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Review

The Role of the Locus Coeruleus in Pain and Associated Stress-Related Disorders

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ABSTRACT

The locus coeruleus (LC)-noradrenergic system is the main source of noradrenaline in the central nervous system and is involved intensively in modulating pain and stress-related disorders (e.g., major depressive disorder and anxiety) and in their comorbidity. However, the mechanisms involving the LC that underlie these effects have not been fully elucidated, in part owing to the technical difficulties inherent in exploring such a tiny nucleus. However, novel research tools are now available that have helped redefine the LC system, moving away from the traditional view of LC as a homogeneous structure that exerts a uniform influence on neural activity. Indeed, innovative techniques such as DREADDs (designer receptors exclusively activated by designer drugs) and optogenetics have demonstrated the functional heterogeneity of LC, and novel magnetic resonance imaging applications combined with pupillometry have opened the way to evaluate LC activity in vivo. This review aims to bring together the data available on the efferent activity of the LC-noradrenergic system in relation to pain and its comorbidity with anxiodepressive disorders. Acute pain triggers a robust LC stress response, producing spinal cord–mediated endogenous analgesia while promoting aversion, vigilance, and threat detection through its ascending efferents. However, this protective biological system fails in chronic pain, and LC activity produces pain facilitation, anxiety, increased aversive memory, and behavioral despair, acting at the medulla, prefrontal cortex, and amygdala levels. Thus, the activation/deactivation of specific LC projections contributes to different behavioral outcomes in the shift from acute to chronic pain.

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PAIN AND STRESS-RELATED DISORDERS

Pain is an unpleasant subjective experience with a sensory and emotional component (http://www.iasp-pain.org/terminology) that warns the body about a real or potential danger to tissue integrity. To exert its effect, pain must be aversive and stressful. Thus, if the main task of the stress response is to mobilize energy resources to meet the specific demands of a given situation, in the case of pain it is specifically orientated to protecting the damaged area and preventing further harm. However, when chronic, pain extends beyond the normal healing period and is no longer protective (1).

It has been noted that chronic pain is very often comorbid with stress-related disorders, which is particularly significant given that chronic pain is thought to affect 20% to 30% of the adult population in Europe and the United States (2,3), more than half of whom display comorbid depression and anxiety (4). In psychiatry, the prevalence of pain complaints is surprisingly diverse, and it is reported by 15% to 100% of patients with depression (5) and 50% to 90% of those with anxiety-related disorders (6,7). It is difficult to establish whether it is the pain that leads to the altered emotional states or whether the affective disorder predisposes an individual to the development of pain (8). However, this comorbidity establishes a vicious cycle that amplifies the negative effects of each alone, often complicating treatment and resulting in poor outcomes (9).

There is a clear biological framework underlying this comorbidity, because both sensory areas and stress-related neural networks are activated in these conditions (10). Bearing in mind that the locus coeruleus (LC)-noradrenergic system regulates a plethora of processes, such as arousal, stress, cognition, and pain, it may be a critical hub driving the comorbidity of pain and stress-related disorders. With the recent development of powerful neuroscience tools, new levels of network organization are coming to light in relation to different events (11), including pain. Therefore, the aim of this work is to provide a comprehensive review and critical analysis of our current understanding of the role of the LC in pain at the level of neuronal efferent circuits, focusing on neuropathic pain and its relationship with stress-related disorders.

NEW TOOLS TO DEFINE LC-NORADRENERGIC NEURAL CIRCUITS

The noradrenergic system consists of clusters of neurons in the brainstem that contain noradrenaline, classified from A1 to A7 (12,13). The A6 cluster is the LC, a small structure in the brainstem that contains approximately 1500 neurons in each hemisphere in mice and 20,000 in humans. The LC innervates and receives inputs from many different brain areas and from the spinal cord (SC) (14). As indicated in a recent review (11), the LC has long been considered a homogeneous nucleus,

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both anatomically and functionally, although more recent findings have revealed a heterogeneous LC organization and function (11,15–20). There is evidence that LC neurons can release dopamine along with noradrenaline at different brain targets (e.g., the hippocampus or thalamus) (21–23). Nevertheless, it is still unclear if all LC neurons or only subpopulations with specific targets release different neurotransmitters (noradrenaline, dopamine, galanin, etc.) and whether specific signals are required for this release.

The classic view of homogeneous and global LC activation has been challenged of late through reports of discrete modes of activation, whereby different modules of noradrenergic neurons enter segregated operational modes with respect to circuit functions within their efferent domains. For example, activation of the LC–basolateral amygdala (BLA) module promotes aversive learning, while the LC–infralimbic cortex module promotes extinction (18). Much of this novel information has come from the use of genetic approaches such as chemogenetics (DREADDs [designer receptors exclusively activated by designer drugs] and PSAMs [pharmacologically selective actuator modules]) and optogenetics (24,25), techniques that use different combinations of viral vectors and transgenic methods to target LC neurons (Figure 1A). Indeed, when combined with positron emission tomography (DREADD-assisted metabolic mapping), the effect of chemogenetic modulation on the activity of the whole brain can be explored in freely moving rodents (26–28). Other innovative tools that are deepening our understanding of the role of LC (photometry, genetically encoded noradrenaline sensors, translating ribosome affinity purification-sequencing, and BiPOLES [bidirectional pair of opsins for light-induced excitation and silencing]) (29–31) (Figure 1B) continue to emerge. Despite their advantages, these new strategies have several limitations. First, these approaches cannot fully mimic the natural activation of the LC or its ensembles. Second, the study of tyrosine hydroxylase–containing neurons may be still limited, because noradrenaline and dopamine co-release by the same neuron cannot be addressed (32). This latter issue may be resolved through the use of transgenic Cre-driver mouse lines in which Cre recombinase expression is driven by the dopamine transporter or dopamine beta-hydroxylase promoters (33,34). Finally, the duration or magnitude of the chemogenetic activation is poorly controlled (35–38), and off-target effects have been reported for the DREADD ligand clozapine-N-oxide, increasing the number of control experiments needed.

In the translational field, noninvasive tools are being used to explore LC function in animals and humans (Figure 1C). Specifically, magnetic resonance imaging sequences sensitive to neuromelanin accumulation, a dark polymer pigment generated by catecholamine metabolism, are being used increasingly (39). The paramagnetic properties of neuromelanin (40–42) are used to estimate the integrity of the LC neurons (43,44), although assessing the interaction between freely moving protons and those bound to macromolecules (45) permits rapid visualization of the LC with a better signal-to-noise ratio and isotropic resolution (46). Given the small dimensions of the LC, these methodological advances enable good-quality and high-resolution LC images to be obtained, free from movement artifacts and partial volume effects. These approaches are currently being used to study some neurologic and psychiatric disorders (47,48), although information on pain is still very limited other than that available in a few studies that have focused on attentional analgesia showing that cognition is a modulator of pain (49). Pupillometry is another noninvasive technique from which information about LC activity can be inferred (Figure 1C). Because LC activity is accompanied by fluctuations in pupil diameter (50), this technique provides an easy estimate of LC activity during demanding tasks (51). Furthermore, pupillometry can be used in freely moving or head-restrained rodents (26,52–54), although there are some technical difficulties associated with this approach because it is necessary to carefully control the luminance, and the pupil diameter is also dependent on other neurotransmission systems (e.g., the cholinergic system) (55).

LC IN ACUTE PAIN

Acute pain is of short duration and it gradually resolves as the injured tissues heal, making it a physiologically useful tool to protect our body from actual or potential tissue injury (Figure 2A). The LC is a structure that receives peripheral ascending nociceptive inputs from the SC through the paragigantocellularis nucleus (56), and it projects to several central nervous system structures such as the thalamus, amygdala, hippocampus, and prefrontal cortex (PFC). Descending LC axons target the SC and medulla, in conjunction with other axons from A5 and A7 (subcoeruleus) (12,57–59), such that the influence of the LC in pain is the result of its action at different levels. Numerous studies indicate that the LC is engaged by acute harmful noxious stimuli, inflammation, or nerve damage, promoting feedback inhibition of pain (60–62). Thus, lesions and pharmacological or chemogenetic inactivation of the LC exacerbate pain responses in several conditions of short-term pain, suggesting enhanced pain sensitivity when the normal LC-noradrenergic circuit is disrupted (63–66) (Figure 2A–C). This analgesic activity is mainly driven by the LC ipsilateral to the site of the pain stimulus (65–68), and it appears to be produced by activation of the descending LC pathway to the SC, which enhances the noradrenaline release that would in turn contribute to the blockade of nociceptive ascending inputs (19,62,65) (Figure 3A). Noradrenaline released from descending pathways in the spinal dorsal horn suppresses pain through the inhibitory influence of α_{2A} adrenoceptors on central terminals of primary afferent nociceptors (presynaptic inhibition), as well as by direct α_2-adrenergic action on pain-relay neurons (postsynaptic inhibition) and by α_1 adrenoceptor–mediated activation of inhibitory interneurons (69,70).

Acute nociceptive insult activates LC, as witnessed by electrophysiological hyperexcitability and higher levels of c-Fos or phosphorylated ERK1/2 (extracellular signal–regulated kinase 1/2) (71–73). Moreover, pupil dilatation can also be observed in rodents and humans (53,74), as evident for other stress modalities (75,76). Chemogenetic activation of LC leads to an anxiogenic and vigilant state that is reminiscent of the natural response to stress (26,77), and activation of the LC → PFC module is aversive and anxiogenic (19). Thus, pain involves an affective-cognitive dimension that leads the affected individual to activate adaptive and dynamic emotional learning. Cognitive experiments explored the effect of painful

stimulation as unconditioned stimulus (footshocks) and of a sensory cue (tone) as a conditioned stimulus in Pavlovian fear conditioning. Optogenetic global inhibition of LC neurons demonstrated that the aversive activation of LC neurons participates in conditioning fear learning, while LC activity during the conditioned stimulus period is important for fear extinction (18). Furthermore, in vivo single-unit recording during fear and extinction learning involves segregated populations of neurons that fire in response to sensory predictive cues during fear and extinction learning (the BLA and infralimbic projecting ensembles, respectively). This functional dissociation of LC demonstrates its heterogeneous engagement in fear learning or extinction models, thereby promoting behavioral flexibility (Figure 3A). It has been seen that both populations of LC neurons (fear and extinction neurons) fire intensely in response to footshock stimuli (18), suggesting that LC is globally activated by intense aversive pain stimuli and that it engages a discrete coding mode in relation to cognitive processes (78). Other chemogenetic studies showed enhanced freezing in response to aversive conditioned cues mediated by LC activation, suggesting that LC projections to the central amygdala are critical for the expression of defensive responses elicited by conditioned threats (78) (Figure 3A). Therefore, acute pain is a robust trigger of LC-mediated acute stress responses, producing SC-mediated endogenous analgesia, while ascending information promotes aversion, vigilance, and threat detection.

LC IN CHRONIC PAIN AND ANXIODEPRESSIVE COMORBIDITY

Chronic pain is recognized as pain that persists beyond the normal time of healing (79). In these cases, pain is no longer a symptom, but rather a complex disorder characterized by sensory, cognitive, and affective symptoms that in many cases coincide with anxiodepressive disorders (Figure 2A). A common type of chronic pain is the neuropathic pain caused by a lesion or disease of the somatosensory nervous system, such as post-herpetic neuralgia, trigeminal neuralgia, painful radiculopathy, diabetic neuropathy, HIV infection, leprosy, amputation, peripheral nerve injury, or stroke. In addition to targeting other structures, many of the drugs used to combat neuropathic pain act at the level of the LC (e.g., duloxetine, venlafaxine, pregabalin) (80,81).

Sensory Symptoms

The transition from acute to chronic pain involves the dynamic activation and deactivation of proalgesic and analgesic mediators (Figure 2A–C). Unlike acute pain, LC alterations in chronic pain are more difficult to define, and as such, analgesia, proalgesia, or a role for the LC in the sensorial dimension of chronic pain have all been proposed (82). These discrepancies might be because the temporal dynamics of neuroplasticity vary in different animal models of pain and because strain differences have been reported in the noradrenergic pain pathways (83–86). Most evidence currently available suggests that the LC does not produce endogenous analgesia and that, by contrast, it may even be a pain generator in chronic conditions. Selective destruction of noradrenergic neurons appears to dampen neuropathic pain in Sprague Dawley rats (87,88), and in this same rat strain, lidocaine administration to the LC contralateral to the lesion dampens the evoked pain, whereas its administration to the ipsilateral LC did not modify sensorial hypersensitivity (66,87). Conversely, the blockade of the LC (ipsi- or contralateral) using DREADDs or lidocaine did not modify sensorial hypersensitivity in Long-Evans rats (65,66), suggesting that there is a lateralized pronociceptive activity of LC associated with chronic pain in Sprague Dawley but not in Long-Evans rats.

Studies Into Neuronal Circuits Involved in Sensory Symptoms

In the SC, weaker dopamine beta-hydroxylase expression has been described in Long-Evans and Wistar rats (65,89), suggesting a loss of noradrenergic innervation. Accordingly, DREADDs inhibition does not modify LC → SC activity in Long-Evans rats in chronic pain (65). However, other proalgesic LC projections seem to activate, at least in some rat strains (Sprague Dawley and Wistar rats). Thus, the expression of phosphorylated CREB (cAMP-response element binding protein) is enhanced in the contralateral dorsal reticular nucleus (DRt) of Sprague Dawley rats (66), a pain facilitatory area (90). Likewise, a persistent attenuation of pain responses is observed when noradrenaline levels are reduced in this area (91). DREADDs-mediated inhibition of LC neurons projecting to the DRt produces pain relief (66), and microinjection of an α_1 adrenoceptor antagonist but not an α_2 adrenoceptor antagonist dampened nerve injury–induced hypersensitivity (91). Thus, the LC may exert an indirect pronociceptive effect due to its projections to the DRt acting on α_1 adrenoceptors.

Exogenous activation of the LC using chemogenetics/optogenetics has also been reported. Chemogenetic activation of the whole LC or the projection to the SC consistently reduces hind-limb sensitization any time after nerve injury (19,65). By contrast, similar activation of the LC projection to the PFC exacerbates spontaneous pain without modifying evoked pain (19). These chemogenetic studies align with prior pharmacological data obtained with the selective noradrenergic reuptake inhibitor reboxetine, the acute intrathecal administration of which suppresses pain behaviors (it relieved evoked pain and it induced conditioned place preference). Acute systemic reboxetine administration also relieved evoked pain, although it was aversive in the conditioned place preference paradigm (92). Hence, it is likely that the beneficial effect of noradrenaline at the SC is counteracted by the activation of other supraspinal LC projections (Figure 3). This might also explain the limited efficacy of antineuropathic drugs and why noradrenergic approaches are usually combined with others, including the use of serotonin (duloxetine, venlafaxine) or opioids (tramadol, tapentadol), to treat pain or pain-depression comorbidity (93,94).

Anxiety, Depression, and Cognitive Symptoms

Sensorial hypersensitivity appears immediately after nerve injury, whereas anxiodepressive and cognitive symptoms arise after several weeks (3–8 weeks depending on the species and animal model) (95–97). Thus, the main phenotype after 1 to 2 weeks of experiencing pain (short-term pain) is sensorial hypersensitivity, while at longer times pain coexists with emotional and cognitive symptoms (long-term pain)

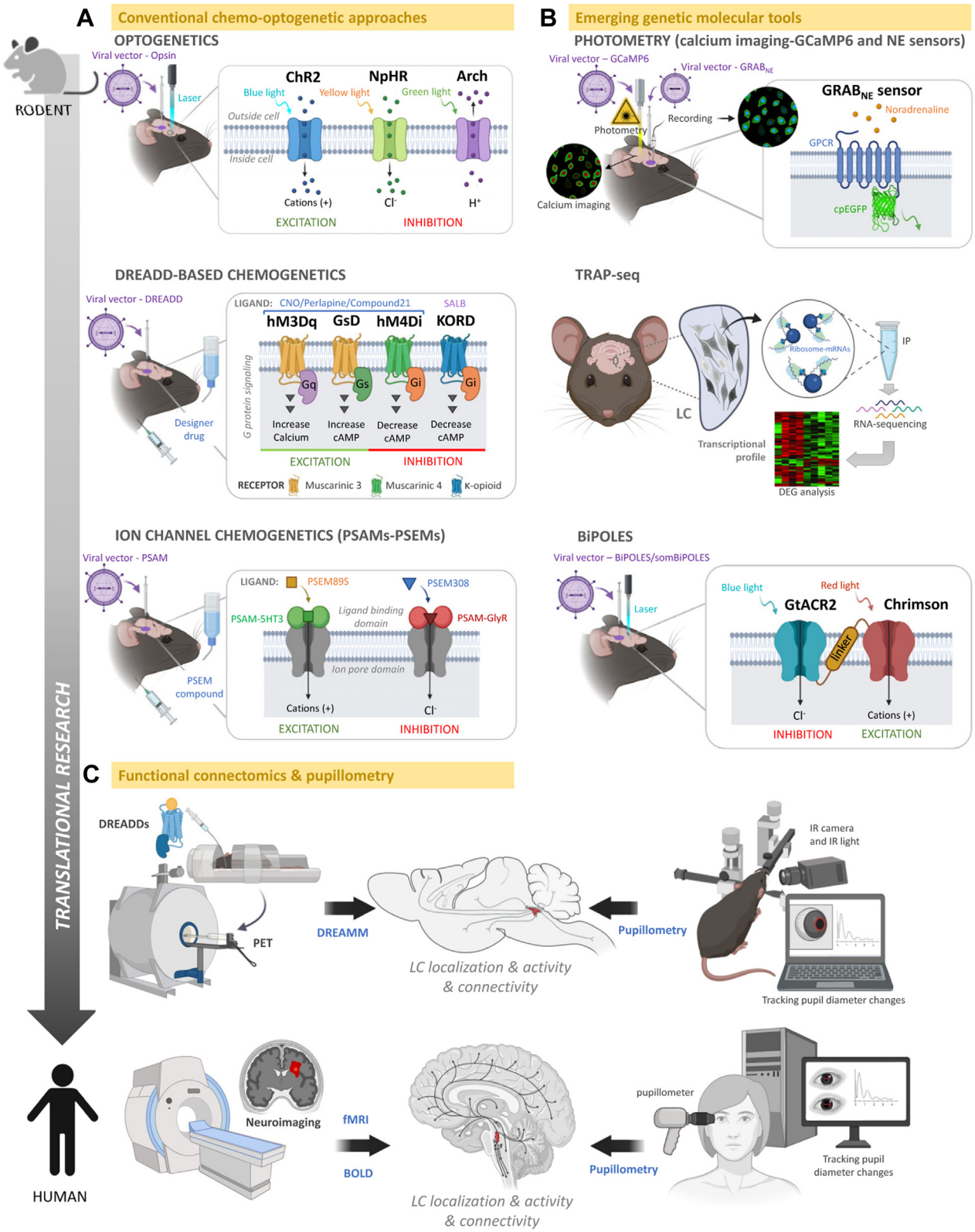

Figure 1. New tools to assess LC function. **(A)** Both optogenetic and chemogenetic approaches can be used to target the expression of light-sensitive ion channels (opsins), DREADDs, or LGICs (PSAM), providing the possibility of modulating specific neurons. These receptors are activated or, in the case of

(Figure 2A–G). The relevance of these latter symptoms is demonstrated in the place/escape avoidance test in which animals must choose between a white anxiogenic area or a dark nonanxiogenic area in which they receive a nociceptive stimulus (Figure 2F). Animals experiencing pain for 1 week spend more time in the white anxiogenic area, while those experiencing pain for 4 weeks avoid it. Because both groups of animals have similar pain thresholds (Figure 2B, C), it seems that the rate of aversiveness associated with anxiety changes as pain persists (98).

In long-term pain animals, alterations to cognitive performance are associated with attentional and working memory deficits, as well as increased aversive emotional learning and memory (99). Animals show deficits in visual attention, visuospatial recognition learning, and memory in the novel object recognition test (100,101) (Figure 2G) and in the object pattern separation test (100). While long-term pain animals display similar fear-conditioned acquisition to sham animals (100,102), they also more commonly freeze in the conditioning phase of these tests, developing stronger and earlier fear behavior when faced with an aversive stimulus (100) (Figure 2H). Animals experiencing long-term pain still freeze when shifted to a nonconditioned context (Figure 2I), suggesting an inability to extinguish contextual fear (100,103). Therefore, long-term pain appears to produce a cognitive bias that affects the processing of aversive stimuli rather than neutral stimuli, probably provoking and maintaining a state of anxiety, apparently similar to what occurs in human disease-related anxiety (104). This comorbidity is also observed in the pain models of fibromyalgia and long-term inflammatory pain (105–108).

LC Neuronal Circuits Involved in Anxiety, Depression, and Cognitive Symptoms

An LC-projecting structure involved in emotions is the anterior cingulate cortex (ACC) (65,96,109), and hyperactivity is recorded in the ACC of patients with depression (110), which has chronic pain-induced anxiodepressive-like consequences (111). Furthermore, a bilateral increase of noradrenaline in the PFC has been associated with long-term pain (112), suggesting overactivation of the noradrenergic system in long-term pain. Stronger c-Fos expression was found in LC neurons projecting to the ACC, and DREADDs-mediated blockade of LC neurons projecting to the ACC completely reverses depressive-like behavior, such as behavioral despair in the forced swimming test (65) (Figure 3B). Site-specific pharmacological blockade indicates that α_1 and α_2 adrenoreceptors within ACC are necessary for this behavior (65). A microinjection of an α_1 adrenoreceptor antagonist in the medial PFC attenuates sensorial hypersensitivity in some models of neuropathic pain (88) although not in others (65), suggesting possible therapeutic benefits of targeting α_1 adrenoreceptors.

When evaluating chemogenetic activation of the LC using DREADD-assisted metabolic mapping, there is a rapid interruption of ongoing behaviors and an increase in exploratory activity and anxiety, in conjunction with synchronized hyperconnectivity in the salient (including ACC) and amygdala networks (26). This suggests that enhanced activation of these two pathways may underlie the symptoms of anxiety associated with pain. Accordingly, chemogenetic activation of the projection from the LC to the PFC (where the ACC is found) increased anxiety in nerve-injured rats (19) (Figure 3B). Both long-term pain-induced anxiety and enhanced fear learning are abolished by chemogenetic blockade of the LC → BLA pathway or intra-BLA administration of a β adrenoreceptor antagonist (100) (Figure 3B). This beneficial effect was also found when β blockers were administered systemically, which may open new interventional options for the treatment of comorbid pain and anxiety. Chemogenetic activation of this pathway has minor effects, which might reflect the ceiling activation achieved in long-term pain (100). Thus, pain would augment noradrenaline in the BLA, enhancing the memorization of negative events. It will be particularly important to determine if the blockade of β adrenergic receptors, which has been shown to have an anxiolytic effect at the BLA level (100), also has a similar beneficial effect at the ACC level. Finally, it is interesting to note that the activation of projections from the LC to the BLA has been implicated in encoding traumatic memories (113), suggesting that a similar dysregulation might occur in posttraumatic stress disorder and long-term pain.

The aforementioned studies were performed on male rodents, and given that the LC is sexually dimorphic (29,114–116), it will be important to take into consideration sex differences. The female LC is larger and contains more neurons (116) with a more complex dendritic arborization (114,115), and sexual dimorphism in gene expression has also been confirmed (29). Contrary to epidemiological data showing

channels, the channel opens, initiating endogenous signaling cascades, excitatory or inhibitory, that regulate the excitability neuron, specifically via light (optogenetics) or in the presence of their respective synthetic ligands (e.g., CNO as DREADD ligand or PSEM as PSAM ligand) (chemogenetics). **(B)** Photometry, TRAP-seq, and BiPOLES are new innovative tools for redefining the role of the LC. Photometry allows for monitoring fluorescence of genetically encoded calcium (GcaMP6f) and $GRAB_{NE}$ sensors in LC neurons as index of activity. TRAP is a technology to isolate the set of ribosome-bound RNA selectively from transgene-expressing cells, referred as their translatome. This technique combined with unbiased RNA sequencing, i.e., with a quantitative assessment of the translatome (TRAP-seq), has become a powerful technique for identification of the transcriptional profile in a cell type–specific manner. BiPOLES is an optogenetic tool for potent neuronal excitation and inhibition with light of two different wavelengths. This technique opens new potential avenues to manipulate specific neuron activity, including potent dual-color spiking and silencing of the same neurons in vivo and dual-color optogenetic control of two independent neuronal populations. **(C)** PET and fMRI are the most important tools to noninvasively visualize neural activity in the rodent and human brain. The PET technique combined with cell type–specific DREADD expression (DREAMM) in transgenic animals, fMRI in humans, and pupillometry provides a profile of brain-wide network activity and a powerful and accessible readout of LC activity in humans and rodents. 5-HT3, serotonin receptor 3; Arch, archaerhodopsins; BOLD, blood oxygenation level–dependent imaging; BiPOLES, bidirectional pair of opsins for light-induced excitation and silencing; cAMP; cyclic adenosine monophosphate; ChR2, channelrhodopsin-2; Cl, chloro; CNO, clozapine-N-oxide; DEG, differentially expressed gene; DREADDs, designer receptors exclusively activated by designer drugs; DREAMM, DREADD-assisted metabolic mapping; fMRI, functional magnetic resonance imaging; GlyR, glycine receptor; GPCR, G protein-coupled receptor; $GRAB_{NE}$, GPCR activation-based norepinephrine; GtACR2, *Guillardia theta* anion channelrhodopsins; H, hydrogen/proton; IR, infrared; LC, locus coeruleus; LGIC, ligand-gated ion channel; NpHR, halorhodopsin; PET, positron emission tomography; PSAM, pharmacologically selective actuator module; PSEM, pharmacologically selective effector molecule; SALB, salvinorin B; TRAP-seq, translating ribosome affinity purification-sequencing.

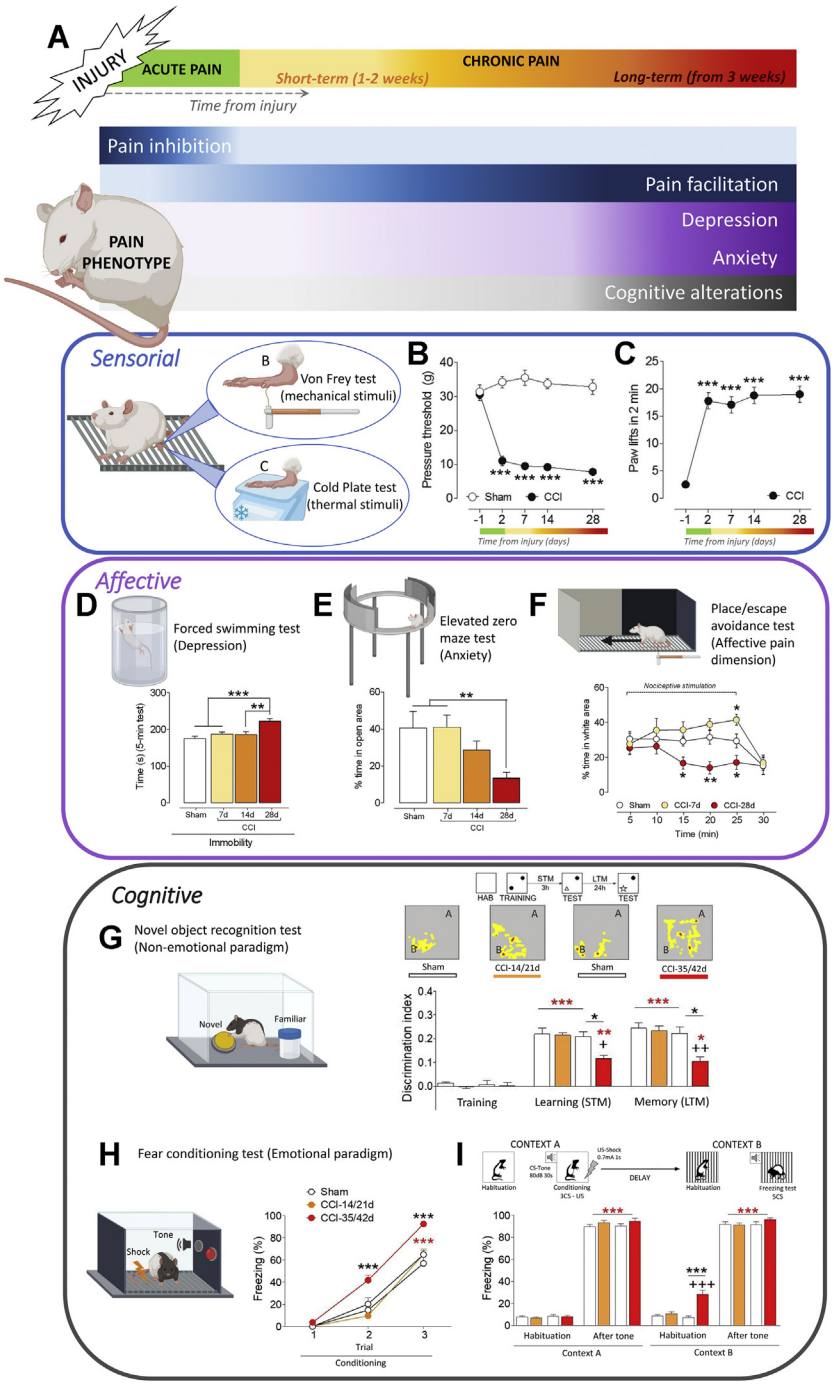

Figure 2. Time-dependent pain phenotypes after nerve injury. **(A)** Timeline representing the phenotypic transition from acute to chronic pain. **(B, C)** Sensorial hypersensitivity appears immediately after nerve injury and it is maintained over the long term. The sensory dimension is commonly evaluated by applying a mechanical (von Frey monofilament) **(B)** or thermal stimulus (cold plate test) **(C)**, producing similar values in the short and long term. **(D–F)** An anxiodepressive phenotype develops several weeks after suffering pain. **(D)** Depressive-like behavior appears in a time-dependent manner, evaluated as an increase in the immobility time in the forced swimming test, with a consistent phenotype in animals experiencing long-term pain. **(E)** Anxiety-like behavior appears in a time-dependent manner, represented as a reduction in the time spent in the open arms, with a consistent phenotype in animals experiencing long-term pain. **(F)** The affective-motivational component of pain is evaluated using the place preference test, in which animals have to choose between a compartment where they receive a nociceptive (black area) or an anxiogenic (white area) stimulus. Animals that experience short-term pain choose the anxiogenic area, while those experiencing long-term pain avoid it. **(G–I)** An altered cognitive phenotype develops after several weeks of pain. **(G)** In the novel object recognition test, a nonemotional paradigm, memory deficits appear in animals experiencing long-term pain through a decrease in the discrimination index. **(H, I)** In the fear conditioning test, an emotional paradigm, animals experiencing long-term pain show a higher percentage of freezing both during the conditioning phase **(H)** and after being exposed to an aversive experience, in context B habituation **(I)**. Hence, long-term pain appears to enhance aversive learning and produce a higher predisposition to fear. Data taken from the studies published previously: **(B–F)** Alba-Delgado *et al.*, 2013 (98); **(G–I)** Llorca-Torralba *et al.*, 2019 (100). CCI, chronic constriction injury model; CS, conditioned stimulus; HAB, habituation; LTM, long-term memory; STM, short-term memory; US, unconditioned stimulus.

that pain and affective pathologies have a higher incidence in females than males (117,118), a recent study showed that although male and female nerve-injured mice developed sensorial hypersensitivity, only the male mice developed anxiogenic-like behavior in the long term (119). Chemogenetic inhibition of the LC projections to the dentate gyrus for 15 days induced anxiogenic-like behavior and reduced hippocampal neurogenesis in female sham rodents (119), and sustained chemogenetic activation of these projections prevented anxiety and increased hippocampal neurogenesis in neuropathic males. Hence, a decrease of LC activity to the hippocampus might trigger pain-induced anxiety in male rodents.

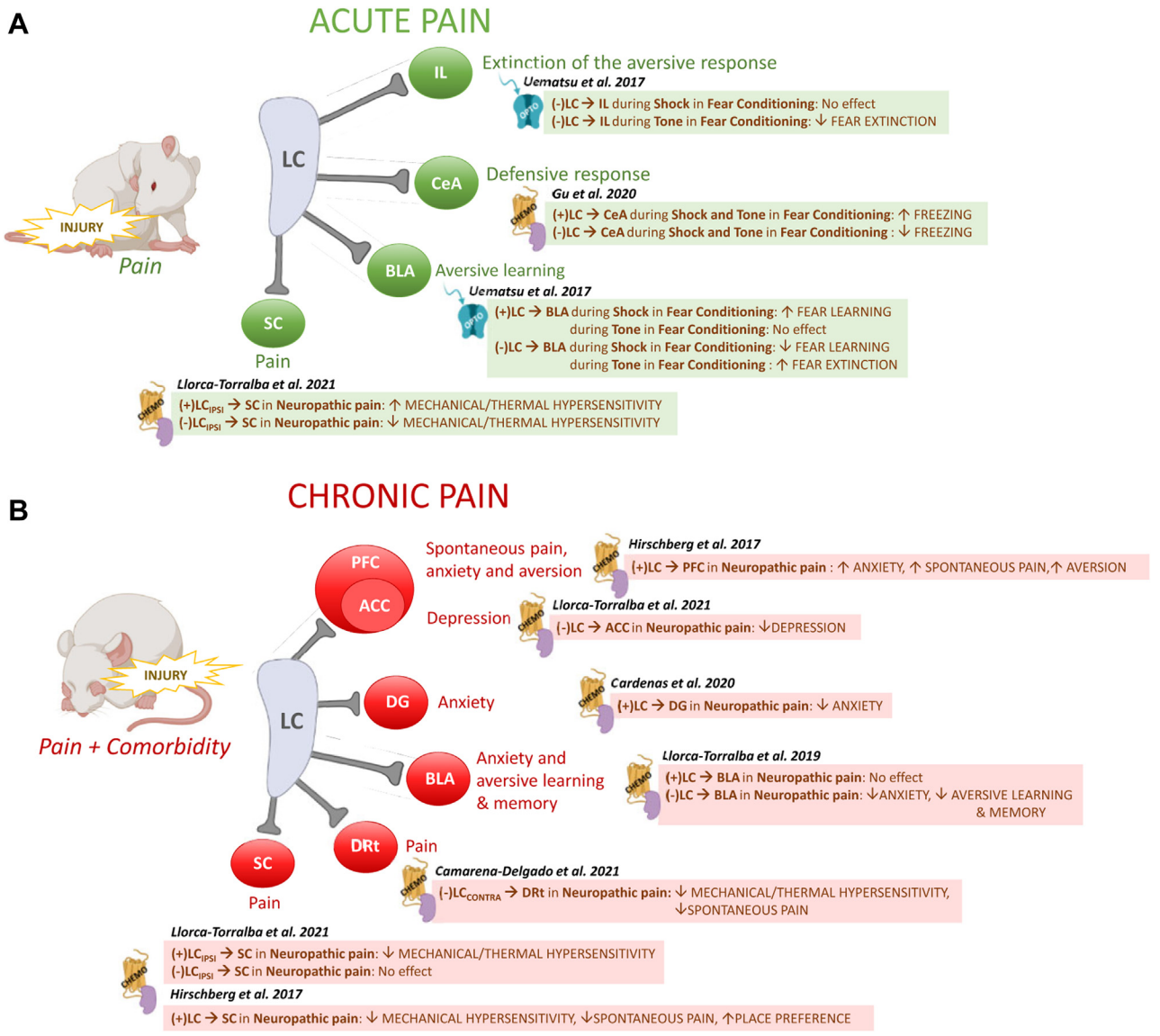

Figure 3. Chemogenetic and optogenetic manipulation of the LC pathways in acute and chronic pain. Summary of studies that through activation (+) or inhibition (−) of specific neural LC pathways in **(A)** acute or **(B)** chronic pain conditions modulate pain-associated behaviors. Note that each box is expressed as following: activation (+)/inhibition (−) of the LC pathway in a specific condition and the resulting behavior. ACC, anterior cingulate cortex; BLA, basolateral amygdala; CeA, central amygdala; CONTRA, contralateral; DG, dentate gyrus of hippocampus; DRt, medullary dorsal reticular nucleus; FC, fear conditioning; IL, infralimbic cortex; IPSI, ipsilateral; LC, locus coeruleus; PFC, prefrontal cortex; SC, spinal cord.

MOLECULAR AND ELECTROPHYSIOLOGICAL CHANGES ASSOCIATED WITH CHRONIC PAIN AND ANXIODEPRESSIVE COMORBIDITY

In terms of markers of activation, weaker phosphorylated ERK expression has been associated with short-term pain (120), although no changes in c-Fos or phosphorylated CREB have been found (87,121). Curiously, c-Fos and phosphorylated CREB expression, as well as firing activity, increase in association with long-term pain (80,91,122,123), suggesting that as pain persists, the involvement of the LC machinery increases, coinciding with the onset of anxiodepressive symptoms. Significant increases in tyrosine hydroxylase/dopamine beta-hydroxylase and noradrenaline transporter expression and enhanced α_2 adrenoreceptor expression have been detected in association with long-term pain but not earlier (98,119,121,123–125). It is intriguing that LC activation has been reported in animals with chronic pain that show an anxiodepressive phenotype (98,122), as in chronic stress (126–129), while in other animals this is a sign of resilience (119,130,131). Thus, an enhanced firing rate of LC neurons is evident in mice that resist chronic social defeat stress (131) but not in other mice (126). Inhibitory enkephalin afferents to LC were engaged in rats that resisted defeat, whereas in rats that

are biased toward subordination, excitatory corticotropin-releasing factor (CRF) inputs to LC were engaged (132). All these stressful conditions implicate a higher demand on LC activity but, depending on the model, lead to resilience or vulnerability. Greater activation before stress exposure might promote resilience, whereas increased vigilance induced by stress would be passive and pathological—for example, hyper-responsivity in posttraumatic stress disorder. It is possible that high levels of natural activation act as a predictor not only of stress resilience but also of pain. Acute pain only transforms into a chronic affectation in some individuals for reasons that are poorly understood (133). Therefore, a robust noradrenergic-LC system normally would be engaged to protect against the development of chronic pain and any associated emotion-related comorbid disorders.

Enhanced mu opioid receptor desensitization in vitro and weaker electrophysiological effects of morphine after noxious injured hind-paw stimulation has been associated with long-term pain (122). This phenomenon is reminiscent of LC adaptations to stress, which are regulated by the opposing influences of excitatory and inhibitory neurotransmitters, as previously discussed (132). Thus, when the opioid tone is weaker, the excitatory component would be predominant, enhancing maladaptive arousal-like states (134). Regarding the excitatory component, the major excitatory neurotransmitter of LC-related stress response is the CRF (135,136). Increased CRF_1 receptor expression in the LC of males with long-term pain and an anxiogenic phenotype has been reported, and curiously, this receptor was weakly expressed in anxiety-resilient female mice with pain (119). This is intriguing because the increased sensitivity of the female LC to CRF signaling (137) would lead to a heightened LC response to nonpainful stress in females (138,139). Finally, it is notable that other excitatory mediators seem to be enhanced in the LC in association with neuropathic pain (140,141). As such, further studies will be necessary to determine if differential CRF modulation is related to stress or pain, while interpreting global data from LC with caution because the activation of different LC clusters can produce a similar global picture.

CONCLUSIONS

In acute pain, the LC promotes feedback inhibition of nociception owing to the activation of the descending pain pathway to the SC, while ascending projections engage stress-related mechanisms. As time passes after injury, endogenous LC analgesia fails, and it promotes pain facilitation through the DRt and PFC. PFC or BLA activity contributes to anxiety, increasing aversive learning and memory and behavioral despair, sharing similarities with stress-related disorders. These data demonstrate the functional heterogeneity of the LC output in pain that will be of interest when designing therapeutic strategies. Thus, exogenous activation of the LC → SC projection can relieve pain, and activation of the projection to the hippocampus can prevent pain-induced anxiety, while the activation of other structures (PFC, BLA) may produce adverse effects, explaining the limited efficacy of antineuropathic drugs.

The axonal arbor of noradrenergic neurons (including the LC) that project to the SC also reaches the neocortex (cingulate and frontal cortices), several nuclei of the thalamus such as the reticular and ventrolateral nucleus, the periaqueductal gray, the inferior olive, and the cerebellum. Conversely, the hippocampus, the nucleus accumbens, and the caudate putamen are not innervated by those noradrenergic neurons (59). Thus, future functional studies will be necessary to elucidate if these SC-projecting neurons also mediate the emotional and/or cognitive aspects of pain. They could be engaged simultaneously by pain stimuli or differentially in segregated populations, so how these different circuits are orchestrated under conditions of acute and chronic pain should be explored.

Finally, because pain has a higher incidence in females than males (117,118), further studies into these sex-related differences are necessary, which, together with noninvasive approaches such as pupillometry and neuroimaging, will help translate the research findings into novel therapies that will eventually become available to patients.

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