Locus coeruleus: a new look at the blue spot

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Abstract | The locus coeruleus (LC), or 'blue spot', is a small nucleus located deep in the brainstem that provides the far-reaching noradrenergic neurotransmitter system of the brain. This phylogenetically conserved nucleus has proved relatively intractable to full characterization, despite more than 60 years of concerted efforts by investigators. Recently, an array of powerful new neuroscience tools have provided unprecedented access to this elusive nucleus, revealing new levels of organization and function. We are currently at the threshold of major discoveries regarding how this tiny brainstem structure exerts such varied and significant influences over brain function and behaviour. All LC neurons receive inputs related to autonomic arousal, but distinct subpopulations of those neurons can encode specific cognitive processes, presumably through more specific inputs from the forebrain areas. This ability, combined with specific patterns of innervation of target areas and heterogeneity in receptor distributions, suggests that activation of the LC has more specific influences on target networks than had initially been imagined.

Research by a handful of anatomists, behavioural scientists and physiologists over the years has provided limited yet tantalizing clues that the noradrenaline (NA) containing nucleus locus coeruleus (LC) plays a critical role in core behavioural and physiological processes. Evidence of LC-NA involvement in multiple clinical conditions further underscores the need for achieving an advanced understanding of the function of this nucleus.

The position of the LC deep in the brainstem and its small size have made it difficult to record LC neuronal activity and correlate this activity with environmental events and behaviour. The LC, translated from Latin as 'blue spot', comprises a mere 3,000 brain cells in the rodent and is similarly diminutive in primates. However, those LC-NA neurons display broad and divergent axonal pathways through the CNS. For more than 50 years, the LC was thought to be homogeneous in its structure and function, such that its primary transmitter, noradrenaline (NA), could be released uniformly and act simultaneously on cells and circuits throughout the forebrain, brainstem, cerebellum and spinal cord. Because the LC projects to a large number of CNS areas with widely divergent functions, researchers have tended to see the LC-NA as a system with unified action on neural networks and behavioural outcomes. Under such a unified conceptualization, however, it has been a challenge to develop testable hypotheses regarding a comprehensive and coherent role for the LC in physiology and behaviour. This struggle to find support for such unified theories is compounded by the fact that the input/output relationships of the LC are indeed complex, and for technical reasons have resisted detailed study.

Recent methodological advances have provided powerful new tools that can be applied to unlocking the mysteries of the LC. Studies utilizing optogenetics and chemogenetics, viral tract tracing, RNA sequencing, and neuron- and molecule-specific labelling methods in combination with behavioural measures have begun to dramatically expand our thinking about LC organization and function (see FIG. 1 for an overview). New discoveries indicate that the nucleus and its neurons are more heterogeneous than had previously been appreciated. Technical advances in functional MRI (fMRI) also promise to soon identify behaviour-specific activity in the LC of humans and other primates, opening a new avenue for future investigation. All these advances are enabling new clinical translational studies and treatment innovations (BOXES 1–3), as well as new directions for basic research (FIG. 1).

One way to accelerate our understanding of the LC is to map leverage points where new approaches and additional expertise could have maximal impact in advancing the field. This report is the result of an intensive 3-day workshop in which participants were tasked with summarizing and synthesizing the current understanding of LC organization and function, with particular emphasis on recent breakthroughs. Contributors focused on identifying opportunities and priorities for exploitation, including pinpointing critical unanswered questions to guide current and prospective investigators in the field. This report is not a consensus document adopting conventions or formalizing a database, nor is it a detailed review of the state of the field. Rather, it is an attempt (1) to identify major issues of LC organization and physiological function, which were previously intractable or poorly conceived, that can now be realistically addressed with our ever-increasing knowledge base and new methodologies; (2) to suggest new ways to think about how the LC is related to major behavioural and cognitive functions or clinical disorders, as well as describe new ways to use such knowledge translationally; and (3) to present major unsolved questions about LC function whose answers will encourage accelerated research in multiple directions.

Anatomy of the LC

The LC is a cluster of relatively large neurons containing NA that is located bilaterally in the brainstem just under the cerebellum and lateral to the fourth ventricle. What we thought we knew about the widely

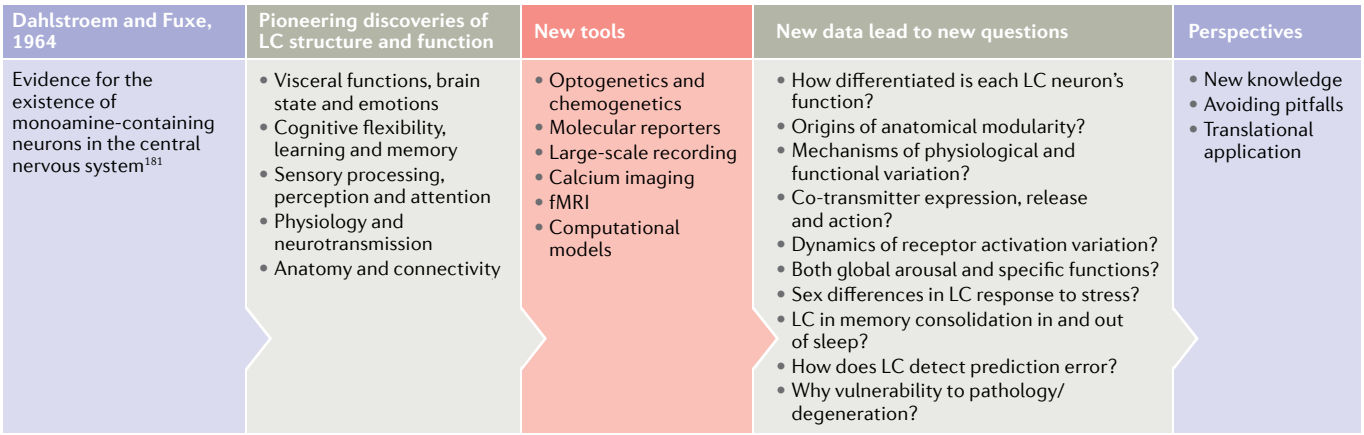


Fig. 1 | **The blue spot: past discoveries and future horizons.** The discovery of the existence of monoamine-containing neurons in the CNS by Dahlstroem and Fuxe in 1964 (REF.[181]) inspired subsequent systematic research on locus coeruleus (LC) structure and function. Use of the available neuroscience methodologies allowed many pioneering discoveries and influential theories of function (second panel). Unprecedented technological advances in recent years have been a catalyst for obtaining new results that confirmed, but also challenged, the existing knowledge about the LC-noradrenaline (NA) system (third panel). Newly revealed complexity of the organization of the LC-NA system has raised many new questions (fourth panel), opening new vistas for future research. fMRI, functional MRI.

projecting efferent and convergent afferent anatomy of these neurons, prior to about 10 years ago, was inaccurate. We will here attempt to explain why this decades-long understanding has recently changed and describe the functional and clinical implications of this new understanding. We present several areas of LC functional anatomy in need of further exploration: its efferent and afferent projections patterns, neurotransmitter identities, presynaptic release profiles and reuptake, and the receptor make-up of its terminal fields.

LC efferent anatomy. Understanding the anatomical organization of the LC is critical to unlocking its function, and this understanding has changed radically over the past 10 years. Nearly a dozen studies from different labs have provided compelling evidence that LC projection patterns may be modular in design, with segregated output channels and the potential for differential release and actions of NA upon its projection fields. Although this work is still ongoing, these new findings have prompted a wholesale shift in our thinking about LC operations and demand a revision of theoretical constructs regarding the impact of the LC-NA system on behavioural outcomes in health and disease. Target-specific projection patterns of individual LC neurons could enable differentiated modulation of diverse behaviours and cognitive functions[1–3]. New strategies employing transgenic animals and viral vector approaches[4–8] are providing a picture of complex, targeted projections from some subsets of LC cells, divergent projections in other subsets, and a mixture of widespread projections with preferred targets in still others.

The distribution of LC-NA axonal fibres is nonuniform across the rat[9] and primate[10,11] neocortex and is characterized by axon varicosities[9]. The axon collaterals of distinct LC neurons appear to be distributed in a coordinated fashion to functionally related target circuits, such as those coordinating aversion and/or anxiety in the anterior cingulate and amygdala versus those promoting analgesia in the spinal cord[4,6,7,12–15]. Thus, clusters of some LC neurons are organized into modules with respect to their efferent targets.

These newly observed organizational features of the LC-NA system are based primarily on studies of a limited set of emotional, cognitive (for example, amygdala versus ventromedial prefrontal cortex circuits for fear learning versus extinction[7]) and sensory regions of the forebrain and spinal cord[12]. However, additional evidence for projections from a subgroup of LC neurons to the caudate–putamen, along with increased NA turnover in striatum and the functional connectivity of striatal–motor networks with chemogenetic LC stimulation, is revising previous conceptions that LC-NA projections do not influence striatal–motor function[16]. Furthermore, the organization of well-known LC projections to other key motor structures (for example, cerebellum, inferior olive, or motor cortex) has largely been ignored. Misconceptions based on gaps in our anatomical understanding still limit theories of LC involvement in basic functions and disorders. However, these initial results point out an enticing area of study and collectively make a strong case for continued investigation of heterogeneity in LC efferent structure. One way forward is to probe the functional consequences of modularity in LC outputs by selective opto- or chemogenetic activation of subsets of terminal-field-specific LC neurons in waking, behaving animals.

The current challenge in understanding LC efferent anatomy is to develop a model that takes into account the limited number of LC neurons and the extensive distribution of LC axons throughout the neuraxis — an arrangement that requires massive arborization of individual axons — while also acknowledging recent findings of focused or modular efferent projections[2] (FIG. 2). New imaging methods that target NA receptor occupancy[17] hold promise for establishing a detailed mapping of the spatial distribution and timing of NA release and receptors in terminal fields relative to sensory responses and behavioural output. Such information will provide an understanding of the LC axonal organization that is functionally relevant.

LC afferent anatomy. Although modularity of the efferent connectivity of LC is an active area of research, the integration of LC efferent modularity and LC afferent patterning has largely been neglected. The LC microcircuitry consists of a dense, cell-rich 'core', where NA cell bodies and processes reside, and a pericoeruleus (peri-LC) 'shell' into which LC-NA dendrites extend and ramify[18]. Distinct populations of GABAergic neurons are also found in the

peri-LC region and provide local regulation of LC-NA cell activity[19,20]. Early anatomical-tracing studies identified prominent afferent inputs from medullary nuclei into the LC core[21], and subsequent work revealed that LC-NA neurons are innervated by many regions of both the brainstem and forebrain[6,20,22]. Inputs targeting peri-LC GABAergic cells are partially distinct from the inputs that directly innervate LC-NA neurons[20].

Multisynaptic viral-tracing approaches have provided a broader analysis of LC input connectivity[23]. Inputs from diverse sources can produce differential effects on LC-NA neuronal output directly and indirectly, by differentially targeting the core nucleus versus the pericoerulear regions. One target of opportunity is to investigate how afferent connections and connectivity within the LC and peri-LC regions regulate discrete LC cell populations that themselves have different efferent connectivity. For example, while some LC cells receive common afferent drive, it is unclear whether cell populations with more specific efferent connectivity receive distinct afferent inputs[6] (FIG. 2). Another mechanism for afferent-specific regulation would be differential expression of transmitter receptors between LC modules. Elucidating how specific afferents are connected with local LC and peri-LC networks and modules will be important for understanding LC's diverse functionality.

LC transmitters and co-transmitters. Another area of LC anatomy that is ripe for investigation is the distribution of neuroactive substances, primarily peptides and dopamine[24], that are colocalized in the NA-containing neurons. To date, galanin, neuropeptide Y, brain-derived neurotrophic factor, cocaine- and amphetamine-regulated transcript, dopamine and other neuroactive substances have been identified as co-transmitters in LC-NA cells[25–31]. Some are expressed in most, but not all, LC-NA neurons, while others are sparsely distributed[29]. There may be species differences as well[32]. The functional neuroanatomy of LC co-transmission is an untapped area of inquiry. For example, there is limited information about the efferent targets and discrete versus global inputs to these cells.

We also know little of the extent to which different neuroactive substances are stored in shared or separate vesicles, under what conditions they are released, and the physiological/behavioural effects of LC-mediated neuropeptide and co-transmitter signalling. Recent technological advances in neuroanatomical mapping, opto- and chemogenetic methods of modulating neuronal activity, the detection of transmitter/modulator release, and genetic ablation can now be brought to bear. For example, to map monosynaptic inputs to LC cells that are co-expressing a specific neuropeptide, mouse lines with Cre recombinase knocked into a neuropeptide gene locus can be infused with a Cre-dependent modified rabies virus into the LC[33]. Similarly, LC-NA/neuropeptide output projection patterns can be identified following intra-LC infusion of neuropeptide gene Cre-recombinase-dependent AAV fluorescent tracers[34].

While measuring neuropeptide release in real time has not previously been possible, the suite of new genetically encoded fluorescent neurotransmitter indicators can be expanded beyond those currently available for small-molecule neurotransmitters to include neuropeptides[35]. Pairing these indicators with optogenetic stimulation or silencing of specific subsets of LC neurons or with calcium-indicated or single-unit electrophysiological recordings of spontaneous activity will reveal, for the first time, the firing-frequency and pattern dependence of LC-NA peptide and dopamine co-transmission. The functional importance of non-noradrenergic LC neurotransmission is illustrated by recent demonstrations that dopamine release from LC terminals in the thalamus and hippocampus has distinct cellular and behavioural effects on stress responses, synaptic plasticity, and learning and memory[36–38].

Combinatorial RNAi[39] and characterization of conditional-knockout animals that lack a neuropeptide specifically in LC neurons should enable identification of the function of these co-transmitters. This approach was recently deployed to define a role for LC-derived galanin in active coping behaviours[40]. Utilizing inducible knockouts that remove the neuropeptide in adulthood rather than throughout development will further refine and improve this strategy.

Finally, we have only just begun to identify the mechanisms governing co-transmitter and co-peptide release and reuptake[41], the dynamics of which determine transmitter concentration and the duration of exposure to membrane-bound receptors. An important future direction will be to fully characterize the dynamics of LC co-transmitter release and reuptake across LC terminal fields. Microdialysis in combination with high-pressure liquid chromatography is available for examining fluctuations in local extracellular concentrations of NA[42,43], and possibly other LC co-transmitters, in anaesthetized and waking animals, but measurements with this technique have poor spatial and temporal resolution. As such, this approach leaves open the question of how rapid transitions in the LC firing rate correspond to transmitter release and to specific

Box 1 | **LC in ageing and neurodegenerative disorders**

Although it was originally reported that locus coeruleus (LC) degeneration occurs over the lifespan, more recent post-mortem studies have indicated that frank LC neuronal loss does not appear to be a component of normal ageing[183]. Some LC-sensitive MRI studies have indicated an age-related reduction in LC integrity, but this signal may reflect changes in neuromelanin, neuron shrinkage and/or the loss of proximal dendrites rather than cell body death[125,184]. LC MRI will be a critical research tool moving forward, as cognitive reserve and memory performance have been linked to higher LC signal intensity and to changes in connectivity in older participants[182,185,186]. Noradrenergic regulation of plasticity in LC terminals also declines with age[125], as does noradrenaline (NA) regulation of hippocampal synaptic plasticity[187], perhaps contributing to the increased difficulty with learning flexibility in healthy ageing individuals, as well as to difficulty with reshaping cortical circuits after brain injury in the aged.

Regardless of the controversy concerning LC integrity during normal ageing, it is well established that LC-NA cells are exquisitely sensitive to pathology and death in neurodegenerative diseases of aging; hyperphosphorylated 'pretangle' tau appears in the LC prior to any other brain region in Alzheimer disease (AD). LC neurons are also among the first touched by α-synuclein inclusions in Parkinson disease (PD), and LC cell loss becomes catastrophic in both late-stage AD and PD[188–190]. Because LC neurons can survive for years despite harbouring inclusions of tau or α-synuclein, and because many prodromal symptoms of AD and PD are consistent with LC dysfunction (for example, sleep disturbances, anxiety, depression and so forth)[191,192], it is critical to understand the consequences of aberrant tau and α-synuclein for LC physiology. Transgenic animals developed using noradrenergic-selective promoters, LC-specific infection with tau/α-synuclein viral vectors, or intra-LC infusion of preformed fibrils represent fresh approaches to overcome the confounds associated with the ubiquitously expressed protein aggregates in earlier models[193–195]. Characterizing the molecular signatures and electrophysiological properties of beleaguered LC neurons and assessing LC-relevant behaviours top the list of priorities in this research area. Further development of sensitive and specific MRI methods to assess LC integrity and the use of such integrity as a biomarker for AD and PD have shown promise but are yet to be fully exploited[184].

physiological outcomes at the cellular, circuit and network levels. Fast-scan voltammetry operates in the range of seconds and has been used extensively in anaesthetized and waking animals for moment-to-moment measurement of extracellular concentrations of dopamine in different brain regions[44,45]. However, this technique has not been exploited to the same extent for NA. What is needed is an electrochemical or other biosensor methodology whereby rapid measurement of NA levels or of other LC co-transmitters can be made simultaneously in multiple brain sites of waking animals engaged in behavioural tasks[46,47]. In particular, NA receptor sensors with subsecond fidelity might hold the key to understanding the dynamics of NA release and receptor activation in LC terminal fields on a timescale capable of providing a link between measured release patterns and observable physiologic action[17]. Such capabilities would permit an evaluation of circuit-specific fluctuations of LC-NA transmitter levels in concert with transitions between behavioural states and concurrent with behavioural and sensory events.

Receptor activation. The net effect of NA release within a given neural circuit is dependent on the presynaptic and postsynaptic complement of α- and β-adrenergic receptors and their subtypes in that region. The nine known receptor subtypes have long been characterized: α1A, α1B, α1D, α2A, α2B, α2C, β1, β2 and β3 each have unique and sometimes overlapping distribution patterns in the brain, different sensitivities to the NA ligand and different actions upon the cells and the network in which they are embedded. For example, β2-receptors are more abundant in the hippocampus. A genetic variant of this β2-receptor subtype prolongs NA binding and is seen more in those who develop post-traumatic stress disorder[48]. The literature on the expression, turnover and function of adrenergic receptors across the brain and spinal cord is extensive, describing the NA receptors on neurons, glia, immune cells and even brain vasculature[49–55]. Any interpretation of the effects of LC output on brain functions and, ultimately, on behavioural outcomes must account for the relative distribution, density, binding properties and physiology of adrenergic receptors on specified cell populations within LC terminal fields. Likewise, information about the distribution of receptors for LC co-transmitters is also critical for a complete understanding of terminal-field responses to LC activation. However, the dynamics of receptor availability and activation are often neglected when considering the net outcome of LC actions on terminal-field operations. Consideration of these factors will provide for a more holistic interpretation of the impact of LC output on CNS function.

Box 2 | **LC and stress**

The locus coeruleus (LC) has been implicated in neuropsychiatric disorders through its role in the stress response[196,197]. Stressors engage the LC-noradrenaline (NA) system through corticotropin-releasing factor (CRF), which biases LC neuronal discharge to favour high-tonic activity and diminishes responses to discrete stimuli[197,198]. These effects could be adaptive in life-threatening environments, where high general arousal and cognitive flexibility would be advantageous. Chemogenetic LC activation in order to mimic acute stress increases brain-wide functional connectivity, particularly in salience networks and amygdala networks, and this is accompanied by decreased exploratory and increased anxiogenic behaviour[16]. A caveat is that it is not known whether chemogenetic stimulation produces the same rate and pattern changes in LC activity that acute stress does, and these results may differ for different types of stressors.

Enkephalin-containing axon terminals converge on some of the same LC dendrites as CRF-containing axon terminals and have opposing effects on LC discharge[199,200] during stress, implicating enkephalin afferents to the LC (acting at μ-opioid receptors) as an important stress-coping and recovery system. Acute stressors engage both CRF and enkephalin afferents to regulate LC activity[201,202]. Although CRF excitation of LC activity predominates during acute stress, μ-opioid receptor antagonism results in a greater magnitude of LC activation and delays recovery[201].

Sex is an important determinant of LC sensitivity to stress, because in rodents the LC neurons of females are more sensitive to CRF and less sensitive to enkephalin than are those of males[203–205]. This increased female sensitivity to CRF has been linked to synaptic CRF receptor and internalization changes in females compared to males[204]. This would translate to a greater LC-NA response to stressors and a decreased ability to adapt through the process of receptor internalization. The role of cycling hormones remains to be thoroughly investigated, but oestrogen and progesterone receptors abound on LC cells and likely have mitigating effects on LC activity[206]. These many sex differences suggest a molecular basis for the higher prevalence of stress-related psychiatric disorders in females[207].

Origin and development of the LC

The notable progress in our understanding of the complexity of LC organization[3] reveals an urgent need to determine the mechanisms that generate such LC cell heterogeneity. We are only beginning to understand how diversity among LC neurons is obtained and maintained across the lifespan. Molecular and genetic tools appear indispensable for exploring the ontogeny of LC-NA neurons and, in combination with other methods, may provide important insights.

LC embryogenesis. One possible clue to the origin of distinct LC modules lies in the finding that different populations of NA neurons originate from molecularly distinct progenitors, with most LC neurons emerging exclusively from a single rhombomere, defined by the expression of a factor named engrailed 1 (REF.[5]). An intersectional genetic fate-mapping method has demonstrated that embryonic origins define features of LC neurons such as their pattern of distribution within the LC complex, their cell morphology and their axonal projection pattern[56]. The development, maintenance and survival of LC-NA neurons are all regulated by complex molecular signalling cascades involving multiple genes and transcription factors[57–61]. A recent exciting study showed that by combining only seven transcription factors, astrocytes and fibroblasts could be converted in vitro into NA neurons by direct reprogramming[62]. A complete set of the genes and transcription factors that are necessary and sufficient for the generation and survival of NA cells in vivo would be helpful for restoring function after neurodegeneration — for example, in Alzheimer disease (where LC pathology begins early) — while the genes and transcription factors that regulate LC cell number and differential circuit formation all need to be catalogued[61] in order to ensure replacement of the proper type and number of LC-NA neurons.

LC postnatal development. At birth, LC-NA neurons are functionally active, yet some of their physiological features undergo postnatal developmental alterations, including changes in membrane properties, receptor expression, cellular coupling (from predominantly gap junctions to more synaptic connections) and responsiveness to sensory inputs[63]. In newborns, the LC's responsiveness to somatosensory stimuli appears enhanced, whereas later in development, LC neurons become less sensitive to innocuous stimuli and more sensitive to noxious stimuli[64].

Enhanced response of neonatal LC neurons to sensory stimulation is thought to underlie some unique features of infantile learning, such as attachment[65]. Indeed, environmental factors in early life can have long-lasting effects on LC function and behaviour into adulthood. For example, high maternal care affects the densities of the benzodiazepine receptor, the α2-adrenoreceptor and the corticotropin-releasing factor receptor in the LC in a way that renders the adult offspring of high-licking mothers more resistant to stress[66].

NA has a protective role for other neuronal cell types in the CNS. A recent study reported that early postnatal NA denervation reduces BDNF protein and the activation of TrkB receptors in the ventral midbrain, possibly leading to dopamine neuron degeneration[67]. The possibility that NA plays a role in preserving neurons in other areas and allowing critical-period plasticity during development has not been adequately addressed.

LC neurons change their electrophysiological characteristics across early development. LC cells in infant rats display slower conduction velocities[63] as well as synchronized subthreshold membrane-potential oscillations and synchronized spiking[64,68]. Interestingly, those LC neurons with oscillatory activity have lower input resistance and a tendency for more electric coupling via dendro-dendritic gap junctions than neurons that do not oscillate. These gap junctions are thought to underlie a high degree of synchrony in the LC of newborns, a synchrony that dissipates by postnatal day 21 (REFS[64,68]). Interestingly, the expression of NA itself fluctuates among LC cells during postnatal development, with some cells exhibiting only transient noradrenergic characteristics[69]. There is also a transiently expressed excitatory synaptic coupling among LC neurons in early infancy that is mediated by α1 adrenergic receptors[70], whereas α2-mediated inhibitory coupling persists throughout the lifespan[68,71]. It is unknown how gap junctions and early activity relate to the final phenotype of LC cells. Better understanding of the molecular signalling mechanisms that control the expression and maintenance of the NA phenotype in the LC will likely lead to ground-breaking approaches for the preservation and restoration of LC function in neurodegenerative and neuropsychiatric disorders.

Electrophysiological properties

The electrophysiological properties of LC neurons have been well characterized by means of a number of intracellular and extracellular recording studies[72,73]. Individual LC neurons in vivo have almost exclusively been measured in adult animals, in which they display a relatively narrow range of discharge rates across varying arousal levels, from almost completely silent in REM sleep to 5–6 Hz in stress[74,75]. LC neurons ex vivo, recorded almost exclusively from very young brains, display spontaneous firing rates in the range of 0.5–5 Hz[72,76]. Although synchronous oscillations between LC neurons, mediated by gap junctional coupling of dendrites, have been reported[72], ex vivo intracellular brain slice recordings have demonstrated that there remains variability among cells in spontaneous firing rate and resting membrane potential[72]. Such physiological variation supports the existence of distinct groups of LC neurons, as has been indicated by anatomical studies (FIG. 2).

Several important questions arise from observations of firing-rate variability among LC cells. First, what, if any, are the transcriptional differences between LC cells that could account for variations in their intrinsic physiological properties? A recent publication demonstrated that the LC neurons that innervate medial prefrontal cortex and ventral hippocampus are not only anatomically distinct but also differ in their physiological responses to clonidine, demonstrating phenotypic variability among cells[77]. The recent development of single-cell sequencing techniques performed in the same cells that have been characterized electrophysiologically offers a powerful window into the molecular determinants of intrinsic physiology. Such electrophysiological and genetic combinations could help reveal why different LC cells behave in unique ways in vivo across behavioural states, both with and without experimental manipulation[78]. Another important question is whether LC cells with different electrophysiological properties also show distinct patterns of anatomical connectivity or neurochemical profiles in order to differentially affect CNS function.

Novel viral-genetic techniques allow investigation of the electrophysiological attributes of discrete assemblies of LC neurons that can be probed according to their connection patterns and/or their co-transmitter profiles, to reveal unique roles in specific behaviours. Early application of such viral-genetic approaches has started to show the compartmentalization of function within LC cells of different electrophysiological profiles, such that the neurons

Box 3 | **Therapeutics**

Dysfunction of the locus coeruleus-noradrenaline (LC-NA) system has been implicated in many neuropsychiatric and neurological diseases, including depression, anxiety, attention deficit hyperactivity disorder (ADHD), post-traumatic stress disorder (PTSD), Alzheimer disease (AD) and Parkinson disease (PD). Even in cases where the LC is not involved in establishing the disorder itself, it is possible that manipulating LC activity could disrupt the feedback loop that supports the dysfunction, thus re-establishing a healthy physiological response pattern and moving the patient towards normal daily activity.

Given the cornucopia of available pharmacological agents targeting NA synthesis, signalling and metabolism, it is surprising how few are currently in use. The selective NA reuptake inhibitors such as atomoxetine, for ADHD[208], and reboxetine, for depression[209], the synthetic NA precursor L-3,4-dihydroxyphenylserine (DOPS; droxidopa), for PD[210], and the α2-adrenergic receptor agonist lofexidine, for opioid withdrawal[211], have all been tried with some success. There are several reasons noradrenergic compounds are not being used more frequently. Although NA dysfunction contributes to many aspects of brain disorders, it is not known to be the specific cause of any symptomatology or known disease process (in contrast to dopamine neuron degeneration, which causes the cardinal motor symptoms of PD and has spawned multiple dopaminergic therapies). Systemic effects of noradrenergic drugs on cardiovascular function prompt caution in their use for treating CNS disorders. Minimally invasive procedures are emerging for manipulating the LC, taking advantage of studies of circuits that regulate LC neurons — for example, the circuit from the suprachiasmatic nucleus (SCN) to the LC via a relay in the dorso-medial hypothalamus[23]. As specialized retinal ganglion cells strongly innervate the SCN, retinal stimulation (for example, using chemogenetics) may modulate the LC for therapeutic benefit[212]. Transcutaneous vagus nerve stimulation is another non-invasive procedure that has positive effects on psychiatric and neurological disorders such as depression[213], epilepsy[214] and cognitive dysfunction[215], all of which are mediated, at least in part, through LC activation. As we learn more about the diversity of LC neurons (for example, efferents, afferents, co-transmitters, survival factors and so forth) and their modulatory organization, and as we develop technologies to target subpopulations of these cells, LC-based therapies for neuropsychiatric and neurological diseases will become more credible and effective while minimizing peripheral side effects.

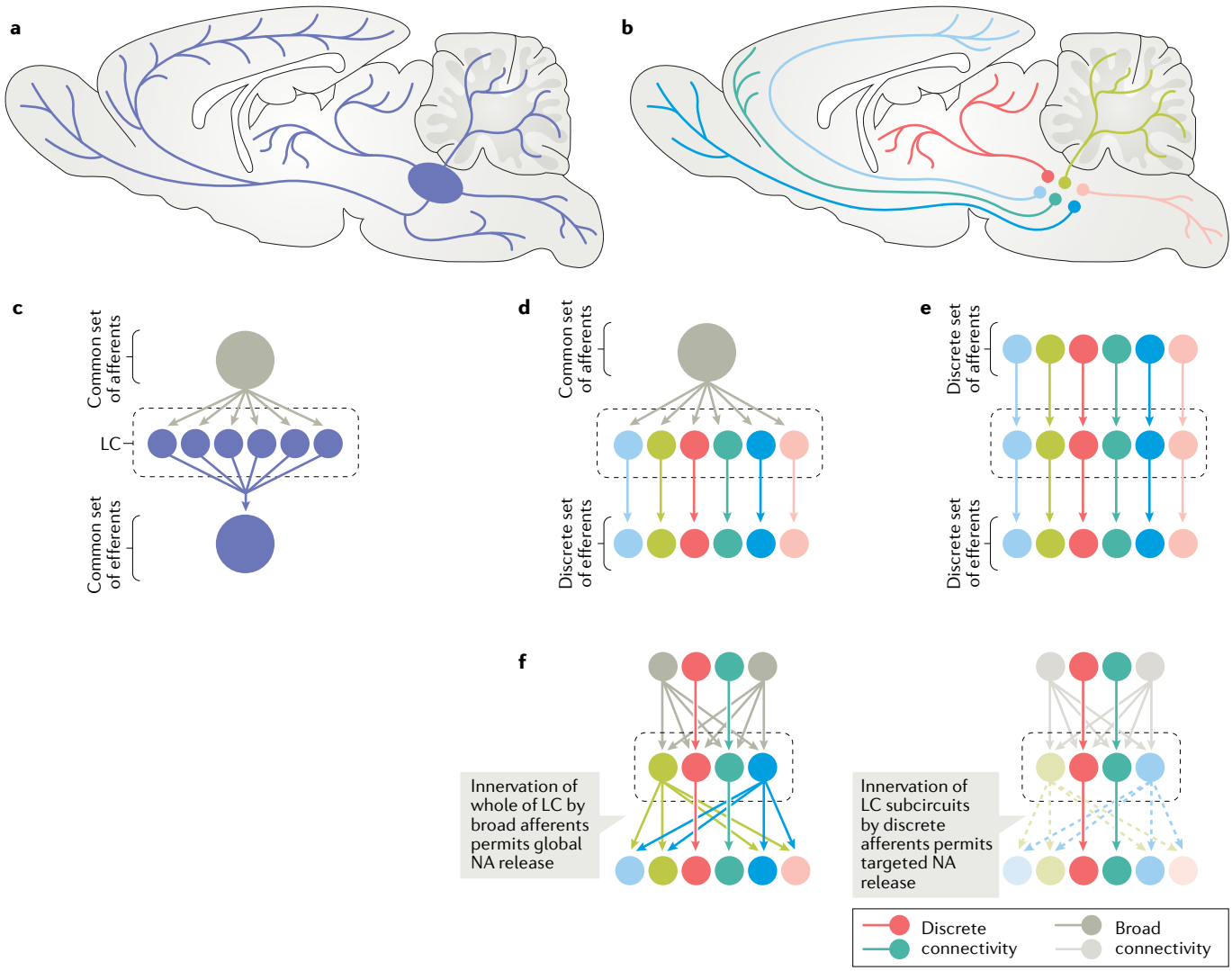


Fig. 2 | **Evolving views of the LC synaptic architecture and functional organization. a** | Historically, the locus coeruleus (LC) had been thought to contain functionally homogeneous neurons (indicated here by a uniform blue colour) whose axons extensively collateralize and indiscriminately innervate functionally diverse terminal fields[21]. **b** | More recently, several studies[4,7,12–14] have shown that the axons of LC neurons are less extensive than had previously been recognized, and instead innervate anatomically and functionally distinct targets. In the schematic, each colour represents a population of LC neurons that projects strongly to a preferred terminal field, including olfactory bulb (medium blue), frontal cortex (medium green), visual cortex (light blue), thalamus and midbrain (red), cerebellum (light green) and spinal cord (pink). Note that this schematic is illustrative only and that there are likely more than six efferent LC pathways. Evidence also suggests that LC neurons also innervate terminal fields beyond their preferred targets, though less densely. **c**–**f** | The organization of the presynaptic inputs to LC is less clear. Early studies suggested that all LC neurons were innervated by a limited set of common afferents, which then went on to innervate all regions of the CNS[16] (part **c**). Another possibility is that a common set of afferents equally innervate all LC neurons, which then transmit information in a selective way to specific terminal fields (part **d**). A more complex arrangement would have unique afferents linked to discrete LC neurons that preferentially innervate functionally distinct terminal fields, such that there is discrete coding of information as it passes through the nucleus (part **e**). Another possibility is that the LC is organized such that some afferents innervate all of the LC, and so can modulate the activity of the nucleus as a whole, while others are more discrete and allow for point-to-point communication[4]. The same holds true for LC efferents: some LC neurons have a preferred terminal field that they densely innervate, while others broadly innervate vast expanses of the CNS[1]. Under this model, the LC is capable of globally broadcasting information to all its terminals simultaneously when its broad afferents are engaged. In some circumstances, however, only discrete afferents may be engaged, to allow for discrete coding of information by LC and transmission only to specific terminals (part **f**). NA, noradrenaline.

innervating different terminal fields can show different input resistances, excitability, after-hyperpolarization profiles, action potential widths, excitability, firing rates and plasticity to afferents. For example, LC efferents to the forebrain (that is, basolateral amygdala and infralimbic prefrontal cortex) have higher input sensitivity and firing rates than the slower-firing LC cells that innervate the motor cortex, which themselves are different from those that project to the spinal cord sensory system. These different electrophysiological properties combine with specific projection targets, co-transmitters and receptor arrays to influence discrete behaviours, such as fear conditioning versus fear extinction and anxiety/augmented responses to noxious stimuli versus analgesia[7,12].

Similarly, high-density electrode arrays have recently enabled large-scale ensemble recording in the LC, allowing discoveries that could not be inferred from patterns of LC single or multiunit activity. The

anaesthetized preparation in these studies permitted the necessary long-duration recording of stable spiking of a large population of LC neurons that has not yet become available for the brainstem of behaving animals. Nevertheless, the LC properties observed under anaesthesia or in vitro require confirmation in awake animals. In anaesthetized rats, spiking among random pairs of LC neurons is predominantly uncorrelated, but on a moment-to-moment basis, clusters of LC neurons may self-assemble to form functional arrays that relay meaningful information[79]. Individual LC neurons do not respond reliably on a trial-by-trial basis to attention-eliciting stimuli[7,79] or to cortical delta waves[79], but output from groups of otherwise inconsistently responding cells may produce a high-fidelity ensemble code that represents LC information processing. These and other results suggest that ensemble-based LC coding underlies the spatially and temporally selective neuromodulation of cognitive, sensory, memory and motor functions[1]. An important open question that is ripe for further investigation is how distinct ensembles arise and are modulated through differences in circuit connectivity and heterogeneous intrinsic physiological properties.

Effects of LC-NA activation

Through its widespread efferent network, the LC-NA system impacts a diverse array of core behavioural processes, including the regulation of waking/arousal and a diversity of state-dependent cognitive and motivational processes. These processes include prefrontal cortex-dependent 'executive' functions like attention and working memory[80,81], sensory processes, synaptic plasticity and long-term memory[82,83], high-arousal/emotional amygdala-dependent memory[74,84], cognitive and behavioural responses to stress (BOX 2) and goal-directed motivated behaviour[85–87], including motivation for drugs of abuse[88]. The diverse behavioural effects of this neurotransmitter system reflect the complexity of the effects of NA receptor activation on neuronal signal processing, gene transcription and neuronal plasticity that optimize behavioural outcomes in complex environments.

LC-NA in sensory processing. Many studies have shown that local administration of NA or activation of LC can modulate the responses of single cells, local circuits and neural networks to external and internal sensory signals[74,89,90]. In anaesthetized and unanaesthetized animals, the application of NA or activation of the LC results in a spectrum of changes in sensory neuronal response properties across thalamic and cortical sensory regions, including changes in the magnitude[91–96] and timing of stimulus-evoked discharges[97,98]. Moreover, NA-mediated actions induce alterations in the receptive-field properties of visual cortical neurons[99,100], modulate odour detection and discrimination thresholds in the olfactory bulb[101], enhance the plasticity of frequency tuning in the auditory cortex[93], enhance auditory perception[102] and improve performance in a visually guided signal detection task[103]. Studies in awake animals have underscored the importance of evaluating LC-mediated effects in terminal fields not only at the single-cell level, but also across ensembles of simultaneously recorded neurons, to account for the heterogeneity of effects on target neurons, leading to net changes in neural circuit and neural network output[104]. Importantly, the experimentally demonstrated modulatory effects of NA and LC activation on cells, circuits and networks follow an inverted-U-shaped function[94,104]; that is, the facilitating effects of NA and LC activation are initially modest, rise to optimal as NA output elevates, and then gradually diminish with further increases in NA concentrations. In vitro studies have shown that, at moderate concentrations, NA acts at α1 receptors to strengthen peri-threshold sensory neuronal responses, whereas at higher concentrations, NA acts via α2 receptors to degrade signal processing[105].

Further experiments in awake, behaving animals will be needed that combine LC manipulations, in vivo recording of neuronal responses to sensory stimulation, and precise psychophysical measures of behavioural responses, to better understand the relationship between differing rates and modes of LC activity, sensory processing, perception and action[94,96,104]. A recent promising study in that direction with human participants combined the pharmacological manipulation of NA with measurement of visually evoked potentials, fMRI activation in visual cortex and the participants' performance on various visual perception tasks. Several aspects of visual perception were improved in tandem with increases in the consistency of the evoked potentials and in the fMRI signal when increasing NA; the opposite results were obtained when decreasing NA[106].

LC-NA state effects. Early correlative observations documented a strong positive relationship between LC firing rate and arousal level[107,108]. Subsequent studies demonstrated that LC neuronal activity and NA receptor activation are sufficient to invoke the alert (conscious) waking state and are necessary for the maintenance of wakefulness[74]. The arousal-promoting actions of NA, at least in part, involve α1- and β-receptors within a network of subcortical sites[75]. The relation between NA and arousal is monotonic, with low LC firing rates being associated with sedation or sleep, and the highest rates being associated with high levels of arousal (however, arousal during REM sleep is an exception; see BOX 4). Recent LC optogenetic and chemogenetic studies have confirmed and extended these earlier observations using a variety of arousal measures, such as pupillary diameter, which is highly linked with LC activity and a diversity of behavioural responses. Taken together, these studies indicate a very tight positive linkage between LC activity, a corresponding NA release and arousal, with LC activity playing a sufficient and necessary role in changing the arousal level[20,109–113].

LC-NA and plasticity. A half century ago, Seymour Kety hypothesized that LC release of NA potentiates the activation of small numbers of neurons that, under the influence of NA, strengthen connectivity as a means to store information and adaptively alter sensory and motor circuits[69]. He suggested that the inhibition of random and spontaneous activity by NA throughout the brain is a characteristic of plasticity-promoting arousal, with smaller, sharply focused and activated circuits mediating learning. Intracellular cAMP-cascade pathways engaged by NA β-adrenoreceptors were proposed to support these learning-associated changes. Developing visual[114,115] and olfactory[116] networks provided direct evidence of such LC-NA-dependent plasticity. NA-induced long-term potentiation (LTP) of entorhinal throughput in hippocampal dentate gyrus provided mechanistic support for the predicted LC-NA effects[117,118].

Since the proposal of Kety's hypothesis that LC mediates learning through circuitry enhancement, we have learned that LC is involved in multiple distinct and learning-critical forms of hippocampal and cortical plasticity, including habituation[119,120], long-term depression (LTD)[121–123] and depotentiation[124]. These contrasting forms of plasticity are

related in part to LC effects on specific synaptic pathways[121,122] and, in the case of depotentiation, to pauses in LC firing[124]. LC activity that occurs in conjunction with hippocampal and cortical synaptic transmission triggers input-specific synaptic plasticity[122,123], and its temporal parameters can determine whether the direction of change is potentiation or depression[122,123].

Glutamate amplification of NA (GANE) is a recent proposal to account for the input-specific potentiating and depressing actions of LC-NA on neural circuitry. GANE proposes that NA concentrations linked to LC activity are modulated locally in target structures by glutamate activity[125] (FIG. 3). Specifically, GANE hypothesizes that informational glutamate circuits compete for metabolic and molecular plasticity resources, with a winner-take-all outcome. Losers are suppressed. The suppression or enhancement depends on the local NA concentrations. NMDA, AMPA and metabotropic glutamate receptors on NA terminals all increase NA release[125]. Nearby astrocytes also play a role in supporting GANE-mediated plasticity (FIG. 3a). These competitive interactions underpin the contrasting cognitive effects of LC activation on concurrent inputs, with salient inputs becoming memorable and others being ignored (FIG. 3b).

Other LC actions are linked to plasticity. LC-NA modulates astrocytes in order to increase cortical perfusion and generate astrocytic calcium waves[126,127]. There is a growing appreciation of the brain-wide modulation of astrocytic signalling that LC-NA produces[127–129], as astrocytes are critically involved in multiple plasticity support functions. The influence of too much or too little NA on the functions of astrocytes for plasticity is open to investigation.

In the hippocampus, optogenetic experiments have revealed a shift in CA1 place cell firing towards a newly rewarded place in an otherwise stable map. Pairing of LC activity with the newly rewarded place is required. The authors suggest that this plasticity is supported by high neuromodulatory tone, changes in local inhibition and astrocytic activation[130]. These results contrast with results from another experiment showing global remapping in CA1 with phasic glutamatergic LC activation[120], strongly supporting a hypothesis of concentration-dependent differences in LC functional effects.

Recent data suggest that long-range glutamate projections play a role in LC plasticity. The pairing of a tone with LC activation both strengthens the representation of the tone in the cortex and leads to the tone now activating the LC through increased synaptic strength of the LC neurons. This tone-evoked LC activation is required to support the enhanced auditory response, which, in turn, improves auditory perception[102].

Taken together, both data and theory speak to a complex and multifaceted LC role in neural plasticity and the resultant adaptive behaviour (FIG. 3). The updated view highlights NA suppression as well as enhancement of neural circuitry and suggests that both local and long-distance interactive feedback loops support plasticity. The selective spatial and temporal activations afforded by optogenetics, combined with the ability to monitor LC output through electrophysiological and imaging methods, will allow rapid progress in testing these hypotheses. One example of a hypothesis ripe for further testing is the discovery that LC-released dopamine in the hippocampus, which mediates novelty-enhanced memory consolidation, is dependent on the reversal of NA transporter activity triggered by high levels of local glutamate and activation of NMDA receptors on NA terminals[41]. Co-release of dopamine in the presence of high local glutamate is a new mechanism consistent with the GANE hypothesis, and its elucidation could increase our understanding of local LC plasticity effects throughout the brain[41].

LC-NA and memory formation. LC neurons fire in bursts in novel environments or when encountering unexpected novelty in a familiar environment[119]. Consequent NA release and postsynaptic activation of adrenergic receptors at LC targets potentiates evoked responses in the hippocampus[131] (see LC-NA and plasticity), changes plasticity-related signal-to-noise ratios[122,132,133], facilitates contextual learning[38] and enhances everyday memory[24]. Beyond the hippocampus, NA action at β- and α1-receptors within the amygdala enhances emotional learning[2,84]. LC activation is critical for offline memory consolidation at discrete time windows after learning in both appetitive and aversive learning[134–137]. LC input may facilitate cross-regional interactions that underlie offline memory consolidation, particularly during sleep[138,139] (BOX 4). NA release impacts various molecular signalling cascades that underlie memory trace stabilization across multiple time windows[140,141]. Questions that are open to future investigation include the factors triggering experience-dependent delayed activation of the LC-NA system and the function of critical silent periods of LC neurons during REM sleep, which could uniquely allow synaptic depotentiation and the updating of memories (BOX 4).

Box 4 | **LC activity across sleep: relevance to waking functions and disease**

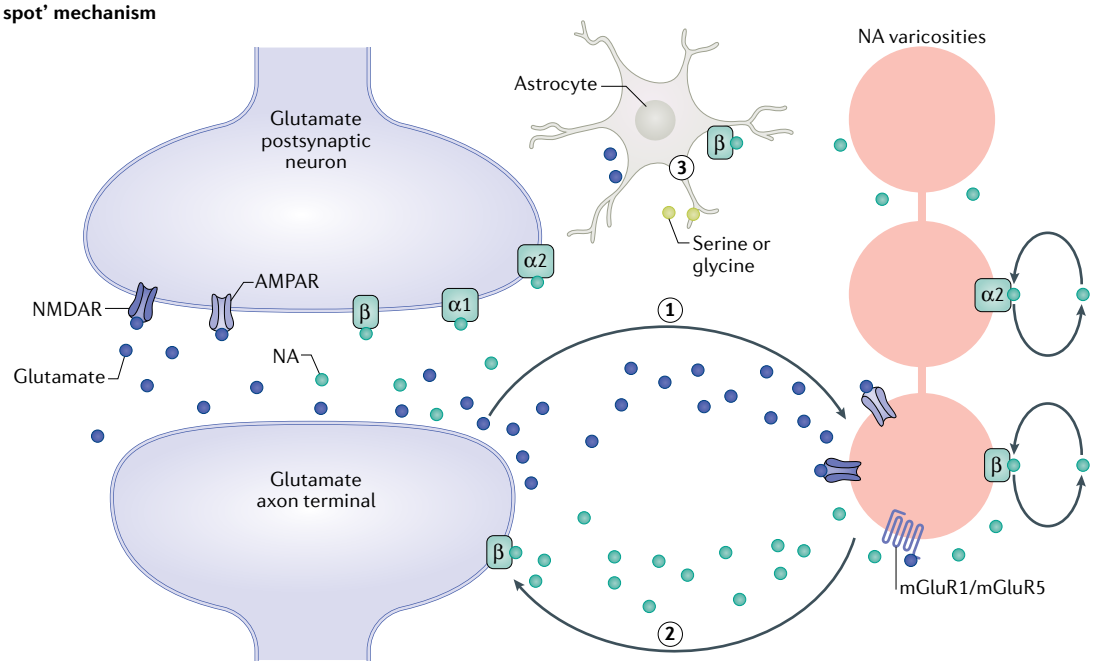
Although the locus coeruleus (LC) firing rate typically slows at sleep onset, within sleep, LC firing rates do not predict either arousal levels or arousal thresholds. In all animals recorded to date, which have included only males, the LC falls nearly silent during the state of rapid eye movement sleep (REMS) and is also silent in the seconds immediately preceding sleep spindles during non-REMS[108,216]. These sleep states show intense brain activity in limbic circuits, ample synaptic plasticity[217,218] and rich cognitive content, in the form of vivid dream reports. LC activity during sleep after learning has been little studied, but the few existing reports reveal an intriguing interplay of activity and silence, both of which could play essential roles in sleep-dependent memory consolidation[216,219,220]. Specifically, LC activity is timed to deliver noradrenaline at forebrain synapses just in time to strengthen or protect circuits during non-REMS memory replay events — that is, at the peaks of slow oscillations[134]; however, LC silences precede non-REMS spindles and REMS replay periods, which would uniquely allow a depotentiation of synapses that would be necessary for memory networks to be reshaped[124,221,222].

Violations either of LC activity at non-REMS slow oscillations or of LC silences before spindles and in REMS could disrupt memory consolidation processes[216,223]. Indeed, the characteristic sleep aetiology of post-traumatic stress disorder (PTSD), insomnia, and opiate withdrawal (BOX 2) suggests an overactive LC during sleep[224], which may underlie the emotional and hippocampal memory consolidation deficits of those disorders[225]. The overactive LC seen in early Alzheimer disease (AD) (BOX 1) may also cause the disturbed sleep apparent at the earliest diagnoses of that disease, and the catastrophic degradation of the LC in late AD could fail to provide NA at forebrain synapses in support of memory preservation during slow oscillation reactivations, contributing to overall memory erasure. These questions have yet to be addressed.

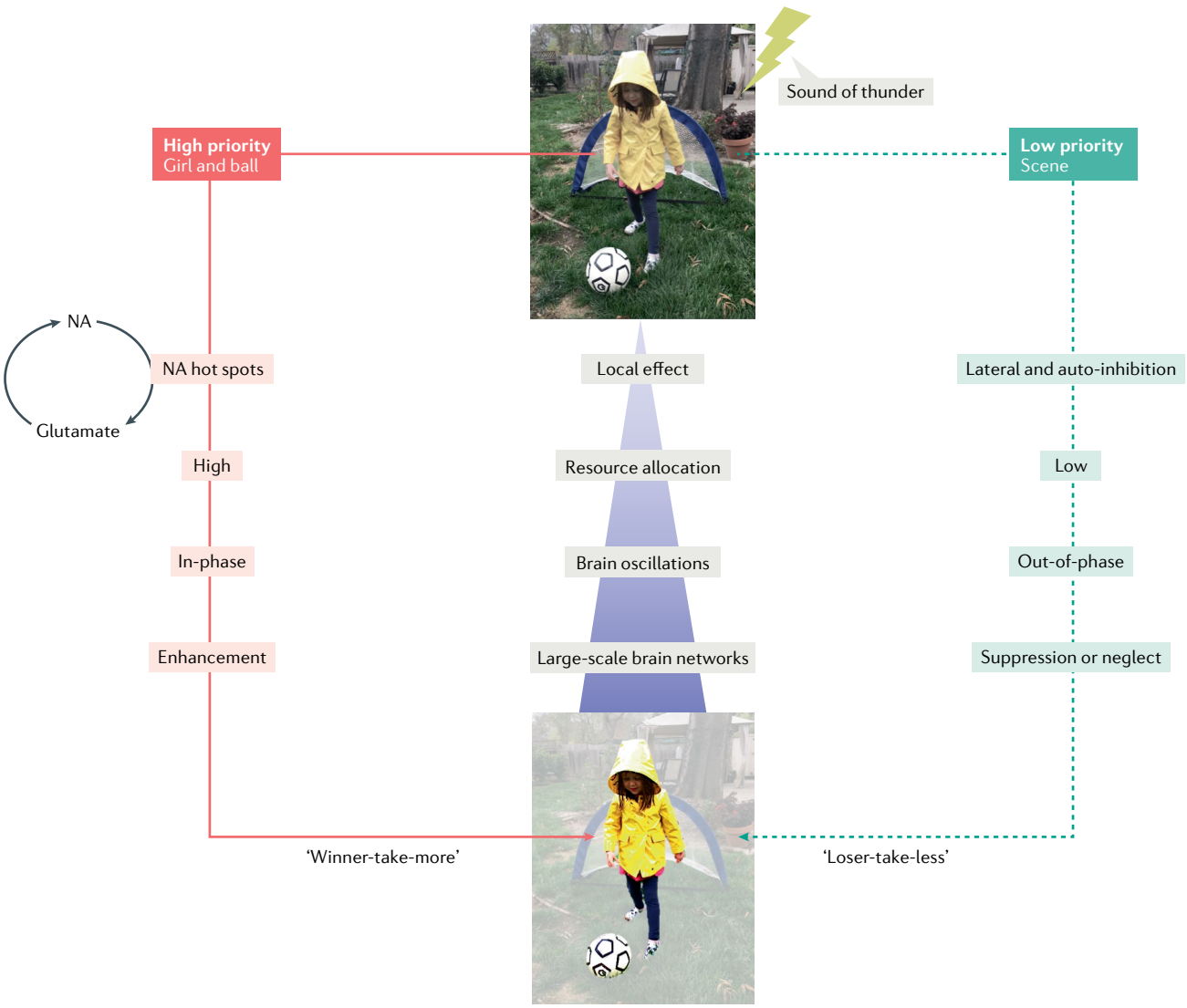
Given the strong effect of the LC-NA on plasticity, one area for future study is the activity of the LC during sleep in females across the oestrous cycle. Increased LC activity during sleep at some phases of the oestrous/menstrual cycle could underlie increased vulnerability to memory consolidation disturbances, opiate use disorder, AD and anxiety-related disorders such as PTSD[226].

The NA 'hot spot' mechanism

a



b



◀ Fig. 3 | **GANE release creates local NA 'hot spots' and alters network processing: the network GANE model. a** | A noradrenaline (NA) 'hot spot'. Local spillover glutamate **(**blue dots) from active glutamate terminals (step 1) interacts with depolarized NA varicosities. These NA varicosities (pink) are depolarized by locus coeruleus (LC) activation. Spillover glutamate acting on three kinds of glutamate receptors — NMDA receptors (NMDAR), AMPA receptors (AMPAR), and metabotropic glutamate receptors 1/5 (mGluR1/mGluR5) — may increase NA release from these varicosities (green dots; step 2). β-Adrenoceptors on glutamate terminals can promote additional glutamate release, in a positive feedback loop. Spillover glutamate also recruits astrocytes (step 3) to release the NMDA co-agonists serine and glycine (grey dots) and additional, astrocyte-sourced glutamate (blue dots). Finally, on the postsynaptic glutamate neuron, local NA activates higher-affinity α2- and α1-receptors. If NA concentrations are sufficiently high, NA also recruits the lower-affinity β-adrenoreceptors to promote long-term potentiation (LTP) at those glutamate synapses. To generate LTP, a high level of β-adrenoreceptor activation is needed, because lower levels of β-adrenoreceptor activation promote long-term depression, attenuating the input strength. Failure to recruit β-adrenoreceptors will leave the glutamate circuitry unchanged. Increased local NA can also act in an autocrine fashion on α2- and β-adrenoreceptors expressed on the NA varicosities, modulating further NA release. β-Adrenoreceptor activation would also increase NA release in a positive feedback loop. **b** | The system-wide effects of glutamate amplification of noradrenaline (GANE) interact with local 'hot spot' effects. Salience-evaluating structures, such as the anterior cingulate cortex and insula, recruit LC firing in order to enable NA to modulate ongoing processing at multiple levels of brain function. Local glutamate–NA memory-enhancing effects occur in parallel with more broad-scale suppression, as NA recruits lateral and auto-inhibitory processes that suppress weaker glutamate signals in lower-priority processing pathways. These noradrenergic mechanisms lead to 'winner-take-more' and 'loser-take-less' outcomes in perception and memory under arousal. For example, if the girl in the yellow raincoat has high priority at the moment, either because of her perceptual salience or because of a goal (for example, rooting for her team), memory for her should be enhanced if something else (for example, loud thunder) induces arousal at that moment, whereas the peripheral inputs are more likely to be forgotten. High-priority input interacts with LC-induced arousal and recruits resources and networks to create new memory circuits, whereas lower-priority input is unsupported. Part **a** adapted with permission from REF.[125], Cambridge University Press. Part **b** image courtesy of David Clewett, UCLA.

Executive function. LC projections to the prefrontal cortex modulate distinct attentional processes, including focused[81], flexible[142] and spatial attention[143]. The modulatory actions of NA on attentional processes follow an inverted-U-shaped curve, with optimal function at moderate NA doses, as was reported for working memory[144]. Interestingly, in contrast to what is seen with working memory, α1 receptor activation promotes both flexible attention and focused attention[81]. The neural mechanisms for promoting focused attention remain to be elucidated and are an area of opportunity. For instance, can we understand the influence of LC activation on attention solely by looking at the actions of the LC-NA system in the prefrontal cortex, or is LC activation related to a more global influence on networks centred on the prefrontal cortex? Also, what are the specific components of attention that are affected by LC activation?

Behavioural and cognitive flexibility. A key to understanding the involvement of the LC in cognitive processes is to determine the environmental stimulus pattern or context that elicits changes in LC neuron activity that are associated with the behavioural response. Here the data are sparse, due to the challenges of recording from this small population of neurons located deep in the brainstem. Nevertheless, existing studies in rats and monkeys engaged in formal conditioning protocols have revealed that LC neurons are engaged from the onset of the learning process, responding to both novel stimuli and reward[85,86,145,146]. Conditioning of LC neurons occurs rapidly, occurring many trials before any behavioural expression of learning or any sign of conditioned responding in cortex[73,145]. Importantly, once the conditioned behaviour is well-established, LC cells no longer respond reliably to the conditioned stimulus. However, any change in reward contingencies that requires a behavioural adaptation, such as extinction or reversal, elicits a robust response in the LC[85,86]. This striking sensitivity of LC neurons to changes in a context led to the hypothesis that the LC-NA system is involved in cognitive flexibility and allows rapid behavioural adaptation to changes in environmental contingencies[73,85,147]. Experiments designed explicitly to test this hypothesis have used pharmacological or genetic manipulation of the NA system and behavioural protocols involving reversal or extra-dimensional shift in rodents[142,148–153]. The results lend strong support to this idea. Recent refinement of fMRI techniques has allowed functional assessment of areas as small as the LC[87], which revealed that this important role of the LC in cognitive flexibility may also operate in humans. These advanced fMRI techniques have opened new vistas for further investigation of the role of the LC-NA system in cognitive and behavioural flexibility[16,139,154,155].

This striking sensitivity of LC neurons to environmental imperatives has been observed across species and within different behavioural situations; nevertheless, new evidence suggests that subpopulations of LC neurons respond in specific behavioural/cognitive contexts[12,156,157]. Two important questions arising from these data are how this type of context-dependent coding is implemented, and whether and how LC neural processing is coordinated within a modular organization (see Anatomy of the LC). Given that small or large numbers of LC neurons can be activated in different contexts, these two questions may be interrelated. Further experiments recording multiple LC single units during behaviour will be needed in order reveal how these subpopulations respond to specific environmental contingencies (see Theories from behavioural correlates for further discussion).

LC-NA in motivation and decision making. LC neurons are phasically activated by the outcome of task-related decision making[145,158]. However, the activation of LC neurons is limited to decisions to act and is not involved in the decision to cancel or prevent action[159]. Recent studies in monkeys have indicated that LC activity correlates with effort production, which is not the case for DA neurons recorded under the same conditions[160]. In an effort–reward task, decreasing the NA level with clonidine specifically affects effort without affecting reward processing[161]. While the site of action of NA-dependent modulation of effort is unknown, these effects are compatible with the idea that NA enhances cognitive control and modulates executive functions[162,163].

NA has been strongly implicated as a motivational factor in drug-seeking behaviour. Activation of adrenergic receptors in select brain regions reinstates a previously extinguished drug-seeking behaviour, whereas NA receptor blockade prevents reinstatement elicited by various stimuli. In particular, engagement of the LC-NA system is required for drug-primed and cue-induced reinstatement, while other noradrenergic nodes (for example, the A2 group) regulate stress-induced reinstatement[164]. At least some of these effects involve actions of NA within arousal-related subcortical structures and are associated with increases in affectively neutral arousal[165].

Theories from behavioural correlates

Early theoretical models articulated a strong relationship between LC activity and arousal, on the one hand, and the inverted-U relation between arousal and performance in cognitive tasks, on the other. Aston-Jones and Cohen[166] developed a model (the adaptive gain theory) centred on modes of LC activity. The phasic mode, characterized by a low baseline firing rate and a strong phasic response to task-relevant stimuli, was seen as enhancing task-related activity, whereas the tonic mode, where LC neurons display sustained activation, would be associated with exploratory behaviour. In early versions of this model, the transient release of NA was proposed to enhance stimulus processing, thereby mediating the role of NA in attention. In later versions, after it was discovered that LC activation was more closely aligned with the behavioural response than with stimulus onset, the NA-induced increase in signal gain in target regions was proposed to facilitate the decision itself — that is, the execution of the action in response to the stimulus. The idea that transient LC activation promotes action execution is compatible with the idea that it enhances effort and is closely related to the notion of cognitive control[90,160]. Altogether, both the results of experimental studies and the model emphasize the specific temporal relation between transient LC activation and the mobilization of cognitive resources to provide an optimal response.

Arnsten[167] provides a neurobiological account of the relation between arousal and cognitive performance through specific sites of NA action. In this framework, optimal performance is achieved at intermediate levels of NA via specific stimulation of high-affinity α2 receptors in the dorso-lateral prefrontal cortex, which supports executive functions. At higher concentrations, NA acts on lower-affinity α1 and β receptors located in posterior, sensory–motor regions, where it promotes fight-or-flight behaviours. Thus, both Aston-Jones and Arnsten provide a noradrenergically based accounting of the inverted-U relation between arousal and performance, with Aston-Jones and Cohen[166] focusing on the dynamics of LC activation, and Arnsten[80] emphasizing different affinities and the heterogeneous distribution of NA receptors. Further refinements of these models will require a better understanding of the factors underlying LC activation and of the consequence(s) of this activation in LC terminal fields.

Another theory of LC function derived from behavioural correlates of LC activity emphasizes the role of NA in cognitive flexibility. Network reset refers to the reorganization of target networks enabling the rapid switch of the cognitive representation of the world to guide behaviour[73,168–173]. Bouret and Sara derived this theory from recordings of LC neurons in rats undergoing learning or extinction[73,85,145] (see above). More recent studies in humans, using fMRI combined with behavioural protocols designed to evaluate cognitive flexibility, have shown that the LC is indeed engaged when shifts in attention or behavioural strategy are required[16,154,155,174]. Pupil dilation, as a correlate of LC activation, has been validated in the monkey[112] and is now widely used in human research. Here again, engagement of the LC in cognitive flexibility has been corroborated[175,176].

A version of this network reset theory of LC function proposed by Dayan and Yu[168] suggests that LC neurons are activated in the context of "unexpected uncertainty", sending a "neural interrupt signal" to prepare the organism for adaptation of behaviour.

These earlier conceptual theories were founded on the idea that LC-NA cells behave homogeneously. However, recent studies have shown that subpopulations of LC neurons can be engaged in a more specific fashion and that their activity patterns, as well as the number and identity of the cells that are activated, can vary depending on the sensory or behavioural context[1,145,157]. Furthermore, emerging evidence suggests that these different patterns of LC activity are coordinated with distinct cell modules that have unique efferent connectivity (from widely divergent to more specific; see FIG. 2). Together, this suggests a new theory of LC function in which the representational (that is, what information is encoded) and modular features are coordinated. One possibility is that in very critical arousal-eliciting situations, such as exploration of a novel environment or exposure to a stressor, all cell modules are recruited to send a unified, widely broadcast NA signal to many brain regions. In other situations, where information is more subtly scattered in space and time (for example, in response to specific task events or during the initiation of movement), smaller populations of efferent-specific or molecularly identified neurons may respond, sending an NA signal selectively to distinct target sites[7]. This situationally dependent targeting of responsive LC cells has been termed "context-dependent modular coding"[2]. Such coding could be implemented through a combination of intrinsic physiological properties that enable LC neurons to switch between coupled and uncoupled modes[177] and between partially distinct afferent inputs onto individual cell modules that either engage many or recruit select LC cell populations[2] (FIG. 2). Determining whether and how context-dependent modular coding is coordinated with the structural organization of the LC will provide important insights into LC function and potentially link theoretical constructs and LC neuronal representations to anatomical and physiological mechanisms. Exploring the dynamics of activity across

Glossary

Chemogenetics
Viral introduction of chemically engineered neurotransmitter receptors into neuronal membranes. These can be subsequently activated by pharmacological ligands that are specific to the receptor.

Co-transmitters
Neuromodulators released from a neuron along with a primary neurotransmitter.

Fast-scan voltammetry
Voltammetry examines fluctuations in current that are driven by variations in voltage/potential. In cyclic voltammetry, after the desired potential is reached, the potential is ramped in the opposite direction to return to the initial potential (time-locked voltage oscillations), causing the substance of interest to be oxidized and reduced in predetermined cycles. The concentration of the substance can be calculated by generating a calibration curve of current against concentration, allowing the relative concentration to be calculated within milliseconds, and thus the real-time detection of neurotransmitter concentration.

Fear extinction
Learning that a context or cue that was associated with an aversive event no longer predicts that event, and thus the fear response to that context or cue is no longer expressed.

Frequency tuning
In the auditory cortex, individual neurons exhibit a specific response pattern based on the sound frequency applied. Delivery of a set of different sound frequencies determines the frequency tuning of the neuron.

Optogenetics
Analysis via the viral introduction of light-sensitive channels or ion pumps into neuronal membranes, which subsequently can be driven by the external application of a specific light wavelength.

RNAi
RNA interference, which comprises the inhibition of gene expression or translation by silencing the target mRNA.

Terminal fields
Neural areas targeted by axonal projections.

these modules as animals experience different sensory and behavioural contexts should provide new insight into the influence of LC-NA on its target regions and its involvement in behavioural adaptation.

Caveats, challenges, conclusions

Until recently, our understanding of the causal relation between LC neuronal activity and behaviour had been limited by an inability to selectively modulate LC-NA neurons in temporally precise and physiologically relevant patterns. This situation has changed dramatically with the advent of optogenetic and chemogenetic approaches, which allow us to activate or inhibit selected subsets of LC projection pathways. However, to provide unambiguous, informative data related to the behavioural consequences of LC activity, it is important that (1) the manipulations accurately reproduce the rate and pattern changes in LC activity that are elicited by physiological stimuli; (2) the efficacy of these manipulations be confirmed through electrophysiological or transmitter-selective sensors under conditions identical to those associated with behavioural testing; and (3) consideration be given to selective (module) versus global (whole-nucleus) activation of LC output channels and their efferent targets.

The establishment of a relation between LC output and behaviour will also require taking timescales into account. LC activity affects behaviour over relatively broad timescales, influencing behavioural states from sleep to arousal, while at the same time, event-related phasic increases in LC activity also influence perceptual processes and promote executive control or single-trial learning at the moment of a decision or at the time of effort production. Many gaps still prevent a full understanding of the local and global dynamics underlying brain-wide NA modulatory effects and the behavioural impact of these actions. We have delineated some of these gaps in each of the sections and subsections of this Perspective, with the aim of orienting current and future investigators.

As we have reviewed, neurophysiological work in rodents and primates indicates that, even if all LC neurons receive inputs related to autonomic arousal, distinct subpopulations of LC neurons can encode specific cognitive processes, presumably through more specific inputs from forebrain areas[6,7,156,178]. This, combined with specific patterns of innervation of target areas and heterogeneity in receptor distributions[16,179], indicates that activation of the LC should have a more specific influence on target networks than had initially been imagined. Future theoretical constructs will need to incorporate not only the fine temporal scale but also the fine anatomical scale of LC function in order to capture the role of the LC in executive function, sensory signal processing, stress responses, motor control and learning, and ultimately in adaptive and maladaptive behaviours. Finally, recent imaging studies in rodents and primates, both human and non-human, have started to unravel the network effect of global LC activation[16,155,180]. Combining such approaches with local pharmacology and behaviour will be critical to better understanding the complex relation between the LC and behavioural outcomes.

Finally, for neural systems such as the LC-NA, with its multiple brain targets and dynamic patterns of behaviourally related activity, behavioural functions cannot be understood while we know only physiological and anatomical properties. The task of fully integrating such complexity requires computational modelling that can take into account multiple dimensions of action in order to make predictions regarding behavioural outcomes and functions, which in turn can be tested in additional biological experiments; this methodology has proven useful in developing prior LC theories[164,166]. The findings of higher complexity across LC neurons and projection targets than had previously been realized indicate an increased need for iterative biological–computational approaches to extend theories of the function of the LC system.

The remarkable advances of the past few years, prompting our 'new look' at the blue spot, have certainly energized and provided new tactics for the field of LC investigation. Such precise and powerful approaches also provide opportunities for investigators focused on any one of the many neural systems that receive LC input, allowing them to effectively attack questions about how that input participates in the operations of their particular system. However, even with recent methodological advances, the unique anatomy and physiology of the LC continue to pose challenges to its precise characterization and to assigning it functional attributes. More data and innovative hypotheses will be needed to develop a comprehensive picture of LC functions. The present Perspective is meant to attract new investigators to help unravel the mysteries of the 'blue spot'.

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