Introduction: revision of an old transmitter

Gregory A. Ordway, Ph.D.

The discovery of norepinephrine dates back to the late 1940s when the Swedish scientist, Ulf Svante von Euler first demonstrated that neurons of the sympathetic nervous system use norepinephrine, rather than epinephrine, as a neurotransmitter. Shortly thereafter in 1947, Peter Wilhelm Joseph Holtz demonstrated that norepinephrine occurred in the brain. Today, we know it is one of three major catecholamine (dopamine, norepinephrine, epinephrine) neurotransmitters found in the central nervous system (CNS). Over 50 years of subsequent research has led to an enormous accumulation of information regarding norepinephrine and its role in physiological and behavioral processes. In addition, drugs that directly manipulate brain norepinephrine have been used therapeutically for over 50 years, and even today, drugs are being developed that target noradrenergic neurons to deliver therapeutic effects. In fact, new disease indications continue to be identified for existing and newer noradrenergic drugs.

Given the revered tenure of this relatively old neurotransmitter and the recent advances and subsequent theories about its contribution to health and disease in the CNS, the authors of this book decided the time was right to bring together historical and recent information about norepinephrine in one book. The intention of this volume is to provide the reader with a thorough understanding of the anatomy, physiology, molecular biology, pharmacology, and therapeutics of norepinephrine in the CNS, including an extensive review of the role of norepinephrine in diseases of the CNS.

The book is divided into four parts, each of which can be read individually for focused information, or collectively to obtain a thorough understanding of the neurotransmitter. The first part of the book, *The neurobiology of norepinephrine*, is a comprehensive review of the anatomy and neurochemistry of norepinephrine neurons in the brain, including discussion of neurotransmitter systems directly affected by noradrenergic input. In Chapter 1, Kimberly Simpson and Rick Lin

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describe in detail the neuroanatomy of the locus coeruleus, the major source of norepinephrine in the brain. Simpson and Lin discuss neuropeptide transmitters that are colocalized with norepinephrine in the locus coeruleus, and provide detailed neuroanatomical descriptions of the afferent and efferent pathways of this nucleus. In Chapter 2, Craig Stockmeier and I provide a review of neurotransmitter systems that provide modulatory input to central noradrenergic neurons. Our focus in this chapter was on transmitter systems that provide input to the locus coeruleus, the specific receptors for those transmitters that are expressed by noradrenergic neurons, and the effects of drugs that activate or inhibit those receptors on locus coeruleus activity. We also describe the reciprocal connections between the locus coeruleus and other brainstem monoamine cell groups such as the dopaminergic ventral tegmental area and the serotonergic raphe nuclei. In Chapters 3 and 4, David Bylund, Ron Duman, and Sam Newton provide a comprehensive review of receptors for norepinephrine as well as their intracellular signaling systems, including the role of norepinephrine in regulating nuclear events such as gene transcription. Bylund also reviews several polymorphisms known to exist in genes encoding noradrenergic receptors, along with their biological consequence. Duman and Newton give due consideration to the effects of norepinephrine, induced as a consequence of norepinephrine uptake inhibition secondary to antidepressant treatment, on gene expression through noradrenergic receptor intracellular signaling pathways. In Chapter 5, Subbu Apparsundaram reviews the norepinephrine transporter, the protein that is largely responsible for the temporal control of noradrenergic transmission through the synapse. This protein is a target of many psychotherapeutic compounds. Considerable recent evidence demonstrates that this protein is not a static entity on the norepinephrine neuron as once believed. Rather, the norepinephrine transporter is regulated in concert with changes in noradrenergic transmission.

The second part of this book, *Norepinephrine and behavior*, is a compilation of reviews of roles that norepinephrine plays in a key output of the brain, i.e. behavior. In Chapter 6, Gary Aston-Jones and coauthors discuss the role of norepinephrine and the locus coeruleus in arousal state maintenance and sleep–wake regulation, and they consider how the locus coeruleus participates in sleep alterations that accompany psychiatric disorders. In Chapter 7, Aston-Jones and coauthors describe different modes of activity that the noradrenergic locus coeruleus exhibits and their relationship to behavioral states. These include a hypoactive mode where locus coeruleus activity is low and relatively unresponsive to external stimuli and behaviorally manifests as drowsiness or inattentiveness. A phasic mode is characterized by phasic activation of the locus coeruleus to discriminated stimuli, associated with high-level performance. Finally, a tonic mode of activity occurs where locus coeruleus neurons do not respond phasically to external (task)

stimuli, but are tonically active. This latter mode is associated with poor performance. Together, the behaviors associated with different modes of activity illustrate that the relationship between locus coeruleus activity and performance in a task is biphasic. Aston-Jones discusses the clinical implications of locus coeruleus activity modes with regard to the biological basis of several disorders of the CNS.

In Chapter 8, Benno Roozendahl reviews the animal and human literature that demonstrates the critical role that norepinephrine plays in the consolidation of long-term memory formation. Roozendahl describes the effects of pharmacological manipulation of the central noradrenergic system on memory and the various areas, particularly the amygdala and hippocampus, of the brain that play a role in mediating norepinephrine's effects on memory. In Chapter 9, David Morilak describes how stress modifies central noradrenergic activity, and how pharmacological agents modulate the effects of stress on the locus coeruleus. Morilak introduces the idea that dysregulation of this stress-sensitive system may be a biological substrate for the vulnerability of certain individuals to stress-related psychopathologies.

Part III of this book, *The biology of norepinephrine in CNS pathology*, is focused on evidence that pathology of the central noradrenergic system is a component of clinical CNS pathologies. In Chapter 10, Leonie Welberg and Paul Plotsky consider animal models that have been used to investigate the neurobiology of psychiatric and neurological disorders and the mechanisms of action of neuropsychiatric pharmacotherapy. Welberg and Plotsky describe animal studies that illustrate that environmental stresses encountered during the prenatal period through adulthood and genetic diversity can alter stress-responsive neural circuitry, particularly the noradrenergic system, and ultimately contribute to psychopathology. In Chapter 11, I consider how research studying brain tissue collected postmortem has contributed to an understanding of the role of norepinephrine in the neuropathology of depression and schizophrenia. I discuss evidence of neurochemical pathology of the noradrenergic locus coeruleus in depression that supports the concept that depression is associated with an elevation in stress-sensitive excitatory input to the noradrenergic locus coeruleus that can be dampened by antidepressant drug administration. In Chapter 12, Pedro Delgado examines human studies (in live patients) that have investigated the role of norepinephrine in the pathophysiology of major depression and mechanisms underlying antidepressant drug action. Delgado reviews studies utilizing peripheral markers and neuroendocrine challenges, and more recent studies that employed catecholamine (norepinephrine and dopamine) depletion to investigate the relationship between mood and brain catecholamine concentrations. This avenue of research has confirmed a very strong association between brain catecholamines and mood.

In Chapter 13, Antti Pertovaara reviews literature that demonstrates modulatory actions of norepinephrine on pain processes in the CNS under normal

physiological and pathophysiological conditions. Pertovaara details the pharmacological basis for the actions of noradrenergic agonists and antagonists on pain. In Chapter 14, Amy Arnsten reviews how norepinephrine influences cortical and subcortical functions that contribute to cognition. Arnsten provides evidence suggesting that norepinephrine acts as a chemical switch that strengthens prefrontal cortical control of behavior under non-stressful alert conditions, but impairs prefrontal function while enhancing posterior cortical and subcortical activity during stress. Arnsten discusses numerous neurological and psychiatric disorders according to whether norepinephrine or noradrenergic activity is low and insufficient, or high and disruptive to normal cognitive functioning. Francesco Fornai reviews the neuropathological findings involving brain noradrenergic neurons in specific neurological disorders in Chapter 15. Several neurological disorders, as well as normal aging, are characterized by degeneration or loss of noradrenergic neurons. Fornai considers studies utilizing experimental animal models that have investigated the neurobehavioral sequelae resulting from noradrenergic neuron loss. In addition, Fornai reviews research investigating the neuropathology of noradrenergic neurons and noradrenergic pharmacotherapy in Parkinson's disease, epilepsy, multiple systems atrophy, dementia, and several other neurological disorders.

In Chapter 16, Inna Belfer and David Goldman provide a thorough description of norepinephrine-related genes, norepinephrine gene variation and the effects of gene variation on the expression and function of norepinephrine genes. The identification of gene variants is a rapidly expanding field and in the time between completion of this chapter and press, several new gene variants have been identified and it is likely that many more will be discovered. Nevertheless, Belfer and Goldman provide a review of the impact of variation of genes encoding proteins that are found in noradrenergic neurons and proteins (e.g. receptors) that receive information, via norepinephrine, from noradrenergic neurons. Detailed tables listing a host of polymorphisms of noradrenergic genes are included in this chapter. The association of polymorphisms of noradrenergic genes to diseases of the brain and periphery are discussed, since, as noted by Belfer and Goldman, "The brain, the body and the genome are not conveniently compartmentalized by disease."

The last part of the book, *Psychopharmacology of norepinephrine*, is devoted to a review of the pharmacology and therapeutics of psychoactive drugs that act on norepinephrine-binding proteins (receptors, transporters, metabolic and catabolic enzymes). This section focuses on the use of noradrenergic drugs in the treatment of depression, anxiety, attention-deficit, eating disorders, and substance abuse. In Chapter 17, Elliot Richelson provides a history of the discovery of antidepressant drugs and how the elucidation of their pharmacological actions historically shaped (and continues to shape) scientific opinion of the biology of affective disease. Richelson reviews the pharmacological actions of drugs that bind

the norepinephrine transporter, including antidepressant and antipsychotic drugs, and also discusses the non-transporter binding effects of antidepressant drugs.

In Chapter 18, Craig Nelson addresses the role of antidepressants that have inhibitory actions at the norepinephrine transporter in the treatment of depression and anxiety disorders. Nelson provides strong evidence for the efficacy of selective norepinephrine uptake inhibitors in the treatment of depression, and also reviews literature suggesting that norepinephrine transporter (uptake) inhibitors share a similar pattern of effects on the core symptoms of depression with selective serotonin uptake inhibitors. Nelson discusses a growing literature that implicates synergistic effects of norepinephrine and serotonin uptake inhibition in the treatment of depression. The lesser role of norepinephrine uptake inhibitors and mixed serotonin/norepinephrine uptake inhibitors in the treatment of panic, obsessive compulsive, posttraumatic stress, and generalized anxiety disorders is also discussed.

The recent success of atomoxetine (a selective norepinephrine transporter blocker) for the treatment of attention-deficit/hyperactivity disorder has reawakened researchers to the importance and role of norepinephrine in attention disorders. To address this, Fred Reimherr and his coauthors in Chapter 19 discuss the use of drugs that modulate noradrenergic transmission in the treatment of attention-deficit/hyperactivity disorder. Reimherr and colleagues provide a detailed review of clinical studies that examine the efficacy of noradrenergic drugs in the treatment of attention disorders. They carefully consider the use of stimulants, tricyclic antidepressants, monoamine oxidase inhibitors, α_2 adrenergic receptor agonists, newer mixed action norepinephrine/serotonin uptake inhibitors, and atomoxetine. Overall, the clinical studies reviewed demonstrate efficacy of medications with norepinephrine actions and these studies provide evidence implicating norepinephrine in the pathophysiology of attention-deficit/hyperactivity disorder.

In Chapter 20, Katherine Halmi and Sun Young Yum address the use of drugs that modulate the central noradrenergic system in the treatment of eating disorders, including anorexia nervosa and bulimia. Halmi and Yum review basic research demonstrating that norepinephrine influences feeding behavior through several complex mechanisms, and examines clinical studies that have investigated various biological and molecular markers of norepinephrine or noradrenergic activity in anorexia and bulimic patients. In addition, Halmi and Yum examine the use of noradrenergic active drugs, in particular tricyclic antidepressants, in the treatment of anorexia and bulimia. The authors conclude that despite robust effects of norepinephrine on feeding behavior, drugs that affect norepinephrine neurotransmission have modest effects in treating bulimia nervosa and essentially no effect in treating anorexia nervosa, although it is worth mentioning here that highly selective norepinephrine uptake inhibitors have not been tested in blinded trials to date.

In Chapter 21, Susan Broom and Bryan Yamamoto provide an overview of the role of norepinephrine in the development and maintenance of substance abuse and summarize the therapeutic potential of noradrenergic drugs to alleviate adverse consequences of substance abuse. Although dopamine is the catecholamine commonly referred to as mediating reward effects of abused drugs, Broom and Yamamoto review evidence that norepinephrine contributes to the dopaminergic system in producing changes in reward pathway activity induced by psychostimulants. The authors discuss evidence that norepinephrine released in select brain regions contributes to withdrawal symptoms and stress-induced drug relapse, and review evidence of the efficacy of drugs that modulate norepinephrine in decreasing adverse consequences of withdrawal.

The chapters in this book reveal a wealth of information about the basic neurobiology of norepinephrine, its role in normal and abnormal behaviors and neurological disease, and its usefulness as a pharmacological target to alleviate symptoms of CNS diseases. The relatively recent development of highly selective drugs to inhibit the norepinephrine transporter further expands the possibility for medical discoveries of the utility of noradrenergic transmission modulation for the treatment of disease. The past 50 years of research elucidating multiple roles of norepinephrine in brain function have simultaneously revealed countless mysteries about norepinephrine's molecular and behavioral actions and these are likely to continue to stimulate research on this transmitter for many years in the future.

Part I

The neurobiology of norepinephrine

1

Neuroanatomical and chemical organization of the locus coeruleus

Kimberly L. Simpson, Ph.D. and Rick C. S. Lin, Ph.D.

University of Mississippi Medical Center, Jackson, USA

Early history of the locus coeruleus

The first description of the norepinephrine (NE)-containing locus coeruleus (LC) dates back to 1809 in a report by Reil.[1] A depression in the anterior floor of the human fourth ventricle was noted that corresponded with a concentration of blue-black substance just below the tissue surface. The pigmented area remained nameless until 1812 when the Wenzel brothers introduced the term "locus coeruleus."[2] Today this designation is widely utilized. However, in early investigations, several other names were applied to this melanin-containing group of cells. Arnold, in 1838 and 1851, referred to the LC as the "substantia ferruginea."[3,4] Jacobsohn, in 1909, coined the name "nucleus pigmentosus pontis."[5] In recognition of a cerebellar subcomponent of the LC, Meynert (1872) and Jacobsohn (1909) assigned the classifications "substantia ferruginea superior" and "nucleus pigmentosus tegmentocerebellaris," respectively.[5,6] Despite its many names, most studies during this period placed the LC within the dorsolateral portion of the rostral mesencephalic and caudal mesencephalic tegmentum of man and higher primates. Localization of the LC in lower species, such as rodents, was more challenging initially because pigmentation was found to be lacking in areas that were readily observed in higher mammals. Consequently, a considerable amount of uncertainty surrounded the actual position of the LC cell group within these animals. The LC, for example, was once considered to be a caudal extension of the nucleus laterodorsalis tegmenti, a region now acknowledged as a major pontine cholinergic center.[7]

An interesting historical perspective, covering the above information in detail and providing a comparative synopsis of the cytoarchitectonic characteristics of the LC, appears in a review written by Russell in 1955.[8] Within the body of this work, Russell presents a thorough timeline of LC-related findings and offers evidence

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for the potential involvement of the LC in trigeminal somatosensation, visceral sensibility, and respiration.

Later progress in the field stemmed from the ability of researchers to further elucidate the chemical composition of the LC. As a result of this advancement, it became possible to identify the LC in several mammalian species, including humans, without visualization of melanin. Monoamine oxidase, aromatic monoamine, ascorbic acid, copper, and acetylcholinesterase were among the first substances to be detected.[9–13] A particularly notable event, however, relating to the demonstration of the LC was the development of the Falck–Hillarp method.[14] This technique, which involved exposing freeze-dried tissue sections to formaldehyde vapor, was found to impart fluorescence to catecholamine-containing cells through the formation of tetrahydroisoquinoline derivatives, enabling subgroups of neurons to be differentiated based upon their color. Cells in the substantia nigra and LC were found to exhibit an intense green fluorescence, while profiles of raphe nuclei appeared yellow. Dopaminergic and noradrenergic neurons were, therefore, classified under the A group designation (A1–A12), while serotonergic neurons were categorized in the B series (B1–B9). In 1964, Dahlstrom and Fuxe[15] more specifically labeled the LC as A6 and noted an additional collection of multipolar cells that extended ventrolaterally in an arch toward A5.

Species comparisons

In most mammals including humans, the LC is generally located within the periventricular gray of the isthmus, medial to the mesencephalic nucleus of the trigeminal nerve. It is composed of a cluster of cells that assemble within the lateral boundaries of the central gray, sometimes extending either dorsorostrally along the roof of the fourth ventricle (IV) or ventrally toward the core of the brainstem. Although studies of the LC have been performed on animals such as the rabbit, chimpanzee, baboon, dog, sea lion, horse, and bat, many investigations have focused on the human, primate, cat, and rodent.[8,16] The following section will provide a brief account of the distribution of the LC nucleus in species that have been more routinely studied and will address architectural characteristics that tend to distinguish one group of animals from another. It is worthwhile to note that many of the features that define the LC were initially assessed in Falck–Hillarp-, Golgi-, or Nissl-stained material. However, a number of findings have been immunohistochemically confirmed using antibodies directed against tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis, or dopamine-β-hydroxylase (DBH), the final enzyme in the biosynthesis of NE.[17–19]

A large majority of studies have utilized rats to determine the basic organization of the LC.[19,20] Reports indicate that, on the basis of cytoarchitecture, this nucleus

can be segregated into as many as four contiguous subregions.[21] Among these neuronal subsets are cell populations that appear in the cerebellum (A4) and the mesencephalic periaqueductal gray (PAG) at a level just behind the decussation of the brachium conjunctivum (BC). However, the most often recognized of these groups is the LC proper and the ventral subdivision. The LC proper extends approximately 1 mm in the rostrocaudal dimension from the genu of the facial nerve to the midportion of the dorsal tegmental nucleus; the ventral subdivision measures just under 500 μm. The former is characterized by densely packed aggregates of somata, which, as a general rule, express NE and localize within rather restricted nuclear subfields.[22] Within the caudal pole, these dorsally distributed cells are separated from the more loosely arranged cells of the ventral division by the medial vestibular nucleus. At rostral levels, the ventral A6 population merges with the principal LC division and becomes less distinct. Together with the NE-containing cells of A7, the ventral portion of the LC is considered by Olson and Fuxe[23] to constitute the subcoeruleus (sub-LC). Grzanna and Molliver, however, point out that the sub-LC lies outside of the central gray and should, therefore, not be included as part of the LC group.[21] They describe the sub-LC as a group of neurons that is situated approximately 1 mm rostral to the cells of the ventral LC subdivision and extends ventrally from the LC proper along the medial aspect of the trigeminal motor nucleus into the region of the rostral superior olivary complex. Despite these minor interpretational differences, regional estimates of cell number in Nissl-stained paraffin sections indicate that one LC nucleus contains roughly 1643 neurons, the ventral and dorsal subdivisions of which contribute approximately 210 and 1430 neurons, respectively.[24] Although results obtained from DBH-labeled material suggest that LC cell populations are slightly smaller (about 1439 neurons, total), these discrepancies can be explained by methodological limitations associated with antibody penetration and the use of thicker tissue sections.

Studies in nonrodent animal models support the idea that the basic structural organization of the LC is conserved across species. However, these same investigations have also revealed that important distinctions exist in the nuclear construct of different experimental models. The cat LC is particularly notable in this regard, due to the fact that it varies from rat LC on several counts.[25,26] One readily apparent feature relates to the diffuse arrangement of cells in the cat LC. Both neurons and glial elements tend to spread out in an irregular fashion over large areas of the periventricular gray and to distribute within deep aspects of the pontine tegmentum. In its entirety, the nucleus extends a distance of 4 mm from the trochlear nucleus to the trigeminal motor nucleus and includes within its boundaries dorsolateral areas surrounding the BC and neuronal territories previously identified as the Kölliker–Fuse nucleus and the magnocellular component of the marginal nucleus of the BC.[25] Unlike the rat LC, which has been described as a collection