labeling after "true blue" injections into SON may be caused by uptake by fibers of passage (but cf. 206). Iijima and Ogawa (196) report that HRP injections into the SON result in dense labeling of a few multipolar cells in both the dorsal and ventral LC. In view of these results, the possibility exists that these few labeled cells result from damage to fibers of passage. Additionally, combined HRP/CA fluorescence data indicate that the NE innervation of the medial preoptic area arises from A1 and A2, not from LC (88), and Palkovits et al. (338) have obtained biochemical data suggesting that A1 is the primary source of hypothalamic NE fibers. McKellar and Loewy (290) have also investigated the NE innervation of the PVN with orthograde transport techniques. They find that LC projects to the posterior, periventricular, parvocellular, and dorsal divisions. Swanson et al. (463) have studied the PVN and SON with antibodies to TH, DBH, and PNMT and found that NE fibers appear to innervate the periventricular zone of the PVN (probably from LC cells of origin) and those parts of the PVN and SON that contain predominantly vasopressinergic neurons (probably from A1 and A2 cells of origin). McNeill and Sladek (293) studied the CA input to magnocellular neurosecretory neurons in rat SON and PVN by examining sections with simultaneous monoamine histofluorescence and neuropeptide immunocytochemistry. Although the major CA innervation to these nuclei did not coexist with the major concentrations of magnocellular perikarya, there was considerable overlap of CA processes and peptide-reactive neuritic processes. Both oxytocin- and vasopressin-reactive profiles were overlapped by CA fibers. Axosomatic contacts were more numerous over vasopressin neurons. Within the PVN, the pattern of CA innervation was heterogeneous with respect to the various subnuclei. This suggested to the authors that "each subnucleus of the PVN with its individual hypothalamic, neurohypophyseal, brainstem, or cortical projection may possibly receive a catecholaminergic innervation by a select group of CA cells or nuclear groups from the brain stem." Pickel et al. (357) and Jones and Moore (206) have described LC projections to other hypothalamic nuclei revealed by anterograde transport methods. There has been a CA fluorescence study (197) of the monkey hypothalamus that is difficult to interpret because both DA and NE fibers are visualized by this method; furthermore it has not yet been determined which hypothalamic fibers originate from LC. In general the morphological char-
acteristics and distribution pattern of CA terminals were similar to those of the rat, but some differences were observed in the suprachiasmatic nucleus, the arcuate nucleus, and the internal layer of the infundibulum.

6. Other brain stem nuclei

Kromer and Moore (245, 246) have studied the LC innervation of the cochlear nuclei, which is bilateral, highly collateralized, and apparently not topographically organized. Injections of HRP into the nucleus tractus spinalis nervi trigemini of the rat produce labeled LC neurons bilaterally and labeled A1 neurons ipsilaterally.

7. Spinal cord

The NE innervation of spinal cord, like that of hypothalamus, is very complex in that the NE fibers have several distinct termination zones, and the innervation of different zones originates in different brain stem nuclei. Nygren and Olson (323) first dissected the portions of spinal cord NE innervation that originate in LC by combined lesions and histofluorescence. After bilateral LC lesioning, profound CA terminal loss was observed in the ventral column, intermediate gray, and the ventral half of the dorsal column. Many terminals in the dorsal half of the dorsal column, CA terminals in the thoracic sympathetic lateral column, and the band of terminals crossing the midline and innervating the central gray were unaffected by the lesion. Subsequent studies have largely confirmed this initial description.

In a study involving HRP injections, lesions, and electrical stimulation, Ader et al. (1) interpreted their findings to indicate that no more than 30% of the NE in the rat spinal cord is contained within LC projections, which they found to be predominantly ipsilateral. This interpretation of laterality disagrees with that of Karoum et al. (214), who found that LC lesions deplete 5% of the NE in contralateral spinal cord and 20–40% ipsilaterally, the exact depletion depending on the level of cord assayed. Commissiong (74), examining CA fluorescence in the cord after various lesions, found evidence that LC does innervate the cord bilaterally and that the decussation occurs at the spinal level. Martin et al. (285) found evidence for a bilateral projection in the opossum.

Commissiong (74) also found that, in the rat, LC does not innervate the lateral column nucleus or the dorsal commissural nucleus of the thoracic cord, and he argues that the LC is therefore unlikely to be involved in the regulation of preganglionic sympathetic neurons. These observations are in general agreement with those of Fleetwood-Walker and Coote (121), who assayed NE levels at segment T3 of the cat spinal cord after lesions of various NE nuclei. The sympathetic lateral column was primarily innervated by A1 (presumably an adrenergic input), with smaller contributions from A2 and
A6 (see 146 for an immunohistochemical description of this innervation). A projection from A5 to the intermediolateral cell column has been demonstrated, and this may well constitute the primary or sole noradrenergic innervation of this area (272). McLachlan and Oldfield (291) have described the NE innervation of the intermediolateral cell column in detail, but they did not address the question of the nucleus of origin of this input.

The LC was also shown to provide the sole NE input to ventral horn (see also 75). An earlier CA fluorescence study of this region found that fluorescent terminals rarely appeared near α-motoneurons, but interneurons were sometimes heavily innervated (209). Using retrograde transport of antibodies to DBH, Westlund et al. (494) found in the rat that spinal cord injections produced labeled cells in LC, subceruleus, medial and lateral parabrachial nuclei, Kolliker-Fuse nucleus, and the region dorsal and lateral to the superior olivary nucleus. There was no labeling of caudal NE groups. These investigations interpret this finding to be compatible with that of Karoum et al. (214), who show that LC alone supplied 70-90% of spinal cord NE at certain levels.

Recently the descending projections of the monkey LC have been investigated in detail by Westlund and Coulter (495). They found that, unlike in the rat, there was a substantial LC innervation of parasympathetic areas such as the dorsal motor nucleus of the vagus, the region of the nucleus ambiguus, and the sacral spinal cord. The subceruleus region was observed to project to the sympathetic intermediolateral cell column of the thoracic cord. In the spinal cord, both LC and subceruleus project to the ventral gray, the dorsal horn, and the region around the central canal.

In the rabbit, double labeling of cells for CAs and HRP after spinal cord HRP injections indicates that spinal NE innervation originates almost exclusively from the pons, the cells of origin being localized to A5, A7, subceruleus, ventral LC, and posterior LC, including the A4 cell group (34).

8. Topographic organization within locus ceruleus

The organization of LC efferents is unusual in that a relatively small number of neurons project to many diverse, sizable brain regions. Presumably, each LC neuron sustains a highly divergent axon that innervates a large terminal field. The organization of these efferents with respect to cells of origin is unclear. For the vast majority of terminal areas, it is unknown whether certain subpopulations of LC neurons preferentially supply given pathways or given terminal fields. Studies of this question have examined only the most rudimentary of possible organizing principles, i.e., that LC neurons are topographically organized (e.g., spatially clustered) according to the target(s) of their projections.

The first terminal region demonstrated to receive an LC-NE projection from a restricted subset of LC neurons was the spinal cord. In the rat (393),
It was shown that after HRP injections into the spinal cord, labeled LC neurons were restricted to the ventral pole of the nucleus and to the subceruleus. Labeling of restricted subsets of LC neurons was also found after HRP injection into other terminal fields by Mason and Fibiger (287). Hippocampal injections, for example, primarily labeled cells in the dorsal portion of LC proper, as did injections into septum. Injections into caudate putamen or cerebellum labeled more ventral populations of cells. There was also differentiation along the anterior-posterior axis of LC: thalamic injections labeled more posterior neurons and hypothalamic injections labeled more anterior subsets of cells. Mason and Fibiger (287) conclude that these results are incompatible with the view that LC is homogeneous with respect to source neurons for projections to different terminal fields. There has as yet been no systematic analysis of this question with accurate, quantified three-dimensional reconstructions of LC (see 126).

Finally, a recent study found that after HRP injection into the ventrobasal thalamus of the rat nearly every cell in LC is labeled, as well as some contralateral cells (132). This finding may indicate an exceptional LC innervation of this diencephalic area.

Double labeling of LC neurons by two different retrogradely transported dyes injected into different terminal areas has been used to study whether individual LC neurons innervate both of the injected target regions. For example, Ader et al. (2) examined the bilaterality of LC forebrain projections with this technique. These authors found double-labeled LC cells after injection of different fluorescent dyes into the two hemispheres at the following sites: cortex-cortex, hippocampus-cortex, thalamus-cortex, thalamus-hippocampus, and in fewer cases bilaterally into hippocampus. These results are similar to those of Room et al. (378), who found double-labeled LC cells after injecting unilaterally or bilaterally any pair of sites in cortex, thalamus, or hippocampus. They also found double-labeled cells after injecting spinal cord or any one of the forebrain sites. Nagai et al. (314) found double-labeled LC cells after one injection into frontal cortex and a second into cerebellar or occipital cortex or into spinal cord. Similar results have been obtained by Steindler (449), who found double-labeled cells after cerebellar and cerebral-cortical injections. All of these results are compatible with earlier antidromic (AD) activation (315) and lesion (333) data suggesting that a single LC neuron could project both to cerebellum and cerebral cortex. Finally, Sawchenko and Swanson (395) have not found double-labeled LC neurons after injections into PVN and spinal cord, injections that single label many LC neurons, including a subregion of LC where the single-labeled cells are intermixed.

Loughlin et al. (274) examined the relationship between individual cells of origin within LC and the geometry and distribution of terminal fields in rat cortex. Simultaneous injections of multiple fluorescent dyes into different cortical regions were used to infer the intracortical arborization patterns of LC axons. Double-labeled cells were found to be more numerous after
injections that were aligned in the rostrocaudal axis than after injections aligned in the mediolateral axis. Double-labeled cells were also evident after simultaneous injections into superficial and deep portions of the same cortical area. The authors interpret these results to indicate that individual LC cells innervate functionally and cytoarchitectonically distinct cortical regions simultaneously and that LC neurons arborize more extensively along the rostrocaudal axis of cortex than along the mediolateral axis. Also, individual LC neurons innervate both superficial and deep layers of a cortical region.

Finally, there is now some evidence that LC-NE projections may not be bilaterally symmetrical. Oke et al. (325) found hemispheric differences in NE concentrations in human thalamus. These concentrations were highest in the ventral thalamus, especially ventralis posterior lateralis and ventral posterior medial nuclei, which had higher NE concentrations in the right hemisphere. In the pulvinar region, NE was higher in the left hemisphere. More recently, Oke et al. (326) have also found biochemical evidence of lateralization of NE levels in rat thalamus.

9. Locus ceruleus innervation of blood vessels

Edvinsson et al. (100) observed NE fibers in close approximation to blood vessels within the brain, even after removal of the superior cervical ganglion. However, these authors point out that approximation at the light-microscopic level does not satisfy the criteria for innervation of brain vessels. Hartman (172) sees NE fibers in close approximation to small intracerebral blood vessels, especially within hypothalamus, medulla, and upper spinal cord. Superior cervical ganglionectomy does not decrease the incidence of such contacts, which therefore presumably arise from brain NE neurons. In cortex, these contacts are not dense or consistent and probably do not represent vessel innervation. Swanson et al. (461) found ultrastructural evidence of central monoaminergic innervation of blood vessels in PVN of animals with the superior cervical ganglia removed. After 5-OHDA treatment, dense-core vesicles were seen making contact with the basal lamina surrounding capillaries. Other investigators have used immunocytochemical methods to label NE fibers but have not observed such ultrastructural contacts of NE fibers with intracerebral capillaries (331).

10. Summary

As techniques for visualizing NE efferents become more sensitive and specific, and as various termination sites for LC-NE axons are investigated more systematically, it is becoming evident that LC-NE arborization patterns are orderly, site specific, and in some cases much more dense than was previously thought. The LC-NE innervation of hypothalamus offers an in-
teresting example in that the projection zone of LC terminations is very limited, and it is very close to termination sites for fibers arising from NE cell bodies in caudal brain stem nuclei. This segregation of NE innervation from different source nuclei indicates that whatever developmental mechanisms exist to specify a particular class of neurons as recipients of NE innervation, they can also specify that these fibers originate from a particular NE nucleus. Finally, there is evidence that the constituent neurons of LC, rather than being randomly distributed within the nucleus, are topographically organized with respect to their efferent projections.

F. Ontogeny of Locus Ceruleus and Its Efferents

The ontogenetic development of the LC and its efferent projections is of special interest for two reasons. First, this system is extremely precocious, its efferents often arriving in terminal areas prior to the arrival of other major afferent systems. Second, LC-NE efferents have repeatedly been postulated to play a crucial role in the development of various brain regions, especially neocortex. Some of these proposals, and the data presently available for evaluating them, are reviewed below.

1. Descriptive studies

The differentiation of rat LC neurons has been studied with tritiated-thymidine autoradiography to date the neurogenesis of these cells (253). Peak LC labeling was evident in those animals injected on days 10–13 of gestation, compared with peaks on days 14–15 for cerebellar Purkinje cells and days 13–18 for hippocampal cells. That is, LC neurons are born well in advance of neurons in these LC target areas. The LC has also been shown to receive synaptic input as early as days 16–20 of embryogenesis, although most synaptogenesis occurs postnatally and continues into adulthood (254, 430, 431). Rat LC neurons exhibit CA fluorescence as early as embryonic day 14 (335, 431). Detailed descriptions of the migration, convergence, rearrangement, and differentiation of the LC into its adult form in the rat are available (335, 422, 431, 445, 446).

In 3- to 4-mo-old human fetuses, CA fluorescence reveals that the LC is well developed, and major axonal pathways are already evident (67, 320). Similar observations of LC have been made by others, with the additional finding that at this age there are CA fibers in neocortex (334). More recently, using TH immunocytochemistry, Pickel et al. (358) have found the LC neurons are not evident at 5 wk of gestation but are at 12 wk. At this age, labeled axons are also visible in CA terminal areas. By 17–21 wk of age, LC and other CA cell groups have coalesced into distinct cell clusters, and neuronal perikarya and processes have become more highly differentiated.

Development of neocortical NE innervation in the rat has been studied
extensively. Schlumpf et al. (399), using CA fluorescence, observed superficial
cortical fibers as early as embryonic day 16. These enter chiefly at the ven-
trorostral portion of neocortex then bifurcate into the deep and superficial
layers of the cortex. Over the next few days, the innervation progresses in
ventral-to-dorsal and rostral-to-caudal directions, covering the entire cor-
tical hemisphere. The segregation between superficial and deep fibers is
generally maintained in the embryo, with fibers only rarely crossing the cortical
plate. Similar observations have been made by Levitt and Moore (260), who
found that the adult pattern of innervation is evident by the end of the first
postnatal week. In the 6-day-old rat, Lidov et al. (264) observed CA fibers
in all layers and regions, although the superficial and deep plexi were still
evident with relatively few fibers traversing the cortical plate. These CA
fibers were eliminated by a midbrain lesion. This is compatible with the
observation that HRP injections into the somatosensory cortex of 2- to 8-
day-old rats label LC and possibly A5 but no other midbrain or hindbrain
cells (242). An earlier study by Loizou (273) traces the anatomical and bio-
chemical development of the monoamine systems during the first weeks of
postnatal development. Many of these CA fluorescence observations con-
cerning the innervation of neocortex have been confirmed with TH immu-
nocytochemistry (445, 446).

At the ultrastructural level, Molliver and colleagues (78, 301) found that
30% of all synaptic terminals in the lateral neocortex of the newborn rat
contain small granular vesicles (SGVs) after treatment with 5-OHDA. Such
terminals were especially dense in layer IV, and some were present in the
marginal zone. Kristt (241), using similar methods, determined that SGV
synapses account for 50-70% of all synapses in the primordium of layer IV
in somatosensory cortex of 1- to 7-day-old rats and mice. The number of
these synapses is markedly decreased by systemic 6-OHDA administration
or by 6-OHDA or mechanical lesion of the ascending dorsal noradrenergic
bundle, indicating their monoaminergic nature and brain stem origin (512).

The NE innervation of the rat hippocampal formation is also substan-
tially developed before birth, being present within this target nucleus while
cytogenesis and cell migration are still active processes (276). The adult
pattern of innervation is essentially present by postnatal day 10 (276). The
NE innervation of spinal cord has also been shown to develop at an early
stage (439). In the rat cerebellum, NE terminals are present at birth and
attain an adult pattern by 4-5 wk of age (504).

2. Manipulative studies: alterations in target regions

Several studies have dealt with the various factors controlling the de-
velopment of neocortical innervation patterns and density.

Caviness and Korde (58) used CA fluorescence to study innervation pat-
terns in normal and reeler mutant mouse embryos. Like others, they observed
fibers appearing rostrally in neocortex of both genotypes within 24 h of the development of the cortical plate. These fibers are distributed to all regions of the neocortex by birth. The laminar distribution of these fibers was different for the two genotypes in a way, the authors argue, that indicates that the same cell types serve as targets in both cortices. First, CA fibers become highly ramified when they enter the layer containing polymorphic cells, whether this is a deep layer as in normal cortex or a surface layer as in reeler. Second, the zones dense with apical dendrites of pyramidal cells, more superficial in normals, are also characterized by CA fiber concentration and branching. In contrast, these fibers pass through the zone of concentration of pyramidal cell somata with little branching. These observations, like those described for the primate visual cortex, suggest a specific neuronal target(s) for cortical noradrenergic input.

In another study of the effects of genetic alteration at a target site, Scholer and Sladek (405) have determined that the SON of the Brattleboro rat has an altered afferent noradrenergic innervation. The Brattleboro, with no vasopressin, had fewer fluorescent varicosities in apposition to the vasopressin-deficient perikarya. The oxytocin-producing neurons in the same nucleus were hyperinnervated, suggesting that the target neuron peptide, vasopressin, is necessary for the maintenance of normal NE innervation patterns. As noted earlier, it is not clear whether this particular NE innervation arises from LC.

The effects of prenatal treatment with the antimitotic agent cyasin on monoamine innervation of cortex have been studied in some detail (202, 203, 208). Hyperinnervation is observed in the atrophied cortex and other atrophied target regions, suggesting that NE axons are programmed to produce a certain quantity of nerve terminal arborizations in target regions, independent of target cell development, which suggests a high degree of intrinsic growth regulation.

The effect of neonatal irradiation on the NE innervation of hippocampus has been studied by Moore et al. (304). Irradiated hippocampi, with a marked loss of dentate granule cells, show a normal innervation of Ammon's horn but a substantially altered innervation of the dentate. In the dentate, there is an apparent decrease in total innervation and a redistribution of the fibers that are present, observations suggesting guidance and growth promotion by the appropriate terminal targets. There was also, however, some evidence that the NE innervation developed without regard to the development of the terminal area, i.e., NE content of the hippocampus was not decreased, and CA3 and dentate molecular layer exhibited apparent increases in innervation.

3. Manipulative studies: elimination of norepinephrine input

Because the NE innervation of target regions occurs so early in development, it has often been hypothesized that NE plays a crucial role in the
subsequent development of neurons in the target region or of other afferents that arrive at a later stage of development.

Kasamatsu, Pettigrew, and colleagues (216, 217) have performed an extensive set of experiments supporting the hypothesis that the NE innervation of primary visual cortex is essential for the plasticity of ocular-dominance characteristics that is seen during a postnatal critical period. If a normal kitten has one eyelid sewn shut, the majority of neurons in primary visual cortex will shift from being predominantly binocular to being driven preferentially by the open eye. This ocular-dominance shift is prevented by any 6-OHDA treatment that eliminates the NE innervation of visual cortex. Local perfusion with NE has been reported to restore ocular-dominance plasticity in kittens older than the critical period and in animals previously treated with 6-OHDA (218, 346). The authors report that NE itself does not alter ocular-dominance properties of neurons; NE effects are evident only when the animal's visual experience is simultaneously altered. In another study (219) this group monocularly deprived kittens at 4 wk of age and then gave them normal visual experience for 1–30 days. Recovery of normal binocular driving of neurons in visual cortex was assessed during this period. Superfusion of NE accelerates recovery, and 6-OHDA administration retards it. This experiment is intended to demonstrate that NE enhances plasticity, even when that plasticity involves an increase in binocularity.

In one of the earliest studies concerned with the possible trophic influence of NE on developing cortex, Maeda et al. (280) lesioned LC at birth, then studied cortical neurons with the Golgi method at 1–2.5 mo of age. Layer VI pyramidal cells in the LC lesioned hemisphere tended to have longer apical dendrites, often reaching layer I. The authors interpreted this to indicate immaturity in the lesioned hemisphere. However, subsequent studies have failed to replicate this effect. Ebersole et al. (97) have injected 6-OHDA into newborn rats and examined subsequent development of the visual cortex. They found no qualitative or quantitative differences in lamination, cytoarchitecture, cell density, or cell size in the animals whose noradrenergic input had been eliminated. Wendlandt et al. (493) and Lidov and Molliver (263) have found a similar lack of effect of noradrenergic lesions on neocortical development. However, Parnavelas and Blue (48, 340) have recently described an increased density of synapses in the visual cortex of rats treated with 6-OHDA during the first week of postnatal life, and they postulate an inhibitory effect of NE on synaptogenesis. Felten et al. (117) administered 6-OHDA systemically to neonatal rats that were subsequently sacrificed at 8 wk of age. In these animals there was a substantial increase in the number of cortical pyramidal cells exhibiting certain abnormalities of dendritic and soma morphology. These effects were evident only in animals that had received four doses of 6-OHDA. Another line of investigation questions whether elimination of the noradrenergic innervation of a particular region enhances or diminishes the innervation of this region by other afferent systems. For example, it has been found that there are no alterations in the development of the mesocortical dopaminergic system after lesions elimi-
nating cortical NE innervation (468). Amaral et al. (10) described the effects of 6-OHDA lesions on sprouting of other afferents in the hippocampus.

4. In vitro studies

Schlumpf et al. (398) have shown that LC neurons in explant cultures from embryonic rat brain exhibited normal appearance in fluorescence microscopy, ultrastructurally defined synapses, and biochemically measured NE. Dreyfus et al. (95) have studied cocultured hippocampal and LC explants from fetal mice. Under these conditions, fibers grew out from the LC explant into the hippocampal explant. These fibers were varicose and exhibited NE uptake. A similar innervation of cocultured dissociated neurons of the spinal cord by LC explants has been observed (284).

5. Regeneration and responses to injury

There have been numerous studies of central noradrenergic neuronal responses to various types of lesions. The regeneration or sprouting observed after such treatments may well provide knowledge about the mechanisms of normal development (e.g., see 400–402). However, because these topics have recently been reviewed extensively (131), we will not discuss them here.

III. LOCUS CERULEUS PHYSIOLOGY

In this section we review current knowledge of the physiology of LC neurons. Beyond the material concerning the afferent and efferent anatomy of this neuronal system, it is necessary to determine what factors are capable of influencing activity in LC neurons to better understand the role of LC in brain functions. The relevant data are organized into three main topics: 1) spontaneous and sensory-evoked activity of these cells as studied in anesthetized or paralyzed preparations, as examined in unanesthetized animals, and as seen for known NE-containing LC neurons in behaving animals; 2) antidromic activation and axonal conduction properties; and 3) pharmacological sensitivity. Many factors influence activity in the LC-NE system, and much research on the physiological characteristics of these cells defines the specific conditions under which the neurons of this nucleus are activated.

A. Spontaneous and Sensory-Evoked Activity

1. Anesthetized or paralyzed preparations

In anesthetized animals, LC-NE neurons discharge spontaneously in a slow, regular fashion. Although most studies have used chloral hydrate
anesthesia (3, 6, 20, 30, 59, 152, 322, 455), a few have used urethan anesthesia (109, 315, 466) or gallamine-induced paralysis (54, 235). The level of spontaneous activity in LC-NE neurons varies with these different treatments, yielding mean rates of 1.1, 2.6, 6–30, and 2.4 Hz for chloral hydrate, urethan, gallamine, and halothane (G. Aston-Jones, unpublished observations), respectively.

A number of experimental manipulations alter LC-NE discharge, indicating that these cells receive information from a variety of central nervous system (CNS) sites. In anesthetized animals the only external sensory stimuli that elicit responses in these neurons are noxious. Thus painful sensory stimuli, such as pinching the tail or paws (59) or electrically stimulating the foot or peripheral nerve (19, 61, 466), transiently alter the slow, tonic pattern of spontaneous discharge seen under anesthesia, whereas nonnoxious strokes on the body, bright flashes into the eyes, or loud auditory stimuli produce little, if any, response in LC-NE discharge, regardless of anesthetic employed (61, 152; G. Aston-Jones, unpublished observations). Such noxious stimuli elicit a transient excitatory response that is immediately followed by a more prolonged decrease in impulse activity. Greater sensitivity to somatosensory stimuli is reported for unanesthetized, paralyzed rats (235), and pronounced responses are seen to stimuli of many modalities in waking animals (see sect. IIIA3). Electrical stimulation of peripheral nerves (vagus, splanchnic, and sciatic) and CNS sites (bed nucleus of the stria terminalis, olfactory bulb, preoptic region, ventromedial nucleus of the hypothalamus, ventral tegmentum, and central gray) has been reported to produce converging orthodromic excitatory and inhibitory influences onto single LC-NE neurons (6, 466). Similarly, with electrical stimulation of tooth pulp, optic, and sciatic nerves, Igarashi et al. (194) reported that convergence of inputs was observed from all three nerves in 23 and 33% of LC and subcerulear neurons, respectively, and from two nerves in 31 and 20% of recorded neurons. Such convergence was rarely observed in nearby parabrachial neurons. Elam et al. (102) found that LC-NE neurons were dose-dependently activated by hypercapnia as a result of central chemoreceptor stimulation. Hypoxia was also found to increase discharge in these cells, but unlike the hypercapnic effect, peripheral receptor activation was responsible. These data “implicate chemoreceptors, centrally but also peripherally located, in the regulation of brain NE neurons in LC” (102). Finally, in a study of the possible correspondence between LC-NE activity and blood pressure, Svensson and Thorén (457) found that there was “no consistent relationship between LC neuronal firing rate on the one hand and blood pressure or heart rate on the other.” These authors did report, however, that intravenous injections of blood slowed LC neurons and that blood extractions increased their discharge rate. Thus blood volume but not blood pressure was effective in altering LC-NE activity. Vagotomy reversed and prevented this response, indicating that physiologically as well as electrically stimulated vagal activity produces responses in the LC-NE system (466).
2. Unanesthetized preparations

Recordings of LC neurons in unanesthetized, nonparalyzed cats have been reported by three independent groups. Hobson, McCarley, and collaborators (181, 289, 450) have described a subpopulation of cells in the cat LC region that exhibits the unusual property of discharging less intensely during rapid-eye-movement (REM) sleep than during waking or slow-wave sleep (SWS). Although nearly all brain neurons studied during REM sleep discharge at rates resembling those observed during waking (for review see 450), these LC cells exhibit slow, regular discharge during waking (similar to activity seen in rat LC under anesthesia), somewhat less activity during SWS, and virtually no impulses during REM sleep. In addition, these authors reported that this subpopulation of "REM-off" LC neurons altered discharge prior to the onset of transitions between stages of the sleep-waking cycle, so that activity characteristic of a stage of sleep or waking was achieved slightly in advance of the onset of that stage. These results are consistent with the hypothesis that LC neurons may play a role in initiating or maintaining stages of the sleep-waking cycle (210, 211). McCarley and Hobson (289) hypothesized that LC-NE neurons may be crucially involved in controlling the REM stage of sleep such that decreased activity in these neurons is required for REM sleep to occur, and increased activity just before REM offset may cause the transition from REM sleep to waking. They also report, however, that other neurons in cat LC do not exhibit this REM-off property with activity increasing just prior to waking and that some REM-off cells were found outside LC. This physiological heterogeneity may reflect the anatomical heterogeneity of cat LC where NE and non-NE neurons are interdigitated. They were therefore unable to determine with certainty whether the subpopulation of REM-off LC neurons were NE or non-NE cells. In recordings from rat LC, which is composed entirely of NE-containing neurons, Aston-Jones et al. (16, 17, 123) showed that LC-NE neurons do homogeneously exhibit similar REM-off characteristics (see sect. IIIA3). Hobson and McCarley have not reported whether REM-off LC neurons alter discharge rates within waking as a function of arousal or sensory stimulation.

Sakai (380) also reported REM-off neurons in cat LC. Such cells were recorded only from regions shown in separate animals to have NE-containing neurons, and areas high in density for NE neurons yielded proportionally greater numbers of REM-off cells than areas containing few NE neurons. However, many REM-on cells were found in the same areas as REM-off neurons, and cytochemistry was not done for cells at recording sites. The activity of REM-off cells during waking or sensory stimulation was not described, except to specify a mean discharge rate of 0.5–2.5 Hz.

Chu and Bloom (68, 69) provide the only other descriptions of LC impulse activity in unanesthetized cat. As in the above cat studies, they found a mixed population of LC neurons, of which only 25% showed their lowest tonic discharge rates during REM sleep. The other 75% exhibited substantial
activity during REM sleep, with some cells phasically active while others were tonically active. A subset of LC neurons exhibited generally enhanced activity during active waking (vigilant surveillance of the environment) as opposed to quiet (nonattentive) waking. These investigators attempted to determine which recordings were obtained from NE-containing neurons by localizing recording sites relative to CA fluorescent neurons. If a recording site (marked by a Prussian blue spot or a microlesion) was within a cluster of fluorescent neurons, that particular recording was considered to be from an NE-containing neuron. Thus, even with direct visualization of recording sites among fluorescent neurons, a physiologically heterogeneous population of cells is found in cat LC. It may be that in cat, LC-NE neurons do not have discharge characteristics in common. However, it seems more likely that even with such careful attempts at localization of recording sites, non-NE cells are still the predominantly recorded neuronal population. This appears to be the case from the correlation found between the percentage of REM-off cells recorded and the corresponding density of NE-containing neurons in areas of cat LC (380), and from recordings in squirrel monkey and rat where recordings from known NE-containing LC neurons yield strikingly homogeneous results (17, 18, 20, 123; see sect. IIIA3). It may be possible to physiologically identify NE-containing neurons in cat LC by AD activation from their target areas. Faiers and Mogenson (109) found that stimulation of cingulate cortex elicited AD spikes exclusively in pontine cells located in rat LC or subceruleus and therefore presumably only in LC-NE neurons. Similar identification of LC-NE neurons in cat may yield a physiologically homogeneous cell population like that found in squirrel monkey and rat (see sect. IIIA9).

A study of single-cell activity in the LC region of rhesus monkey primarily investigated whether recorded cells were antidromically activated from forebrain sites shown to support self-stimulation (144). Presumed LC cells had slow (5 ± 3 Hz) discharge rates during quiet waking, whereas neurons in the subceruleus exhibited higher rates (15 ± 2 Hz). [In anesthetized rat, however, no difference was found between physiological characteristics of LC and subceruleus neurons (163).] No change in monkey LC impulse activity was found during operant responding for applesauce reward. Activity during sleep or sensory stimulation was not studied. The identification of recorded cells in this study may be uncertain due to the large number of electrode penetrations made in each of the two animals studied (40, 45) and the long interval between recording sessions and sacrifice (2 and 6 mo, respectively).

In summary, recordings in unanesthetized cat demonstrate the existence of REM-off cells in LC whose distribution approximately matches that of NE-containing neurons. These cells exhibit discharge characteristics that are consistent with the hypothesis that the LC system plays a role in tonic control of the sleep-waking cycle. The most specific theory resulting from these cat studies is that LC-NE neurons play a critical but permissive role...
in REM sleep, allowing REM sleep to occur by virtually ceasing to discharge, and subsequently terminating this stage by increasing impulse activity near the end of REM-sleep episodes (289). However, the dispersed nature of NE-containing neurons in cat LC prevents confidently ascribing any of these recordings to either NE or non-NE neurons. In such species, with cytochemically heterogeneous cell distributions within LC, criteria such as AD activation from cortex with slow conduction velocity and localization of recording sites to areas dense in NE-containing neurons may yield more positive identification of LC-NE neurons. In addition, none of the above studies in unanesthetized animals measured LC activity during waking as a function of arousal changes, sensory stimulation, or specific behaviors.

3. Recordings from known norepinephrine-containing locus ceruleus neurons in behaving animals

Studies by Foote and colleagues (123, 124) in squirrel monkey and by Aston-Jones and colleagues (16–18, 123, 207) in rat have recently sought to determine the factors that influence impulse activity of known NE-containing LC neurons in behaving animals. Studies by others (9, 85, 192) as well as by our group (127; unpublished observations) indicate that the LC in these species is composed entirely of NE-containing neurons. This property allowed us to confidently ascribe discharge characteristics to known LC-NE neurons. Cells were recorded during spontaneously occurring stages of the sleep-waking cycle, during presentation of a wide array of sensory stimuli, and during various naturally occurring as well as experimentally induced behaviors.

In both species, LC-NE neurons exhibited slow spontaneous activity that varied from 0 to 20 Hz. Discharge rates were found to fluctuate systematically with spontaneous changes in the levels of vigilance of the animals. Even within unambiguous waking, activity was significantly greater during periods of intense cortical desynchronization and behavioral alertness than during periods of drowsiness and electroencephalogram (EEG) synchronization. During inattentive waking and SWS, periods of a few seconds without a single action potential were common, especially in monkey (123, 124). During SWS, discharge activity was significantly reduced compared to activity during drowsy waking, with a mean rate in rats of 1 Hz (17). Furthermore it was found in rats that even during this period of relatively sparse impulse activity, LC-NE discharge varied phasically in close relation to EEG spindles, so that during and immediately after spindles these cells exhibited more activity than between spindle episodes (17). Perhaps most striking in the recordings by Aston-Jones and colleagues (16, 17, 123, 207) of spontaneous LC-NE discharge during the sleep-waking cycle in rats is the nearly complete absence of impulses during REM sleep. Although this and other characteristics are similar to REM-off cells recorded in cat LC (see sect. IIIA2), one important difference between these results and those reported by McCarley and Hobson (289) is apparent during the transition from REM sleep to wak-
As in the previous study in cat, rat LC neurons change their discharge rates in anticipation of most stage transitions during the sleep-waking cycle, so that impulse activity decreases during waking just prior to SWS, decreases further still during SWS in anticipation of REM sleep, and increases markedly during SWS 0.5-1.0 s before the onset of waking. These cells also resemble those reported for cat by increasing their activity during REM sleep before waking, when this transition is measured by the return of skeletal muscle tone. However, it was found in rats that this transition in discharge did not precede but was approximately coincident with the termination of θ-activity in the EEG that characterizes the transition from REM sleep to waking (16, 17). Therefore, Aston-Jones (16) concluded that LC-NE discharge cannot be a primary determinant of REM-sleep termination; rather, LC activity during this transition is controlled by other factors that simultaneously control other brain activity characteristic of the transition from REM sleep to waking (see also 17). As θ-activity in the rat EEG is probably generated in nearby hippocampal structures, in-depth recordings from cat hippocampus may be necessary to reveal a similar relationship with LC discharge in this species.

One possible mechanism for the pronounced decrease in activity found for LC-NE neurons during REM sleep is proposed by Aston-Jones (16) from simultaneous recordings of unit activity and field potentials (FPs) in rat LC (see also 17, 18). Field potentials appear as low-frequency, discrete events 100-300 ms in duration that occur spontaneously in rat LC, synchronized with bursts of unit activity from the same electrode. These potentials presumably reflect concerted excitatory postsynaptic potentials in LC-NE neurons generated by barrages of excitatory afferent activity. Aston-Jones et al. (16, 17) found that although FPs in LC are coincident with bursts of unit activity during waking and SWS, during REM sleep they exhibit their highest spontaneous rate in the virtual absence of unit activity. Other investigators have reported low-frequency events similar to these FPs (although without unit recordings) in the area of rat LC (114, 221, 282). These events resemble pontogeniculooccipital (POG) waves, in that they become most frequent just before and during REM sleep, and have been proposed to represent PGO waves in the rodent (114, 221, 282). Although these FP events may reflect PGO-generator activity impinging on LC-NE neurons, it is unlikely that these cells are responsible for the propagation of the PGO signal to other brain areas during REM sleep, because they exhibit no impulse activity during this stage. The dissociation Aston-Jones observed between FPs and unit activity in the LC during REM sleep led him to hypothesize that LC-NE neurons are tonically inhibited by a potent set of inhibitory afferents during REM sleep, preventing impulse activity in response to simultaneous phasic barrages of excitatory afferent influences onto these cells (16-18). Such a mechanism, resembling influences on motoneurons during REM sleep (65, 147, 316), implies that LC-NE activity would disrupt this state of sleep and that efferent impulse flow from the LC-NE is actively inhibited in order for this state to occur. However, as mentioned in section IIIA2, LC-NE activity
July 1983
LOCUS CERULEUS SPECIFICITY
873

does not have the proper temporal relationship with REM-sleep offset to be the primary generator of the transition from REM sleep to waking.

Although LC-NE neurons in squirrel monkey and rat are not apparently associated with simple isolated movements, certain holistic behaviors are found to correspond to altered activity in these cells. In monkey, LC-NE discharge dramatically increases when animals orient toward a syringe filled with a preferred drinking solution (123). In rats, epochs of grooming or consumption of a preferred water solution result in decreased discharge (17, 20). Grooming and consumption are behaviors that, like sleep, are associated with decreased vigilance; i.e., animals are less attentive to the external environment during such behavioral activity. In both species the most intense activity in LC-NE neurons was observed in association with either spontaneous or sensory-evoked orienting behaviors, times when surveillance of the external environment (i.e., vigilance) was suddenly and dramatically increased. Thus it would appear that LC-NE neurons vary their spontaneous activity in relation to vigilance levels. Increased vigilance, as during spontaneous or sensory-evoked arousal or during orientation to an unexpected or preferred stimulus, is associated with tonically enhanced LC-NE discharge, whereas decreased levels of vigilance, as during sleep, grooming, or consumption behaviors, correspond to periods of diminished activity in the LC.

In both species, LC-NE neurons responded to simple (tone, light flash, touch) and complex (food, novel objects) stimuli with short bursts of impulses. A more prolonged period (300–700 ms in rat) of decreased activity followed these excitatory responses, resembling the biphasic response pattern seen in anesthetized rats to orthodromic or AD stimulation (see sects. IIIA1 and IIIB). However, in sharp contrast to their relatively minimal sensory responsiveness in anesthetized animals (see sect. IIIA1), LC-NE neurons in waking preparations are very responsive to mild, nonnoxious stimuli in the environment. Poststimulus-time histograms from rat data indicate response latencies of 15–20 ms to auditory stimuli and 50–70 ms to visual stimuli. Interestingly the vigor of these responses decreased with decreasing levels of vigilance, such as occur during sleep, grooming, or consumption behaviors. Conversely the most intense responses occurred for stimuli that interrupted any of these behavioral states, resulting in increased vigilance and orienting responses. During uninterrupted REM sleep, stimuli failed to elicit responses, although FPs (reduced in magnitude) continued to be evoked (16, 18). Thus the tonic inhibition of LC-NE neurons proposed to be responsible for the reduction of spontaneous activity during REM sleep (16, 17) may be sufficiently intense to prevent discharge in response to excitatory influences elicited by external sensory stimuli (18). Aston-Jones (16) hypothesized that this inhibitory influence on LC-NE neurons may also be active during other periods of decreased vigilance, such as during SWS, grooming, or consumptive behaviors, because sensory-evoked as well as spontaneous discharge is reduced during these periods as well (see also 18).

Finally, we have found that the physiological characteristics of LC-NE
neurons are generally homogeneous throughout the nucleus and that multiple-unit populations exhibit markedly synchronous discharge during periods of intense activity, as well as being homogeneously quiescent during REM sleep. Thus LC-NE neurons apparently function in concert, varying their discharge as a function of vigilance and sensory stimulation, and perhaps reflect activity in two classes of afferent systems, as proposed by Aston-Jones et al. (16, 18).

B. Antidromic Activation and Axonal Conduction Properties

The LC-NE neurons have been antidromically activated by electrical stimulation of many of their projection areas. Because these cells also receive afferents from diverse sources (see sect. II C), it is imperative that driven spikes be subjected to rigorous criteria for antidromicity. The test of choice determines whether driven activity is occluded by spontaneous impulses that occur within a critical period relative to the stimulation. This procedure, called the collision test, has been described in detail elsewhere (133). Briefly, spontaneously occurring impulses are used to trigger the stimulation after a controlled delay. If driven spikes are blocked at delays less than the sum of the conduction latency plus the refractory period, it can be assumed that both spontaneous and driven impulses travel along the same fiber, and therefore driven spikes are AD. Data from studies that did not apply this test or that did not histologically verify recording sites to known LC-NE neurons are not included in this section.

The AD technique has been used to help identify LC-NE neurons during recording sessions (109) and should be especially useful in identifying NE cells in the LC of species where this nucleus is composed of non-NE cells as well (see sect. III A2). Antidromic stimulation has also been used to study subpopulations of LC-NE neurons. In a study of those projecting to the spinal cord, Guyenet (163) found that ceruleospinal neurons possess an anteriorly directed collateral that may lie outside the main noradrenergic bundle in the midbrain. He further reported that cells projecting to the spinal cord were indistinguishable from those driven antidromically from the midbrain noradrenergic bundle in terms of conduction velocity, A-B break on repetitive stimulation, and refractory periods. However, spontaneous-discharge rates were lower for ceruleospinal neurons: 1.2 Hz vs. 2.6 Hz. A similar distinction in spontaneous activity for cells in the area of rat LC dense in spinally projecting neurons was also reported for unanesthetized behaving preparations (17).

In addition to identifying LC-NE neurons, AD activation also permits analysis of various aspects of the physiology of these cells. The LC-NE axons are reported to exhibit an absolute refractory period in the range of 2–20 ms in studies with double-pulse AD stimulation (109, 163, 466). The longer refractory periods in some of these studies may have resulted from a failure
to discern presumed initial-segment (or "A") spikes driven by the second pulse in pairs delivered at high frequency. The presumed soma-dendritic (or "B") component of driven spikes begins to fail at much lower frequencies (i.e., ~30 Hz; 163) than the smaller initial-segment component. This is to be expected because the safety factor would be very low for a fine axon to provide sufficient current from its impulse activity to bring the disproportionately larger somata to threshold. However, driven A spikes must be taken as evidence of stimulated activity in the fiber, and therefore it seems reasonable to presume that the absolute refractory period of LC-NE axons is on the order of 1-4 ms. In rat, LC-NE fibers conduct impulses slowly, as expected for thin, nonmyelinated axons (estimated to be 0.1-1 μm diam) (186, 331), with conduction velocities reported ranging from 0.20 to 0.86 m/s (6, 19, 20, 109, 163, 466). The mean conduction velocity estimated from stimulation of the cingulate cortex is 0.35-0.45 m/s, whereas somewhat faster velocities are observed with stimulation of midbrain fibers (0.5-0.6 m/s) or of spinal projections (0.65 m/s). This raises the possibility that fibers may change conduction velocity as they project from the pons to the cortex (109) and that fibers destined for spinal terminal areas may be somewhat larger than those projecting to cortical terminal fields. There is a surprising amount of variability among cells in their impulse-conduction latencies to a given target area. This wide range of latencies is not due to differences in technique or stimulation sites among different investigators; for example, the conduction times to cingulate cortex are reported to range from 23 to 82 ms by Faiers and Mogenson (109) and from 28 to 90 ms by Aston-Jones et al. (20). The significance of this marked spread in latencies to reach target areas is not understood but may result from activating fibers of passage as well as terminal fibers (presumably smaller in diameter) from the same stimulation site.

Antidromic stimulation at 1 Hz elicits driven spikes at a nearly constant latency in an individual LC-NE neuron. Driven activity is typically followed by a pronounced reduction in impulse activity for a few hundred milliseconds. This period of decreased activity occurs even when the cell being recorded is not one that is driven by the stimulation (61). The pause in activity after AD stimulation is similar to that seen after orthodromic driving of LC-NE neurons (19, 485). Inhibitory pauses after either type of activation are altered by pharmacological agents in a manner suggesting mediation by recurrent collateral inhibition among LC-NE neurons (6, 61). Recent evidence obtained from extracellular (106) and from intracellular in vivo recordings of LC neurons indicates that a hyperpolarization of membrane potential mediated by an α2-adrenoceptor underlies these inhibitory pauses (7). It is not yet clear whether this putative interaction is mediated by axonal or by dendritic release of NE. Groves and Wilson (158) reported that dendrites in the rat LC contain SGVs, a marker for NE-containing vesicles, after pretreatment with 5-OHDA. Koda et al. (229) have presented evidence of dendritic SGVs in rat LC without such pretreatment and found that the intensity of post-
AD inhibition was greater when soma-dendritic spikes were elicited than when AD stimulation elicited initial-segment spikes only. Also, the magnitude of activity decrease is proportional to the magnitude of the preceding evoked response for either antidromically or orthodromically driven activity (485). Although these results indicate that LC-NE neurons may be interconnected by dendritic inhibitory contacts, at least one other possible explanation exists for such inhibition. Other CNS neurons exhibit a Ca$^{2+}$-dependent K$^+$ conductance that results in pronounced hyperpolarization of the soma for hundreds of milliseconds (189, 409). If such a conductance exists in LC-NE neurons, it may be sufficient to produce the postexcitatory pause in activity characteristic of these cells, independent of any interneuronal connections. This potential mechanism for postexcitatory inhibition of LC-NE discharge remains to be tested. In any case, it appears that LC-NE neurons do share common inhibitory as well as excitatory inputs, as indicated from cross-correlational studies of pairs of neurons recorded simultaneously (486) and from the marked homogeneity of sensory-response properties in waking rats (18; see sect. IIIA3).

Aston-Jones et al. (20) found an interesting property of LC-NE impulse conduction in AD tests that utilized patterns of activity characteristic of those in behaving animals (see sect. IIIA3). During a train of 10-Hz activity, early impulses exhibit an increase in conduction velocity, whereas later impulses in the same train yield a more pronounced decrease in velocity. This type of conduction-velocity fluctuation implies that LC-NE axons modulate the transmission of their own impulse activity and that short-duration, high-frequency bursts of impulses should be the most effective pattern of LC-NE activity for affecting target cells (20). These findings also suggest that short bursts of activity, such as those observed for LC-NE neurons in response to nonnoxious sensory stimuli in behaving animals (see sect. IIIA3), may reflect periods of maximal information flow in the LC-NE system. These descriptions of fluctuations in LC-NE impulse conduction are also useful for defining the optimal parameters for electrical stimulation of LC-NE pathways in experiments on postsynaptic or behavioral effects of LC-NE activity.

C. Locus Ceruleus Pharmacology

Many studies have investigated the sensitivity of LC neuronal activity to various transmitter and pharmacological agents. This line of experimentation has yielded data relevant to specific afferent systems that may mediate response characteristics of the cells described in sections IIIA and IIIB. First we review the effects of putative transmitter agents shown by separate anatomical experiments to exist in LC, or of drugs thought to interact specifically with such systems, and then we summarize the results of studies with agents not yet shown to involve systems endogenous to the LC. With the exception of two reports with paralyzed preparations, the studies discussed
below were performed in anesthetized rats. The effects of pharmacological agents on the electrophysiological activity of LC neurons has recently been reviewed in detail elsewhere (154).

1. Monoamines

Cedarbaum and Aghajanian (59, 60) reported that iontophoretic epinephrine or NE inhibits spontaneous discharge in the LC of chloral hydrate-anesthetized rats and that these inhibitions are blocked by the α-adrenergic antagonist piperoxan but not by the β-antagonist sotalol. These authors concluded that E and NE achieve their inhibitory effects by acting on presynaptic α₂-receptors (thought to be localized on somata of LC-NE neurons but exhibiting pharmacological characteristics like those of receptors localized on presynaptic terminals in peripheral and central systems), rather than on more conventional postsynaptic α₁-receptors. One important aspect of these NE-induced, α₂-mediated inhibitions is the possibility that they may be responsible for the poststimulus suppression seen after activation of LC-NE neurons (see sects. IIIA and IIIB). This hypothesis is supported by the observation that iontophoretic or systemic piperoxan but not sotalol reduced poststimulus suppression after AD activation of LC-NE neurons from the dorsal noradrenergic midbrain pathway (6). Also, the postexcitatory inhibition appeared to depend on activation of CA fibers because injections of 6-OHDA but not of 5,7-dihydroxytryptamine block both AD responses and the periods of poststimulation depression that normally occurred even for cells that were not driven. Piperoxan was also reported to decrease the similar epoch of reduced activity seen after orthodromic activation of these cells (61).

As this hypothesis would predict, clonidine, a putative α₂-agonist, inhibits LC-NE neurons when administered systemically (3, 454, 455) or iontophoretically (163, 455). Chronic administration of clonidine also slows these cells, and there is a rebound excitation on withdrawal (454). In vivo intracellular studies with clonidine and piperoxan also indicate that an α₂-mediated hyperpolarization underlies postburst pauses in discharge (7). The β-agonist isoproterenol also inhibits LC-NE neurons (60), and some β-antagonists decrease discharge rates after chronic treatment (84, 454).

One of the earliest studies of the chemical sensitivity of LC-NE neurons found that intravenous amphetamine in chloral hydrate-anesthetized rats decreased spontaneous impulse activity in these cells (152). A subsequent pharmacological analysis of this effect found that the amphetamine-induced inhibition of LC-NE neurons was blocked by systemic yohimbine, a putative presynaptic blocker of α₂-receptors or autoreceptors (109). These authors concluded that “the amphetamine-induced inhibition of NE neurons in the LC is an indirect effect, mediated via activation of central α-receptors of presynaptic character.”
Segal (413) found that electrical stimulation of the dorsal raphe blocked excitatory responses of LC-NE neurons to noxious peripheral stimuli and that these effects were prevented by pretreatment with p-chlorophenylalanine (PCPA), 5,7-dihydroxytryptamine, or methysergide. These results indicate a strong serotonergic influence on LC from the dorsal raphe (see also sect. II.D).

2. Opiates

Studies of the responsiveness of LC-NE neurons to opiates were largely motivated by the finding that the LC is dense in opiate receptors (21) and that &beta;-endorphin as well as enkephalin pathways innervate this nucleus (44, 356). The endogenous opiate methionine-enkephalin has been iontophoresed onto LC-NE neurons in two studies (165, 507), resulting in inhibition of spontaneous activity. Furthermore, this inhibition by methionine-enkephalin was found to be produced independent of ACh or substance P systems, presumably by acting on a separate set of receptors (165).

A study in slices of guinea pig LC found that opiates and opioid peptides administered in the slice bath by perfusion hyperpolarized LC neurons with an increase in membrane conductance (343). These effects were stereospecific and naloxone reversible. However, guinea pig LC consists of interdigitated NE and non-NE neurons (G. Aston-Jones, unpublished observations), and cytochemical identification of recorded neurons was not pursued in this study; thus the question remains whether these results were obtained for NE or non-NE neurons.

Korf et al. (235) were the first to report effects of opiates on LC neurons. In both paralyzed and anesthetized rats, they found that systemic morphine reduced spontaneous LC-NE impulse activity in a naloxone-reversible manner. Bird and Kuhar (30) reported that iontophoretic morphine and levorphanol depressed spontaneous activity of LC neurons, that this effect was reversed by naloxone and levallorphan, and that the opiate-induced decrease in firing was a stereospecific effect.

Aghajanian (3) studied the effect of chronically administered morphine on LC-NE neuronal activity and found that these cells became tolerant to the inhibitory effects of morphine after 4–5 days of treatment. Iontophoretic naloxone in tolerant animals more than doubled the spontaneous-discharge rate. Also, iontophoretic morphine depressed LC-NE-discharge rates in control and to a lesser extent in dependent animals. Clonidine depressed LC-NE firing in normal as well as tolerant animals, even during naloxone activation in tolerant rats. Clonidine antagonism of abstinence-induced LC hyperactivity was blocked by piperoxan but not by naloxone, whereas piperoxan was ineffective on the morphine-induced depression of LC-NE activity. These results indicate that separate opiate and &alpha;-adrenergic receptors mediate the responses to morphine and clonidine, respectively.
In addition to the above effects of opiates on LC spontaneous discharge, Segal (413) reported that morphine blocked responses of LC-NE neurons to noxious stimuli in anesthetized rats in a naloxone-reversible fashion.

3. Acetylcholine, substance P, and neurotensin

Rat LC-NE neurons stain positively for ACh (9, 262). Such staining is localized predominantly to somata and proximal processes. Acetylcholine has been found to strongly excite LC-NE neurons in several studies (30, 107, 163-165). Substance P has also been reported to excite these cells (164, 165). Guynet and Aghajanian (165) conclude from iontophoretic studies of the potencies of a series of cholinergic agonists and antagonists that all cholinergic transmission in LC is muscarinic. This study also showed that ACh, substance P, and Met-enkephalin operate at independent sites of action in the LC. Although Engberg and Svensson (107) obtained excitatory effects of systemic nicotine on LC-NE neurons, they conclude that this excitation is indirect because iontophoretic nicotine was ineffective on these cells and muscarinic but not nicotinic antagonists block the effects of iontophoretic ACh.

Iontophoretic neurotensin has been reported to inhibit spontaneous as well as glutamate-induced firing in some LC neurons (510) but was found ineffective in another study (164).

4. Ethanol

There have been three studies of the effects of acute ethanol administration on LC-NE impulse activity. Using unanesthetized but paralyzed rats, Pohorecky and Brick (362) reported that a 2 g/kg dose of ethanol decreased spontaneous-discharge rates in a majority of the cells they recorded. However, Svensson and Engberg (456) found no effect of ethanol (2 g/kg) on spontaneous-discharge rates of NE-LC neurons in chloral hydrate-anesthetized rats. Also, Aston-Jones et al. (19) found no effect of ethanol (up to 3 g/kg) on spontaneous activity of LC-NE neurons. This same study did find, however, that doses of ethanol as low as 0.5 g/kg significantly disrupted sensory responses in these cells, such that the variability of response latencies increased and the intensity of responses decreased in a dose-dependent manner. It seems possible that the decrease in LC-NE spontaneous activity seen by Pohorecky and Brick may be related to the high mean predrug rate (17 Hz) in their study, a rate much higher than that seen in either anesthetized animals or waking preparations (see sects. III A and III B). If this unusually high rate is due to increased orthodromic excitation of LC-NE neurons resulting from the stress of paralysis, then the ethanol-induced decrease in spontaneous activity would be consistent with the finding that ethanol disrupts orthodromic responses in these neurons (19).
fect a stress-alleviating action for ethanol, as suggested previously (456). Aston-Jones et al. (19) also found that ethanol increased the magnitude of postexcitatory suppression of LC-NE discharge. This may indicate that ethanol increases recurrent collateral inhibition among LC-NE neurons or that ethanol enhances a calcium-dependent potassium current, whichever is responsible for the period of decreased activity after driven responses in LC-NE neurons. In any case, an enhancement of this period of suppressed activity could at least partly account for ethanol’s disruption of sensory-evoked responses in these cells.

5. Other pharmacological agents

In addition to the inhibitory effects of opiates on LC-NE neurons mentioned above, many agents are found to depress activity in these cells. Iontophoretic γ-aminobutyric acid (GABA) and glycine are reported to inhibit LC-NE discharge (60). Benzodiazepines have been reported to inhibit LC-NE activity (153), as have tricyclic antidepressants (322). Chronic imipramine (458) or desipramine (190) treatment is effective in inhibiting LC-NE spontaneous activity (322). One nonamphetamine stimulant, amfonelic acid, has also been found to reduce spontaneous discharge of LC-NE neurons in anesthetized rats (145). The hallucinogens mescaline and LSD also suppress spontaneous firing of LC neurons in anesthetized animals, but unlike amphetamine, desipramine, and clonidine, which also suppress spontaneous activity, these two hallucinogens augment the responsiveness of LC-NE neurons to peripheral stimuli (4).

Vasopressin has been reported to excite LC-NE neurons (327). Young et al. (510) and G. Aston-Jones and M. Segal (unpublished observations) have also found excitatory effects of iontophoretic glutamate on LC-NE neurons. Vincamine, piracetam, and Hydergine, drugs used to treat geriatric mood and cognitive disorders, all activate LC-NE neurons in chloral hydrate–anesthetized rats when injected intraperitoneally (329).

D. Summary and Conclusions

In anesthetized preparations, LC-NE neurons tend to discharge in a slow invariant manner and are responsive only to noxious or extreme nonphysiological stimuli. On the other hand, a broad spectrum of stimuli is very effective in eliciting responses in these neurons in waking preparations. Physiological results agree with anatomical studies indicating that LC-NE neurons receive inputs from a wide variety of sources. Electrical stimulation of various peripheral as well as central sites yields converging excitatory and inhibitory influences onto these cells. These neurons are also influenced by both central and peripheral chemoreceptors that mediate responses to
July 1983    LOCUS CERULEUS SPECIFICITY  881

hypercapnia and hypoxia, respectively. Stimulation of peripheral volume receptors but not of baroreceptors is also reported to affect LC-NE discharge. Recordings in unanesthetized cats show a subpopulation of LC cells in this species that vary their impulse activity with stages of the sleep-waking cycle. The most outstanding characteristic of these recordings is the lack of impulse activity during REM sleep. These same REM-off cells also anticipate stage transitions in the sleep-waking cycle, so that activity characteristic of a succeeding stage becomes apparent just preceding the onset of that stage. The numbers of REM-off cells recorded in a particular portion of LC correlate well with the corresponding density of NE-containing cells in that area of cat LC. However, cat LC is interdigitated with NE and non-NE neurons, and many REM-on cells are found in areas highly populated with fluorescent cells, whereas REM-off cells are found outside such areas. It is impossible from these studies to confidently ascribe discharge characteristics to either NE or non-NE neurons. Future studies of LC activity in such species with cytochemically heterogeneous LC cell distributions may benefit from AD identification of NE-containing neurons from stimulation of target areas (e.g., cerebral cortex) unique to LC-NE neurons.

Recordings from known NE-containing LC neurons in squirrel monkey and rat have revealed strikingly homogeneous cell populations. These neurons cause spontaneous-discharge rates to vary as a function of stages of the sleep-waking cycle, becoming virtually silent during REM sleep, much like the REM-off LC neurons reported for cat LC. Rat studies (17) found that LC-NE activity during the transition from REM sleep to waking does not precede changes in other brain activity at this time (e.g., EEG θ-offset, probably generated in hippocampal structures), and therefore these neurons are unlikely to be primarily responsible for REM-sleep offset, in contrast to previous suggestions (181, 289).

This marked correlation between LC-NE activity levels and stages of the sleep-waking cycle in these rat and monkey studies reflects a more pervasive correspondence with levels of vigilance. Thus the most intense LC-NE discharge occurred for spontaneous or sensory-evoked disruptions of low-vigilance episodes such as sleep, grooming, or drinking behaviors, whereas minimal activity and sensory responsiveness is observed during uninterrupted epochs of these behaviors. These data indicate that the excitability of LC-NE neurons may be strongly modulated by a system of inhibitory afferents, decreasing the outflow of activity in the LC-NE system when stimuli in the external environment may produce undesired disruptions of ongoing behavioral states. These characteristics of LC-NE neurons appear to be quite specific to these cells compared to other neurons in nearby pontine areas, both from the heterogeneous physiological results found in cat LC and from recordings obtained outside the area of known LC-NE neurons in rat.

Rat LC-NE axons conduct impulses slowly, ranging from 0.20 to 0.86 m/s. Faster conduction may occur for fibers of passage (e.g., the midbrain
noradrenergic dorsal bundle) than for terminal areas (e.g., cingulate cortex). There seems to be a relatively low safety factor for spike transmission from the initial segment of the axon into the soma, as evident from the long refractory period for the B component of AD spikes (~30 ms). This is consistent with the waveform of spontaneously occurring impulses, which exhibit a pronounced initial-segment component in the rising phase of the spike. On the other hand, LC-NE axons appear to have a much shorter refractory period (~2 ms) than the soma. One striking aspect of LC-NE impulse conduction is the extremely wide distribution of conduction latencies to terminal fields such as cingulate cortex, where some cells take more than three times longer to transmit impulses than others. The significance of this temporal divergence in impulse-conduction times is obscure but may result from concurrent stimulation of terminal axons and fibers of passage. Another unusual feature of LC-NE impulse conduction is the marked variability in conduction velocity found for individual fibers. These results indicate that LC-NE axons, perhaps because of their small diameter, may easily become tonically depolarized, causing spike-conduction velocity to fluctuate during trains of activity and perhaps eventually leading to failure of conduction for long trains of high-frequency activity (e.g., over ~50 Hz for more than a few seconds). These results suggest that short, high-frequency bursts of impulses (e.g., ~10 spikes at 50 Hz) may be maximally effective on target cells. These parameters should be kept in mind for future studies of the effects of electrical stimulation of the LC-NE system on target cells and behavior.

Physiological and pharmacological evidence suggests that LC-NE neurons may be interconnected with inhibitory recurrent collaterals. Thus AD or orthodromic excitation is shortly followed by inhibition for a few hundred milliseconds, which is reduced by iontophoretically or systemically administered piperoxan. Ultrastructural and other physiological evidence indicates that dendritically released NE may be at least partially responsible for such recurrent collateral inhibition in LC. However, the possibility that impulse activity in LC-NE somata engages long-lasting hyperpolarizing currents independent of a recurrent collateral mechanism (such as a Ca2+-dependent K+ conductance) remains to be tested. A more complete elucidation of the membrane events controlling LC discharge awaits the development and utilization of intracellular recording techniques beyond the pioneering studies recently reported (7, 174, 343).

The LC-NE neurons are sensitive to a wide range of pharmacological and putative transmitter agents. The monoamines NE, E, and serotonin are all inhibitory on these cells. Other inhibitory transmitter candidates reported to be effective in reducing LC-NE activity are Met-enkephalin, GABA, and glycine. The inhibitory response to morphine exhibits tolerance with chronic treatment, and these cells exhibit enhanced impulse activity during opiate withdrawal. Excitatory putative transmitters effective on these cells include ACh (via muscarinic receptors), substance P, and glutamate.
In addition to these transmitter candidates, various psychotherapeutic agents alter discharge in LC-NE neurons. Amphetamine and the hallucinogens mescaline and LSD depress activity in these cells, as does morphine. The enhanced activity seen during withdrawal from chronic opiate administration can be antagonized by clonidine. Benzodiazepines, tricyclic antidepressants, and chronic desipramine also inhibit these cells. Low doses of ethanol, although producing no apparent effect on spontaneous discharge, significantly disrupt sensory responses in the LC-NE system.

In conclusion, these results indicate that there are many specifiable factors that are capable of influencing impulse activity in LC-NE neurons. These cells alter their activity in a highly predictable manner as a function of behavioral state and sensory stimulation in behaving animals. They conduct impulses reliably to their target areas and exhibit basic physiological properties similar to those reported for other CNS neurons. Finally, they are sensitive to a number of putative transmitter agents, a characteristic compatible with innervation by many CNS areas utilizing diverse transmitter substances. Other nearby pontine cells that have been examined are easily distinguished from LC-NE neurons in terms of these characteristics. Thus these cells represent a component of the pontine reticular system with well-defined physiological and pharmacological characteristics, whose activity can be specified as to many sensory, behavioral, and pharmacological factors that control it.

IV. SYNAPTIC STRUCTURE AND FUNCTION OF LOCUS CERULEUS SYSTEM

In this section we review the data currently available concerning the impact of LC innervation on target neurons in various brain regions. This includes the ultrastructural evidence concerning the morphology of LC-NE nerve endings and the data concerning LC-NE effects on target neurons. Neither body of data is without controversy, and our interpretations, which incorporate recent data, are offered here. The experimental observations demonstrating that NE is a neurotransmitter for LC neurons is not explicitly reviewed, but much of it is referred to in addressing the questions posed in this section.

A. Ultrastructure of Locus Ceruleus Terminals

One of the most controversial aspects of LC-NE anatomy is the nature of LC axonal connections with potential target cells and the interpretation of whether these fibers form true synapses or end in a diffuse endocrine type of relationship with many unspecified target cells (see 25, 40, 41, 303, 511). The controversy stems from differences in the methods used to visualize the terminals and the region of the brain in which they are studied. In areas known to be innervated by LC axons, and in which there is little or no other
CA innervation, such as the cerebellum (47, 250), hippocampal formation (230), brain stem (423), and parietal cortex (90, 91, 252), NE terminals have been demonstrated ultrastructurally by one or more selective histochemical methods (see 35, 41, 303). These terminals typically contain pleomorphic, small vesicles (~500 Å diam) but often also large, dense-core vesicles. When the material is prepared by the permanganate (230) or 5-OHDA method (78, 241), the small vesicles are also dense cored or granular (SGVs).

Descarries and associates (90, 91, 252) used autoradiographic techniques to study NE axons and terminals that were selectively labeled by superfusion of the cerebral cortex with tritiated NE. Although the cortex was exposed to high amine levels, raising the possibility of nonspecific labeling, control experiments demonstrated that this labeling was blocked by pretreatment with 6-OHDA or by desmethylimipramine, a blocker of NE uptake. The labeled axons appear oriented parallel to the pial surface in layer I and perpendicularly or obliquely in layers II-IV. This agrees with fluorescent and immunohistochemical studies (137, 259, 307). In a topometric ultrastructural analysis with few terminals followed through more than two or three serial sections, Descarries et al. (91) concluded that only 5% of the labeled terminals make synaptic complexes with postsynaptic elements. The remaining labeled terminals were not observed to make identifiable synaptic complexes. This is in contrast to other unlabeled terminals in the sections, 50% of which made typical synaptic complexes.

The interpretation of these observations is not straightforward. First, it may simply imply that there is no specific contact between LC axons and their receptive postsynaptic elements. In this case, LC stimulation would presumably result in the release of NE from all of the terminals, and the effects of this stimulation would depend on the presence of appropriate receptors in nearby neural elements. This type of organization, which would not be unlike the relationship between peripheral sympathetic axon terminals and the tissues they innervate (186), has also been inferred for some parts of the hypothalamus (71). Other alternatives should also be considered (303). A wholly different morphological analysis of neocortical NE fibers, as revealed by light-microscopic immunocytochemistry with anti-DBH staining (162, 307, 330), led to quite a different interpretation. These latter data indicate that the fibers in the outer molecular layer, which were labeled most heavily by the topically applied exogenous NE in the studies by Descarries et al. (91), may not be typical terminal NE fibers, but rather fibers in passage that are tangentially oriented in the medial-lateral or anterior-posterior directions to provide specific innervation of deeper cells. With immunocytochemical staining, which does not require an external labeling procedure, NE terminals (i.e., those positive for anti-DBH immunoreactivity) exhibit typical synaptic specializations in more than 50% of the cases examined in several brain regions, and in neocortex they are much more frequent in layers V and VI (330, 331).

In the cerebral cortex of the neonatal rat, after parenteral injection of 5-OHDA (while the blood-brain barrier is permeable to CA analogues), those
presumptive NE terminals identified as SGV-containing also show a very high incidence of specialized synapselike contacts (78, 241, 301). These contacts were also seen mainly in the deeper cortical layers, rather than in the superficial layers (241, 301), which were most densely labeled in the studies by Descarries et al. (91) in the adult rat cortex. An ultrastructural study in kittens suggests that there may be a significant species difference in which laminae are preferentially innervated (198).

In other areas whose CA innervation is solely from LC axons, ultrastructural studies also demonstrate conventional synapselike terminations. In the hippocampus of the rat, Koda et al. (230-232) have examined in detail those boutons containing endogenous NE as revealed by permanganate fixation without the necessity of superfused or topical NE. With this fixation procedure, NE terminals contain the characteristic SGVs and become relatively easily identified ultrastructurally. The conclusion that the boutons exhibiting SGVs are equivalent to NE-containing boutons is derived from several observations. The distribution of SGV boutons is correlated quite closely with the topographic distribution of glyoxylic acid–induced fluorescent boutons in the dentate gyrus and adjacent hippocampal layers (230); both the fluorescent boutons and the SGV boutons disappear after treatments that decrease brain NE, such as reserpine administration, intracisternal injections of 6-OHDA (230), surgical transection of the ascending dorsal NE bundle, or electrical stimulation of the LC (232). Discrete LC lesions after microinjections of 6-OHDA produce a dramatic but incomplete loss of glyoxylic acid–induced fluorescence in axon varicosities and of permanganate-reactive boutons containing SGV (231). The residual reactive fibers suggest that there may well be significant innervation from the contralateral LC. When the incidence of specialized contacts for SGV-containing boutons was compared with all other boutons in the same fields of the rat dentate gyrus, the NE boutons showed specialized junctional contacts (in ~18–20% of the boutons) as frequently as did all other boutons (231).

Most recently, immunocytochemical techniques have been used to visualize and examine the nature of NE terminal contacts with target neurons (330, 331). With an antiserum directed against rat DBH, Olschowka et al. (331) examined labeled terminals in several brain regions. No DBH-positive varicosities were observed to contact intracerebral capillaries. Approximately 58% of all labeled varicosities examined in a single electron-microscopic thin section demonstrated synaptic membrane specializations. Postsynaptic elements were usually small- to medium-sized dendrites.

As in all other cases of attempted ultrastructural-physiological correlation, the assumption that the morphologically specialized contact zone is the site of synaptic transmission remains to be confirmed. It is clear that sympathetic fibers of the peripheral autonomic nervous system transmit to smooth muscle without such specializations but do show them at intraganglionic (neuron-to-neuron) contacts. In view of the observations reviewed by Bevan (28), which suggest that receptiveness, receptor distribution, and sensitivity are closely correlated with the distance between release and response...
sites, accurate localization of central adrenoreceptors with reference to identified adrenergic boutons (337, 508, 509) may be the ultimate criteria for demonstrating NE transmission sites. Although there are data indicating the existence of neuronal, glial, and vascular noradrenergic receptors by radioligand displacement assays (81, 83, 87, 98, 175, 345), the actual location of functional receptors among these binding sites, their spatial relationship to NE axons and their varicosities, and the firing frequency parameters under which these receptors could be occupied by endogenous NE have not yet been determined. Additionally, both α- and β-noradrenergic receptor subtypes are detected in terminal fields innervated by LC fibers, although there is still no clear demonstration of the significance of the two receptor types. Finally, there is not a compelling correspondence between various types of data; for example, consider the demonstration of sites that bind the highly selective β-antagonist dihydroalprenolol in the striatum (337), which is virtually devoid of NE fibers, or the presence of β-antagonist binding on cerebral microvessels (83) despite the lack of change in cerebral blood flow or metabolism after 6-OHDA LC lesions (170, 220; also see 429). Nevertheless the autoradiography of ligand binding sites definitely seems an improvement over fluorescent probes of such sites (see 295), which can be difficult to discriminate from intracellular autofluorescent granules (23, 77).

B. Postsynaptic Effects of Locus Ceruleus Efferents

1. Control of norepinephrine release

The earliest studies to correlate structure and function for central NE projections were those combining the classic electrophysiological methods of ablation or stimulation with measurements of regional NE and its primary metabolic product 3-methoxy,4-hydroxy-phenethylamine glycol (MHPG). These biochemical studies (see 14, 15) supported estimates of local transmitter turnover by histochemical methods (15) and confirmed that the LC and its dorsal ascending connections were the sole source of neocortical and limbic NE fibers. Although NE metabolism in cortex was globally enhanced by prolonged exposure to stressors (233, 471) and decreased by electrolytic lesion of LC (14, 234, 236), more refined stimulation parameters suggested that LC projections are predominantly unilateral (79, 234, 236, 433). More recent studies of release in vivo (467) confirm the histologically detected (see sect. II) existence of bilateral projections. Stimulation experiments also helped define the effects of different activity levels on synthetic metabolism (for review see 382) and suggested that the system would be maximally activated at rates above 20 Hz. Subsequent studies utilizing this general paradigm indicate that, while terminal release and synthesis rates generally reflect levels of LC activation, local factors may increase release in terminal fields independent of LC activation (see 199, 319); however, intense sensory activation of cortex may also depress local NE release (370). Also LC activation may release peripheral NE indirectly (80).
In summary, these studies indicate that NE release at forebrain sites is primarily determined by levels of activity in LC neurons. In the following sections, we review data generated by three different methods of determining the postsynaptic effects of NE.

2. Effects of norepinephrine microiontophoresis on spontaneous activity of target neurons

One method commonly used to determine potential postsynaptic effects of the LC-NE system is microiontophoretic application of NE. Although this procedure has its interpretive limitations, it has usually yielded effects on target neurons similar to those elicited with other methods, e.g., stimulation of the source neurons in LC. Several previous reviews have documented the accumulating evidence that iontophoretically applied NE generally depresses the spontaneous activity of test cells in vivo in virtually every region of the brain (41, 42, 303, 464, 481). These effects are antagonized by β-adrenergic blockers and, in most cases, by prostaglandins (183, 184), certain antipsychotics, acute exposure to Li²⁺ (408, 410, 438), and certain metallic ions (317). More variation in NE effects has been encountered in cerebral cortex, where—although depressant actions are also of the β-type (92, 119, 318, 328, 349, 451, 470)—an excitatory α-adrenergic effect can also be found with iontophoretic tests (see 29, 424, 464, 488). Recent evidence suggests that the excitatory actions of iontophoretic NE on principle cells of the rat LGN (222, 375–377) and facial nucleus (480) are antagonized by α-adrenergic blockers, as are responses of mitral cells in the olfactory bulb (46). It remains to be determined whether the localization of α- and β-responsive sites represents multiple options on the same neuron (see 464) or indirect (e.g., presynaptic) versus direct (postsynaptic) actions. Indirect suppression of tonic inhibitory effects could explain some NE excitations (see 151, 385–389); however, many depressant responses to NE have not yet been characterized pharmacologically (55, 63, 119, 131, 368, 371, 498).

A principle advantage of studies on the NE system accrues from the ability to study target cells whose NE or LC innervation can be confirmed by morphological observations. Thus recent reinvestigations of the responsiveness of spinal neurons to NE iontophoresis (201, 209, 249, 392) confirm earlier reports (491, 492) that interneurons receiving noradrenergic input (see 209) are more responsive than motoneurons that are only sparsely innervated.

3. Effects of locus ceruleus activation on spontaneous activity of target neurons

Tests of the iontophoretic action of NE in vivo can at best simulate synaptic release, and often the simulation does not accurately reflect the cellular topography of NE boutons on the target cell. Thus release from the
pipette is often localized to perikarya during iontophoretic tests, whereas axodendritic innervation is a more common innervation pattern (see 36, 40, 303, 331). Therefore tests of the effects of LC stimulation on target cell activity can in some respects more directly determine the qualitative effects of NE circuits. The prototypic example of such tests is the cerulocerebellar pathway to Purkinje neurons (129, 184, 185, 298, 300, 435, 486, 499). Purkinje cells show remarkably uniform inhibitory responses to LC stimulation, with a latency of ~125 ms (185). Antipsychotic phenothiazines (128), cobalt, lanthanum, lead, and lithium (317, 408, 438), as well as prostaglandins (185) are able to antagonize these effects. Intracellular recording from some Purkinje cells during stimulation of LC with single shocks reveals long-latency (~150 ms) relatively small hyperpolarizations. With trains of pulses to LC, large hyperpolarizations are observed that outlast the stimulation period. Input resistance either increases or does not change during the LC-evoked hyperpolarizations. Identical effects (hyperpolarization of the membrane potential with either minimal change or increased input impedance of the membrane) also characterize the actions of iontophoretically applied NE as assessed by intracellular recordings from cerebellar Purkinje cells (185, 434, 436), spinal motoneurons (283), and hippocampal pyramidal neurons (179). Stimulation of the LC input to facial motoneurons produces slight depolarizing effects with minimal changes in membrane impedance (288, 480), and LC stimulation has been shown to enhance the discharge rates of dorsal lateral geniculate neurons (222, 376). A correlation between innervation and responsiveness to LC stimulation is seen in dorsal cochlear nucleus (innervated and responsive; 66) versus lateral vestibular nucleus (uninnervated and unresponsive, but see 224), documenting the importance of target cell selection in functional testing (see 36).

In physiological studies on cerebral cortex (22, 328, 349, 470), hippocampus (417, 418), septum (411), spinal trigeminal nucleus (388, 389), spinal cord (182), olfactory tubercle (444), thalamus (373), and tectum (465), the effects of LC activation are generally comparable with those described in the cerebellum regarding overall rate changes or responsiveness to orthodromic activation. To summarize simply, the most obvious effect of LC stimulation or NE iontophoresis on spontaneous activity is reduction of discharge rate (also see 244, 277, 487, 488), although there are exceptions to this general rule. When presumed target neurons are systematically activated and LC-NE effects on this elicited activity are assessed, more complex effects become evident, as is discussed in section ivD.

4. In vitro tests of norepinephrine effects

Recently methods for assessing central transmitter action have come to include in vitro methods of pharmacological analysis, employing slice or culture preparations for longer-term intracellular recordings of membrane...
potentials while transmitter agonists are applied by droplet or by perfusion of known drug concentrations. The NE target region studied most intensively in vitro has been the rat or guinea pig hippocampus in the form of a slice preparation. Although some results replicate the in vivo effects of hyperpolarization with increased membrane resistance (414), a variety of nonconforming effects has also been reported. These range from hyperpolarizations with decreased resistance (251) to depressant α-receptor actions and excitant β-receptor actions (312, 313). Although NE generally is depressant in vivo, it has been reported to increase release of somatostatin in vitro (at both α- and β-sites; 108). Furthermore, in contrast to modest inhibitory actions on spinal motoneurons in vivo, spinal motoneurons cocultured with disaggregated fetal LC neurons respond to NE or LC stimulation by depolarization (284).

C. Molecular Mechanisms of Central Norepinephrine Synapses

After the demonstration that rat cerebellar Purkinje neurons as well as hippocampal and cerebrocortical pyramidal neurons are contacted by LC axons and give uniform inhibitory β-type responses to NE and LC stimulation, studies were conducted to further delineate the mechanism of this inhibitory response. In all three terminal fields, cyclic 3',5'-adenosine monophosphate (cAMP) mimicked the ability of NE to depress spontaneous activity (183, 185, 415, 416, 434, 436, 451). Additional experiments pursued the possibility that this action of cAMP in the CNS might reflect the operation of a “second messenger” (453) at central noradrenergic synapses (37, 38, 41, 183–185). Biochemical evidence had already suggested that NE could elevate cerebellar cAMP levels through β-adrenergic receptors (see 367; also see refs. in 38). This effect is even more pronounced in primate brain (8, 440), especially in the human brain (134, 476). Recently methods for direct microscopic localization of receptors showed that Purkinje neurons in the cerebellum and pyramidal neurons in the hippocampal and cerebral cortex seem to be the major cell classes with β-receptors (337, 508, 509). Furthermore, parenteral administration of phosphodiesterase inhibitors such as aminophylline or theophylline potentiated NE depressions of Purkinje cells, whereas iontophoretic administration of these methylxanthines and of papaverine converted weak excitant actions of iontophoretic cAMP into pronounced depressions (37, 41, 183–185, 435). These observations led to the proposal that certain actions of NE (183), and later those of the NE-mediated LC synaptic projections to Purkinje cells (434, 436), could be mediated by cAMP.

Although questioned repeatedly (see 36, 149, 350), the proposal has subsequently been strengthened by observations that NE, the NE pathway, and the cyclic nucleotide all hyperpolarize Purkinje cells (185, 433–435, 487) and hippocampal pyramidal cells in vitro (414) through similar membrane actions in which conductance to passive ion flow is decreased or unchanged.
The cAMP mediation of NE actions is also supported by observations that prostaglandins and nicotinate selectively block NE effects on Purkinje cells (435, 437) and hippocampal pyramidal cells (415, 416) as they do the cAMP-mediated adrenergic responses of trophocytes (453). In fact all substances shown to block the responses of cerebellar Purkinje neurons to iontophoretic NE also show potent antagonism of the NE activation of cerebellar adenylate cyclase (see 317). Even more direct confirmation of the second-messenger hypothesis stems from the observation that NE and stimulation of the LC-NE pathway will increase the number of Purkinje cells reacting positively to an immunocytochemical method detecting bound intracellular cAMP (45, 432). In hippocampal slices, cAMP generation in response to NE appears to occur in both glial and neuronal cells and to be mediated by \( \beta_2 \) and \( \beta_1 \) receptors, respectively (419).

An alternative suggestion that cAMP actions are mediated by conversion to adenosine has been challenged by observations in cerebellum and cerebral cortex that methylxanthine phosphodiesterase inhibitors potentiate NE and cAMP effects yet block the effect of adenosine or 5'-AMP (185, 435, 451). With cultured Purkinje neurons, Gahwiler (138) has observed potentiation of NE and cAMP depressions with phosphodiesterase inhibitors. He also observed that the response thresholds to NE were 100~1,000 times lower than for responses to cAMP (applied by superfusion), which agrees with predictions from other instances of second-messenger mediation (see 38, 317, 453).

Finally, cAMP is known to activate a class of enzymes termed protein kinases (see 155, 156), which phosphorylate specific protein substrates; functional changes in these protein substrates may be the mechanism for expressing altered cAMP levels (see 155). In cerebellum, chemical analogues of cAMP mimic the inhibitory effects of NE in direct correlation to their ability to activate brain cAMP-dependent protein kinase (433). Likewise, simulations of the postsynaptic effects of NE have recently been achieved in invertebrate nervous systems by injecting purified subunits of the protein kinase into the postsynaptic neuron (57, 212). The question arising at this point is whether the alteration in cyclic nucleotide levels and in enzymes regulated by cyclic nucleotides can be considered to mediate the synaptic actions of NE or whether these chemical changes and effects are an epiphenomenon or a simple associated effect of \( \beta \)-receptor activation associated with some NE synapses. The hyperpolarizing effect of NE and LC with increased resistance is in direct contrast to changes seen with classic inhibitory postsynaptic potentials (IPSPs) or with iontophoresis of GABA (see 436). The classic inhibitory pathways and inhibitory amino acid transmitters (see 243) are thought to operate exclusively through mechanisms that increase conductance to ionic species whose equilibrium potentials are more negative than the resting membrane potential. In such cases the hyperpolarization is associated with a decrease in membrane resistance. Intracellular recordings indicate that the hyperpolarization produced by NE and LC stimulation, on the other hand, is accompanied by and perhaps generated by decreases.
In passive ionic conductance across the membrane (i.e., an increase in membrane resistance) (437). In this respect the NE effect differs from conventional postsynaptic responses, which are mediated by increased ionic conductance. This effect may be due to a decrease in conductance to an ion such as Na⁺ or Ca²⁺ or to activation of an electrogenic pump (350, 352, 502). Clearly the depressant actions of NE do not require extracellular Ca²⁺ (142); inhibitory substances, which also antagonize Ca²⁺ actions, also do not interfere selectively with NE inhibitory effects (128).

There are also substantial reasons to consider the possibility that an electrogenic pump-type mechanism may be activated by these LC inputs (see 435-437). This pump activation could produce hyperpolarization with a resistance increase. Phillis and Wu (352) recently reviewed this evidence in depth. Such a mechanism could produce both presynaptic and postsynaptic NE effects. For example, according to this hypothesis the influx of Ca²⁺ occurring during the presynaptic action potential inhibits Na⁺-K⁺-ATPase in the nerve terminal and thus triggers the release of transmitter. Agents such as CAs, which are known to inhibit the release of transmitter from some nerve terminals, might do so by activating the Na pump, reducing terminal depolarization. Activation of Na⁺-K⁺-ATPase might also mediate NE effects within target neurons. Stimulation of Na⁺-K⁺-ATPase by NE was first described in brown adipose tissue (176). The effect could be blocked by propranolol, a β-adrenergic antagonist. Subsequent to this finding, a number of investigators reported biogenic amine stimulation of Na⁺-K⁺-ATPase in various brain preparations. Schaefer et al. (396, 397) were the first to demonstrate that CAs, including NE, DA, and isoproterenol, could stimulate particle-bound Na⁺-K⁺-ATPase from rat brain synaptosomal membranes. Phenolic amines, tyramine, hydroxyamphetamine, metaraminol,amphetamine, and phenylephrine did not stimulate the enzyme. Godfraind et al. (149) favored a disinhibitory action to account for the stimulation of Na⁺-K⁺-ATPase by CAs, suggesting that they could be chelating Ca²⁺. If, however, CA stimulation of Na⁺-K⁺-ATPase is merely the result of removal of inhibitory divalent metal ions, the stimulating effect should be abolished in the presence of high concentrations of chelating agents. This does not appear to happen (501). The other proposed mechanism for CA-induced stimulation of Na⁺-K⁺-ATPase is that a specific CA receptor is involved. A number of investigators have reported antagonism of the stimulatory effects of CAs on brain Na⁺-K⁺-ATPase with both α- and β-adrenergic antagonists. For example, Wu and Phillis (500, 502, 503) have shown that Na⁺-K⁺-ATPase in rat cerebral cortical homogenates or synaptosomal preparations is stimulated by NE, at <10⁻⁷ M threshold. The responses to NE could be antagonized by both the α-antagonist phentolamine and the β-antagonist propranolol. These results clearly demonstrate that Na⁺-K⁺-ATPase activation evoked by NE can be blocked by adrenoceptor antagonists and may possibly involve both α- and β-receptors in the activation process. In this respect, CA stimulation of cerebral cortical Na⁺-K⁺-ATPase is similar to the CA-evoked ac-
tivation of cAMP, which is also mediated by both α- and β-receptors (86).
In order to evaluate the role of NE in regulating the activity of Na⁺-K⁺-ATPase in vivo, enzyme activity in the rat brain was measured after
electrical stimulation of LC. Increased Na⁺-K⁺-ATPase activity was found in LC
projection areas such as the hippocampus and hypothalamus but not in
corpus striatum (see 131 for LC effects on caudate). Increased activity was
limited to target regions ipsilateral to the stimulating electrode. Although
cAMP does not directly affect Na⁺-K⁺-ATPase activity, it could do so sec-
ondarily via a protein kinase cascade effect. Interestingly the enzyme is most
sensitive to NE during the developmental stage (7–21 days), which corre-
sponds to the most rapid period of synaptogenesis (5) and the appearance
of adrenergic receptors (169, 361). The responsiveness of adenylate cyclase
to NE follows a similar developmental pattern: barely detectable during the
1st wk after birth and reaching adult levels at ~14 days (344).

As discussed above, intracellular recordings show that iontophoretically
applied NE hyperpolarizes spinal motoneurons (283, 351), cerebellar Purkinje
cells (437), hippocampal pyramidal neurons (414), and cerebral cortical neu-
rons (99, 348), and decreases in membrane conductance frequently accom-
pany these hyperpolarizations (99, 283, 437). In some instances, as with
cerebellar Purkinje cells, the increases in resistance arc dramatic (184, 437)
and have been proposed as the effect of transmitter action responsible for
the hyperpolarization (308). The increase in membrane resistance that ac-
companies CA-evoked hyperpolarizations may account for the enhanced ef-
fect of other excitatory and inhibitory synaptic inputs observed during CA
application or LC stimulation (see 41, 303, 499). The most detailed analysis
of the effects of NE on membrane potential and resistance to date involved
spinal motoneurons (283) and hippocampal CA1 pyramidal cells in vivo (179)
or in vitro (414). Hyperpolarizations of the resting membrane potential by
NE were usually 5–10 mV in amplitude and were often accompanied by 10–
20% increases in membrane resistance. Hyperpolarization enhanced and
depolarization reduced the NE-evoked hyperpolarizations. The hyperpolar-
ization was still enhanced at potentials far in excess of the equilibrium
potential for K⁺. Reversal of the NE-evoked potential into a depolarization
occurred at membrane potentials between −5 and −20 mV. Intracellular
injections of Cl⁻ that reversed IPSPs evoked by nerve stimulation caused
little or no change in the amplitude or time course of the NE hyperpolar-
ization. Similar observations were first made in studies of the hyperpolar-
ization of Purkinje cells (437). Although reversal potentials were not ob-
served, the pattern of increased amplitude of the hyperpolarization during
increased polarization of the membrane by injected current was seen, and
Cl⁻ injection did not alter the amplitude or time course of the response.

Hyperpolarizations are evoked by NE (2–10 mV) in neurons of the cat ce-
rebral cortex, and an accompanying small increase in membrane resistance
(<10%) is detectable in about half of the neurons tested (99, 349). Segal
(414) has recently studied the ionic basis of NE hyperpolarization of CA1
neurons in hippocampal slices. Topical application of NE caused a hyperpolarization of 3–4 mV, associated with an 8–13% decrease in input resistance. Intracellular Cl\(^-\) injection or a reduction in extracellular Cl\(^-\) had little effect on the initial phases of the NE hyperpolarization, although there was some reduction in its duration. When experiments were conducted at 22°C (instead of the normal 34°C), the hyperpolarizing action of NE disappeared and was replaced in some instances by a depolarization. Cyclic AMP also hyperpolarized CA1 cells, with little or no change in membrane resistance.

An analysis of all the evidence regarding CA-evoked hyperpolarizations, however, suggests that activation of an Na\(^+\) pump could be a plausible explanation disregarding undetected presynaptic effects. A simultaneous reduction in membrane Na\(^+\) and K\(^+\) permeability (283) could account for a hyperpolarization with a reversal potential of −10 to −20 mV, but disregarding undetected presynaptic effects fails to account for the often poor correlation between hyperpolarization and membrane resistance increase. Yarbrough (505) examined the ability of ouabain to antagonize NE and GABA inhibition of caudate and cerebellar neurons. Ouabain exhibited antagonistic activity toward amine inhibitions on both types of cells, while leaving the amino acid inhibition intact. The depressant effects of NE but not of GABA or cAMP on spontaneous firing of rat cerebral cortical neurons was antagonized by a number of known Na\(^+\)-K\(^+\)-ATPase inhibitors, including ouabain, digitoxin, digitoxigenin, K-strophanthin, thevetin A and B, etacrynic acid, and harmaline (391). Ouabain also antagonized the hyperpolarizing actions of NE and cAMP on CA1 cells in rat hippocampal slices (414).

Another possible postsynaptic effect of NE and other CAs might be to alter voltage-sensitive conductances. Cyclic nucleotide effects on voltage-sensitive conductances have been established in at least two invertebrate neuronal systems. Klein and Kandel (225) have shown that serotonin acts to broaden the spike in a sensory neuron in *Aplysia* and that this broadening is due to an effect on voltage-sensitive Ca\(^{2+}\) conductance. They have presented evidence suggesting that this effect may be mediated by cAMP (also see 477).

In a voltage-clamp study, CA-induced inhibition of an *Aplysia* burst-firing neuron was shown to be mediated by effects on voltage-sensitive conductances normally underlying burst generation (see 248). This study demonstrated that DA (or ACh) changed the current-voltage relationship of the cell from one showing a negative-resistance region (apparently necessary and sufficient for pacemaker activity) to one lacking the negative-resistance region. Moreover, Levitan and Treistman (258) have shown that cyclic nucleotides modify bursting activity in invertebrate neurons. These findings indicate that, at least in some systems, CAs may act through direct effects on pacemakers and suggest that effects may be mediated by cyclic nucleotides. Of these three mechanisms, current evidence favors the possible effect on endogenous pacemaker activity.

The effect of NE might therefore be mediated in one of three ways: 1) a decrease in resting Na\(^+\) or K\(^+\) conductance, which should hyperpolarize the
cell membrane; 2) an electrogenic ion pump might be activated to produce the hyperpolarization; or 3) in analogy with the effects of NE on cAMP-mediated pacemaker mechanisms in the heart, NE could slow firing by altering the voltage-sensitive conductances involved in pacemaker generation. All three possible actions of NE could be considered candidate functions, which could in turn be the primary molecular action of increased cAMP.

D. Locus Ceruleus–Norepinephrine Effects on Target Neuron Functional Activity

The studies summarized above were generally performed in anesthetized animals, and LC-NE effects were assessed in target neurons that were not being activated (or inhibited) in any way relevant to their function. Adequate characterization of the functional properties of the LC-NE system in the operating brain requires that the structure and function of the LC system be considered with other neural systems projecting to the same targets or to immediately connected target cell systems. That is, in order to understand the impact of LC-NE innervation on a particular target cell, LC-NE effects on functional activity, rather than spontaneous activity, of the target neuron must be assessed. This requires the simultaneous activation of other inputs to target neurons while iontophoresing NE or activating the LC-NE system. In the past several years, many such experiments have been conducted aimed at determining whether LC-NE differentially affects different aspects of target cell activity. The target areas most intensively studied have been the cerebellum (129, 298–300, 506), hippocampus (415–418), and cerebral cortex (125, 487, 488). Effects similar to those described in detail below have also been observed in the lateral geniculate nucleus (222, 375–377), spinal motoneurons (496), and olfactory bulb (200).

1. Effects of norepinephrine or of locus ceruleus stimulation on cortical neuronal activity

The first demonstration of differential NE effects on spontaneous versus elicited target cell activity was observed in a study by Foote et al. (125) of the activity of auditory cortex neurons in the awake monkey. In this preparation, iontophoretically applied NE reduced spontaneous-discharge activity to a greater extent than activity elicited by acoustic stimulation. The authors noted that this resulted in an enhancement of vigorous responses relative to weak responses or background activity, providing greater contrast or discrimination between various types of input. Recently a similar enhancement of evoked activity relative to spontaneous activity has been observed in cat visual cortex neurons during NE iontophoresis (215). Water-
house and colleagues (487-490) have intensively investigated the effects of iontophoretic NE and of LC stimulation on neocortical neuronal activity in rat. They observed a similar reduction of background activity relative to stimulus-elicited activity in the rat somatosensory cortex (490). Additionally, evoked excitatory responses were enhanced by NE application in some cells. In 82% of the cells tested, NE augmented stimulus-bound inhibition and postexcitatory suppression of activity (490). In other studies, Waterhouse et al. (487, 488) have demonstrated that iontophoretic NE enhances responses to iontophoretic GABA and ACh and that enhanced excitatory sensory responses are produced and blocked by α-adrenergic but not β-adrenergic agents (see also 29, 371). Enhancement of synaptically driven excitatory responses via α-adrenergic receptors has also been observed in the LCN (222, 375-377).

2. Effects of norepinephrine or of locus ceruleus stimulation on spontaneous and evoked Purkinje cell activity

Freedman et al. (129) performed the initial study of LC-NE interactions with synaptically elicited Purkinje cell activity. Norepinephrine was iontophoresed onto Purkinje cells that were systematically activated by climbing fibers (cerebral cortex stimulation), mossy fibers (peripheral limb and vibrissae stimulation), and cerebellar interneurons (off-beam stimulation). Spontaneous activity was reduced by NE more profoundly than either simple-spike or complex-spike synaptic excitation. Climbing fiber responses were often actually enhanced (this was the first demonstration of simultaneous enhancement and reduction of different types of activity by NE), and inhibitory responses to basket and stellate cell input were often potentiated. These effects were often observed at doses of NE that had minimal effects on spontaneous-discharge rate. Thus effects similar to those seen in cortical neurons were observed. Moises et al. (298–300) have shown that LC stimulation, at intensities that do not affect spontaneous-discharge rate, enhances complex-spike excitation of Purkinje cells elicited by sensorimotor cortex stimulation. Postexcitatory reductions in activity were also potentiated. These effects were blocked by a β-adrenergic receptor blocker. These authors have also demonstrated that NE potentiates the depressant effect of iontophoresed GABA, but not of glycine, and the stimulant effect of iontophoresed glutamate on Purkinje cells. Further studies by Yeh et al. (506) suggest that the potentiation of GABA effects by NE is selective in that β-alanine and taurine inhibitions are not similarly enhanced. Finally, Woodward et al. (499) find that the NE enhancement of GABA or synaptic inhibition is mediated by β-adrenergic receptors in both cerebellum and neocortex.
3. Effects of norepinephrine or of locus ceruleus stimulation on hippocampal neuronal activity

In a series of studies, Segal and Bloom (415–418) investigated the effects of iontophoretic NE and of LC stimulation on activity of hippocampal pyramidal neurons. Both treatments inhibited pyramidal cell spontaneous activity, and numerous control experiments suggested that these effects indicated the existence of an LC-to-pyramidal cell synaptic pathway. In freely moving rats, either the presentation of a loud tone to the animal or electrical stimulation of LC produces pyramidal cell inhibition. These tone responses are inhibited by drugs that interfere with noradrenergic transmission, possibly reflecting their generation by LC discharge. Furthermore LC stimulation delivered as the unconditioned stimulus in a classic conditioning paradigm restored previously habituated inhibitory responses to the tone. Finally, LC stimulation preceding a tone stimulus potentiated inhibitory and excitatory responses to a tone that had been paired with food presentation. Thus these target neurons exhibited effects similar to those observed in neocortical and cerebellar neurons. Additional evidence that LC modifies hippocampal responses to afferent stimulation has been generated in studies of hippocampal evoked responses to interhemispheric stimulation (412).

4. Hypotheses of locus ceruleus–norepinephrine effects on target neuron functional activity

The material reviewed earlier clearly indicates that LC activity produces a reduction of target cell spontaneous activity in several brain regions. The fact that the mechanism of synaptic action of LC on its target cells has some rather unconventional aspects, such as coupling to cAMP formation and possible selective effects on ionic channels and pacemaker activity, may be the reason that LC has more complex consequences for the postsynaptic integration of synaptic signals than conventional synaptic inhibition. Various hypotheses describe the net impact of the cellular effects produced by the LC system. Some of these emphasize the enhancement of evoked activity relative to spontaneous activity for afferent systems simultaneously active with LC (125, 418, 499). Others describe this process as enabling, 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 (41, 303). As noted above, Yeh et al. (506) and Moises et al. (298) present evidence that such enhancement by NE may occur only with certain transmitters. Dismukes (93) has presented an extensive consideration of the terminology used to describe various types of transmitter effects on target neurons. Schulman (406) has noted that one possible ramification of this signal enhancement is sharpened spatial localization of activity, within any area of diffuse afferent activation, to targets convergent with those of LC.
In cerebellum, for example, because NE appears to potentiate both on-beam excitatory effects and off-beam GABA-mediated inhibition, LC may serve to regulate the strength of lateral inhibition in the cerebellar cortex. Activation of LC might therefore restrict the area of cerebellar cortex activated by a given mossy fiber input and enhance the spatial precision with which mossy fiber systems could act on the cerebellar cortex. Flicker et al. (122) have related aminergic activation to response plasticity of target neurons, suggesting the involvement of cAMP as a second messenger.

The molecular and ionic mechanisms responsible for these complex effects of NE on various aspects of target neuron activity are not yet understood, although certain possibilities were described in section IV C; i.e., the LC effect on Purkinje cells involves an apparent decrease in membrane ionic conductance. It has been shown in Aplysia sensory neurons (213) and in neurons of the bullfrog sympathetic ganglion (407) that generation of decreased conduction by one synaptic pathway results in increased transmission via other synaptic pathways, if the latter function by classic conductance-increase mechanisms. However, such effects may not be fully explained by the observed conductance decrease. It has been shown that facilitatory effects of NE and of LC stimulation outlast effects on firing rates and can be demonstrated even when LC stimulation does not detectably alter the firing rate of the cerebellar Purkinje cell. Moreover, facilitatory effects of NE in cerebellum hold for some transmitters (e.g., GABA endogenous to cerebellum) but not others (e.g., glycine or taurine not found in cerebellum) (499, 506). The mechanisms mediating these heterosynaptic interactions are not known, and the possible intermediaries are numerous. For example, it has recently been demonstrated that NE induces glycogenolysis in cortical slices (281), an effect that could mediate changes in neuronal responsiveness.

Finally, 6-OHDA lesions of the dorsal bundle do not prevent the occurrence of neocortical low-voltage fast activity or hippocampal rhythmical slow activity (374). This raises the question of how to reconcile the numerous demonstrated cellular effects of the LC-NE system with the failure to demonstrate obvious effects on “summary” electrical activity of forebrain regions.

V. CONCLUSIONS

A. What is Specificity?

The data reviewed here require new perspectives on certain fundamental functional characteristics of the LC-NE system. The most important recent observations are: 1) LC neurons in the unanesthetized animal exhibit systematic changes in discharge rate as a function of specified changes in brain activity or in the external environment, 2) LC-NE neurons form specialized
synaptic contacts with other neurons, 3) these target neurons are specifiable subsets of the neurons constituting the target nucleus, and 4) NE affects the electrophysiological activity of postsynaptic neurons in specified ways that influence their functional activities. Taken together these observations strongly suggest that the effects of the LC-NE system on target neurons are characterized by temporal and spatial discreteness. That is, the LC-NE system acts on a discrete, specifiable set of neurons under a particular set of circumstances to produce a defined set of effects. This is the anatomical and physiological specificity referred to in the title of this review. However, there are clearly ways in which this system is not specific. These are largely reflected in the variety of physiological and anatomical afferents to the LC and in the diversity of its efferents. We suggest that the LC is not specific in terms of the sensory modality to which it is responsive or in terms of communicating with a restricted, easily defined set of brain nuclei. Rather, the LC-NE system is specific in that at certain times it alters, in a functionally relevant way, the input-output characteristics of its target neurons. For example, visual cortex neurons have been shown to exhibit systematic changes in receptive-field properties as a function of arousal (270; see also 64). It is possible that the LC-NE system participates in producing such changes, as shown by the effects of iontophoretic NE on visual cortex receptive fields (215). One effect of the massive efferent system of the LC might be the simultaneous imposition of such state changes on many brain regions.

R. Why is Specificity Important?

The anatomical and physiological specificity recently demonstrated for the LC-NE system is important because it indicates that this neuronal system is orderly and the appropriate object of neurobiological studies. For example, these neurons produce action potentials in accordance with specified stimulus-response rules, they faithfully transmit action potentials along their axons, and they release a specified transmitter at synaptic sites to exert defined postsynaptic effects. These findings indicate that the powerful hypotheses relevant to action potentials, axons, and synapses are also applicable to the LC-NE system. The description of this specificity of the LC-NE system is also important because it suggests that the LC is an integral component of brain information-processing systems and participates in the control of behavioral events that are themselves temporally discrete, involve many brain systems, and reflect the occurrence of substantial information processing. If NE were released continuously, or if it were released in a spatially random manner, it would matter little when the cells were electrophysiologically active or how their fibers were distributed. In fact both of these arguments have been made. If these fibers innervated only or primarily blood vessels, the effects of NE on neurons would be of little interest, and in fact this argument has also been made. On the contrary, it can be argued that these neurons are understood better than many others in the
brain and that they therefore constitute prime candidates for intensive study.

C. Toward Testable Hypotheses of Locus Ceruleus Function

Because the importance of any neuronal system must ultimately be assessed in terms of its impact on its target neurons, it is most important at this time to determine the effects of LC-NE activity on target neurons under the most naturalistic conditions possible. This must, of course, be done with guidance from the most refined anatomical data available, suggesting appropriate target neurons for study. Although there is a strong presumption that LC-NE activity alters the discharge properties of neurons in many brain regions, this has never been demonstrated directly under physiological conditions. In such a demonstration, a well-delineated hypothesis concerning the functions of the target neuron(s) would be desirable so that the ultimate impact of LC-NE activity on brain functioning, at least in that target area, could be reasonably interpreted. The data summarized in this review suggest the possibility that the LC-NE system acts at many target sites to somehow enhance the reliability and efficiency of feature extraction from sensory input. Recordings from behaving animals indicate that the extent to which this function is engaged varies from second to second, fluctuating in a systematic way in response to environmental and organismic conditions.

In addition to determining the effect of synaptic NE on brain operations, the effects of this system on the development of various brain regions must be assessed. The tantalizing hypothesis that NE is essential for cortical plasticity has generated little experimentation, presumably because of technical difficulty. Perhaps new anatomical and physiological insights will help reduce this general hypothesis to manageable proportions so that it can be adequately tested. The most exciting possibility is that there may be a unifying hypothesis of LC-NE function integrating developmental and physiological functions of this system into a broader scheme of brain organization in which the long- and short-term postsynaptic effects of the LC-NE system are seen as different aspects of the same function.

Paul Sawchenko, John Morrison, and Menahem Segal provided helpful information concerning parts of the literature review. Our work was supported by Public Health Service Grants AA 03504, NS 16209, and NS 18023.

Present address of G. Aston-Jones: Dept. of Psychology, State University of New York, Binghamton, NY.

REFERENCES

4. AGHAJANIAN, G. K. Mescaline and LSD facilitate the
activation of locus coeruleus neurons by peripheral stim-
5. AGHAJANIAN, G. K., AND F. E. BLOOM. The formation of
synaptic junctions in developing rat brain: a quanti-
tative electron microscopic study. Brain Res. 8: 716-727,
1967.
6. AGHAJANIAN, G. K., J. M. CEDARBAUM, AND K. Y.
WANG. Evidence for norepinephrine-mediated collat-
eral inhibition of locus coeruleus neurons. Brain Res. 136:
7. AGHAJANIAN, G. K., AND P. VANDERMAELEN.
α1-Adrenoceptor-mediated hyperpolarization of locus
coeruleus neurons: intracellular studies in vivo. Science
8. AHN, H. S., R. K. MISHRA, C. DEMIRJIAN,
AND M. H. MAKMAN. Catecholamine-sensitive adenylate
cyclase in frontal cortex of primate brain. Brain Res 116:
9. ALBANESE, A., AND L. L. BUTCHER. Acetylcholines-
terase and catecholamine distribution in the locus cer-
10. AMARAL, D. G., C. AVENDANO, AND W. M. COWAN.
The effects of neostigmine 6-hydroxydopamine treatment on
morphological plasticity in the dentate gyrus of the rat following
entorhinal lesions. J. Comp. Neurol. 191: 171-
191, 1980.
11. AMARAL, D. G., AND W. M. COWAN. Subcortical dif-
erentia in the hippocampal formation in the monkey. J.
12. AMARAL, D. G., AND H. M. SINNER. The locus coe-
ruleus: neurobiology of a central noradrenergic nucleus.
13. ANDEN, N. E., A. DAILITROM, K. FUXE, K. LARSON,
L. OLSON, AND U. UNGERSTEDT. Ascending monoamine
eurons to the telencephalon and diencepha-
14. ARBUTHNOTT, G. W., J. E. CHRISTIE, T. J. CROW, D.
ECCLESTON, AND D. E. WALTER. Loci of the locus coeruleus
and noradrenaline metabolism in cerebral cor-
15. ARBUTHNOTT, C. W., T. J. CROW, K. FUXE, L. OL-
SON, AND U. UNGERSTEDT. Depletion of catechol-
a mine in vivo induced by electrical stimulation of cen-
16. ASTON-JONES, G. The Behavioral Physiology of Locus
Coeruleus Neurons (PhD thesis). Pasadena: California
17. ASTON-JONES, G., AND F. E. BLOOM. Activity of
norepinephrine-containing locus coeruleus neurons in
behaving rats anticipates fluctuations in the sleep-wak-
18. ASTON-JONES, G., F. E. BLOOM. Norepinephrine-
containing locus coeruleus neurons in behaving rats ex-
hibit pronounced responses to non-noxious environmen-
19. ASTON-JONES, G., S. I. FOOTE, AND F. E. BLOOM.
Low doses of ethanol disrupt sensory responses of brain
noradrenergic neurons. Nature London 296: 857-860,
1982.
20. BATTISTA, A., K. FUXE, M. GULLSTRAND, AND M.
UGAWA. Mapping of central monoamine neurons in the
21. BEAUFRE, AND L. DESCARDES. The monoamine
innervation of rat cerebral cortex: synaptic and nonsyn-
22. BECKSTEAD, K. M. Afferent connections of the entorhi-
nal area in the rat as demonstrated by retrograde cell-
labeling with horseradish peroxidase. Brain Res. 152:
23. BELLAND, R. M., Y. B. DOMESICK, AND W. J. H.
NAUTA. Efferent connections of the substantia nigra
and ventral tegmental area in the rat. Brain Res. 175:
24. BELLAND, R. M., Y. B. DOMESICK, AND W. J. H.
NAUTA. Efferent connections of the substantia nigra
and ventral tegmental area in the rat. Brain Res. 175:
25. BEVAN, P., C. M. BRADSHAW, AND E. SZABADI. The
pharmacology of adrenergic neuronal responses in the
cerebral cortex: evidence for excitatory and inhibitory
26. BIRD, J. J., AND M. J. KUHAR. Immunohistochemical
application of opiates to the locus coeruleus. Brain Res. 122:
503-583, 1977.
27. BJÖRKLUND, A., AND U. STEN&Euml;V. Regeneration of
monoaminergic and cholinergic neurons in the ma-
malian central nervous system. Physiol. Rev. 59: 68-100,
1979.
28. BLACKSTAD, T. W., K. FUXE, AND T. KÖPFELT. Nor-
adrenaline nerve terminals in the hippocampal region of
the rat and the guinea pig. Z. Zellforsch. Mikrosk. Anat.
29. BLESSING, W. W., J. P. CHALMERS, AND P. R. C.
HOWE. Distribution of catecholamines containing cell
bodies in the rabbit central nervous system. J. Comp.
30. BLESSING, W. W., A. K. GOODCHILD, R. A. L. DAMP-
NEY, AND J. P. CHALMERS. Cell groups in the lower
brain stem of the rabbit projecting to the spinal cord,
with special reference to catecholamine-containing neu-
31. BLOOM, F. E. Ultrastructural identification of cate-
cholamine-containing central sympathetic terminals. J. His-
32. BLOOM, F. E. To spritz or not to spritz: the doubtful
value of aimless iontophoresis. Life Sci. 14: 1819-1834,
1974.
33. BLOOM, F. E. Central monoaminergic transmission. In:
Colpol Centennial Symposia: Perspectives in Neurobiol-
y, edited by M. Santini. New York: Raven, 1975, p. 489-
495.
34. BLOOM, F. E. Role of cyclic nucleotides in central syn-
103, 1975.
35. BLOOM, F. E. Physiological assessment of pre- and post-
synaptic receptors. In: Presynaptic Receptors, edited by
36. BLOOM, F. E. Central noradrenergic systems: physiology
and pharmacology. In: Psychopharmacology—A Genera-
tion of Progress, edited by M. A. Tipton, K. C. Killam,
37. BLOOM, F. E. Chemical integrative processes in the cen-
tral nervous system. In: The Neurosciences: Fourth Stud-

LOCUS CERULEUS SPECIFICITY


108. EPHELBAUM, J. L. TAPIA-ARANCIBIA, AND C. KOR- DON. Noradrenaline stimulates somatostatin release
from incubated slices of the amygdala and the hypotha-

109. FAIERS, A. A., AND G. J. MUGGENSUN. Electrophysi-
ological identification of neurons in locus coeruleus. Exp.

110. FALK, H., N.-A. HILLARP, G. THIEME, AND A. TUKP.
Fluorescence of catecholamines and related compounds
condensed with formaldehyde. J. Histochem. Cytochem.

111. FALK, B., AND C. H. OWMAN. A detailed methodolog-
ical description of the fluorescence method for the cel-
lar demonstration of biogenic amines. Acta Univ. Lund

112. FALLOW, J. H., D. A. KOZIELLA, AND R. Y. MOORE.
Catecholamine innervation of the basal forebrain. II.
Amygdala, suprachiasmatic and entorhinal cortex. J.

113. FALLON, J. H., AND R. Y. MOORE. Catecholamine in-
nervation of the basal forebrain. III. Offlagy bulb, an-
terior olfactory nuclei, olfactory tubercle and piriform

114. FARBER, J., G. A. MARKS, AND H. P. ROFFWARC.
Rapid eye movement sleep PGO-type waves are present
in the dorsal pons of the albino rat. Science 209: 615-617,
1980.

115. FELTEN, D. L. Dendritic monoamines in the squirrel

116. FELTEN, D. L., AND K. A. CRUTCHER. Neuronal-vas-
cular relationships in the raphe nuclei, locus coeruleus,
and substantia nigra in primates. Am. J. Anat. 155: 497-

117. FELTEN, D. L., H. HALLMAN, AND G. JONSSON. Ev-
idence for a neurotrophic role of noradrenaline neurons
in the postnatal-development of rat cerebral-cortex. J.

118. FELTEN, D. L., A. M. LATIES, AND M. B. CARPENTER.
Monoamine-containing cell bodies in the squirrel monkey

119. FERRON, A., L. DESCARRIES, AND T. A. READER.
Altered neuronal responsiveness to biogenic amines in
rat cerebral cortex after serotonin denervation or deple-

120. FINLEY, K. H., AND G. CROCH. The capillary bed of the

121. FLEETWOOD-WALKER, S. M., K. A. CRUTCHER, AND
J. H. COOTE. The
histochemical characterization of a neocortical projection
from the locus coeruleus of the squirrel monkey. J.

122. FREE, L., AND R. J. HOPPER. Phenoethamine an-
tagonism of the noradrenergic inhibition of cerebellar

123. FREE, L., H. J. HOPPER, D. J. WOOGWAR, AND D. PURK.
Interaction of norepinephrine with cere-
bellar activity evoked by money and climbing fibers.

124. FROGIN, K. P., D. T. O'CONNOR, AND G. L. LEVINE.
Human dopamine-b-hydroxylation: comparison of the en-
zyme from plasma, adrenal medulla, and pheochromocytoma
by radioimmunoassay. Mol. Pharmacol. 19: 444-
460, 1981.

125. FUJIMOTO, S., M. SAIDA, AND S. TAKAKORI. Inhibition
from locus coeruleus of mammalian neurons activated by

126. FUKUSHIMA, T., AND F. W. L. KERR. Organization of
trigeminothalamic tracts and other thalamic afferent systems
of the brainstem in the rat: presence of gelati-
unae neurons with thalamic connections. J. Comp.

127. FULLER, J., AND J. SCHLAG. Determination of anti-
ergic excitation by the collision test: problems of inter-

128. FUMAGALLI, R., V. BERNAREGGI, B. FERTRI, AND M.
TRABUCCI. Cyclic AMP formation in human brain in an
in vitro stimulation by neurotransmitters. Life Sci. 10:

129. FUKE, K. Evidence for the existence of monoamine neu-
rons in the central nervous system. IV. The distribution
of monoamine nerve terminals in the central nervous

130. FUKE, K., D. GANTZ, T. HÖKFELT, AND P. BOLME.
Immunohistochemical evidence for the existence of an-
gicinergic II-containing nerve terminals in the brain and

131. FUKE, K., B. HAMMBERGER, AND T. HÖKFELT. Dis-
tribution of noradrenaline nerve terminals in cortical areas

132. GAHWILER, B. H. Inhibitory action of noradrenaline
and acetylcholine on ischemia in hippocampal and
dentate pyramidal neurons. Exp. Neurol. 39: 494-
495, 1972.

133. GARVER, D. L., AND J. R. SLADEK, Jr. Monoamine
distribution in primate brain. I. Catecholamine-contain-
ing perikarya in the brain stem of Macaca ssp. Brain

134. GARVER, D. L., AND J. R. SLADEK, Jr. Monoamine
distribution in primate brain. II. Brain stem catechol-
amineergic pathways in Macaca ssp. (cercopithecoid).

135. GATTER, K. C., AND T. P. S. POWELL. The projection
do loco coeruleus upon the mesocortical area in the

136. GELLER, H. M., AND B. J. HOPPER. Effect of calcium
removal on monoamine-salicited depressions of cultured

137. GERMANN, D. C., AND M. BOWDEN. Locus coeruleus
in rhesus monkey (Macaca mulatta): a combined histo-
chemical fluorescence, Nissl and silver study. J. Comp.

138. GERMANN, D. C., AND H. E. ELLIS. Responses of primate
locus coeruleus and subcoeruleus neurons to stimulation at
reinforcing brain sites and to natural reinforcers.
pocapal neurons recorded in vivo displays four different reaction-mechanisms to iontophoretically applied transmitter agonists. Brain Res. 212: 351-363, 1981.


283. MARSHALL, K. C., AND I. ENGBERG. Reversal poten-


311. NATHANSON, J. A. Cyclic nucleotides and nervous sys-


318. NELSON, C. N., R. J. HOFFER, N.-S. CHU, AND J. K.

BLOOM. Cytochemical and pharmacological studies on poly-


319. NELSON, M. F., R. ZACZEK, AND J. T. COYLE. Effects of

sustained seizures produced by intrahippocampal in-


320. NOBIN, A., AND A. BJÖRKLUND. Topography of the

monoamine neuron system in the human brain as re-


321. NORITA, M., AND K. KAWAMURA. Subcortical affer-


322. NYBACK, H. V., J. R. WALTER, G. K. AGHAJANIAN,


324. O'CONNOR, D. T., R. P. FRIGON, AND R. A. STONE. Ac-


325. OLSCHOWKA, J. A., R. GRZANNA, AND M. E. MO-

LLIVER. The distribution and incidence of synaptic con-


326. OLPE, H.-R., A. GLAUT, J. LASZLO, AND A. SCHEL-

LENBERG. Some electrophysiological and pharmacolog-


327. OLPE, H.-R., AND M. W. STEINMANN. The activating action of vincamine, piracetam, and hydrgine on the activity of the noradrenergic neurons of the locus coe-


328. OLSCHOWKA, J. A., R. GRZANNA, AND M. E. MOLL-

LIVER. The distribution and incidence of synaptic con-


329. OLSCHOWKA, J. A., M. E. MOLLIVER, R. GRZANNA,

F. L. RICE, AND J. T. COYLE. Ultrastructural demon-

stration of noradrenergic synapses in the rat central nervous system by dopamine-β-hydroxylase immunocyto-


330. OLSON, L. O. BOREUS, AND A. SEIGER. Histoch-

emical demonstration and mapping of 5-hydroxytrypt-


331. OLSON, L., AND L. FUXE. On the projections from the


334. OTTERSEN, O. P. Afferent connections to the amygd-


335. PALACIOS, J. M., AND M. J. KUHAR. Beta-adrenergic recep-


336. PALMQUIST, M., L. ZADBONSEK, A. FEMINGE, E.

MENEZ, M. I. K. PEKETRE, J. P. HERMAN, B. KAN-


337. PALMER, M. R., AND R. J. HOFFER. Catecholamine


339. PEARSON, J., L. BRANDIS, AND M. GOLDSTEIN. Immu-


341. PEPPER, C. M., AND M. HENDERSON. Opiate and

342. PERKINS, J. T., AND M. M. MOORE. Regulation of the
dopamine cyclic 5,3-monophosphate content of rat cere-

bral cortex: ultrastructural development of the respons-

343. PEROUTKA, S. J., M. A. MOSEKOWITZ, J. F. REIN-

HARD, Jr., AND S. H. SNYDER. Neurotransmitter re-

344. PETTIGREW, J. D., AND T. KASAMATSU. Local per-


345. PFISTER, C., AND H. DIANKE. Fluorescence histo-

chemical and neurohistological investigations on the loc-


348. PHILLIS, J. W., N. LAKE, AND G. YARBROUGH. Cal-

cium mediation of the inhibitory effects of biogenic


385. SASA, M., S. FUJIMOTO, S. IGARASHI, K. MUNEKIKI, AND S. TAKAORI. Microiontophoresis studies on noradrenergic inhibition from locus coeruleus of spinal


400. SEGAL, M., AND S. C. LANDIS. Afferents to the septal area of the rat studied with the method of retrograde


456. SVENSSON, T. H., AND G. ENGBERG. Antagonism of ethanol's central stimulation by catecholamine receptor


