Role of the locus coeruleus-norepinephrine system in arousal and circadian regulation of the sleep–wake cycle

Gary Aston-Jones, Ph.D., Monica Gonzalez, and Scott Doran
University of Pennsylvania, Philadelphia, USA

Introduction

A variety of previous results indicate that the norepinephrine (NE) locus coeruleus (LC) system is integrally involved in regulation of sleep and waking. In particular, LC activation promotes wakefulness. This appears to be one of the major arousal systems in the brain. The LC was also found to be critical for rebound sleep following sleep deprivation, and increased sleep that occurs following a stressor. We recently identified a circuit from the suprachiasmatic nucleus (SCN) to the LC, in which the dorsomedial hypothalamus (DMH) serves as a relay. The functionality of this circuit was confirmed in our studies, showing that LC neurons have a circadian rhythm in their firing activity and that this circadian fluctuation in the LC requires an intact DMH. Other recent studies have also shown that lesions of the LC decrease the amplitude of the circadian rhythm in the sleep–wake cycle. These results indicate that the LC may be an important component in SCN efferent circuitry for driving circadian rhythms in sleep–wake cycles. Other recent results in our lab have revealed that light deprivation produces a profound loss of NE in the frontal cortex in rats, and this NE loss in dark-maintained animals is associated with decreased amplitude of the circadian sleep–wake rhythm. The dependence of the LC system on light for normal function has implications for clinical disorders such as seasonal affective disorder. Other disorders that are comorbid with sleep anomalies and associated with LC dysfunction are discussed.

Brain systems involved in sleep–wake regulation

Waking, slow-wave sleep and paradoxical sleep

Three naturally occurring, distinct vigilance states have been identified in all higher vertebrates (birds and mammals) according to cortical, muscular, autonomic, and
behavioral activities: wakefulness (W), slow-wave sleep (SWS), and paradoxical sleep (PS). During W, individuals interact with the external environment using a great variety of volitional responses. Cortical activity as measured with the electroencephalogram (EEG) is dominated by high-frequency, low-amplitude signals, with a predominance of beta (20 to 30 Hz) and gamma (30 to 60 Hz) frequencies. Theta activity (5 to 8 Hz) appears during quiet waking and focused attention, and may facilitate learning during wakefulness. Postural muscle tonus, as measured by the electromyogram (EMG), is variable depending on the posture or locomotor activity of the individual. Autonomic indices of W include an elevated cardiac rhythm and a rapid and irregular respiratory frequency. During sleep onset and SWS, EEG-recorded cortical activity slows and becomes more synchronized until high-amplitude spindle waves (12 to 15 Hz) intermingle with increasingly dominant high-voltage delta waves (1 to 4 Hz) and slow waves (<1 Hz). Both voluntary muscular and cardiorespiratory activities decrease, and the brain temperature reaches a nadir.

Paradoxical sleep (also referred to as rapid eye movement or REM sleep) is always preceded by SWS, except during narcolepsy, idiopathic hypersomnia, and some drug/alcohol withdrawal periods. It is characterized by low-amplitude high-frequency desynchronized cortical EEG activity similar to EEG recordings during W. In fact, the EEGs of W and PS are sometimes difficult, or impossible, to distinguish without other polygraphic signals, especially the EMG. Paradoxical sleep is also characterized by pronounced postural muscular atonia and body temperature dysregulation. Periodic reemergence of high-frequency low-amplitude EEG activity during sleep with atonia inspired Jouvet to rename the previously described “Rapid Eye Movement” sleep of Aserinsky and Kleitman as “Paradoxical Sleep,” which he classified as a distinctive sleep state. Other physiological indices of PS include penile erections, myoclonic twitches, irregular cardiorespiratory rhythms, and unique extracellular field potentials found in the pons, lateral geniculate nucleus, and occipital cortex (PGO waves).

**Networks involved in regulation of the sleep–wake cycle**

**Neuronal groups**

Figure 6.1 illustrates the main neuronal groups involved in sleep–wake cycle regulation. Neurons associated with cortical activation during waking are concentrated in the pontine and midbrain central tegmentum, posterior hypothalamus, and basal forebrain (Figure 6.1a), primarily within the area described as the “reticular activating system” by Moruzzi and Magoun. In particular, the noradrenergic LC system in the pons; the cholinergic neurons located in the brain stem reticular formation (pons and midbrain) and the basal forebrain (substantia innominata, diagonal band nuclei and septum); the glutamatergic neurons of the mesencephalic reticular
formation; the dopaminergic neurons of substantia nigra and ventral tegmental area
in the midbrain; the orexin neurons from the lateral and perifornical hypothalamus;
the histamine neurons of the posterior hypothalamus; and serotonergic neurons
(5-HT) of the dorsal raphe nucleus (DRN) are all prominently implicated in arousal
and waking.5,16–24 Figure 6.1b shows that the SWS-generating systems are concen-
trated within the midline brain stem, dorsolateral medullary reticular formation,
the anterior hypothalamus–preoptic region and basal forebrain.19 Pioneering stud-
ies done by Jouvet showed that rhombencephalic structures were responsible for
generating PS.12 Subsequent transection studies identified the region caudal to
the midbrain and rostral to the spinal cord (primarily the lateral portion of the

Figure 6.1 Simplified schema of the circuits involved in the vigilance states. MC, nucleus reticularis
magnocellularis; LC, locus coeruleus; LDT/PPT, laterodorsal and pedunculopontine tegmen-
tal nucleus; PAG, periaqueductal gray; NTS, nucleus tractus solitarius; DRN, dorsal raphe
nucleus; PH, posterior hypothalamus; POA, anterior preoptic area; IL, intralaminar nucleus;
RET, reticular nucleus; BF, basal forebrain; MRF, mesencephalic reticular formation; peri-
LC, peri-LC area; PGO, ponto-geniculo-occipital (waves); CGL, lateral geniculate nucleus.
Other abbreviations: Glu, glutamate; Asp, aspartate; Gly, glycine; ACh, acetylcholine; NE,
norepinephrine; 5-HT, serotonin; His, histamine; Orx, orexin; GABA, γ-aminobutyric acid; Gal,
galanin; N, nicotinic receptor, M, muscarinic receptor. Modified from Gonzalez (1997).117
nucleus reticularis pontis oralis) to be the core network responsible for generating PS (Figure 6.1c).14

Mechanisms involved in sleep–waking cycle stage changes

Daily transitions into sleep from wakefulness appear to rely on circadian and sleep homeostatic cues from the hypothalamus that inhibit wakefulness-promoting activity from the brain stem. Tonic firing of NE-, 5-HT-, and histamine-containing neurons decrease during SWS and become practically silent before and during PS.25–29 Active inhibition of this waking network to allow sleep is proposed to be mediated by sleep-generating systems including γ-aminobutyric acid (GABA)ergic and galaninergic neurons of the preoptic area, periaqueductal gray, prepositus hypoglossi, and the solitary tract nucleus.23,30–35 A PS-on or executive system has been proposed to inhibit the monoaminergic waking systems (PS-off or permissive system) which is indispensable for the genesis and maintenance of PS.36,37 This PS executive system is proposed to include cholinergic or cholinoceptive systems of the mesopontine tegmentum, the ventromedial medulla, and part of the nucleus reticularis parvocellularis (see Figure 6.1).22,28,36,38–40

LC involvement in sleep–wake regulation

Activity of LC neurons during sleep and waking

Tonic LC activity varies with behavioral state

The LC is the major source of NE in the brain, and the sole source of NE in the cerebral cortex, cerebellum, and hippocampus. Although the LC also contains non-NE neurons, which are particularly numerous in certain species such as cats and dogs, our focus will be on the NE neurons of this nucleus.

Spontaneous activity of NE neurons in the LC of rats and cats has been found to co-vary with stages of the sleep–wake cycle, firing most rapidly during W, more slowly during SWS, and becoming virtually silent during PS.25,41,42 State-dependent LC firing has also been found in monkeys, although some differences exist as described below.43–45 Although average LC impulse activity co-varies with arousal state, it is notable that LC firing changes markedly in anticipation of most state changes. Thus, for example, LC neurons decrease their activity during W in anticipation of SWS. In addition, LC neurons engaged in low tonic firing during SWS increase average spike rates dramatically just prior to a transition into W.25,41 Locus coeruleus activity is also reported to increase just prior to the PS-to-W transition if arousal state is delineated by measuring changes in EMG.41 This observation led to the view that LC activation was a primary signal causing the PS-to-W transition.36 However, in our recordings, LC activation did not precede but, rather, followed the change
in EEG activity during the PS-to-W transition. This discrepancy results from the fact that EEG indices of this state transition precede the corresponding EMG transition. These findings also indicate that, although LC neurons may participate in producing most transitions between sleep–waking cycle stages, LC activity is not the primary signal terminating PS episodes.

Primate LC activity during sleep and waking

Very few reports are available that detail how LC activity varies across or within arousal states in primates. The only previous data recording LC activity during arousal state changes in primates reported distinct LC slowing from about 2 Hz during active waking to less than 1 Hz (often inactive) during naturally occurring drowsiness. Locus coeruleus activity was very low or absent during PS, as was found in other species (described above). More recent studies reveal that monkey LC activity is primarily associated with wakefulness, and varies less with different stages of drowsiness and sleep than in other species: during quiet wakefulness (defined by the presence of alpha waves in the EEG) impulse activity of the monkey LC neurons was not significantly different from that during either SWS or PS. Furthermore, LC activity during SWS and PS was not significantly different. Thus, monkey LC activity may be less graded during different sleep–waking states than in rats or cats, and appears to be active primarily during periods of high cognitive engagement. However, at present this is based upon a limited data set, and additional study of LC activity during sleep and waking in the monkey is needed to confirm these initial results.

Monkey LC activity during W exhibits different modes of activity (including changes in the tonic levels of discharge as well as in phasic responses to task stimuli) that correspond to the current behavioral strategy. These properties are described in detail in Chapter 7 of this volume and are not considered further here. However, it is noteworthy that this system appears to have functions in both state control and in behavioral performance within waking.

LC activity and cortical arousal

The midbrain lesion and stimulation experiments performed by Moruzzi and Magoun pointed to the relevance of the ascending tracts of the brain stem in arousal and established the idea of a “reticular activating system” located in the mesopontine tegmentum. It is notable that the LC is located centrally within this arousal system. As reviewed above, state-dependent LC activity and the location of the LC within the reticular activating system are highly suggestive of a role for the LC in regulating arousal. However, this evidence was not definitive of a causal role of the LC in arousal. Experiments by Berridge and Foote using direct stimulation of the LC provided evidence for such a causal relationship. This research
showed that LC stimulation via direct microinfusion of cholinergic agonists desynchronized the normally synchronized EEG of halothane-anesthetized rats. This effect was shown to be NE-mediated as it was blocked by pretreatment with the β-adrenoceptor antagonist propranolol. Conversely, inhibition of LC activity via microinfusion of an α2-agonist into the LC of more lightly anesthetized rats caused an increase in EEG slow-wave activity. Of importance, this effect required bilateral LC inactivation, indicating that even a small amount of LC activity was sufficient to prevent EEG slow waves. Additional studies in unanesthetized rats indicated that the wake-promoting actions of the LC appear to involve NE actions at α1- or β-receptors within the medial septum or medial preoptic area.

Effects of locus coeruleus lesions on sleep and waking

Spontaneous Sleep

Because the NE-LC is part of the sleep–waking network and is thought to be a major arousal system (see Figure 6.1a), lesions of the LC would be expected to decrease waking and arousal. However, early studies of lesions of the LC did not find substantial changes in total amount of waking. In addition, different authors have described an increase, a decrease, or no effect on PS duration following LC lesions. Inconsistent findings on this topic could be a consequence of differences in lesion research techniques, leading to inconsistent damage to the brain’s aminergic nuclei between studies.

N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) is a tertiary haloalkylamine that induces long-term degeneration of noradrenergic fibers coming exclusively from the LC, with a concomitant depletion of NE levels in principal LC target areas. Lesions produced by DSP-4 have been shown to produce minimal impact on any peripheral or central aminergic neurons other than the NE-LC system at 2 weeks post-DSP-4 administration. Thus, DSP-4 is a useful tool to evaluate the specific role of the LC in sleep–waking mechanisms. Monti et al. were the first to evaluate the effects of DSP-4 on spontaneous sleep in rats. However, that study was restricted to the light period, and measures were taken at 5 to 6 days after DSP-4 treatment, a time when the toxin also affects other amine systems that are potentially involved in the sleep–wake cycle (e.g., dopamine and 5-HT neurons). Thus, the increase in PS observed by these authors could not be ascribed exclusively to a depletion of NE from the LC.

Later, Gonzalez and colleagues found that at one month after DSP-4 administration rats showed significantly decreased PS during the day (inactive period) and increased SWS during the night (active period). These results indicated a differential involvement of the NE-LC in the SWS and PS mechanisms depending on the light–dark cycle. Subsequent circadian analyses of DSP-4 lesions confirmed and extended these findings, as described below.
Sleep Rebound

In 1960, Dement observed in humans an increase of both SWS and PS during the night following sleep deprivation. This enhanced sleep duration above baseline level after sleep deprivation was designated “sleep rebound,” and considered to be a component of normal sleep regulation. Since then, different sleep deprivation methods (water-tank, methylamphetamine administration, forced locomotion, disk-over-water method) have been used to produce enhanced PS to study the mechanism underlying PS.

The water-tank method (Wt) is the most widely used technique to sleep-deprive laboratory animals. This method consists of placing the animal on a small pedestal surrounded by water. At the onset of each PS period the loss of postural muscle tonus causes the animal to contact the water, which awakens him and prevents PS episodes. After one or two wettings, the animal avoids further water contact by periodic awakening and light SWS only, resulting in a total suppression of PS and a partial loss of deep SWS.

During sleep deprivation, cholinergic, peripheral and central monoaminergic systems are activated. As most of these studies point out, the traditional Wt not only triggers a sleep rebound mechanism but also triggers peripheral and central responses similar to those elicited by stressors. For example, Wt treatment induces oxidative damage, immunosuppression, gastric ulcers, enlargement of the adrenals, and increases in corticosteroid levels. Water-tank treatment also decreases the number of β-adrenoceptors in the brain and reduces both thymus and body weight. Cerebral NE and 5-HT synthesis and turnover are enhanced, as is tyrosine hydroxylase (TH) gene expression in LC neurons. Tyrosine hydroxylase activity and extrahypothalamic and hypothalamic corticotropin-releasing factor (CRF) levels also increase.

As the NE-LC system is strongly activated by stress, the stress inherent in most sleep deprivation methods must be taken into account when interpreting sleep deprivation results designed to study the role of the LC in spontaneous sleep mechanisms. In addition, a stressor stimulus without sleep loss, such as immobilization stress, produces a sleep increase on the following night, similar to that seen following sleep deprivation. To characterize the role of the NE-LC in sleep rebound mechanisms after sleep deprivation, Gonzalez and colleagues evaluated the effect of NE-LC lesion by DSP-4 on sleep that was induced exclusively by stress alone. One month after DSP-4 administration, neurotoxic NE-LC lesions reduced or eliminated the SWS and PS increases typically recorded after immobilization stress (Figure 6.2). Thereafter, the same authors studied the effect of DSP-4 NE-LC lesions on sleep rebound following sleep deprivation. Ten hours of Wt treatment induced significant SWS and PS rebound during the first dark period following sleep deprivation in control animals, while DSP-4 treatment significantly attenuated both PS and SWS rebound (Figure 6.3). Water-tank treatment did not induce sleep rebound in BALB/c mice, a genetic strain with 38% of the NE-LC...
perikarya missing and decreased density of cortical NE terminals. Given the stressful nature of Wt treatment and the requirement for an intact NE-LC to provoke increased sleep after immobilization stress, it is possible the NE-LC participates in sleep increases via stress responses triggered by sleep deprivation. The view that sleep rebound is stress-related, and not necessarily a concomitant of sleep loss per se, is consistent with recent findings that the wake-enhancing drug modafinil increases waking without causing a subsequent sleep rebound.

**Role of corticotropin-releasing factor in noradrenergic regulation of sleep and waking**

Anatomical, physiological and behavioral studies have shown that CRF plays a key role in the stress response. In particular, CRF is a key neurotransmitter involved in the NE-LC activation by certain stressors. Immunohistochemical studies in the rat describe CRF-immunoreactive axons in the LC and peri-LC.
Neurophysiological experiments showed that activation of LC neurons by hypotensive stress is dependent upon CRF neurotransmission within the LC.\textsuperscript{101} Also, biochemical studies reveal that both acute and chronic stress can double the concentration of CRF in the LC.\textsuperscript{102} Finally, CRF application in the LC produces a number of stress-related effects in behavior and physiology. For example, local administration of CRF generates anxiogenic behavior, induces EEG activation, suppresses immune responses, and increases plasma corticosterone concentration.\textsuperscript{103–106}

To evaluate the participation of CRF in sleep rebound, experiments were conducted using the specific CRF receptor antagonist, α-helical CRF (9–41) (ahCRF).\textsuperscript{107,108} This compound has been shown to prevent behavioral stress responses caused by endogenous CRF activation while having no effect on unstressed animals.\textsuperscript{86,96,109–114} Intracerebroventricular (ICV) injection of ahCRF
Figure 6.4 Changes in hourly mean duration of the vigilance states up to the next dark period following immobilization stress (IS). The groups were: distilled water (vehicle), distilled water after immobilization stress (vehicle + IS) or α-helical CRF (9–41) after immobilization stress (ahCRF + IS). From Gonzalez and Valatx (1997).
abolished the PS increase typically induced by stress while stress-induced SWS increase was unchanged (Figure 6.4). When administered ICV throughout 10 hours of sleep deprivation by Wt, ahCRF decreased PS rebound in rats without altering SWS rebound (Figure 6.5). These results support the view that stress, acting via CRF activity, is a main factor inducing PS rebound following sleep deprivation. Moreover, ahCRF treatment was more effective than specific lesions of the NE-LC system in decreasing PS rebound, suggesting that neural systems activated in parallel to the NE-LC system by CRF, but spared from DSP-4 neurotoxicity, may be involved in PS rebound following sleep deprivation.68,116 Because ahCRF had no effect on spontaneous vigilance states, these studies indicate that endogenous CRF is involved in the PS mechanism only under stressful conditions, and that the substrates involved in spontaneous PS differ from those in PS rebound.115,117

Brain regions showing the largest depletion of NE after DSP-4 administration include the neocortex, hippocampus and amygdala, all of which are important inputs to the sleep-generating network (i.e., preoptic area, hypothalamus). Sleep rebound could result from modulation of spontaneous sleep networks by structures related to stress but not directly involved in sleep mechanisms (i.e. the amygdala), implying that rebound sleep differs significantly in mechanism from spontaneous sleep.115 Based on this hypothesis, Charifi and colleagues found that a local injection of DSP-4 in the central nucleus of the amygdala decreased sleep rebound induced by Wt without altering spontaneous sleep.119

**Role of LC in circadian regulation of sleep and waking**

Sleep and waking are expressed as a circadian rhythm that is under the control of the primary endogenous circadian pacemaker. The SCN appears to orchestrate diurnal variation of most physiological and behavioral variables in mammals.120,121 Hypothalamic projections from the SCN appear to be prominently involved in the control of circadian sleep–waking rhythms, but precisely which projections and neurotransmitters exert circadian control is unclear. Arginine vasopressin (AVP)-and vasoactive intestinal peptide (VIP)-containing SCN efferents to the hypocretin/orexin arousal system of the posterior hypothalamus have been described in rats.122 Inhibitory (GABAergic) and excitatory (glutamatergic) inputs from the SCN modulate the activity of ventromedial and ventrolateral preoptic areas, both known to be involved in thermoregulation and the induction and maintenance of sleep and waking.123,124 Lesioning direct SCN targets, such as the ventral area of the subparaventricular zone, abolished the circadian rhythm of sleep.125

**Indirect projection from the SCN to the LC**

As part of our program of research into afferent regulation of the LC, we employed the transsynaptic retrograde tracer pseudorabies virus (PRV) to study indirect
Figure 6.5  Hourly mean duration of sleep states up to 48 hours after the end of sleep deprivation. Rats were repeatedly treated during sleep deprivation with vehicle (vehicle + Wt) or α-helical CRF (9–41) (ahCRF + Wt). From Gonzalez and Valatx (1998).\textsuperscript{116}
inputs to the LC.\textsuperscript{126} We, and other researchers, have developed methods for using PRV with central injections to study chains of neurons connecting identified brain nuclei.\textsuperscript{127,128} The Bartha strain of this virus is a very powerful tool in neuroanatomy because it moves only in a retrograde fashion and only across synapses to produce specific labeling of direct and indirect afferents to the nucleus injected. Injection of PRV into the LC resulted in strong labeling of the SCN after approximately 60 hours of survival, a time long after known direct inputs are well labeled (Figure 6.6).\textsuperscript{129} This, in addition to previous studies and our own unpublished results, has shown that the SCN does not directly project to the LC. These findings indicate that the SCN is an indirect input to the LC. At 60 hours after PRV injection into the LC, the great majority of ipsilateral SCN neurons were labeled, indicating that the SCN is potentially a major indirect input to the LC. This finding prompted a series
of double-labeling and lesion studies to identify the relay in the SCN–LC circuit. Results indicated that the DMH is a major relay in this circuit, as significantly fewer SCN cells were labeled with PRV following injection in the LC of animals with ibotenic acid-induced DMH lesions.129

Circadian rhythm in LC impulse activity

The above findings prompted the hypothesis that LC neurons may exhibit a circadian rhythm in their activity, a possibility that had not previously been tested. Although a prior report found a circadian rhythm in brain NE levels,130 LC neurons vary their activity strongly with sleep and waking (as reviewed above), and therefore any changes in activity seen in behaving animals over the circadian cycle would be confounded by coincident changes in sleep and waking. To circumvent this possible confound, we recorded impulse activity of LC neurons from anesthetized animals taken from their active (dark cycle) or rest periods (light cycle). Anesthesia ensured that animals taken from either period were in a similar state of arousal when recordings were obtained, so that state-dependent changes in LC activity (e.g., waking vs. sleep) were eliminated. These experiments revealed that tonic LC neural activity was significantly faster during the active period compared to the rest period (Figure 6.7).129 This was true even in animals maintained in continuous darkness in the absence of extrinsic circadian cues for whom active and rest periods were defined by spontaneous locomotor activity. This finding supports our hypothesis that activity in LC neurons reflects an endogenous circadian signal. Finally, we found that this rhythm was absent in animals with ibotenate lesions of the DMH, showing that the SCN–DMH–LC circuit defined with PRV tracing is a functional circuit for efferent transmission of circadian information.

LC lesions decrease circadian amplitude of sleep–waking cycle

As activity in the LC is associated with arousal, the finding of a circadian rhythm in LC activity indicated that the LC may contribute to circadian rhythms in the sleep–wake cycle. To test this hypothesis, we recently extended the previous study of effects of DSP-4-induced LC lesions on sleep and waking,68 and analyzed the effect of specific lesions of the NE-LC system on the circadian rhythm of the sleep–waking cycle in rats. For this circadian study, we included cosinor analysis of results, as well as recordings while the animals were kept in continuous darkness (DD). Three weeks after DSP-4 administration, we recorded electrographic sleep signs by telemetry (EEG, EMG, temperature), and quantified the amount of W, SWS, and PS in lesioned versus nonlesioned control rats maintained in normal lighting conditions (12 hours:12 hours light–dark), or in DD. In the light–dark (LD) group, DSP-4 treatment caused a significant decrease in W and increase in sleep at night, while W increased and sleep slightly decreased during the day (Figure 6.8).
Figure 6.7  Histograms showing the distributions of LC firing rates during different epochs of the circadian cycle. Three paired groups of rats were maintained in either 12 h/12 h dark/light (panels a and c) or 24 h darkness (panel b). One paired group received bilateral ibotenic acid lesions of the DMH (panel c). Impulse activity was recorded in LC neurons during either the dark or light photoperiod (panels a and c) or the active or inactive epoch of the rat’s circadian cycle in 24 h darkness (panel b). There was a significant difference in LC firing rates in animals taken from their dark vs. light periods (panel a), and from their active vs. inactive periods when maintained in continuous darkness (panel b; \( p < 0.0001 \), each). Lesions of the DMH eliminated the difference in LC firing rates during the dark vs. light photoperiods (panel c). The scale on the y-axis only corresponds to the histogram bars. The solid lines on each of the graphs represent best-fit normal distribution curves for the histogram data. A one sample Kolmogorov-Smirnov test revealed that in all cases the data fit a normal distribution with \( a > 0.725 \) probability. The following is a tabulation of the number of animals and LC neurons used to calculate each histogram. Panel a – dark: \( n = 52 \) neurons from 10 animals; light: \( n = 61 \) neurons from 12 animals. Panel b – active: \( n = 17 \) neurons from 3 animals; inactive: \( n = 33 \) neurons from 3 animals. Panel c – dark: \( n = 35 \) neurons from 6 animals; light: \( n = 30 \) neurons from 5 animals. Taken from Aston-Jones et al. (2001).129
We hypothesize that less W occurs in the active period of lesioned rats because there is less LC influence to drive arousal from the SCN. We also propose that the decreased sleep (increased waking) observed in the rest period reflects a decreased homeostatic sleep need due to decreased W during the preceding active period. Decreased W/increased sleep during the night coupled with increased W/decreased sleep during the day produced a flattening of the circadian rhythm of sleep and W expression following LC lesioning. These results confirmed that LC lesions reduce the amplitude of the circadian rhythm of the sleep–wake cycle, as expected if LC activity contributed to waking in a circadian fashion. Interestingly, although there was a significant decrease in circadian amplitude of the sleep–waking rhythm, there was no overall change in the absolute amounts of sleep or waking in the lesioned animals. This may explain the minimal or inconsistent effects of LC lesions on total amounts of sleep and waking in previous studies that did not employ a circadian analysis. Another important finding was that sleep onset, and body and
Role of LC in arousal and sleep

Figure 6.9  Hourly mean duration (in min) of each vigilance state during the active period and rest period under constant darkness; n = 7; vertical bars = SEM.

brain temperature were not affected by DSP-4 treatment in either LD or DD animals compared to controls.68,131

Light deprivation-induced loss of cortical NE

Contrary to the above findings for decreased amplitude of the sleep–wake rhythm after LC lesions in animals maintained in LD conditions, animals maintained in DD conditions did not exhibit a significant alteration of the sleep–wake rhythm following LC lesions, as compared to nonlesioned rats maintained in DD (Figure 6.9).131 This was initially puzzling, as loss of a circadian signal following LC lesions should have resulted in decreased sleep–wake cycle rhythm amplitudes in DD conditions. However, analysis of NE fibers revealed that control (nonlesioned) DD-maintained animals exhibited substantially reduced NE fiber staining density in the frontal cortex when compared to control LD animals. This finding was confirmed with high-performance liquid chromatography analysis, which showed a decrease in frontal cortex NE of 45%.132 We hypothesize that LC lesions in the DD animals were ineffective in modifying the circadian rhythm of sleep and waking because the control (nonlesioned) DD-maintained animals had less cortical NE, reducing the apparent effect of lesions by reducing the functional contrast with DD-lesioned subjects. Indeed, we observed that DD control rats (nonlesioned) exhibited reduced amplitude in the waking and sleep rhythms, as previously reported.133 Furthermore, this reduced amplitude is indistinguishable from that observed in lesioned animals recorded during LD. In addition to offering a possible explanation for the lack of effect of LC lesions in the DD group, the finding that light deprivation caused substantial decreases in NE neurotransmission in the frontal cortex raises a number of important questions. Does this change reflect degeneration of LC efferent fibers or only a decrease in dopamine-β-hydroxylase and NE in otherwise intact fibers? Are there behavioral changes (aside from sleep–wake rhythms) associated with this change in NE projections? For example, do light-deprived animals exhibit a depressive behavioral phenotype? If so, this might correspond to clinical problems such as
seasonal affective disorder. Finally, can these anatomical and associated behavioral changes be reversed by light exposure or other treatments? These questions are being actively pursued in our laboratory at present. In any case, these results indicate that LC-NE projections depend upon light for maintenance of normal function.

Clinical perspectives: LC participation in sleep alterations due to clinical pathologies

Many psychiatric disorders are prompted by, or include, stress responses. This may be important for understanding the neurobiology of corresponding sleep disturbances, because LC neurons are strongly activated by several physiological and visceral stressors. As described above, activation of the NE-LC system increases wakefulness and arousal. We hypothesize that hyperactivity of the LC via enhanced stress responses may help to explain sleep disruptions emerging secondary to many clinical conditions, some of which are detailed below. Long-term up-regulation in NE production, release, or receptor regulation could contribute to sleep disruptions simply by increasing the drive towards wakefulness.

Depression

Disordered sleep is a cardinal symptom of both unipolar and bipolar clinical depression required for clinical assessment of chronic depression according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; 1994). Patients with major depressive disorder (MDD) typically report insomnia, a claim supported by polysomnographic recordings showing increased W, decreased SWS, and increased PS in people with depression compared to age-matched controls. Increased W and light sleep typically replaces SWS in people with MDD. A sleep EEG taken during MDD contains significant amounts of high-frequency (10 to 35 Hz) EEG intruding into normally slow-wave (0.5 to 4 Hz) EEG during sleep. Paradoxical sleep abnormalities during MDD include shortened time to PS from sleep onset (i.e., reduced PS latency), increased total PS time, and an increase in the phasic eye movements and muscle twitches characteristic of active PS. Findings of persistent insomnia, reductions in SWS, high-frequency sleep EEG, and increases in PS support a hypothesis that generalized physiologic activation (heart rate, body temperature, metabolism, mental activity) is a source of disordered sleep during MDD. Disordered sleep, especially involving PS changes, is predictive of depression relapse, recurrence, and increased risk of suicide. Conversely, insomnia itself is a significant risk factor for the development of depression and is prodromal in people at risk for depression. This bidirectional relationship between depression and sleep has long suggested that intrasleep variables may provide diagnostic benefit. Altered PS during MDD (primarily short
PS latency) is so prevalent that it was once considered a reliable trait of people prone to depression. Unfortunately, shortened PS latency is also found in several other disorders, including narcolepsy, borderline personality schizophrenia, and eating disorders, limiting diagnostic use of PS measures in depression. Sleep disruptions are found during the chronic phase of numerous psychiatric disorders, which also limits their diagnostic utility but strongly suggests common disruptions of the neural mechanisms responsible for sleep expression.

NE and depression

A large body of literature has implicated the LC-NE system in depression. This view arises, in part, because several clinically effective antidepressants have actions, sometimes specifically, on the NE system (including the new selective NE reuptake blockers). However, measurements of NE and its metabolites in depressed individuals have been inconsistent, and therefore inconclusive, regarding alterations in the NE of depressed individuals. This may reflect NE involvement in some subtypes of depression but not others. For example, one study found that cerebrospinal fluid (CSF) levels of 3-methoxy-4-hydroxyphenylglycol (MHPG) were higher among patients who were cortisol nonsuppressors than among either patients who were cortisol suppressors or controls. Also, urinary outputs of NE and normetanephrine were significantly higher among patients who were cortisol nonsuppressors than among controls. These results suggest that dysregulation of the noradrenergic system and hypothalamic–pituitary–adrenal axis occur together in a subgroup of depressed patients, consistent with a previous hypothesis that depression is associated with dysregulation of the NE-LC system. These data also imply that depression associated with stress disorders may be particularly associated with NE-LC dysregulation. Given the role of the NE-LC in sleep regulation, such dysregulation may account for some of the sleep disturbances in depression.

Stress, depression, CRF, NE-LC system and sleep

There are several links between stress, CRF, the NE-LC system and depression. Behavioral alarm and stress response were reduced in rhesus monkeys given a CRF receptor antagonist. Both patients with MDD and animal models of depression show increased levels of circulating cortisol and CRF compared to nonstressed controls. Hyperactivity of CRF-containing neurons appears to account for several symptoms of depression, including disrupted sleep, blunted daily temperature fluctuation, and altered growth hormone-releasing hormone to CRF ratio. Administration of CRF in normal adults increases W and reduces SWS but not PS. Stress, depression, and sleep may interact via the NE-LC system because effects of stress on sleep have been shown (as described above) to involve the NE-LC system, probably via CRF. Moreover, the LC receives a CRF input and CRF
potently activates LC neurons.\textsuperscript{88,99,139} It seems possible that alterations in the CRF influence on LC neurons may underlie sleep alterations associated with depression as a by-product of stress responses. Altered sleep associated with depression may involve specific stress mechanisms and LC functioning distinct from those involved in normal sleep regulation, as indicated in animal studies of the role of NE in stress effects on sleep.\textsuperscript{68,117}

Sleep deprivation and depression

Sleep deprivation itself is a surprisingly potent strategy for alleviating symptoms of depression. Total sleep deprivation, partial sleep deprivation, and PS deprivation all improve mood, albeit transiently, especially for patients with increasingly severe depression.\textsuperscript{157} Partial sleep deprivation is most successful when enforced for the second half of the night and/or when PS is preferentially reduced. Mood elevation due to any type of sleep limitation only occurs for about 50\% of patients tested and typically lasts only until the next sleep period.\textsuperscript{158} Given the links between NE, depression, and sleep, it seems possible that the LC could be involved in the antidepressant effect of sleep deprivation. Consistent with this possibility, Schrieber et al. found significant increases in plasma NE after sleep deprivation in healthy adults compared to baseline.\textsuperscript{159} Likewise, patients with MDD have increased MHPG after sleep deprivation, indicating increased NE neurotransmission.\textsuperscript{160}

Several methodologies of neuroimaging have recently been reviewed in order to better understand the functional neurology of the antidepressant response to sleep deprivation. Gillin et al.\textsuperscript{158} found, in general, that patients whose moods improved after either total sleep deprivation or partial sleep deprivation tend to have higher brain activity in the ventral anterior cingulate cortex (ACC), a localized increase in brain activity that normalized after sleep deprivation treatment. Both the ventral ACC and the orbital frontal regions were most often recognized as modified by sleep deprivation if researchers were careful to sort the data into “responders” vs. “nonresponders” (>50\% improvement on Hamilton Depression (HAM-D) ratings). Both the ACC and orbital frontal cortices are strongly innervated by the NE-LC system in primates, and our recent data (see Chapter 7 of this volume) also indicate that both are prominent afferents to the primate LC.\textsuperscript{161–163} Thus, dysfunctions in these areas could affect sleep and depression, in part, via interactions with the NE-LC system.

Anxiety

Abundant symptomatic similarities exist between diseases of excessive anxiety and major depression. As in depression, both types of insomnia (difficulty initiating sleep and difficulty staying asleep) are reported to persist during several anxiety disorders, e.g. panic attacks, attention-deficit/hyperactivity disorder (ADHD),
posttraumatic stress disorder (PTSD), and obsessive-compulsive disorder (OCD). Generalized anxiety disorder (GAD) is comorbid in patients diagnosed first with depression in an estimated 58% of cases. Subjectively, 50% to 70% of patients with anxiety tend to report disturbed, unsatisfying sleep with morning drowsiness. Objectively, the polysomnographic study with the largest number of patients with GAD reported decreased total sleep time, decreased sleep efficiency (i.e. more time awake once sleep was attained), and more early morning awakenings. Selective 5-HT uptake inhibitors are as effective in treating GAD and other disorders of excessive anxiety, as they are in treating depression. Similar neurobiologic disruptions in NE, 5-HT, and CRF systems are thought to contribute to both depression and anxiety. A review by Ressler and Nemeroff suggests that down-regulation of 5-HT and up-regulation of NE activity may occur in both anxiety and depression. According to their model, the NE-LC system primarily sensitizes the amygdala and hippocampus to increase the salience of, and conditioned responding to, stressful or fearful stimuli. Although Ressler and Nemeroff note that both 5-HT and NE dysregulation likely contribute to depression, NE is more directly implicated in the fear and stress responses contributing to persistent clinical anxiety.

A noradrenergic contribution to GAD has been difficult to ascertain because GAD is often comorbid with depression and other anxiety disorders. Conditioned anxiety disorders (PTSD and panic disorder) are marked by increased peripheral sympathetic arousal. Work in animals reveals that LC-NE neuronal activity often correlates strongly with sympathetic activity, probably reflecting the major inputs to the LC from the medulla. Thus, these disorders may also be associated with elevated LC activity. Ordway summarized the neurology of mood disorders to suggest that stress-induced CRF hyperactivation of the LC and ventral tegmental area (VTA) alters the balance of those monoamines with 5-HT. In addition, experimentally stressed macaque monkeys show higher CRF and depressed cortisol in the CSF while being hyperresponsive to yohimbine. Higher levels of CRF are also reported for patients with panic disorders and PTSD. Thus, chronic NE-LC hyperactivity via elevated CRF inputs may help sustain GAD and related disorders, and this LC hyperactivity may occur primarily as a conditioned response to stress that does not naturally ameliorate.

Postmortem evidence for changes in the human LC is not as available for clinical anxiety as for depression. However, there is an abundance of data in animal models linking LC activation to stress, pain, learned helplessness, hyperarousal, and nearly all face- and construct-valid animal measures of human anxiety. Activation of the amygdala has been linked to CRF-mediated LC hyperactivation as an explanation for the link between conditioned anxiety, hypothalamic activation, and subsequent NE-LC up-regulation.
Posttraumatic Stress Disorder (PTSD)

Posttraumatic stress disorder is an anxiety disorder arising from the conditioned emotional or injury-based responses to a singular or repeated salient event. Sleep disturbances are a common symptom reported by people who experience an acutely stressful episode. Polysomnographic assessment of patients with PTSD has produced a literature of mixed results. The symptoms of PTSD – recurrent anxiety, increased startle reflex, and sleep problems – do not occur in all who experience a traumatic event. Prevalence of PTSD varies widely, depending on the age of the subject, the severity of the event, and other unrecognized factors. Furthermore, comorbidity with other psychiatric, putatively emergent, disorders (e.g., depression) has been reported to be as high as 85%. A wide variety of events can cause PTSD, and the heterogeneity of responses make subject selection in studies so difficult that generalities about their sleep are difficult to support. For example, Hefez et al. found that some PTSD patients have decreased sleep efficiency, but that the sleep of other patients is not different from age-matched controls. Lavie reviewed the literature on sleep in PTSD patients to summarize that “patients with PTSD appear to have deeper sleep and lower rates of dream recall than normal persons.”

Locus coeruleus activity itself cannot be measured in people with PTSD, but there is evidence for disruptions in the NE-LC system contributing to the symptomatology of PTSD. Excessive startle reflexes and behavioral hypervigilance have been suggested to result from an increase in NE production from the LC, which may be a response to decreased NE receptor sensitivity following an acute stress event. Norepinephrine levels in the CSF of PTSD patients have been evaluated using an indwelling catheter to allow for serial recordings without sampling stress. Geracioti et al. reported higher CSF norepinephrine levels across 6 hours of wakefulness in men with PTSD as compared to healthy controls. Notably, CSF NE correlated with PTSD symptom severity while plasma NE levels did not. Increased chronic NE production in PTSD patients can explain increased arousal and disturbed sleep by virtue of increasing the drive to wakefulness via LC hyperactivity. Circadian variation of NE levels has not been seen when measured in people with PTSD or depression compared to control subjects. The tendency towards wakefulness at all times of day, not dysregulation of sleep state control, appears to arise from LC hyperactivity that increases nightmares and the perception of poor sleep during clinical PTSD.

The neurobiology of PTSD suggests a functional connection between behavioral hyperresponsivity, the NE-LC system, and stress-evoked CRF increases. Heim and Nemeroff suggest that early trauma chronically increases CRF reactivity leading to subsequent supersensitivity to stressful events. Chronic stress in animals has been shown to increase NE levels in the hippocampus and amygdala, which may lead to
increased memory consolidation during periods of stress. Conditioned anxiety responses to specific triggers (in cases of PTSD or panic disorder), or generalized to many triggers, would thereby help maintain NE hyperactivity in the central nervous system, leading secondarily to sleep disruptions of excessive arousal.

**Attention-deficit/hyperactivity disorder (ADHD)**

Impulsive, hyperactive, and inattentive behavior identifies a (typically young) person as having ADHD. These same types of behavior are seen in nonhuman primates during the tonic mode of activity in monkey LC neurons (as described in Chapter 7 of this volume). This finding is consistent with the possibility that this mode of LC activity contributes to the disorder. Treatment of ADHD with amphetamines is effective clinically but is not sufficient to explain underlying mechanisms.

Arousal state dysregulation, primarily nocturnal insomnia and wakeful hyperactivity, are common in as many as 75% of young people with ADHD, according to parental reports. Polysomnography studies of children with ADHD are few in number but shortened REM latency and an increase in motor activity are most commonly reported. Comorbidity between ADHD and nocturnal motor activity has been seen in several studies of ADHD, with a strong trend towards excessive sleep movements, including clinical levels of period limb movements in sleep (PLMS). Sleep instability measured with actigraphy in 38 students with ADHD found greater sleep onset variability and shorter average sleep duration than age-matched controls.

Solanto surveyed existing data to surmise that ADHD, like anxiety, occurs due to dysregulation of more than one catecholamine to manifest the full range of cognitive and behavioral signs of ADHD, specifically implicating both NE and dopamine (DA). Up-regulated NE release may contribute to both the sleep and behavioral symptomatology of ADHD, as described above for anxiety. In vivo microdialysis finds larger increases in local NE levels than local DA levels in response to amphetamine-like stimulants, further suggesting that increased NE levels contribute to both distractible behavior and more disrupted sleep. However, it has proved difficult to specify the role of NE changes in ADHD. Reports of NE levels measured via metabolites in ADHD patients are so mixed as to be unusable for understanding its role in this disorder. Amphetamines, the primary drug treatment for ADHD, activate the NE-LC system but also alter dopaminergic activity in the VTA. Dopamine transporter knockout mice, normally hyperactive and impulsive, show reduced hyperactivity following methylphenidate and dextroamphetamine, behavioral responses similar to the behavioral responses seen for patients taking these medications. Because DA transporter knockout mice do not have DA transporters, these drugs must operate through another neurotransmitter system. The LC is a good candidate, but confirmation requires further examination.
Aging

Aging represents a “natural disorder” that typically causes an increase in sleep problems, including significant loss of SWS and PS, increased awakenings, and less total sleep time. Czeisler and colleagues suggest that aging disrupts the ability to maintain sleep and reduces the sleep response (homeostatic sleep response) to increased waking periods. The dominant theory at this point is that comorbid health problems conspire to disrupt sleep but that the elderly may retain their capacity for good sleep if external health and lifestyle sleep impediments are eliminated. However, older subjects measured in the “forced desynchrony” protocols showed significantly worse sleep than younger subjects when they were forced to sleep at nonoptimal circadian periods.

The symptomology of aging suggests a gradual diminution of NE-LC basal activity and reactivity. Aged primates and humans show a profound loss of LC neurons (particularly in Alzheimer’s Disease), and aged monkeys have forebrain catecholamine depletion. Memory problems that emerge with age in rhesus monkeys can be temporarily offset by administration of α2-agonists, but sleep effects of this treatment have not been studied. One possibility is that sleep–wake state instability results from a loss of wake-drive promotion due to LC insufficiency. Locus coeruleus insufficiency, in contrast to other clinical disorders reviewed here, could reduce daily sleep drive secondary to a reduction in active wakefulness. In contrast to these signs of decreased LC function, one set of studies found that aged rats produced higher levels of plasma catecholamines during both acute and chronic stress than did young controls, implying that elevated NE could contribute to sleep problems in the elderly. However, much more work is needed in this area before conclusions can be drawn regarding an LC role in sleep problems of the elderly.

Insomnia

Two types of insomnia exist: difficulty falling asleep or difficulty staying asleep. Together they result in chronic sleep problems reported by one-third to one-half of all Americans at some time in their lives. Subjective insomnia is often overstated when compared with polysomnographic signs of sleep, but clearly at least 10% of people have great trouble falling asleep or staying asleep, and this disruption can last for months at a time. Furthermore, although insomniacs sleep less and report being sleepier, they do not tend to fall asleep faster if given multiple chances to sleep across the day.

The neurobiology of insomnia remains a mystery even though several therapeutic strategies find good success treating insomnia in its variants. Benzodiazepines (transient insomnia), cognitive behavioral therapy (chronic insomnia), phototherapy (seasonal insomnia), and stimulus control therapy (conditioned insomnia)
Role of LC in arousal and sleep

are capable of providing both subjective and objective sleep improvements. Few of these pharmacobehavioral strategies to treat insomnia have received adequate basic neurobiology research.

Notably, abundant clinical evidence suggests that most chronic insomnia develops subsequent to stress-related transient insomnia. People with insomnia have been shown to perceive minor stressful events as more difficult to cope with than people with the same number of stressful events who have normal sleep. Nocturnal cortisone levels are higher in insomniacs than controls suggesting that insomnia results from increased hypothalamic–pituitary axis activity. Increased hypothalamic – pituitary–adrenal activation, as in the other disorders, is capable of increasing both central and peripheral release of epinephrine and NE. In addition, one study found that circulating levels of NE were higher in insomniacs than patients with depression or age-matched controls, while another found that circulating levels of NE correlated with sleep disturbance frequency. Furthermore, levels of nocturnal catecholamine correlated with the amount of sleep disruption found for insomniacs but not for patients with depression. In a recent clinical review, Hauri notes that signs of hyperarousal can be recorded in patients with primary insomnia: increased body temperature, high-frequency EEG during sleep, and metabolism, and they tend to be “sensation avoiders,” suggesting they feel generally overstimulated all the time. Physiologic overstimulation coupled with stress responses may be adequate to stimulate stress-sensitive input to the LC and cause NE hyperactivity to result in sleep disruption as described earlier in this chapter for other disorders.

Conclusions

Arousal state maintenance and sleep–wake regulation clearly involves the NE-LC system. Participation of the LC system in several clinical disorders implies that this brain nucleus may contribute to the sleep disturbances associated with those disorders, as outlined above. One theme that emerges from our review of this clinical literature is that sleep disturbances in psychiatric disorders are often associated with enhanced stress response. This could be significant because the LC plays a large role in such responses. Thus, CRF release in the LC, and consequent LC activation during stress appears to be sufficient to evoke both sleep disturbance and aberrant PS expression found in many clinical mood disorders. However, CRF is not the only messenger to communicate stress-related information to the LC. For example, glutamate inputs from the medulla are also involved in LC activation by somatic or visceral stressors. Whatever the input that drives the LC, increased NE output consequent to stress may participate in sleep disturbances associated with a variety of psychiatric problems. Further work along these lines may yield new approaches
to treating these sleep disturbances and sleep improvement may, in turn, facilitate clinical resolution of the primary psychiatric disorder.

REFERENCES


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