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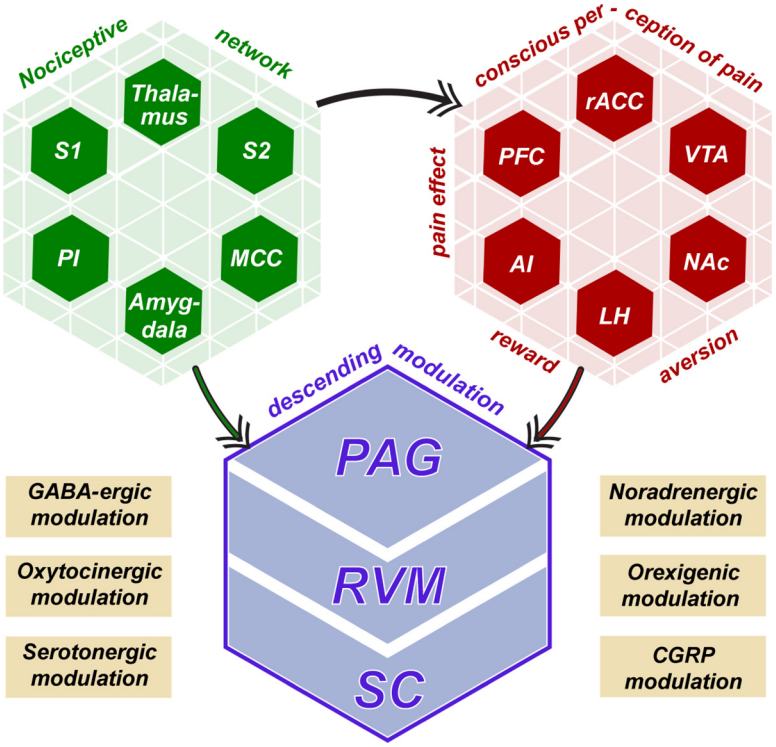
Review Article

CELLULAR CIRCUITS IN THE BRAIN AND THEIR MODULATION IN ACUTE AND CHRONIC PAIN

GRAPHICAL ABSTRACT



CLINICAL HIGHLIGHTS

This article reviews recent advances in understanding of precise sub-neocortical circuits mediating sensory, affective, and motivational components of pain, their interactions with reward pathways, and how they regulate descending pathways that control spinal processing of sensory inputs. Modulation of these circuits by GABAergic, serotonergic, noradrenergic, and major peptidergic pathways in the brain is discussed.

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KEYWORDS

aversion; functional circuits; imaging; nociception; optogenetics

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CELLULAR CIRCUITS IN THE BRAIN AND THEIR MODULATION IN ACUTE AND CHRONIC PAIN

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Kuner R, Kuner T. Cellular Circuits in the Brain and Their Modulation in Acute and Chronic Pain. *Physiol Rev* 101: 213–258, 2021. First published June 11, 2020; doi:10.1152/physrev.00040.2019.—Chronic, pathological pain remains a global health problem and a challenge to basic and clinical sciences. A major obstacle to preventing, treating, or reverting chronic pain has been that the nature of neural circuits underlying the diverse components of the complex, multidimensional experience of pain is not well understood. Moreover, chronic pain involves diverse maladaptive plasticity processes, which have not been decoded mechanistically in terms of involvement of specific circuits and cause-effect relationships. This review aims to discuss recent advances in our understanding of circuit connectivity in the mammalian brain at the level of regional contributions and specific cell types in acute and chronic pain. A major focus is placed on functional dissection of sub-neocortical brain circuits using optogenetics, chemogenetics, and imaging technological tools in rodent models with a view towards decoding sensory, affective, and motivational-cognitive dimensions of pain. The review summarizes recent breakthroughs and insights on structure-function properties in nociceptive circuits and higher order sub-neocortical modulatory circuits involved in aversion, learning, reward, and mood and their modulation by endogenous GABAergic inhibition, noradrenergic, cholinergic, dopaminergic, serotonergic, and peptidergic pathways. The knowledge of neural circuits and their dynamic regulation via functional and structural plasticity will be beneficial towards designing and improving targeted therapies.

aversion; functional circuits; imaging; nociception; optogenetics

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This article reviews recent advances in understanding of precise sub-neocortical circuits mediating sensory, affective, and motivational components of pain, their interactions with reward pathways, and how they regulate descending pathways that control spinal processing of sensory inputs. Modulation of these circuits by GABAergic, serotonergic, noradrenergic, and major peptidergic pathways in the brain is discussed.

I. INTRODUCTION

A. Acute Pain and Chronic Pain Disorders

Acute pain is the cornerstone of the body's defense against potentially harmful stimuli and critically prevents tissue damage in day-to-day life. Lack of pain, given largely by genetic mutations, leads to a short life span and a life full of medical visits and treatment.

Pain, however, has the ability to persist and become chronic in a variety of pathological states, driven by long-term inflammatory tissue damage or nerve injury via trauma, metabolic dysfunction, pathogenic infections, or cancer growth. Importantly, physical and chemical factors act alone, but there is a combination of physical with psychosocial factors in driving the transition from acute to chronic pain. Chronic pain is manifest in a variety of ways, including spontaneous pain; enhanced sensitivity to painful stimuli (hyperalgesia); pain in response to normally innocuous stimuli, e.g., gentle brushing of skin (mechanical allodynia) or mild cool temperatures (cold allodynia); and aberrant referral of pain to unaffected body parts. Chronic pain is a major health problem worldwide and is responsible for prevalent human suffering (40, 135). A major problem re-

mains that conventional therapy is largely unsatisfactory in treating and eradicating chronic pain, particularly of neuropathic origin with more than 50% patients reporting less than 50% pain relief (64, 136). It is also widely acknowledged that the diversity of pain disorders with differing pathophysiology that are encountered clinically as well as the strong psychological and social influences that are observed render it unlikely that a single cure can be widely efficacious alone (44, 317). Therefore, research on the causes and mechanisms underlying the chronicity of pain arising from different origins remains a necessity.

B. The Multidimensionality of Pain

Like other senses, pain is a highly specialized sensory experience. Acute somatic pain, as we commonly experience in day-to-day life in response to a physical or chemical stimulus, can be precisely localized in somatotopy and described in modality and intensity. However, pain is different from other senses by virtue of the strength of its unpleasant affective-motivational components. Pain thereby reflects a multidimensional composite created by complex interactions between afferent sensory inputs and their processing throughout the nervous system from the periphery to the brain and affective brain circuitry, with an additional layer of complexity given by memory, expectations, attention, and mood. This multidimensionality has prompted a discussion between the delineation of the underlying sensory dimension of pain, with its bodily specificity, and the percept of "conscious pain," which integrates all of the aforementioned components (18, 101). The former has been suggested to recruit a basic nociceptive pathway, spanning peripheral nerve terminals, their somata in the dorsal root ganglia (DRG) or trigeminal ganglia, second-order nociceptive neurons and excitatory as well as inhibitory interneurons in the spinal dorsal horn, diverse ascending pathways that carry processed information to a multitude of brain centers involved in sensory-motor processing, and the efferent arm of the network which enables the appropriate motor responses to be carried out (101, 159). Conscious pain, on the other hand, has been suggested to involve a higher order network that enables the generation of the percept under the influence of learning, expectation, and current affective state of the individual (18, 99, 101, 196).

Thus the emerging pain percept is not a direct one-to-one correlate of the sensory input but represents a dynamic state that is shaped by diverse psychosocial factors. This not only accounts for large interindividual and cross-situational differences in pain perception, but also plays a key role in whether pain becomes chronic despite a healing of the initial injury or cause. Moreover, the concept entails the view that an individual has the inherent power to augment or suppress pain perception, with multiple clinical implications, including predisposition or resilience to pain chronicity, placebo and nocebo responses, neurostimulation-based analgesia, conditioned pain modulation, among others (70, 249, 310). To the sufferer, pain represents an invasion in the psyche, leading to alterations in mood, anxiety, and depression as pain becomes chronic. Unsurprisingly, these come about as frequent comorbidities of chronic pain disorders and thereby further mutually complicate therapy.

C. Neural Plasticity as a Mechanistic Basis

One widely accepted view is that nociceptive pathways change in an activity-dependent manner, i.e., show plasticity, which is a general and fundamental property of all neural systems (23, 29, 94, 164, 229, 287). The concept of functional plasticity provides mechanistic links between specific changes in molecules, synapses, microcircuits, and systems and thereby links a variety of modulatory factors to a change in perception and behavior (18, 23, 164, 165, 229, 313).

Over the last three to four decades, studies in animal models of chronic pain have established that peripheral afferents sensitize in response to a variety of molecules secreted by nonneuronal cells, such as immune cells, keratinocytes, and blood vessels, including inflammatory cytokines and growth factors (87, 138). Structural reorganization of peripheral afferents and their connectivity has been described in certain types of chronic pain. Great strides have also been made in the molecular understanding of peripheral plasticity, encompassing diverse ion channels that transduce and propagate noxious inputs, such as the transient receptor potential (TRP) channels, Piezo channels, and sodium channels, and factors that modify their expression and sensitivity, such as signaling via nerve growth factor (NGF), among others (12, 26, 144). At the level of the spinal cord, literally hundreds of molecular alterations have been described, spanning not only critical changes in key neurotransmitters and modulators of synaptic transmission, but also the tremendous impact of spinal glial mechanisms that drive and maintain central sensitization (56, 246). Importantly, the past decade has been highly productive in yielding an understanding of the complex spinal circuitry, comprising different types of afferent- and inter-neuronal connectivity (12, 37, 98), which determines the balance between excitation and inhibition (231). Major insights have come in the delineation of nociceptive circuitry from tactile processing and crossovers therein during the establishment of mechanical allodynia (156, 190). We refer our readers to the aforementioned, excellent reviews for gaining an overview on rapid advances in peripheral-spinal circuitry. These have been paralleled with breakthroughs in our understanding of brain circuitry in acute and chronic pain, which constitutes the main topic of this review.

Plasticity and its role in pain chronicity can vary depending on the type of pain and relative contributions of peripheral and central factors and can therefore differ across diverse

pain disorders. Cancer metastatic pain and osteoarthritis, for example, have larger contributions of peripheral cell-cell interactions and sensitization, as opposed to disorders such as fibromyalgia and chronic neuropathic pain, which are believed to entail large contributions of plasticity at central circuits. It is also implicit that plasticity in one locus in the nociceptive pathway can drive alterations in other avenues, e.g., by feed-forward propagation of activity, releasable factors, and adaptive changes in circuits.

The research and clinical community of pain has also come to appreciate that polymorphisms and genetic determinants can account for variations in basal pain sensitivity among individuals and animal species employed in pain models. A large body of literature, however, supports the view that the significance of epigenetic modulation in chronic pain is paramount (80, 82), providing yet another avenue for rationalizing how mind set, life style, as well as social and learning experiences can drive pain chronicity versus resilience. Moreover, it has been increasingly clear that sex and gender differences also define the experience of pain and pain chronicity (31, 188). A theme gaining prominence is therefore to understand pain as an individual experience, which can provide scope for implementing personalized medicine in the future.

D. Rationale for the Review and Its Scope

This review is dedicated to assessing literature and concepts on brain circuits and their contributions to pain and pain chronicity. Herein, we would like to start by discussing the urgent need and importance of dissecting and understanding circuitry. Starting with a short overview on fundamental insights gained from large-scale macroscopic studies in patients/human subjects and animal models, we will make a case for the critical need for a cellular understanding of circuits. Moreover, we will discuss the inevitable need for causal analyses to escape the conundrum of whether the myriads of alterations described in chronic pain represent a cause or a consequence of persistent pain, a factor which is critical for the success of therapies. Before plunging into the actual studies addressing circuits, we will therefore describe tools for interrogation of circuits, including optogenetics, chemogenetics, viral tracing, and advances in cellular and population imaging analyses.

Given the wealth of scientific literature on brain circuits, we have set a primary focus on studies in rodent models that interrogate the function of specific neuronal subtypes. Wherever possible, we will make a reference to human data, but the focus is placed here on detailed insights gained at a cellular level, which are currently enabled by mouse genetics and rodent-based technologies. We will discuss key avenues in nociceptive networks, laying a particular emphasis on structures below the neocortical level, and then go on to explore at length second-order networks, particularly limbic areas. We will discuss how pathways of reward and aversion participate, interact, and intercalate with pain circuits. As a mechanistic basis for neuromodulatory influences on pain perception, we will discuss current knowledge on diverse key neurotransmitter systems and hormone/peptidergic modulators, such as dopaminergic, noradrenergic, serotonergic, cholinergic, and peptidergic pathways that are operational in brain circuits. Wherever possible, we have tried to point out the therapeutic significance of basic findings on brain circuits.

The authors acknowledge, with a large degree of awareness of self-limitations as well as constraints of space and scope, that despite our best efforts, this work will not be comprehensive. Although we will endeavor to provide a balanced representation, we concede that realistically, the review will not do justice to a large body of literature and we humbly apologize in advance to colleagues whose work has not been mentioned here. Moreover, we fully acknowledge that neocortical domains, such as the insula and the somatosensory, prefrontal, and cingulate cortices, are critically important in processing of sensory, emotional, and cognitive aspects of pain. With each representing a very large area of research in its own right, they do not constitute a major focus of this review. However, as far as possible, we have endeavored to point out well-studied and salient interactions between sub-neocortical regions and certain neocortical areas, such as the prefrontal cortex (PFC).

II. NATURE OF BRAIN REGIONS RECRUITED DURING PAIN

A. Insights from Human Imaging Studies

An excellent starting point in understanding how the percept of pain is created in the brain is given by imaging analyses that provide a macroscopic snap-shot of activity across diverse brain regions in the context of an acute painful stimulus, resting activity changes in chronic pain states, or alterations in representation to pain-relieving drugs in the human context. Most human functional magnetic resonance imaging (fMRI) studies employ local changes in blood flow (BOLD signals; see sect. II*A*) as a surrogate parameter of neuronal activity and integrate changes over a period of several minutes. There is widespread consensus in the field, based on a myriad of imaging studies, that origin of pain is not restricted to a single area, but rather that pain results from integrated function across brain networks (315). However, like with every method, drawing unequivocal interpretations of a brain map for pain and using it to interpret pain perception has been fraught by several difficulties (128). These are compounded by technical caveats, differences in analyses, and interpretation of BOLD signals as well as striking interindividual as well as inter-trial variations in both resting and evoked activity within and across studies.

One approach that has been particularly gainful in this regard was developed by Wager et al. (298), who employed a machine learning-based regression model for pain perception to decipher a neurologic signature of acute pain that was consistently activated in heat-evoked acute pain, delineated from pain-unrelated aversion and inhibited by a pain-relieving drug (Remifentanil). The signature included both somatic-specific areas such as the ventrolateral thalamus, the secondary somatosensory cortex, and the dorsal posterior insula as well as regions related to affect and mood, such as the anterior insula, the dorsal anterior cingulate cortex, and the medial thalamus (298).

The nature of brain activity patterns and regions underlying the affective dimension of pain, i.e., its unpleasantness, remains a topic that is characterized by both dynamic rise in insights gained as well as considerable debate. While some notable human imaging studies have implicated the involvement of specific brain structures, particularly the rostral anterior cingulate cortex (234) and the posterior insula (253), others have argued against localization of pain affect to individual brain structures (75). Apkarian and colleagues (18) have postulated that recruitment of key elements of the limbic brain, namely, the amygdala, the hippocampus, the ventral striatum, and the medial prefrontal cortex, which govern emotion, expectation, and salience, is required. During acute pain, subjectively perceived by human subjects, Segerdahl et al. (253) did not find consistent activation of key elements of the limbic brain (253). In support, the neurologic signature of acute thermal pain (see above) also does not identify these key nodes of the limbic circuitry in acute pain, although the anterior insula and the dorsal anterior cingulate cortex are included. There is, however, broad consensus that the limbic circuitry plays a key role in emotional modulation of pain and holds significance in the transition to several forms of chronic pain.

Two intriguing models on the temporal dynamics and spatial organization of nociception to conscious pain are particularly noteworthy. In the first one, considering the wealth of literature on imaging studies and analyses of mesocortical and mesolimbic networks, Baliki and Apkarian (18) have suggested that activity in cortico-mesolimbic-cortical loops is key for reaching the threshold for eliciting conscious pain perception as opposed to nociception. They hypothesize that the control of the incorporation of sensory afferent input into higher cortical centers involved in conscious states lies with corticostriatal circuits, drawing parallels to other sensory modalities. Moreover, they extend this hypothesis to chronic pain, proposing that corticolimbic risk factors determine inter-individual differences as to whether pain will persist chronically after acute injury or the normal state of physiological pain will be recovered. A parallel model has been put forth by Garcia-Larrea and colleagues (25), who have incorporated data from intracranial electroencephalographic (EEG) recordings in 16 brain areas performed during the percept of pain, which is particularly noteworthy because it enables taking critical temporal aspects into account that are lacking in magnetic resonance imaging (MRI) analyses. Bastuji et al. (25) noted that activity within 1 s after application of a painful stimulus in human subjects, a time frame that is sufficient for conscious pain perception, can be broken down into three consecutive waves. The first set of regions that activated before conscious pain perception include classical parts of the nociceptive network, such as posterior insula, operculum, and the mid-cingulate cortex, as well as the amygdala (25). Regions that were activated during conscious perception and the initiation of voluntary motor reactions included the anterior insula and prefrontal cortex. Thus the regions identified by Bastuji et al. (25) largely match the neurologic signature of pain identified by Wager et al. (298), although the thalamus and somatosensory cortex were not compared. The study also noted that regions such as the hippocampus and pregenual and posterior cingulate cortices are activated after conscious pain perception and likely represent processes linked to memory and self-awareness. Overall, there is consensus that noxious information reaches both sensory networks and several parts of limbic networks via diverse ascending pathways and that orchestration of activity over several elements of both networks is observed across diverse studies.

There is a wealth of literature on structural and functional changes in brain networks in chronic pain patients. This is a massive field of study, and we would like to refer to several excellent recent reviews covering this topic (18, 45, 163, 165, 183, 286). It is also noteworthy that a majority of these instances of functional plasticity and structural reorganization in chronic pain share commonalties with other affective disorders, which interestingly are also frequent risk factors or comorbidities of chronic pain, such as post-traumatic stress disorder, fear, and anxio-depressive disorders (131, 243). As far as possible, we have endeavored to refer to key insights from human studies as we discuss cellular circuits in animal models in the remaining parts of this review.

B. Insights from Imaging Studies in Animal Models

MRI imaging studies in rodents are important to build a translational bridge between the human imaging studies and mouse models. A caveat thereof, however, remains that animal MRI imaging is largely performed in the anesthetized state as opposed to human studies performed in the awake state, often in conjunction with behavioral tasks. Nevertheless, a considerable commonality is observed in brain regions recruited during nociceptive stimulation in rodent and human imaging analyses. BOLD signals have been reported in the medial and lateral posterior thalamic nuclei, diverse cortices, including the sensory-motor, cingu-

late, insular and retrosplenial cortices, pretectal area as well as the descending modulatory centers, i.e., the hypothalamus and the periaqueductal grey (PAG), upon hindpaw thermal stimulation in rats (121). In a model of inflammatory pain, heat-evoked signals were amplified in all of these areas (121). These studies are summarized in a recent survey of the literature (71a).

There has been considerable interest in mapping both structural and functional changes in brain networks over the course of pain chronicity in rodent neuropathic pain models. Three studies are particularly noteworthy (19, 28, 130), since each tested unique aspects longitudinally over diverse phases post spared nerve injury from as early as 1 wk to as late as 20 wk (allodynia lasts lifelong in mice with spared nerve injury). Baliki et al. (19) performed a whole-brain connectivity analysis and observed that by 4 wk post-injury, widespread local changes were observed in several brain regions. However, in terms of connectivity alterations, they only observed changes in the connectivity between limbic regions and between the nociceptive network and limbic regions. These results support the view emerging from several human imaging studies that as pain becomes chronic, there is a progressive shift from nociceptive to emotionally governed networks (71a, 165). Hubbard et al. (130) tested activation of networks to a cold stimulus in control and neuropathic rats to address the basis of cold allodynia, which is a debilitating problem in neuropathic pain. They report sustained changes in multiple brain areas, including the thalamus, the somatosensory and cingulate cortices, and the PAG over the course of chronic cold allodynia in neuropathic rats. Finally, Bilbao et al. (28) sought to test the mouse parallels of the large-scale volume changes that have been reported in chronic pain patients in voxel-based morphometry. They report robust decreases in volume following pain induction in a number of brain regions that form a part of the nociceptive network as well as mesolimbic pathways; importantly, they report that nearly all effects were progressively reversed over time until 12 wk post-injury, although allodynia persisted, as was also the case for global changes in connectivity. This has important implications for the scope of structural brain changes in both allodynia as well as alterations and mood and affect which are known to accompany neuropathic pain. Emerging literature on the latter in rodent models points to a manifestation of anxiety and depression-related behaviors within specific time windows following nerve injury, which are progressively resolved and normalized over time (131, 279). Notably, the study found that nucleus accumbens took on a more prominent role in connectivity, which is consistent with the hypotheses proposed by Apkarian and colleagues (18) on emerging mesolimbic dominance over pain chronicity based on human imaging studies. Moreover, Bilbao et al. (28) report a sustained decrease in connectivity of the prefrontal cortex and hippocampus, which is consistent with the widely reported deactivation of the medial prefrontal cortex as well as deficits in working memory in chronic pain patients.

We conclude therefore that although there are individual points of divergence, overall, the patterns of changes reported in rodent models with respect to functional activation, large-scale structural alterations and connectivity have much in common with the principles emerging from imaging analyses on human pain conditions.

III. ADDRESSING CELLULAR SUBSTRATES AND THEIR FUNCTIONAL CONTRIBUTIONS IN BRAIN CIRCUITS

A. Cause, Consequence, or Epiphenomenon?

Pain happens in the brain, but how do we know which circuits are mediating the perception of pain? With any of the methods available to study brain circuits and behavior, how do we know if activity recorded in certain neurons directly produces a specific behavior observed in this moment or whether it reflects another process such as an internal representation? The activity could simply be an epiphenomenon, related to any other processes happening in the brain at this very moment and may reflect computations of internal states, sensory input, motor action, or any combination of these. The activity may also be triggered by the pain stimulus, but yet subserve functions other than pain perception, for example, processing unconscious homeostatic functions. Furthermore, the activity could be the consequence of pain perception, an immediate response of the brain to the percept of pain. Even if we could record the behavior of all neurons of the brain simultaneously, and thereby know all cells are active at a given moment, and if one would know the entire connectivity of theses neurons, one might not be able to unequivocally establish causality. This is because synaptic connections are stochastic (158) and thereby may signal along a certain pathway of neurons in one instance, but not another. Hence, defining causality is not an easy task when solely relying on imaging approaches that observe activity levels of neurons. A complementary approach to causality is to specifically stimulate a single neuron, or an ensemble of neurons, or an entire brain region. If direct stimulation of a single neuron triggers pain within a few milliseconds, one could safely conclude that this neuron causes the percept of pain. How likely is it that activity of single neurons causes a percept? While single action potentials in a single neuron can produce behavior, for example, a small displacement of a whisker after stimulating cortical layer 5 neurons (39), this is unlikely to account for more complex behaviors or subjective percepts the brain generates. If stimulation of an ensemble of neurons with a defined activity pattern causes the percept of pain, one could quite safely conclude that this subset of neurons, using that specific activity pattern, can trigger the percept of pain. However, it remains unclear if these very

neurons or a downstream connected set of neurons produces the percept. Furthermore, the same ensemble could theoretically produce another percept or behavior when stimulated with a different activity pattern or when stochastically connecting to a different subset of target neurons. Therefore, one would ideally attempt to both selectively induce defined activity patterns in ensembles of neurons and simultaneously record activity from as many neurons as possible at the same time. This is a daunting task, however, not impossible when using appropriate model systems. Another issue to consider is how to define if a pain percept is present in model systems that cannot verbalize? In rodents, pain states reliably trigger behavioral responses that can be determined and that allow indirect inferences on the presence of a pain percept. For example, conditioned place preference/aversion, grimace scale, operant behavior (278), or a combination of several tests can be used as a proxy to infer that the animal is experiencing pain. To support observations made in model systems, we consider it important to cross the translational bridge and compare observations made in rodents with human data. This could be achieved by first relating the activity patterns found on the level of neuronal ensembles to mesoscopic imaging achieved with fMRI. In turn, these could be compared with human fMRI, finally connecting cellular activity patterns with subjectively perceived pain. However, the caveats discussed above will have to be kept in mind while crossing the translational bridge. In conclusion, rigorously establishing the causal relationship of neuronal ensembles with a simultaneously produced pain percept is a feasible, yet challenging, but finally highly rewarding task.

B. Tools Used for Interrogating Pain Circuits in the Brain

1. Imaging

Imaging techniques can visualize neuronal activity on different spatial and temporal scales. Ideally, a combination of different imaging tools will be used to comprehensively address the questions discussed in the previous section. Generally, the term *imaging* involves a wide range of techniques, ranging from microscopic fluorescence imaging, via mesoscopic X-ray imaging to macroscopic MRI. Here, we will focus on imaging approaches that are of particular interest to address the contribution of brain circuits to pain perception, and we will first consider MRI imaging and then move to the cellular level.

A) MAGNETIC RESONANCE IMAGING. This very widely used technique offers noninvasive imaging of the whole brain in the intact organism. It can be done in humans (96) and in rodents (79) and thereby offers a powerful translational bridge to relate findings made in both models to each other. During image acquisition, the subjects have to remain immobile, hence precluding extensive motor actions, yet allowing sensory stimulation and limited motor responses. Rodents typically need to be anesthetized to ensure immobilization, but also imaging in awake rodents trained to stay in the narrow tube of the MRI device has been achieved (84). MRI data can be acquired using a plethora of different modes and can be analyzed with a diverse set of methods. For the scope of this article, two MRI techniques will be briefly elaborated on.

Functional MRI can be used to assess spatio-temporal activity levels of the brain at rest or in response to certain stimuli or self-generated motor actions (96). This technique provides an indirect readout of neuronal activity based on the BOLD MRI signal that essentially reflects different magnetic properties of oxygenated versus deoxygenated hemoglobin. Hence, if neurons in a certain volume are active, they will consume more oxygen and thereby change the BOLD signal (123). This mechanism also defines the rather low spatial and temporal resolution of this imaging method, in humans typically a cubic millimeter and minutes, respectively. In summary, this method is ideally suited to determine activity patterns across the entire brain at a spatiotemporal resolution that allows inferences on the brain region that is activated and to relate these activities to certain brain states (e.g., the subject consciously experiences something, receives a certain stimulus, or generates a movement).

Voxel-based morphometry (VBM) is an MRI technique that focuses on brain structure and its dynamical changes over time (15). VBM typically reports volume changes in grey matter, white matter, and liquor spaces. Such changes could be observed in certain disease conditions and thereby could reveal areas that might underlie chronic, structural changes of the brain (see below for examples). However, the cellular correlates of grey or white matter volume changes remain poorly understood, although correlative studies using parallel fluorescence and MRI in a longitudinal fashion in mice and ex vivo studies have provided new answers (13). VBM can be done at voxels with a width of 150 μm in rodents. In summary, VBM can identify regions undergoing structural changes that may accompany chronically changed function, such as chronic pain.

B) FLUORESCENCE IMAGING. Fluorescence imaging describes a wide range of highly versatile imaging techniques using fluorescent molecules to readout cellular structures and functions (248), and only a small selection of techniques will be highlighted here. Fluorescence imaging can resolve single molecules and their dynamics and spatial arrangement at the scale of nanometers, but also large networks of neurons and their activity at a scale of hundreds of micrometers. Fluorescence is typically imaged in rodent model systems, but has been applied also to other species including nonhuman primates (143). Imaging can be done in a variety of preparations, including ex vivo preparations and intact anesthetized or awake rodents, either head-fixed or freely

moving. Cellular signals are typically visualized using genetically encoded reporters that change their fluorescence upon detecting ions or voltage (32, 226). These indicators can be expressed using a plethora of modern genetic tools, allowing expression in a cell type-specific manner, in single neurons, in an activity-dependent manner, thereby capturing neuronal ensembles. The most frequently used indicator of neuronal activity are GECIs, genetically encoded Ca^{2+} indicators that report Ca^{2+} signals generated in response to action potentials, hence representing an indirect readout of action potential activity (241). Variants of GCaMP are most commonly used, with GCaMP6 and very recently GCaMP7 being principally capable of reporting single action potentials (72). Slow binding kinetics of Ca^{2+} limit the temporal resolution so that rapid bursts of action potentials cannot be discriminated. Genetically encoded voltage indicators are in the process of evolving into practically useful tools, yet this will have to wait a few more years, but hold considerable promise. Taken together, fluorescence imaging is a versatile technique that allows structural and functional imaging even in behaving animals, allowing correlations of structure and function with behavioral states and, possibly, even perceptual states.

Wide-field mesoscopic fluorescence imaging through the intact skull can visualize the activity of neurons expressing GCaMP (292), allowing a mesoscale (resolution of tens of micrometers, areas of several millimeters) imaging of entire cortical regions in head-fixed mice. For example, this approach is capable of visualizing the flow of activity across cortical regions in response to sensory stimuli.

Endoscopic imaging with portable miniaturized cameras (miniscopes) using implanted GRIN lenses allows visualization of subsets of neurons expressing GCaMP even deep in the brain (hypothalamus, hippocampus, thalamus, prefrontal cortex) (103). Ensembles of typically 50–100 neurons can be imaged in freely moving animals. The positional stability of the imaging site allows repetitive imaging of the very same neuronal ensemble over time periods of weeks, a unique feature allowing to follow the composition and dynamics of ensembles over time. Activity can be sampled at a speed limited by the GECI used, typically a frame rate of 10 Hz is used. This imaging approach can be combined with actuators such as channelrhodopsin, enabling studies of distinct connectivity and their functional readouts from synaptically connected neurons.

Two-photon (2P) imaging (81) is one of the most widely used techniques for imaging cellular activity in intact brain of anesthetized or awake rodents through a permanently implanted transparent cranial window (126). While 2P imaging is typically done in head-fixed animals, researchers have also achieved 2P imaging in freely moving rodents using portable 2P microscopes, although this has not yet become standard procedure. 2P microscopy routinely allows imaging depths of ~500 μm (126). Depending on the brightness of the fluorescent protein used, its expression levels, and emission wavelength (500–650 nm), the achievable depth can vary and reach up to 1,000 μm under certain conditions (282). Cellular structure can be imaged deeper than functional readouts. When using low-magnification, high-numerical aperture objectives, the volume of accessible brain tissue can be up to 700 μm $\times$ 700 μm $\times$ 700 μm. However, when scanning every voxel in such a large volume, a long acquisition time is needed because the focus of the laser light needs to be placed at every voxel (in this example several hours). To overcome this problem, random access scanning using acusto-optical devices were developed that randomly position the imaging focus at arbitrary positions within the imaging volume (2, 199). This way, not the whole volume is scanned, but only several hundred points of interest, thereby speeding up temporal resolution to the millisecond range. With this technique, activity of several hundred neurons can be determined at a rate of ~50 Hz.

2P in vivo microscopy through cranial windows enables the use of longitudinal imaging approaches, a powerful method that allows repetitive imaging of a defined region in the same animal over extended periods of time (1 yr is easily possible). Thereby, dynamic changes can be observed over time, decreasing the number of animals required to detect these changes compared with using an ex vivo population approach.

Three-photon (3P) microscopy is a variant of 2P microscopy that uses three photons for the excitation of the fluorophores, permitting the use of longer wavelengths for excitation (e.g., up to 1,500 nm), and thereby deeper penetration and less scattering of the excitation light (216). Recently, the development of new lasers made this technology practically accessible for microscopy applications. While the slow repetition rate of the laser is still limited, an imaging rate of typically 4 Hz can be achieved for functional imaging. However, imaging depths of 1,500 μm have been demonstrated, thereby extending the depth limit of 2P microscopy significantly.

Sculpted light imaging is a novel 2P imaging technique that uses the trick of illuminating large focal volumes by employing light sculpting techniques (230). Larger focal volumes limit spatial resolution, but strongly increase temporal resolution and signal-to-noise of the fluorescence signal because many more photons can be collected in a short time from a larger volume. If this volume is tuned to the size of an average neuron, activity-dependent Ca^{2+} signals can be recorded from that neuron. This imaging technique allows imaging of several thousand neurons at a time resolution of a few milliseconds, thereby providing unprecedented access to study population activity of large neuronal networks.

This section summarized the current state of the art of imaging techniques available to study the activity of pain circuits on different spatial and temporal scales, required to address fundamental questions concerning the relationship between neuronal ensemble activity and pain perception illustrated above.

2. Optogenetics

Optogenetics uses genes encoding light-activated ion conductances or biochemical signaling proteins that can be targeted to genetically identified cells (78, 91, 151). Briefly, opsins can either depolarize cells and generate action potentials, or they can hyperpolarize cells and prevent action potential firing with light stimulation (91). A multitude of opsin variants have been developed that differ in spectral properties, conductance, activation kinetics, and expression properties. Depending on the question at hand, the most suitable opsin needs to be identified. For example, excitation and inhibition of cells can be achieved using opsins with a different spectral profile and coupled to a depolarizing or hyperpolarizing conductance (channelrhodopsin and halorhodopsin; Ref. 91), such that blue illumination excites and yellow illumination inhibits the neuron. Light can be applied via implanted optical fibers or by direct illumination through cranial windows (90, 91, 151). When using these approaches, a large volume of tissue is illuminated and causes a temporally synchronized activation of large neuronal populations that may not necessarily correspond to the activity pattern such populations of neurons would generate in the native state. Nevertheless, activity stimulated in defined subsets of neurons allows inferences of causality. More elaborate techniques of light application use three-dimensional holography and thereby achieve controlled stimulation of individual identified neurons with high temporal accuracy, allowing to induce more natural activity patterns in neuronal ensembles (9, 258, 272).

In addition to precise optical control of neuronal activity in vivo, optogenetics in conjunction with molecular genetics provides an additional layer of experimental freedom. The expression of opsins (91) in neurons can be controlled by many means. For example, opsins can be expressed only in a limited subset of neurons that is defined by a specific promotor. For example, only parvalbumin-positive interneurons can be targeted by using a mouse line expressing Cre recombinase under control of the parvalbumin promoter and breeding this line with a line driving Cre-dependent opsin expression from a ubiquitous locus (91, 151). Further spatial specificity can be achieved by expressing the opsin from a Cre-dependent virus injected into a defined target area of the brain. This limits expression of the opsin to cells located in a specific cortical volume at a defined time point and only in parvalbumin-positive interneurons. Having access to an ever-increasing selection of Cre-driver lines and constructs for viral gene transfer, this Lego system allows highly versatile control of defined neuronal populations. When Cre-lines are not available for a given target cell type, even green fluorescent protein (GFP)-expressing mice can be used to drive opsin expression by using Flippase-dependent on GFP (Flp-DOG), a molecule that binds GFP and thereby activates its Flp recombinase function that in turn can be used to activate opsin expression (277). For example, Flp-DOG can be expressed together with a Flp-dependent construct using viral vectors within a brain region containing GFP-expressing cells of interest. Only cells that express GFP, Flp-DOG, and FLEX^{FRT}-ChR2 (a Flp recombinase-dependent expression cassette) will selectively express ChR2 and therefore can be stimulated with light (277). Another highly promising avenue is to use activity-dependent promoters to tag neuronal ensembles representing a certain behavior or internal state. This approach can be used to tag an ensemble of neurons and subsequently control its activity by opsins expressed in these neurons. This approach provides a powerful approach to link the activity of neuronal ensembles to behavior. Finally, neuronal subsets can also be labeled via their connectivity. To this end, a virus specifically taken up in nerve terminals such as AAVretro (281) will be injected in the target region of interest and will then label only neurons in other locations of the brain that extend their axon to the injected target region. Interestingly, this approach can be combined with the tools described above so that only a genetically identified cell type projecting into a defined target area can be optically controlled. To achieve this, AAVretro can mediate expression of Cre recombinase, Flip recombinase, GFP, or any other protein of interest in the neuron forming a synapse within the injection site. Depending on the strategy, this can lead to a knockout of floxed alleles, activation of a floxed reporter, activation of a flexed gene, or activation of a recombinase via Flp-DOG recognizing GFP.

Optogenetic manipulations and behavioral paradigms need carefully designed control experiments and critical interpretation. Behavioral responses can often be affected by other nonsensory effects, including motivational status, memory, or the modulation of motor responses. Furthermore, optogenetic stimulation often causes highly synchronized activity in neuronal networks that are unlikely to occur in vivo and may therefore affect the behavioral outcome.

3. Chemogenetics

Chemogenetics strategies provide another powerful option for dissection and functional interrogation of circuits in animal models. Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) are the most commonly employed chemogenetic tools (43, 192, 242). DREADDs operate on the principle of activation of a genetically modified G protein-coupled receptor (GPCR), which can be expressed in neurons using transgenic or viral means, which is specifically activated via an exogenous ligand, namely, clozapine-*N*-oxide (CNO), and not by any endogenous ligand of the receptor. DREADDs come in many varieties; the

most commonly used ones are variants of excitatory human muscarinic receptors that couple to canonical $G_{q/11}$ signaling in the cell (hM3Dq, an excitatory DREADD) or to inhibitory $G_{i/o}$ signaling (hM4Di, an inhibitory DREADD). Thus exogenous supplementation of the ligand CNO permits activation or inhibition of neuronal activity reversibly over the period that CNO is available in the brain before being metabolized. At doses of CNO that are typically used, this affords a time window of a few hours over which functional consequences of DREADD-mediated neuronal activation or silencing can be studied in behavioral, electrophysiological, or imaging paradigms (47).

The DREADD technology offers several advantages. One, given that neuronal activation and silencing can be performed via systemic delivery of a drug, use of this method does not require major specialized equipment, e.g., as opposed to lasers and optic fibers employed in optogenetics. Animals are free to move and are untethered, whereby implementation in complex behavioral paradigms is facilitated. Second, as compared with optogenetics, the longer time frame of neuronal silencing or activation afforded by DREADDs enables testing prolonged paradigms in experiments, and by supplementing CNO via food, water, or implantable pumps, impact of manipulations can be studied over days to weeks. Third, unlike the use of lasers in optogenetics, there are fewer concerns about heating of tissue or toxicity to neurons with reasonable doses of CNO. As with optogenetics, regional or cell-type specific targeting is possible via regionally restricted expression of DREADDs using viral injections or cell type-specific Cre-expressing mouse lines.

Disadvantages of DREADDs include the fact that CNO is not the active principle (105), but breaks down to clozapine, which can have effects by itself owing to blockade of multiple neurotransmitter receptors (dopamine, serotonin, α1 adrenergic, histamine H1); in any case, every experiment with DREADDs has to be accompanied by a control group expressing a nonfunctional control protein, e.g., enhanced GFP (EGFP), and CNO delivery. A substantial source of variability is contributed by the inter-individual differences in kinetics of CNO breakdown and clearance of clozapine. A few studies have reported effects on DREADD expression in the absence of CNO, likely because G proteins and GPCR signaling effectors are sequestered away by expression of DREADDs in large quantities, thereby hindering endogenous GPCR signaling (245). Furthermore, the efficacy of a DREADD in activating or silencing neurons depends on the type of neuron and the availability of GPCR effectors, e.g., expression of GIRK channels determines whether $G_{i/o}$ signaling induces hyperpolarization. This could be a reason for a lower efficacy of DREADD-mediated silencing in cortical networks compared with optogenetics, but also disinhibition following prolonged neuronal silencing in cortical circuits could account for this observation.

In conclusion, the DREADD approach provides advantages for global, noninvasive, and long-term interrogation of freely behaving mice and can be considered a tool complementary to optogenetics.

IV. THALAMUS

A. Peripheral and Cortical Connectivity

1. Thalamus and pain

In the early 20th century, pain was believed to arise in the thalamus (117), consistent with the observation that cortical stimulation of somatosensory areas rarely caused pain (223). Today, we believe that pain is a percept created by the cortex with thalamic contributions; however, we still do not understand the cellular and circuit mechanisms, nor do we understand how cortex interacts with thalamus to produce the pain percept. This paragraph revisits the organization of the thalamus, the input it receives from the periphery, and its bidirectional communication with the cortex. Importantly, the thalamus is not just a relay for sensory information to the cortex, but also receives cortical inputs that outnumber peripheral inputs (108, 260, 261). More recent studies have shown a contribution of the thalamus to modulating cortical activity in perceptual decision-making, executive control, and attention (113). Yet, the mechanisms acting on the level of circuits remain poorly understood.

A thalamic function in acute pain is well documented by microstimulation and imaging studies in humans (170, 191, 298, 315), while its contribution to chronic pain is less clear-cut. Imaging studies typically do not reveal prominent contributions of the thalamus in chronic pain, which may relate to the event-based nature of the fMRI method (75). To circumvent this limitation, connectivity and default network analyses as well as other types of analyses of MRI data have been used, but typically do not reveal a consistent and prominent thalamic role (21, 118, 182, 71a). However, the following observations support a thalamic contribution to chronic pain states: *1*) chronic neuropathic pain was found to be associated with an ongoing decrease of thalamic activity (191) accompanied by an altered thalamocortical rhythm (301); *2*) chronic back pain was correlated with bilateral activity in the posterior thalamus (20); *3*) central post-stroke pain has been shown to be mediated by thalamus (153, 266, 293); *4*) thalamic microstimulation experiments revealed pain sensations in both nonpain and chronic pain patients (76); *5*) chronic pain was associated with a reduced thalamic volume and transmitter imbalances consistent with the loss of neurons (11, 110); and *6*) loss of thalamic somatosensory neurons affect the activity of tha-

lamic reticular nucleus neurons and ultimately may disturb the thalamocortical rhythm, thereby affecting pain perception (119). Hence, the available evidence suggests that the thalamus plays a role in generating chronic pain states, but it remains unclear which nuclei and circuitry are involved.

The evidence discussed so far entirely arose from studies in humans. How does this relate to rodents? Surprisingly, in mice, the anatomy of pain-related systems such as the spinothalamic tract, thalamic relays, and projections into cortex have been described by only a few studies (74, 250), while in rats, a more detailed description is available (e.g., see Ref. 318). To use rodents, and in particular mouse models, as a translational bridge, the thalamic contribution to pain needs to be understood in much more detail. Systematic connectivity studies (1, 132) will support this aim and provide a basis for systematic interrogation of circuit function in relation to behavior.

2. Sensory inputs to the thalamus and cortical targets

Nociceptive stimuli reaching the spinal cord via C or Aδ fibers formed by DRG cells are synaptically transmitted to dorsal horn neurons and relayed in multiple parallel pathways to multiple targets in the brain stem, diencephalon, and possibly even cortex (309). The logic behind these parallel information streams and their multiple targets remains incompletely understood, but may reflect the requirement to feed nociceptive information into different subsystems of the brain to orchestrate an overall homeostatic response. This needs to be kept in mind when considering the origination of different subjective qualities of the pain percept.

Nociceptive dorsal horn neurons send their axons via the spinothalamic tract to the posterior, medial, and lateral thalamus, but also to several other targets, including brain stem areas such as the reticular formation, A1 noradrenergic group, parabrachial nuclei, intercollicular area, and the mesencephalic periaqueductal grey (86, 100, 309) **(FIGURE 1)**. In the lateral thalamus (nomenclature according to Refs. 160, 193), ~5% of these fibers terminate onto neurons of the somatosensory ventral posterior lateral thalamic nucleus (VPL) and ~30% target the ventral posterior inferior nucleus (VPI) (86). Relay neurons of the VPL send their axons to the S1 cortex, while those of VPI target the S2 cortex. The VPL-S1 projection mediates the sensory-discriminative aspect of nociception ("when, where, how strong") **(FIGURE 1)**. The VPI-S2 connection contributes sensory-discriminative functions too but may also be involved in higher-order cognitive functions. The largest fraction of spinothalamic tract fibers (40%) targets the posterior group of thalamic nuclei **(FIGURE 1)**, including the posterior nucleus (PO), suprageniculate nucleus (SG), and the anterior pulvinar nucleus (PuA). The latter is also known as nucleus ventrocaudalis portae in primates and corresponds to the lateral posterior nucleus (LP) (329) or possibly the posterior medial nucleus (POm) (71) in rodents. PuA was found to be the most likely area underlying central post stroke pain (293) and revealed the largest responses when delivering painful stimuli to the skin (24). The posterior nuclei have widespread cortical projections, but relay neurons receiving spinothalamic tract inputs project to the posterior insula (86). This projection seems to contribute to nociceptive processing, cognitive aspects of pain, and the generation of the pain percept. With regard to the posterior thalamus, an area referred to as the posterior extension of the ventral medial thalamic nucleus (VMpo) was postulated to be the main mediator of nociception and thermoception (30, 68). The existence of the VMpo area has been intensely debated (142, 311), yet it appears that this area is not a distinct nucleus, but that it forms a continuum of a spinothalamic tract-innervated areas that extend from the lateral to the posterior thalamus. This debate has been eloquently summarized as a brief history of the "nociceptive thalamus" (100). Finally, ~25% of the spinothalamic tract fibers terminate in the medial thalamus, including the mediodorsal nucleus (MD), central lateral nucleus (CL), and the parafascicular nucleus (PF). Relay cells of these nuclei receiving inputs from the spinothalamic tract project mainly to motor areas of the cingulate cortex (86), but also to anterior parts of the cingulate and the PFC. Hence, these projections mediate motor aspects, attentional orienting, and the emotional component of the pain percept. The MD nucleus also receives nociceptive signals via the reticular formation, also referred to as the spinoreticular tract. Together, this "medial system" triggers activity in the ACC that contributes to the emotional and aversive quality of the pain percept.

Importantly, the pattern of cortical regions identified by connectivity analyses of the spinothalamic tract, its thalamic relays, and the cortical target areas corresponds perfectly well with the activation patterns found in a large meta-study on brain regions activated during acute pain (298, 315): thalamus, posterior insula, mid-cingulate cortex, and S2. The S1 cortex was possibly not evident in fMRI because of the small number of spinothalamic tract fibers contacting VPL relay neurons not producing a sufficiently strong signal in S1.

Another layer of complexity is added to the spinothalamic system when considering axonal projections of individual dorsal horn neurons situated in different laminas. Superficial lamina I and also lamina V neurons tend to project to lateral and posterior thalamic areas, while deep lamina VI-VIII mainly project to the medial thalamus (309). Given that action potentials triggered in dorsal horn projection neurons by synaptic inputs from Aδ fibers will reach the thalamus within tens of milliseconds after peripheral stimulation and that C fibers-triggered signals will need hundreds of milliseconds (24), any processing of coincidence in regions receiving both types of input will be difficult to achieve. Interestingly, the pain percepts triggered by the two

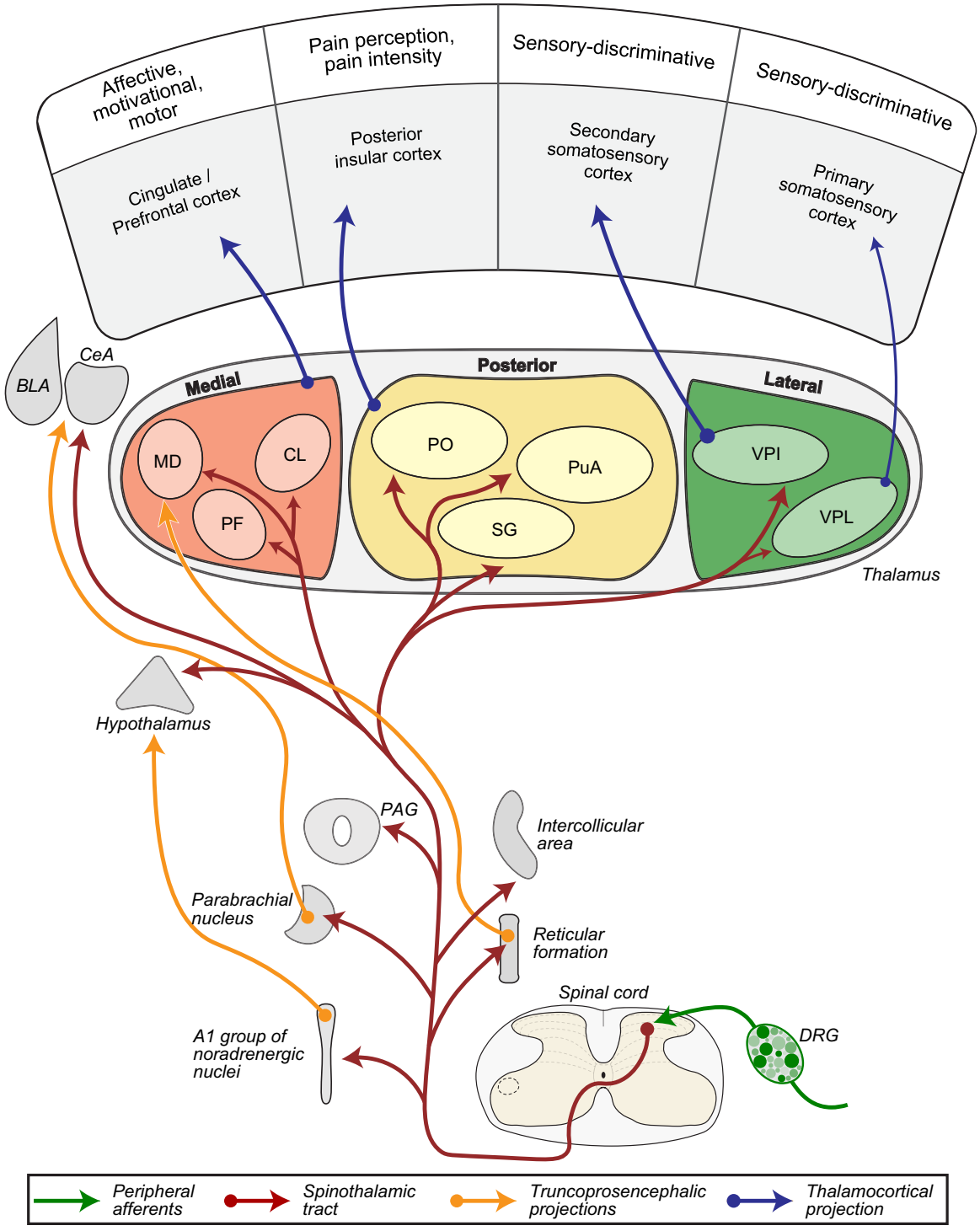

FIGURE 1. Scheme depicting spinothalamic tract signaling to thalamus and cortex. *Bottom*: the peripheral sensory inputs, the spinothalamic tract, and its terminations in the brain stem, including pain-relevant projections of brain stem nuclei to prosencephalic areas. *Middle*: the thalamic targets of the spinothalamic tract and connections to the amygdala. Only thalamic nuclei relaying spinothalamic tract inputs are shown. The trigeminothalamic tract and ventral posterior medial thalamic nucleus (VPM) are not shown for clarity. *Top*: the thalamic relay to cortex and functional modalities. CL, centrolateral nucleus; MD, mediodorsal nucleus; PF, parafascicular nucleus; PO, posterior nucleus; PuA, anterior pulvinar nucleus; SG, supragenicular nucleus; VPI, ventral posterior inferior nucleus; VPL, ventral posterior lateral nucleus.

systems differ, with A∂ fibers eliciting a sharp pain sensation and C fibers triggering dull pain. Whether this arises from different patterns of connectivity or neuronal activity remains unclear. The timing of nociceptive signals in the lateral (VPL), posterior (PuA), and medial (CL) thalamus has been determined in response to controlled noxious stimuli

in patients (24). Interestingly, voltage signals could be recorded with an identical onset latency of ~120 ms in all three regions, consistent with Aδ fibers mediating these inputs. Thus nociceptive signals are likely to reach their cortical destinations within a similar time window, predicting that this activity elicits the percept of sharp pain. Whether the delayed C-fiber response has similar properties remains to be studied.

Information carried by the spinothalamic tract indirectly reaches additional diencephalic and cortical areas via numerous brain stem targets **(FIGURE 1)**. Spinoreticular and spinomesencephalic axons may mediate attention and arousal. The spinoparabrachial axons relay nociceptive information via the parabrachial nucleus to the amygdala (27), thereby providing a direct pathway into the limbic system. A spinolimbic projection may even deliver nociceptive information directly to the amygdala (62). Furthermore, spinohypothalamic (324) and truncohypothalamic axons (220) may affect autonomic and endocrine functions via the hypothalamus.

The dorsal funiculus delivers epicritic information from DRG neurons via the gracile and cuneate nuclei and the medial lemniscus to the VPL, with axons terminating on neurons situated in the core region (309). These neurons project to the medial aspects of the S1 cortex. The core zones are embedded in the matrix zone that is populated by neurons receiving input from the spinothalamic tract, with leminiscal fibers largely outnumbering the spinothalamic fibers. One might speculate that this arrangement gives opportunities for a crosstalk between the nociceptive and the epicritic systems, although action potentials elicited in the periphery at the same moment in time will reach the thalamus at different time points, with C-fiber responses trailing A-fiber responses by hundreds of milliseconds (24).

A similar design described here for somatosensation from the main body applies to the skin covering the head and parts of the neck. Here, the ganglion cells corresponding to the DRG neurons of the spinal ganglion are situated in the trigeminal ganglion and synapse onto neurons of the spinal nucleus of the trigeminal system. These in turn project to the contralateral ventral posterior medial nucleus (VPM), from where the nociceptive information is ipsilaterally relayed to the lateral region of the somatosensory cortex.

In conclusion, nociceptive information is relayed by multiple parallel streams involving multiple regions **(FIGURE 1)**. As a simple approximation, two nociceptive streams contribute two major aspects of the cortical pain percept: the lateral system that mediates discriminative features and the medial system that mediates emotional aspects generating an aversive state and triggering motor responses (108). Following the segregation of the spinothalamic tract on the level of thalamus into three main components, one could also define a medial system for motor action and emotional aspects, the lateral system for the sensory discriminative aspects, and the posterior system for producing the primary pain percept and intensity of perceived pain. The latter proposal is consistent with the observation that microstimulation of the posterior insula is so far the only known cortical area resulting in the perception of pain (185, 214). The direct and indirect connections to the amygdala further contribute to the aversive/emotional representation of pain **(FIGURE 1)**. Importantly, numerous other systems such as the inputs to the hippocampus, hypothalamus, and potentially other pathways via the brain stem may also contribute to the multifaceted pain percept generated in the cortex **(FIGURE 1)**.

3. Cortical inputs to the thalamus

While this section focuses on the sensory inputs to the thalamus, another, more neglected, category of inputs to the thalamus needs to be strongly emphasized: corticothalamic projections (113, 259–261). These numerous projections typically originate in primary sensory cortices and project via two types of synaptic connections (see sect. IV*B1* for more details) to higher order nuclei of the thalamus. In turn, the relay cells of the higher order nucleus project back to the primary and secondary somatosensory cortices as well as primary motor cortex. The contributions of such corticothalamocortical loops to pain processing remain unknown, yet these loops are in a key position to compute differences between an initial representation generated in the primary somatosensory cortex and the ongoing stream of sensory inputs that reach the thalamus. It remains to be seen how thalamic nuclei receiving sensory input intersect with those fed by the cortex.

B. Pain-Related Thalamic Circuit Activity Studied in Rodents

1. Corticothalamocortical interactions

Somatosensory information relayed via the VPM and VPL nuclei to the S1 somatosensory cortex is processed in the upper layers and finally distributed through layer 5b pyramidal neurons to the posteromedial thalamic nucleus (POm) **(FIGURE 1)** with collaterals reaching the thalamic reticular nucleus as well as other targets outside of cortex and thalamus (268). The corticothalamic projection of layer 5 pyramidal neurons conveys activity to the POm relay neurons via driver synapses (259, 260). These are large synapses containing multiple release sites that can trigger large excitatory postsynaptic currents (107), which in turn depolarize the membrane and activate T-type Ca^{2+} channels (255). Therefore, a single presynaptic action potential can trigger several postsynaptic action potentials that are then relayed back to the S1 cortex, but also to the S2 and M1 cortices. Hence, somatosensory information

processed in the S1 cortex gets routed through the POm nucleus to the higher order S2 cortex and the primary motor cortex. In parallel, layer 6 neurons extend their axons to ventro-posterior thalamus (VP) and POm, yet forming small modulatory synapses preferentially on the distal parts of the relay cell dendritic tree.

Such corticothalamocortical loops originate in primary sensory cortices and in the prefrontal cortex, yet their function is far from clear. They provide another means of transcortical processing (261), but they could also integrate ongoing sensory inputs with information already processed by the cortex. For pain processing, it remains unclear to what extent layer 5 pyramidal neurons and subsequently POm neurons get activated by pain stimuli; the same is true for layer 6 projections. Changes in corticothalamic information transfer during acute pain states have been described using wire arrays implanted at S1, ACC, VP, and MD (305), suggesting that cortical neurons in the deep layers may have a role in pain processing that is waiting to be identified on the cellular level. In this context, it seems promising to study the mouse homolog of PuA, which could be part of the LP (329) or POm (71).

A similar loop circuit is established between the MD thalamic nucleus and the prefrontal cortex. As mentioned above, the MD nucleus receives nociceptive input via the medial pathway from the periphery and relays it to the cingulate cortex. A recent study has pointed to major differences in the functional role of the midcingulate and rostral pregenual domains of the cingulate cortex, showing that the former mediates nociceptive sensory hypersensitivity via its direct connectivity to the posterior insula, while the latter is pivotal for aversion and pain affect (276). From there, corticothalamic projections reach back to the MD nucleus. Interestingly, this specific pathway has been recently implicated to mediate chronic pain-related aversion (186). Optogenetically activating MD inputs elicited pain-related aversion in a sciatic nerve injury model and a chemotherapy-induced model. In these models, excitatory responses of ACC layer 5 neurons were smaller than controls. Intriguingly, inhibition of layer 5 neurons produced pain-related aversion. This study could demonstrate a specific role of corticothalamic projections to the MD in generating the aversive aspect of the pain percept in chronic neuropathic pain (186).

2. Intrathalamic interactions

Information processing within the thalamus is dominated by inhibitory interactions mediated by the thalamic reticular nucleus (TRN). Relay neurons of the VP projecting to S1 send collaterals to TRN GABAergic neurons. Likewise, layer 6 neurons in S1 that project to the VP send collaterals to TRN (108). This connectivity pattern constitutes a feedback (excitation from VP towards the cortex excites TRN neurons and therefore inhibits neurons in the VP) inhibition and could serve to attenuate incoming sensory information. The cortical layer 6 projection also forms a feedback system; however, in this instance, a feedforward inhibition onto thalamic relay neurons is established, resulting in an attenuation of cortical outputs flowing back to the cortex.

Chemogenetic and optogenetic activation of parvalbumin positive neurons in the rostro-dorsal TRN increased pain sensitivity by inhibiting neurons in the anterior dorsal nucleus and in the paratenial thalamic nucleus (176). These findings suggest an interaction of TRN and the limbic system, which in turn affects pain sensation. Another study found relieved thermal hyperalgesia in chronic inflammatory pain when optogenetically stimulating TRN neurons projecting to the VPL and VPM (321), while reduced GABAergic transmission promoted pain. These results are consistent with a study using voxel-based morphometry in chronic neuropathic pain patients, which found a significant loss in somatosensory thalamus volume concomitant with decreased activity in the TRN and S1 cortex as well as a reduction in thalamic inhibitory neurotransmitter content (119).

In addition to inhibitory inputs from TRN, several nuclei situated in the vicinity of the thalamus provide inhibitory projections: the zona incerta and the anterior pretectal nucleus (104). These inputs use giant GABAergic terminals harboring multiple synaptic contacts and can produce large postsynaptic signals; however, any relation to pain processing of these connections remains unknown. What kind of processing could be going on in thalamic relay neurons, given that there is barely any connectivity within individual thalamic nuclei? Relay neurons can switch between a tonic firing mode and a burst mode, a switch which is mediated by the setting of the resting membrane potential. At hyperpolarized potentials, the relay cells fire bursts, while at depolarized potentials they fire tonically. This switch in output characteristics could be modulated by the inhibitory connections mentioned above and the small modulatory synapses arising from layer 6 neurons. Such mechanisms may also modulate pain processing and will have to be addressed by future work.

3. Thalamic interactions with other brain areas

The anterior nucleus of paraventricular thalamus (PVA) is highly connected with diverse brain areas (see below). The PVA has been shown to produce mechanical hyperalgesia in chronic neuropathic and inflammatory pain models (54). Chemogenetic inhibition of PVA activity decreased hyperalgesia in pain models, while optogenetic stimulation of PVA neurons induced persistent hyperalgesia. PVA receives inputs from the central amygdala, and optogenetic stimulation of these inputs yielded hyperalgesia too. While these results are straightforward to interpret, it will be more challenging to understand the role of PVA neurons on a circuit level, because PVA receives input from many pain-related

areas (PB, mPRF, ACC, MD, PAG) and in turn projects to such areas (ACC, MD, and PAG) (54). Smart application of circuit mapping tools in mice will aid in dissecting such questions.

Optogenetic stimulation of the NAc alleviated chronic neuropathic pain induced by chronic compression of DRG (149). This effect is mediated by a striatothalamocortical pathway that normalizes activity in the VPL and may therefore represent a mechanism filtering information flowing into the thalamus via the spinothalamic tract en route to primary somatosensory cortex. A similar effect was found when optogenetically stimulating inhibitory ACC neurons, which resulted in a reduced activity of cells in the VPL/VPM (109). In addition to such "classic" thalamic filtering mechanisms, the thalamus can also redirect information from the amygdala to the secondary somatosensory cortex. For example, the central amygdala sends GABAergic projections to the PF, which in turn sends glutamatergic projections to the secondary somatosensory cortex (332). Interestingly, this connection has been shown to selectively mediate comorbid pain in depression (332). Here, the inhibition of the GABAergic cells in the central amygdala is enhanced in the depressed state, thus decreasing excitatory output from PF to S2, resulting in comorbid pain. When using chemogenetic or optogenetic approaches to interrogate this circuit, reducing central amygdaloid nucleus (CeA) activity or stimulating PF alleviated comorbid pain as quantified by a battery of behavioral tests (332).

V. ROLE OF LIMBIC AND REWARD CIRCUITS

The limbic brain, comprising the amygdala, the hippocampus, the ventral striatum, and cingulate cortex, is a key network regulating emotions, mood, and affect. Together with the ventrotegmental area (VTA) in the midbrain, these centers form the "reward pathway," called this based on the finding that rodents are willing to work and undergo behavioral tasks for electrical stimulation of these areas, similar to their behavior towards seeking a food or drug reward (92).

A. Duality of Reward and Aversion in Mesolimbic Circuitry

Surprisingly, both aversion and reward are mapped to these areas (92). At the macroscopic level of human fMRI studies, extensive overlap is observed between brain areas, including the mesolimbic circuitry, that are activated by pain signals and reward. The corticolimbic circuitry was also postulated to serve as a risk factor for developing chronic pain in clinical studies. A landmark finding in this regard was reported by Baliki et al. (18), who imaged patients with acute back pain patients and in a follow-up observed that the strength of the connection between the nucleus accumbens and the prefrontal cortex precede and predicted the transition of acute lower back pain to chronic pain, and was inversely proportional to resilience against pain chronicity. A subsequent study longitudinally tested subacute back pain patients over 3 yr and observed among factors promoting transition to chronic pain as opposed to recovery of the strength of long-distance connectivity across three macroscopic networks, namely, the dorsal medial prefrontal cortex-amygdala-accumbens, the ventral medial prefrontal cortex-amygdala, and the orbitofrontal cortex-amygdala-hippocampus networks (291).

Mesolimbic pathways involving both aversion and reward involve a dense interconnectivity between the hippocampus, amygdala, the nucleus accumbens (NAc), and the prefrontal cortex (106, 297). The striatum represents a large and complex hub, with diverse functionally heterogeneous subregions orchestrating processing of sensorimotor information, motor control, motivation and affect, and stress, among others. The NAc is located in the ventral striatum and receives dense convergent excitatory glutamatergic axonal input from the amygdala, hippocampus, and the prefrontal cortex and dopaminergic input from the VTA (106, 297). The VTA, with its dopaminergic projections to the cortex, the amygdala, and nucleus accumbens, comprises the center of the reward pathway (106, 297) **(FIGURE 2)**.

Apart from dopamine, several neuropeptide modulators, such as galanin, nociceptin, among others, act within the shell or core regions of the NAc to modulate diverse types of behavior (52) (see sect. XII). Medium spiny neurons of the shell of the NAc demonstrate increased firing during anticipation of reward, and the level of dopamine in the NAc shell is a key determinant of the reward-seeking behavior of the organism, with low levels leading to reduced motivation towards reward. The medium spiny neurons of the NAc shell are GABAergic projection neurons, which inhibit the activity of the globus pallidus in the basal ganglia, ultimately impacting on thalamocortical neurons **(FIGURE 2)**. The ventral pallidum is also a major output center of the NAc and projects, in turn, to the lateral habenula and VTA.

Accordingly, these cortico-mesolimbic loops have been implicated in a number of affective disorders, including addiction, depression, fear, and post-traumatic stress disorder. Their intricate links to pain were only appreciated more recently, since they were initially thought to be largely distinct from pain circuits. There were at least two salient but puzzling findings that changed the course of their analysis in the pain field, namely, *1*) that human imaging studies demonstrated significant parallels between alterations found in brains of drug addicts and brains of chronic pain patients, and *2*) a significant proportion of midbrain dopaminergic neurons, which were hitherto thought to be only recruited during reward prediction and expectation, were found to be directly activated by pain-eliciting cues. Today, it is appre-

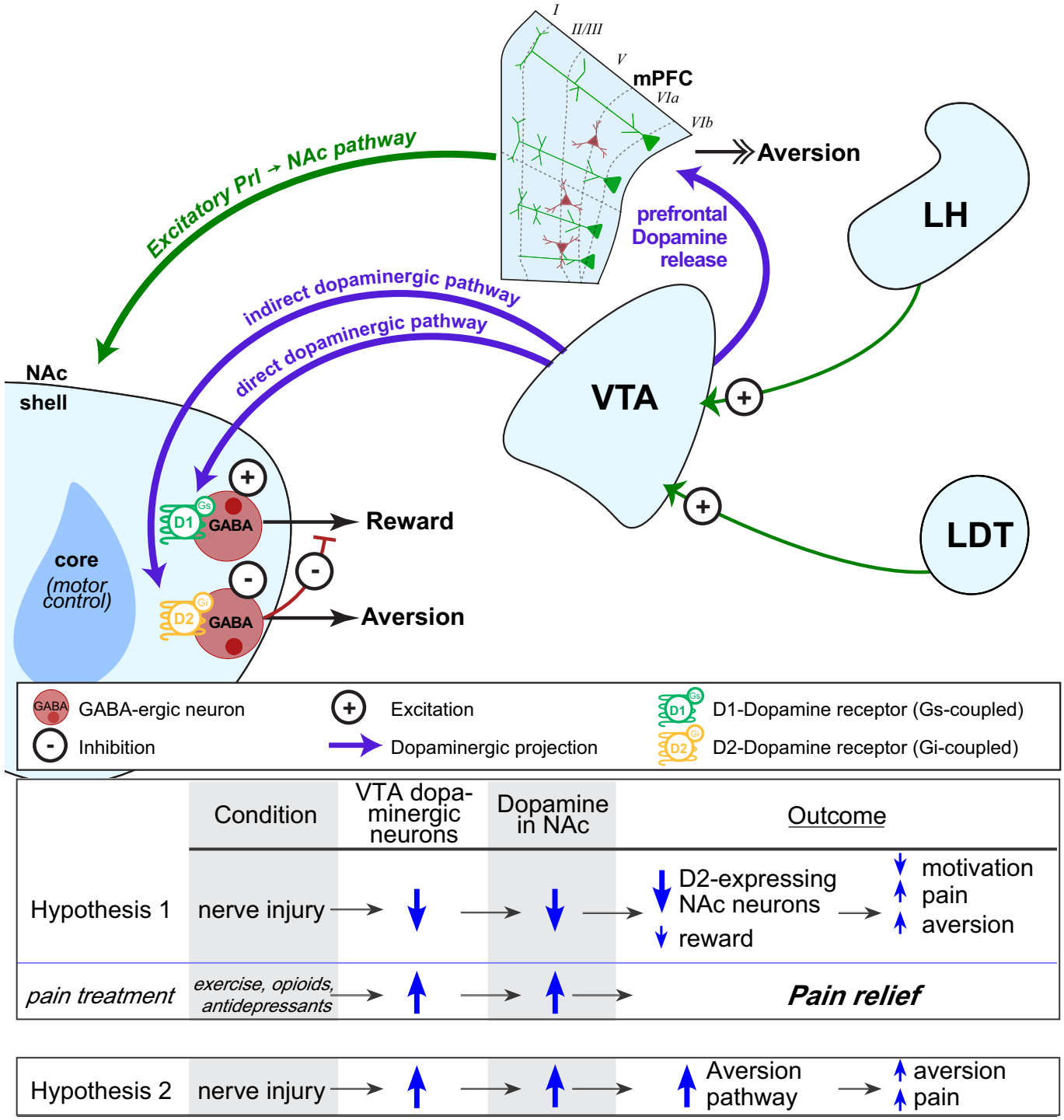

FIGURE 2. Duality of reward and aversion in mesocorticolimbic pathways and its impact on pain chronicity and motivation. Shown are dopaminergic pathways (blue arrows), originating from the ventrotegmental area (VTA), which regulate activity of nucleus accumbens (NAc)-pallidal circuits and prefrontal cortical activity, and their control, in turn, by loops with the lateral habenula (LH) and laterodorsal tegmentum (LDT). Ultimately, alterations in dopamine content in the NAc govern the balance between aversion and reward. D1/D2, dopamine receptor 1 or 2; mPFC, medial prefrontal cortex.

ciated that there are two parallel but functionally segregated circuits that mediate aversion and reward, demonstrating thereby the importance of elucidating pathways and circuits at the cellular level. Over the recent years, there has been an explosion of studies investigating cellular circuits in limbic and striatal pathways in animal models of

chronic pain. Below, we review recent progress made in animal studies on delineating cellular identity and functional specificity of the individual circuits in the main participating regions.

B. Ventrotegmental Area-Nucleus Accumbens Circuitry and Loss of Motivation in Chronic Pain

Understanding how mesolimbic circuits encode and delineate the mutually opposing functions of aversion and reward is a major current challenge in the neurosciences and a field marked by dynamic breakthroughs as well as controversial views. A major clue as to how specificity is reached came from a landmark study that demonstrated using optogenetics and transgenic tools with genetically labeled cell types that distinct subgroups of midbrain dopaminergic neurons are recruited in these opposing states (166). This duality of dopaminergic neuron populations of the VTA arises from two distinct input pathways to the VTA, namely, from the laterodorsal tegmentum and the lateral habenula **(FIGURE 2)**. The study found that VTA neurons projecting to the NAc shell receive projections from the laterodorsal tegmentum and elicit reward. In contrast, VTA neurons involved in aversion receive inputs from the lateral habenula and project to the PFC (166).

Another viewpoint for the encoding of both aversion and reward in VTA-NAc circuits is given by a new study which demonstrated that dopaminergic terminals of VTA projections in the ventral part of the NAc medial shell are selectively activated during exposure to aversive stimuli, while the corresponding dopaminergic terminals in the lateral part of the NAc shell were excited by cues predicting reward (77). Additional ground for duality in the opposing behaviors related to aversion and reward is given by two types of NAc GABAergic neurons receiving dopaminergic inputs, namely, neurons expressing either the excitatory G_s-coupled dopamine receptor D1 or the inhibitory $G_{i/o}$-coupled dopamine receptor D2. D1-expressing NAc neurons promote reward-seeking behavior while D2-expressing neurons inhibit it and promote aversion (297). However, a recent study has challenged the belief of segregation of encoding of reward or aversion via D1-expressing or D2-expressing medium spiny neurons of the NAc, respectively, suggesting instead that both cell populations can play either role depending on the duration of their stimulation (265). Brief versus prolonged stimulation of either population altered activity patterns in target regions, such as the VTA and the ventral pallidum, suggesting that caution should be exercised when comparing studies with diverse protocols and deriving inferences for real life situations from simulated experimental conditions (265).

Another breakthrough, particularly in understanding its significance in pain, was achieved by Schwartz et al. (252), who studied glutamatergic transmission at D1-expressing or D2-expressing NAc neurons, which receive excitatory projections from the PFC and the amygdala. Using a mouse model of chronic pain, they observed a marked reduction in the motivation of mice to work towards a food reward, although there was no change in basic food consumption. This was associated with a weakening of glutamatergic receptor-mediated transmission selectively in D2-expressing neurons, resulting in their long-term depression. Interestingly, this modulation of the synapse between glutamatergic incoming axons on cells expressing dopaminergic D2 receptors in the chronic pain state was mediated by galanin 1 (see sect. XII), thus demonstrating the intricacy of layers of modulation and interplay between glutamatergic, dopaminergic, and peptidergic systems. Of note, another neuropeptide, namely, prepronociceptin, was recently reported to be expressed in a subset of paranigral VTA neurons, whereby the peptide as well as this class of peptidergic VTA neurons were found to limit motivation for reward seeking (222); the significance of these findings to pain is not elucidated so far.

A number of subsequent studies validated the involvement of VTA-NAc circuits in acute and chronic pain. By directing the calcium sensor GCaMP6 to genetically identified dopaminergic neurons and recording their activity in awake, behaving mice using fiber photometry, Moriya et al. (195) further verified that acute aversive stimuli rapidly increase the activity of dopaminergic neurons in the VTA (195). The activity of the dopaminergic reward pathway from the VTA to the NAc shell is decreased in models of chronic pain, such as neuropathic pain and cancer-associated pain. Electrophysiological analyses have revealed that the intrinsic neuronal excitability of dopaminergic VTA neurons projecting to the NAc is reduced in mice with chronic pain (306). Directly optogenetically stimulating dopaminergic VTA neurons or their projections in the NAc transiently alleviated neuropathic allodynia, suggesting that loss of reward-related output in the VTA-NAc pathway contributes to the overall neuropathic pain state (306).

Inputs and outputs in the VTA or NAc in both reward and aversion circuits are also reported to be subject to modulation by pain relief strategies (204). Interestingly, consistent with the results of Lammel et al. (166) described above, Su et al. (267) found that administration of an AMPAkine, i.e., a drug that augments AMPA receptor function, in the NAc shell ameliorated mechanical hypersensitivity as well as depression-related behavior in a model of postoperative pain. Furthermore, the laterodorsal tegmentum-VTA pathway mediating reward is strengthened in exercise-mediated antinociception in rodent models of neuropathic pain (146), and accordingly, chemogenetic inhibition of VTA-NAc pathway was found to suppress exercise-induced antinociception (300). Pregabalin, a drug used to treat some types of neuropathic pain, was found to enhance dopamine levels in

the NAc at 17–20 days after spinal nerve ligation, but the effect tapered off later, suggesting that involvement of the reward pathway may change over distinct temporal phases of neuropathic pain (147). Moreover, there has been a string of new studies suggesting that the mesolimbic dopaminergic circuitry robustly alters efficacy of opioids and antidepressants used in the treatment of chronic pain in mouse models (189, 204).

However, in contrast to the studies discussed thus far, other studies have associated ongoing activity of the VTA-NAc pathway with maintenance of pain. Chang et al. (53) reported multiple adaptive changes, largely comprising macroscopic connectivity and gene expression of dopamine receptors in the NAc in correlation with mechanical allodynia in neuropathic mice; at the same time, however, acute local inhibition of the NAc via lidocaine injection alleviated allodynia, suggesting a role for ongoing NAc activity in promoting neuropathic pain. Moreover, in contrast to studies reporting decreased activity of the VTA-NAc pathway and decreased content of dopamine, a study reported higher extracellular dopamine levels and reduced expression of D2 in neuropathic rats (244). The same study found using electrophysiological analyses burst activity of VTA neurons was enhanced in neuropathic animals (244), which was also observed via c-Fos staining as a surrogate parameter for neuronal activation by Zhang et al. (322). The latter study also reported suppression of nerve injury-induced thermal hyperalgesia upon optogenetic inhibition of VTA neurons projecting to the NAc, proposing a role for brain-derived neurotrophic factor (BDNF) in the NAc in promoting neuropathic pain (322).

It remains unclear what factors lead to these apparent discrepancies across studies, which can include differences in models of neuropathy employed, types and modalities of behavioral readouts, and time points of analyses post-injury. Moreover, one study has found differences in molecular alterations in the NAc following neuropathy across mice with pain alone or mice with pain and defects in social behaviors (16), suggesting large variations in motivational-emotional states across mice with nerve injury. Importantly, given the knowledge on the mutually opposing roles of the different subgroups of VTA dopaminergic neurons and NAc neurons with D1 versus D2 receptor expression in reward and aversion, i.e., the so-called "direct" and "indirect pathways," respectively, it will be quintessential for studies on pain models to stringently adopt strategies that permit selectively studying these individual cellular subtypes and their connections rather than bulk activity. Along these lines, an elegant recent study by Ren et al. (237) observed that only the NAc neurons of the indirect pathway, i.e., those whose activity is repressed by VTA dopaminergic afferents via D2 inhibitory receptors, show enhanced intrinsic excitation following nerve injury. They went on to suggest that this occurs as an adaptation to reduced dopamine levels in neuropathic state. Selectively inhibiting these neurons chemogenetically alleviated neuropathic allodynia, and vice versa, their activation worsened allodynia. These results suggest that hyperactivity of D2 receptor-expressing neurons, which are involved in aversion, comes about after nerve injury and contributes to neuropathic pain.

A noteworthy new twist in the knowledge on the role of NAc shell neurons in pain and aversion was introduced by a recent study which showed that NAc shell neurons expressing the opioidergic neuropeptide dynorphin are both necessary and sufficient to drive negative affect in response to noxious stimuli (184). These neurons had been known to promote aversive behaviors and reduce rewards, and the study by Massaly et al. (184) uncovered their seminal relevance to pain.

How does the output of the NAc shell ultimately modulate pain? Much work is still needed to clarify the circuits downstream of the NAc. A few studies have employed optogenetic manipulations of the ventral pallidum, a target region of the NAc, to dissect circuits involved in reward and aversion. A model has been proposed in which glutamatergic neurons of the ventral pallidum promote aversive processing, while GABAergic neurons play a role in reward-associated behaviors (314). Both cell types project to the lateral habenula and the VTA, thereby "closing the loop" and suggesting that flow of activity in these mesolimbic loops ultimately enables expressing the appropriate adaptive response.

Thus, although critically important questions remain open regarding the differential possible roles of the VTA-NAc dopaminergic projections in pain relief (via augmenting reward) versus pain maintenance (via facilitating aversion), this appears to be an exciting area of research with fast-paced discoveries and significant potential for promoting treatment options.

C. Interactions of the Lateral Habenula with Circuitry for Reward and Aversion

The lateral habenula is a highly connected structure in the dorsal thalamus. It receives inputs from diverse forebrain structures, the basal ganglia, and the hypothalamus. In turn, it sends efferent projections to the PAG and the raphe nuclei in the brain stem and has accordingly been postulated to play a modulatory role on descending inhibition and facilitation of nociception (256). Based on its connectivity pattern, the habenula has been suggested to play a role in evaluation of external stimuli and processing of appropriate adaptive responses. Owing to its small size, it is not often included in human imaging analyses. However, a recent study has demonstrated its activation in response to noxious heat using high-field MRI in human subjects, which also revealed a significant connectivity with the basal gan-

glia and the PAG during processing of the noxious stimulus (257).

However, circuits within the lateral habenula have not been studied widely at the cellular level in the context of pain. A recent study using in vivo electrophysiological recording and fiber photometry in awake mice found that neurons in the lateral habenula are recruited in response to a wide variety of stress cues, including aversive and painful stimuli (302), while responsivity to reward cues was less efficient. Importantly, habenular neurons demonstrated experience-dependent plasticity, showing increased excitation in conditioning tasks associated with learning of stressors. In the context of repetitive exposure to noxious signals, this may place the habenula in an ideal position to sense potentially painful cues more quickly and activate circuits to develop adaptive or escape strategies.

Indeed, as described in the preceding section, the lateral habenula is known to be an important point of origin of the aversion pathway which projects to the mPFC over the VTA. Conversely, a recent study has shown that optogenetic stimulation of glutamatergic projections from the VTA to the lateral habenula elicits conditioned place aversion (240), suggesting that recurring activity between the lateral habenula and VTA loop may potentiate the organismal ability to respond to potentially harmful stimuli **(FIGURE 2)**. Overall, however, much remains to be dissected and discovered in the lateral habenula in terms of specific connectivity and functionality in pain. This would be promising given especially that it has been known for decades that electrical stimulation of the habenula induces robust analgesia (63).

D. The Hippocampus and Pain-Related Cognitive Decline

The hippocampus is the most-studied structure in the context of mechanisms of learning and memory and is believed to be the seat of contextual and short-term memories. Because chronic pain patients frequently demonstrate cognitive impairments and defects in working memory, much attention has been paid to characterizing hippocampal alterations. In an elegant study combining analyses in both patients and rodent models of chronic pain, Mutso et al. (198) reported reduced hippocampal volume in patients with two types of chronic pain, which was complemented by observations of reduced adult neurogenesis in animals with neuropathic pain. Reduction in hippocampal neurogenesis is exacerbated by chronic stress in neuropathic animals (238). Interestingly, Galhardo and colleagues (51) performed chronic multichannel electrophysiological recordings in hippocampal place cells of rats and observed that place cells become instable in neuropathic pain. This is complemented by reduced performance in spatial memory tasks and impairment of connectivity and coherence of activity between the hippocampus and the prefrontal cortex (50, 198) as well as reduction of some synaptic plasticity-related markers (198). In another study, although cognitive defects and impairment in memory tasks were reproduced in another model of neuropathic pain, no changes were observed morphologically in the expression and localization of synaptic proteins (194). A recent study has reported defects in long-term potentiation (LTP) and a loss of perineuronal extracellular matrix nets surrounding inhibitory interneurons in neuropathic animals, suggesting that disinhibition via extracellular matrix aberrations may underlie cognitive defects in chronic pain (273).

A new twist has been introduced by newer studies which suggest that, by regulating memory processes, the hippocampus may play an active role in modulating the perception of pain itself. Using antimitotic drugs and mouse lines with alterations in neurogenesis, a study reported causal associations between neurogenesis and behaviors related to persistent neuropathic pain (10). However, this needs additional verification owing to the lack of specificity of the manipulations, both in terms of anatomical locus and cell types affected. Along these lines, bilateral hippocampal injection of a mixed Aβ solution, which induced hippocampal pathology, and a learning impairment also reduced hypersensitivity in a model of inflammatory pain (180). This is also supported by imaging data from Bilbao et al. (28), who reported that glutamate levels are increased in neuropathic pain and stay high over the course of pain chronicity. It will be interesting in future studies to pinpoint the role of dorsal hippocampal circuits in pain chronicity using specific and cell-directed tools. Furthermore, neuroinflammatory mechanisms shared between the hippocampus and the spinal cord, such as involvement of tumor necrosis factor-α, have been reported and suggested to contribute to the frequent co-occurrence of pain and depression (177).

Analgesic drugs, such as opioids and cannabinoids, critically modulate diverse aspects of hippocampal function. One avenue of modulation occurs at the level of oscillatory activity, such as theta and gamma frequency rhythms, which are critical for the role of the hippocampus in cognitive processes. In the CA3 region of the hippocampus, as with the neocortex, these oscillatory rhythms are evoked by synchronous firing of the parvalbumin (PV)-expressing GABAergic interneurons, which are positively modulated by cholinergic inputs they receive (127, 270, 288). However, in the CA1 region of the hippocampus, an independent mechanism involving cholecystokinin (CCK)-expressing GABAergic interneurons was suggested to mediate theta oscillations in response to cholinergic stimulation (200). Interestingly, this role of CCK-positive interneurons is sensitive to modulation by cannabinoids and opioids (200). In CCK-positive GABAergic neurons, activation of type 1 cannabinoid receptors (CB1), located presynaptically on axon terminals, suppresses GABA release by inhibiting presynap-

tic N-type Cav2.2 calcium channels (271). Another avenue of modulation has been recently reported at the level of astrocytic function and its role in regulating LTP in the Schaffer collateral pathway (201). In contrast to the classical inhibitory actions of opioids on neuronal excitability, activation of M-opioid receptors in astrocytes of the Schaffer collateral pathway was found to boost LTP by enhancing release of astrocytic glutamate, and thereby stimulate memory acquisition as seen in behavioral conditioned place preference paradigms (201). Thus these studies suggest that diverse mechanisms of modulation of hippocampal function may contribute to the effects of cannabinoids and opioids on memory and cognition; testing their impact on pain-modulatory functions of opioids and cannabinoids would therefore be timely and relevant.

VI. ROLE OF THE AMYGDALA AND ITS CONNECTIVITY WITH THE PREFRONTAL CORTEX AND THE PAG

The amygdala is pivotal to emotion and motivational behavior. Two divisions of the amygdaloid complex bear particular relevance to pain, namely, the CeA and the basolateral amygdala (BLA) **(FIGURE 3)**. By sending projections via the ventral amygdalofugal pathway, they govern the activity of a number of regions, including the thalamus, the hypothalamus, the prefrontal cortex, the basal forebrain, the nucleus accumbens, the septal nucleus and brain stem centers that send descending information to the spinal cord and regulate autonomic outflow.

The lateral capsular region of the CeA is critical for the negative emotional aspects of pain and is also termed as the "nociceptive amygdala" (207). Although it receives inputs from various regions, two are of particular significance: *1*) excitatory inputs from the parabrachial nucleus (see sect. XII*A*) and *2*) inputs from the neighboring lateral BLA **(FIGURE 4)**. Information processing in the CeA is governed by interplay between local excitatory and various types of inhibitory neurons. The CeA forms the major output nucleus of the amygdala and is critical for providing information back to brain stem areas that control the expression of innate behaviors **(FIGURE 4)**.

The BLA is a major site of integration of polymodal sensory information, receiving input from the auditory pathway as well as a variety of noxious and non-noxious somatosensory stimuli. It plays a critical role in generating the fear response following exposure to a variety of potentially hazardous stimuli and is paramount for memory consolidation of cued fear. By conveying information to the CeA, it helps form responses to avoid injury or pain, which are then executed by brain stem centers. In addition, the BLA sends dense glutamatergic projections to the nucleus accumbens, the CA1 of the ventral hippocampus, and the prefrontal cortex, all of which are important in the regulation of motivated behavior **(FIGURE 4)**.

Here, we will review recent advances in our understanding of how the amygdaloid nuclei, in orchestration with the PAG and the prefrontal cortex, mediate and modulate distinct dimensions of pain.

A. CeA

The circuitry of the CeA in aversive learning and pain has been the focus of several recent studies, although major gaps still exist in our knowledge. The CeA comprises several distinct populations of GABAergic neurons, defined by their neurochemical identity (see sect. VIII), which have been the intense focus of optogenetic and chemogenetic dissection in the field of research on fear and anxiety. Input

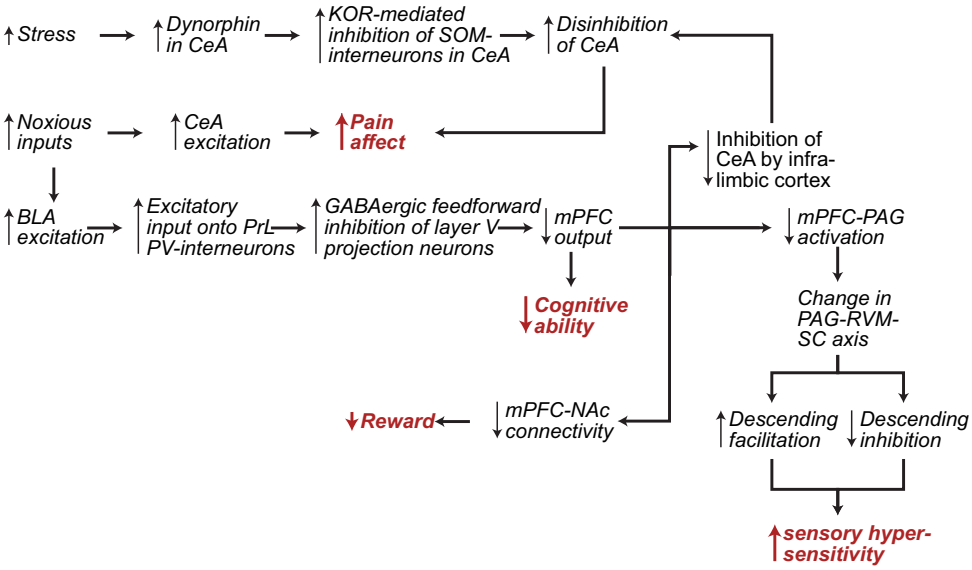

FIGURE 3. Flowchart demonstrating relationships between the activity status of the central and basolateral amygdaloid nuclei, output of the medial prefrontal cortex, and the brain stem axis for descending modulation. Their outcomes with respect to changes in sensory hypersensitivity, pain affect, reward, and cognitive functions in chronic pain are shown. BLA, basolateral amygdala; CeA, central amygdala; KOR, kappa opioid receptor; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; PAG, periaqueductal grey; PV, parvalbumin; RVM, rostroventral medulla; SC, spinal cord.

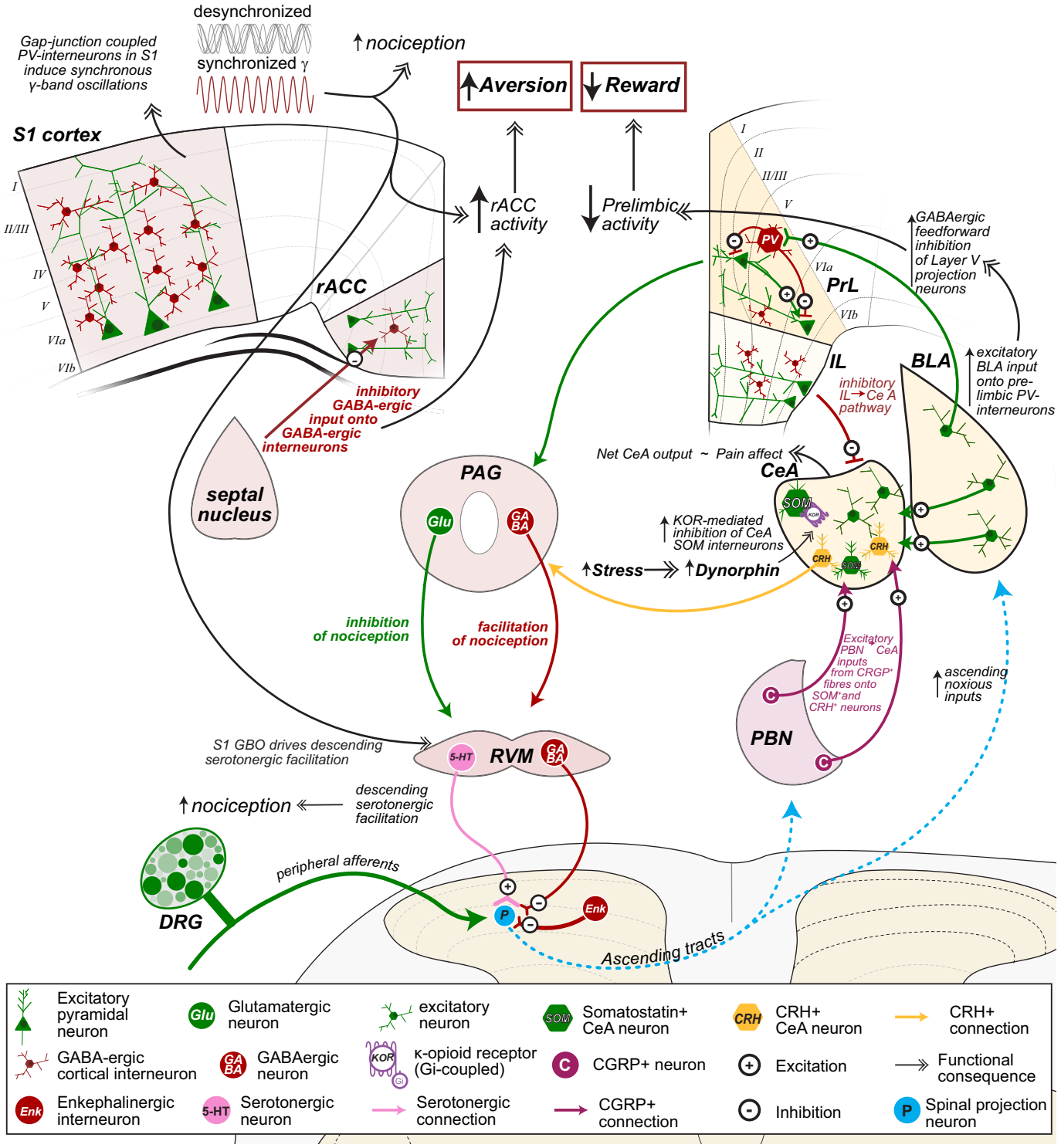

FIGURE 4. Current concepts on GABAergic modulation of brain circuits in acute and chronic pain. GABAergic neurons modulate neuronal activity locally in diverse cortical and subcortical areas involved in sensory and affective components of pain, generate gamma oscillatory rhythms, and participate in fine-tuning the balance between descending facilitation and descending inhibition of nociception via the periaqueductal grey (PAG)- rostral-ventral medulla oblongata (RVM)-spinal cord axis. The figure also shows integration of key GABAergic neurons in amygdala circuits mediating threat detection, threat memory, and pain affect. Abbreviations: BLA, basolateral amygdala; CeA, central amygdala; CGRP, calcitonin gene-related peptide; CRH, corticotropin releasing hormone; DRG, dorsal root ganglion; GBO, γ-band oscillations; Glu, glutamine; IL, infralimbic prefrontal cortex; NAc, nucleus accumbens; PrL, prelimbic prefrontal cortex; PV, parvalbumin; rACC, rostral anterior cingulate cortex; S1, primary somatosensory cortex; SC, spinal cord; SOM, somatostatin.

on noxious stimuli is brought to the lateral capsular region of the CeA by afferents from the parabrachial region (PBN), which is the main target of spinal lamina 1 projections (see sect. XII*A*). A very recent study by Li and Sheets (172) delivered seminal insights into the functional organization of the PBN-CeA pathway in terms of cell type specificity. Using a combination of optogenetic stimulation of incoming PBN afferents and molecular markers to identify diverse types of GABAergic interneurons in the CeA, the authors mapped direct monosynaptic connections from the PBN to lateral capsular, lateral, and medial subdivisions of the CeA. They found remarkable specificity of PBN inputs to two types of CeA neurons, those expressing somatostatin (SOM) and corticotropin releasing factor **(FIGURE 4)**. Noxious inputs are thus processed in the CeA as a part of a broader threat appraisal system (see sect. XII*A*). How inputs from BLA are processed in relation to inputs from the PBN has not been studied so far in the context of pain, to our information.

The net output of the CeA is determined by the balance between excitatory and inhibitory transmission, and emerging evidence suggests that this is subject to several types of modulatory influences and alterations in chronic pain **(FIGURE 3)**. The general principle is that aversive or potentially harmful experiences generate memory traces, and the strength of the memory increases in relation to the intensity of the aversive experience. Therefore, in chronic pain, enhanced inputs from the periphery and spinal networks provide scope for scaling up synaptic transmission in the PBN-CeA pathway. Analysis of membrane properties as well as synaptic transmission were indeed indicative of enhanced excitation in the CeA in models of inflammatory as well as neuropathic pain (115, 172, 262) **(FIGURE 3)**. Second, balance between excitatory and inhibitory transmission in the CeA is regulated by dynorphin, an endogenous opioidergic peptide that induces aversion and dysphoria in contrast to enkephalins and endorphins. Interestingly, dynorphin content is reported to be increased in the CeA following stress (203), suggesting that processing in the CeA may contribute to the observed exacerbation of pain by chronic stress. Dynorphin signals via kappa opioid receptors, and pharmacological experiments with locus-specific application have shown that dynorphin signaling promotes disinhibition in the CeA (205) **(FIGURES 3 AND 4)**. Behaviorally, this was shown to lead to a specific enhancement of the aversiveness of pain, while there was no effect on sensory hypersensitivity, e.g., mechanical allodynia seen in neuropathic rodents. This suggests that the CeA is pivotal in the generation of percepts related to the negative emotional component of pain.

A recent study, however, suggests that the role of the CeA in pain is not mono-dimensional, but rather that the CeA can bidirectionally modulate nociception (312). Specifically, these dual and opposing functions were shown to be associated with two distinct classes of GABAergic neurons that receive inputs from the PBN, namely, protein kinase C-delta-expressing neurons, which enhance nociception, and somatostatin-expressing neurons (SOM), which have antinociceptive functions (312). The study demonstrated that following nerve injury, the activity of protein kinase C-delta-expressing neurons is enhanced, while that of SOM neurons is suppressed in the CeA, leading to a net increase in nociceptive sensitivity (312).

Another route via which the CeA may contribute to chronic pain is via its paramount role in anxiety. Anxio-depressive behaviors not only accompany chronic pain as frequent comorbidities, but also serve as predisposing factors for pain chronicity. It remains to be determined how the CeA contributes to mutual interactions between pain and anxiety.

The PAG and the RVM are critical for mediating behavioral coping responses in the face of threat and thereby constitute key target structures of CeA output activity (285) **(FIGURE 4)**. Descending modulation of spinal nociceptive processing by CeA-PAG connections has been implicated in acute stress-related analgesia as well as antinociceptive effects of opioids acting locally in the BLA, and modulation of noradrenergic pathways has also been discussed (181). Like the PBN-CeA input pathway, the CeA-PAG output pathway is also subject to context-dependent plasticity. For example, Ozawa et al. (217) report that expectation can diminish the strength of aversive memory by activating a descending feedback circuit from the CeA to the PAG. The CeA-PAG pathway is also altered in states of alcohol dependence and mediates alcohol withdrawal-induced hyperalgesia (17). Moreover, membrane properties of CeA-PAG synapses are altered in mouse models of inflammatory pain (171). Another pathway via which the CeA regulates descending inhibition is via its projections to the locus coeruleus (LC); specifically, suppression of activity of CeA neurons expressing corticotropin releasing factor (CRF) and their projections to the LC have been linked to enhancement of descending inhibition and reduced allodynia, suggesting, via extrapolation, that potential enhancement of activity of CRF neurons in the CeA may lead to a loss of descending inhibition in chronic pain (7).

Taken together, these results suggest that the CeA is not only a critical mediator of the emotional component of pain, but can also contribute to adapting spinally mediated behavioral responses by recruiting descending pathways mediating endogenous analgesia.

B. BLA

As with the CeA, the BLA directly contributes to the emotional responses to noxious stimuli and can indirectly influence pain chronicity by virtue of its involvement in fear

memory. Although it is widely accepted that the BLA is activated by noxious stimuli as well as by other sensory inputs, there are conflicting reports on whether it actually modulates the sensory component of chronic pain, i.e., hyperalgesia and allodynia. One study noted that complete chemical ablation of the BLA, but not of the CeA, by site-specific stereotactic injections of an excitotoxin reduced mechanical allodynia when it was performed before nerve injury; however, when the BLA was ablated post-establishment of allodynia, there was no effect (175). Other studies with reversible modulation of BLA activity have not observed changes in sensory sensitivity; however, since only time points post-injury were tested, this point remains to be resolved. A noteworthy study by Corder et al. (67) employed in vivo imaging to track changes in activity of populations of neurons in the BLA and identified subsets that are specifically activated in correlation to pain affect **(FIGURE 3)**. Following nerve injury, these subsets could be activated by innocuous stimuli, suggesting plasticity of encoding in the BLA. Silencing their activity attenuated behavioral correlates of pain affect without modulating sensory hypersensitivity (67). These findings emphasize the importance of the BLA in encoding pain-related negative affect. Since the BLA was not silenced in total in this study, it remains unclear whether other neurons in the BLA may contribute to sensory hypersensitivity.

Apart from the CeA, an output target of the BLA that is pivotal to pain perception is given by the medial prefrontal cortex (mPFC) **(FIGURE 4)**, which processes ascending noxious inputs and is widely implicated in both sensory and affective aspects of pain. Seminal work from Neugebauer and colleagues (137) has established that hyperactivity of BLA neurons in chronic pain states leads to deactivation of the mPFC. In a model of chronic arthritic (inflammatory) pain, the authors noted that activity of pyramidal neurons of the prelimbic cortex, the mouse homolog of the human dlPFC, is decreased in a manner that could be reverted by blocking BLA activity. This was accompanied by improvement of chronic pain-associated cognitive decline (137) **(FIGURES 3 AND 4)**. This principle has been tested in several additional studies, and it was revealed that PV-type GABAergic neurons in the prelimbic cortex are activated by inputs from BLA projections, i.e., feed-forward inhibition (327) **(FIGURE 4)**. A very recent study has further elucidated the underlying mechanisms, showing that reduced endocannabinoid modulation in the mPFC accounts for enhanced feedforward inhibition of mPFC neurons receiving BLA inputs (129). Neugebauer and colleagues (154, 269, 283) have demonstrated that signaling via metabotropic glutamate receptors also plays an important role in amygdala-driven inhibition of the PFC. Electrophysiological experiments demonstrated that mGluR1 receptors in the PFC decrease excitability of prefrontal pyramidal neurons in a glutamate- and action potential-dependent manner, indicating feed-forward inhibition (269). In contrast, selective pharmacological activation of mGluR5 increased pyramidal neuron activity in the infralimbic PFC via an endocannabinoid-dependent mechanism (154). Enhancing endocannabinoid signaling in the infralimbic PFC, but not in the anterior cingulate cortex, restored PFC output and alleviated both pain and cognitive deficits in arthritic rats (154).

The amygdala and the mPFC reciprocally modulate each other, and it has been suggested that the deactivation of the mPFC in chronic pain reciprocally attenuates inhibitory control within the amygdala, thereby enhancing its activity. A recent study has clarified the underlying circuitry, showing that the infralimbic division of the mPFC inhibits the CeA to a stronger extent than the prelimbic division; therefore, a loss of this inhibition in chronic pain leads to enhanced function of the CeA, resulting in enhanced pain affect (155) **(FIGURE 4)**. These findings would indeed support the concept proposed from human imaging literature in chronic pain, that limbic circuits mediating emotion gain prominence over nociceptive circuits (165).

A major subpopulation of mPFC neurons project to the PAG **(FIGURE 3)**. Because the PAG is involved in both descending inhibition and descending facilitation, a change in mPFC activity in chronic pain can potentially have multiple implications. Cheriyan and Sheets (60) directly tested the alterations in mPFC neurons projecting to the PAG in a model of neuropathic pain, using uncaging of glutamate to test activation properties of PAG-projection neurons of the mPFC, identified via retrograde labeling. In electrophysiological experiments, they observed layer-specific changes, including a significant reduction in the excitability of layer 5 neurons projecting to the PAG in mice with nerve injury. As with many studies on cortical circuits, they observed bilateral changes despite the unilateral nature of nerve injury, consistent with many clinical neuropathic pain states. In an elegant series of experiments, Zamponi and colleagues (129) demonstrated that simulating the activity of the BLA-mPFC-PAG pathway via optogenetics facilitates nociceptive hypersensitivity in neuropathic mice by engaging descending noradrenergic and serotoninergic pathways to the spinal cord.

These preclinical studies bear significant clinical relevance, since altered connectivity of the amygdala, prefrontal cortex, and the PAG has also been reported in clinical studies in diverse forms of chronic pain. This includes episodic migraine and migraine without aura (58, 174), which was further consolidated in a rat model of recurrent headache (139), trigeminal neuralgia (325), fibromyalgia (289), and chronic back pain (319). Moreover, they provide a mechanistic model for explaining how altered amygdala activity and connectivity with the PFC-PAG pathways in chronic pain states can augment both the sensory component of pain as well as pain affect. Moreover, these results implicate

the amygdala in cognitive comorbidities in chronic pain. It will be interesting to test whether similar principles apply to anxio-depressive behaviors, which also accompany chronic pain. Although amygdala circuits are paramount in fear and anxiety, their implications in anxio-depressive behaviors as pain comorbidities in models of chronic pain have not been widely tested. A recent study by Zussy et al. (335) is noteworthy in this regard. Reporting that the metabotropic glutamate receptor 4 ($mGlu_4$) is expressed in the CeA, but not in the BLA, the authors employed an optopharmacological approach to address whether $mGlu_4$ regulates sensory and emotional behaviors. By selectively and repeatedly manipulating activity $mGlu_4$ activity in the CeA with light, they observed that activation of $mGlu_4$ abolished not only inflammatory mechanical allodynia, but also reduced anxiety and depression-like symptoms (335). The specific localization pattern and electrophysiological analyses suggest that this results from modulation of polymodal sensory information from the thalamus by $mGlu_4$ rather than by modulation of purely nociceptive information from the PBN (335).

It is also important to clarify that the BLA-driven feedforward inhibition is not the only driver of prefrontal deactivation in chronic pain. Several other mechanisms have been proposed, including intrinsic changes in pyramidal neurons, such as changes in hyperpolarization activated cyclic nucleotide-gated currents (66), structural rearrangements such as reduced dendritic branching in layer 5 neurons of the PFC (150), involvement of the enzyme cdk5 (303), changes in endocannabinoid signaling (129), and reduced cholinergic inputs (233) among others. For most of these, it remains to be determined whether they result from alterations in amygdala inputs to the mPFC or arise from other circuit or cell-autonomous alterations.

VII. ROLE OF THE FUNCTIONAL CONNECTIVITY OF THE PREFRONTAL CORTEX WITH NUCLEUS ACCUMBENS

In the preceding sections, we discussed how NAc plays a critical role in reward and aversion in chronic pain. We then scrutinized how the BLA plays a critical role in determining the activation status of the mPFC in chronic pain conditions. With this knowledge in hand, one can now "close the loop" and discuss how these alterations in the mPFC impact back on cortico-striatal functional connectivity and ultimately distort activity of the NAc and reward pathways **(FIGURE 3)**. Two studies from Wang and colleagues (169, 328) are particularly insightful. To directly test the predictions made by the findings of Baliki et al. (22) regarding alterations in strength of mPFC-NAc connectivity as a predisposing factor for pain chronicity, Lee et al. (169) employed optogenetic tools to directly activate the prelimbic division of the mPFC or projections from the mPFC to the NAc in rats with nerve injury and observed alleviation of sensory hypersensitivity as well as behaviors related to pain affect. To address whether this pathway is recruited endogenously during acute pain, the group also optogenetically inhibited mPFC-NAc projections and observed augmentation of nociception as well as aversion; moreover, pain affect in a neuropathic model was further enhanced (328). These results are noteworthy for a number of reasons. First, they demonstrate the utility of rodent models and novel circuit dissection strategies towards causally supporting the correlative observations made in human studies. Second, they strengthen the hypotheses on pivotal involvement of the corticostriatal circuitry in conscious pain as well as pain chronicity (18).

Since then, additional studies have implicated PFC-NAc connectivity in alterations in reward processing in pain states. Recently, CRF expressing neurons in the mPFC and their projections to the NAc, which activate NAc neurons, have been linked to facilitating opioid-induced reward in neuropathic pain (145). Thus CRF acting on CRF receptor 1 on NAc neurons sensitizes behavioral responses to morphine (145), which may have implications for opioid dependence in chronic pain patients. Moreover, projections from the infralimbic part of the PFC to NAc have been implicated in governing the balance between drives to avoid pain and obtain reward during decision-making, which is modified in chronic pain states (251).

Thus the PFC acts as a convergence point of the BLA-mPFC-PAG circuitry, which elicit prefrontal deactivation and regulates nociception, pain affect and cognition, and the PFC-NAc circuits, which modulate reward and avoidance. It will be interesting in future studies to test whether PFC neurons participating in these pathways are segregated or shared.

VIII. GABAERGIC CONTROL OF BRAIN CIRCUITS IN PAIN

Flow of signals in local neuronal circuits as well as large cross-regional networks in the brain are sculpted by GABAergic neurons. GABAergic inhibition can come about via an intriguing diversity of GABAergic interneurons with distinct properties, connectivity, and function, not only at the local level, but also via long-range axonal contacts between brain regions (49).

GABAergic neurons constitute ~20% of cortical neurons, and at least five main classes of GABAergic neurons are known in the cortex, including those expressing parvalbumin (PVs), somatostatin (SOMs), vasoactive intestinal peptide, as well as neurogliaform cells (127, 270, 288). The largest class is represented by PVs, which are further subdivided into basket cells that target soma and proximal dendrites of excitatory neurons and chandelier cells that target the initial axon segment (127, 270, 288). In several cortical

layers, the probability of connection of GABAergic neurons to neighboring principal cells (excitatory) is close to 100%, explaining why these cells can profoundly shape activity despite their relative sparsity (127, 270, 288). PVs receive direct input from the thalamus, local excitatory neurons, and other types of GABAergic neurons and are capable of firing rapidly and with a high temporal precision, stabilizing activity in cortical networks (127, 270, 288). The somatostatin-expressing class of GABAergic interneurons (SOMs), including Martinotti cells, constitute another major class (127, 270, 288). They receive input from local principal neurons, but not directly from the thalamus (127, 270, 288). SOMs target dendritic tufts of principal cells and inhibit other interneurons, particularly PV neurons (127, 270, 288). Fast-spiking PV cells are the most characterized subtype of GABAergic neurons (127, 270, 288). They demonstrate the intriguing property of being highly interconnected via gap junctions (49). In the neocortex, gap junctional transmission between individual pairs of interneurons results in a net increase in excitatory drive as well as enhanced timing of action potential generation (49, 127).

GABAergic interneurons play a critical role in shaping various brain functions, including learning and memory, anxiety, sleep, and cognition (49, 127, 288). In the spinal cord, disinhibition has been proposed as a mechanism for chronic pain, particularly in the context of nerve injury-induced mechanical allodynia. In contrast, the understanding of how inhibition shapes neuronal activity in the brain in pain states is still sparse. Although previous studies have tested microinjection of drugs acting at GABA receptors in some brain areas, a circuit and cellular understanding of the nature and functions of specific local and long-range GABAergic circuits in the brain in pain states is only recently beginning to emerge. Here, we will largely focus on new studies addressing cell-specific tracing of connectivity and cell-specific tools for interrogating GABAergic circuits, such as optogenetics and chemogenetics, with an emphasis on subneocortical structures, such as the thalamus, VTA, PAG, and RVM. However, given that GABAergic inhibition is most intensely studied in the neocortex, we have also touched upon a few salient insights gained from recent studies on GABAergic circuits linking the neocortex with subneocortical structures.

Some recent studies have delivered salient insights on the contribution of GABAergic inhibition in the nociceptive arm of pain networks, including the thalamus, and the descending pathways affecting spinal nociceptive processing, including the PAG and the RVM **(FIGURE 4)**. As discussed in sections IV and V above, GABAergic control of activity in the thalamus is tantamount for its key function as a filter as well as relay station. There is a remarkably high density of GABAergic neurons in the adjoining TRN, which receives input from the cerebral cortex and thalamus and sends inhibitory GABAergic projections back to the thalamus. Specific chemogenetic activation of GABAergic neurons in the TRN has recently been reported to facilitate nociception, suggesting that the TRN participates in a pro-nociceptive loop instead of inhibiting pain (176). In contrast, in another study, optogenetic activation of afferents from the TRN to one of its target zones, the ventrobasal thalamus, inhibited inflammatory hypersensitivity, putatively via restoration of thalamic GABA levels that are depleted in inflammatory pain (321). Overall, it is evident that multiplexed modulatory mechanisms are in play over diverse thalamic centers, and a precise elucidation of the highly complex circuitry of excitatory and inhibitory neurons is still pending.

A large body of literature indicates that GABAergic interneurons are key determinants of the integrity of synchronous activity patterns in the brain and have been implicated particularly in the emergence of slow brain rhythms, including both theta and gamma oscillations (49). Although this may appear counter-intuitive, given that GABAergic transmission depresses neuronal firing, gamma activity entrained by PV neurons involves synchronous activity of excitatory (pyramidal) neurons **(FIGURE 4)**. Gap junction coupling between PV neurons and the resulting synchronicity of their firing is critical for this modulation (49). This is relevant for pain processing, because in human subjects, oscillatory activity rhythms in cortical networks are emerging as a critical correlate of pain perception and processing (196, 227); however, underlying mechanisms and causal contributions were unknown so far. Unexpected insights have come from a study on activity patterns in the S1 neocortex and the role of local PV fast-spiking interneurons. Tan et al. (275) demonstrated a high level of preservation of changes in oscillatory activity across rodents and humans in pain in the S1 cortex and reported that gamma band activity in the S1, but not other oscillatory rhythms, are specifically elevated in power when a particular stimulus was noxious and elicited nocifensive behaviors, independently of motor activity (275). Moreover, gamma activity could be elicited by subthreshold stimuli in the context of mechanical allodynia, and nociceptive stimulus-induced gamma rhythms were strengthened in inflammatory pain. Importantly, using optogenetic activation of PV neurons to entrain gamma rhythms, the study went on to directly demonstrate that gamma activity initiated by local GABAergic neurons in the S1 facilitates nociception (275). Viral tracing and pharmacological manipulations revealed that this pronociceptive modulation results from recruitment of descending serotonergic facilitatory pathways originating in the RVM by the enhanced output of the S1 cortex **(FIGURE 4)** (276).

GABAergic mechanisms are being increasingly studied in prefrontal regions that have been functionally linked to emotion, aversion, and affective states, namely, the rostral ACC and the prelimbic cortex **(FIGURE 4)**. GABAergic inhibition with the medial septum and its downstream modulation of activity in the ACC were shown to participate in

the pain-induced conditioned pain aversion, suggesting a modulation of pain affect (8). In the rostral ACC, frequency of miniature and spontaneous inhibitory postsynaptic currents in pyramidal neurons was depressed in inflammatory pain (157), suggesting that presynaptic GABAergic plasticity promotes ACC activity in inflammatory pain. In contrast, a number of studies have reported inhibition of the medial prefrontal cortex in chronic pain via GABAergic feedforward inhibition, which is linked to pain affect. The most direct evidence for GABAergic contribution is given by experiments performed by Zhang et al. (327) showing that optogenetic stimulation of PV-type GABAergic interneurons in the prefrontal cortex elicits behaviors associated with pain affect **(FIGURE 4)**. Following nerve injury, the activity of prefrontal GABAergic neurons is fostered by incoming projections from the basolateral amygdala that are under cannabinoidergic control, which in turn elicits decreased firing of pyramidal neurons leading to prefrontal deactivation (327) **(FIGURE 4)**. Notably, optogenetically entraining gamma oscillatory activity in the S1 cortex via synchronous activity of S1 PV-type GABAergic interneurons was also found to elicit conditioned place aversion and alter activity in the rostral ACC and the prelimbic cortex (275) **(FIGURE 5)**. This suggests that activation of the primary S1 cortex via ascending nociceptive thalamocortical activity can further recruit prefrontal cortical circuits via gamma oscillatory rhythms.

GABAergic modulation in the amygdala and hippocampus was discussed in the preceding sections. Moreover, as already introduced in the sections on reward circuitry, GABAergic mechanisms are increasingly coming into focus in areas such as the VTA, the DRN, and the NAc, where they modulate the balance between aversion and reward. GABAergic neurons modulate VTA function to drive aversion via at least three different mechanisms. *1*) Within the VTA, GABAergic neurons get activated in response to painful stimuli, such as foot shock, and elicit conditioned place aversion by inhibit dopaminergic neurons of the VTA via $GABA_A$-mediated hyperpolarization (274). *2*) GABAergic neurons in the NAc are targets of dopaminergic projections from the VTA, which co-release glutamate (232). Stimulation of these VTA dopaminergic-glutamatergic fibers drives aversion by activating PV-type GABAergic neurons in the NAc, which, in turn, inhibit medium spiny output neurons of the NAc (232). *3*) GABAergic neurons of the rostral VTA project to the DRN and preferentially inhibit local GABAergic neurons in the DRN, leading to disinhibition of DRN serotonergic neurons and thereby to aversion (173).

GABAergic connectivity across the VTA-DRN has also been implicated in opioid dependence. Li et al. (173) demonstrated that in contrast to the aforementioned GABAergic neurons in the rostral division of the VTA, GABAergic neurons of the caudal VTA directly synapse on and inhibit serotonergic neurons of the DRN. Optogenetic stimulation of this pathway promotes reward. Morphine depresses GABAergic inhibition on serotonergic neurons in this caudal VTA-DRN pathway, thereby suggesting a role in opioid dependence (173).

Another brain region in which GABAergic circuits have been studied in the modulation of nociception is the ventrolateral periaqueductal grey (vlPAG), the origin of descending bulbospinal inhibitory control. Consistent with a large volume of literature from pharmacological manipulations and electrophysiological analyses, a recent study showed that global chemogenetic manipulation of vlPAG neurons suppressed nociception (247). Intriguingly, employing cell type-specific chemogenetic modulation in the vlPAG revealed that excitatory neurons inhibit and inhibitory neurons promote nociception, suggesting that balance between excitation and inhibition within the microcircuitry of the PAG is of paramount significance (129, 247) **(FIGURE 4)**. Because the vlPAG directly determines the activity of the NRM and the LC, alterations in the net vlPAG output may play a pivotal role in the dysbalance between descending inhibition and descending facilitation via noradrenergic and serotonergic pathways that has been linked to nociceptive hypersensitivity (see sects. IX and X). Indeed, there are indications that GABAergic inhibition in the vlPAG is altered in rodent models of chronic pain. In rats with neuropathic pain, presynaptic GABA release was reported to be increased, which should, by projection, lead to a reduction in the strength of vlPAG-mediated descending inhibition and analgesia (112). In contrast, another study reported that in conditions of inflammatory pain, tonic $GABA_A$ currents are decreased in female, but not in male, rats (284). While Hahm et al. (112) did not see changes in morphine-induced inhibition of GABA currents in electrophysiological experiments in the vlPAG of inflamed rats, Tonsfeldt et al. (284) observed stronger morphine-induced antinociception in female rats than in males in behavioral experiments (112, 284). Modulating the strength of GABAergic inhibition via positive allosteric modulators or antagonists, depending on the nature of the pain state, may thus offer a therapeutic window.

Finally, we would like to discuss two conceptual peculiarities of GABAergic circuits, which are only beginning to be addressed in the context of pain. First, although a majority of GABAergic interneurons modulate local activity, there is increasing evidence that long-range GABAergic neurons couple brain regions that are functionally related. The coupling is frequently found to be bidirectional, e.g., such as between the septum and hippocampus or the entorhinal cortex and the hippocampus (49). Although not well-studied so far, long-range GABAergic inhibition may play a major role in pain-related networks since long-range inhibitory networks are ideally suited to coordinate the activity between several brain areas. An intriguing property of this cell type is that long-range GABAergic projections typically

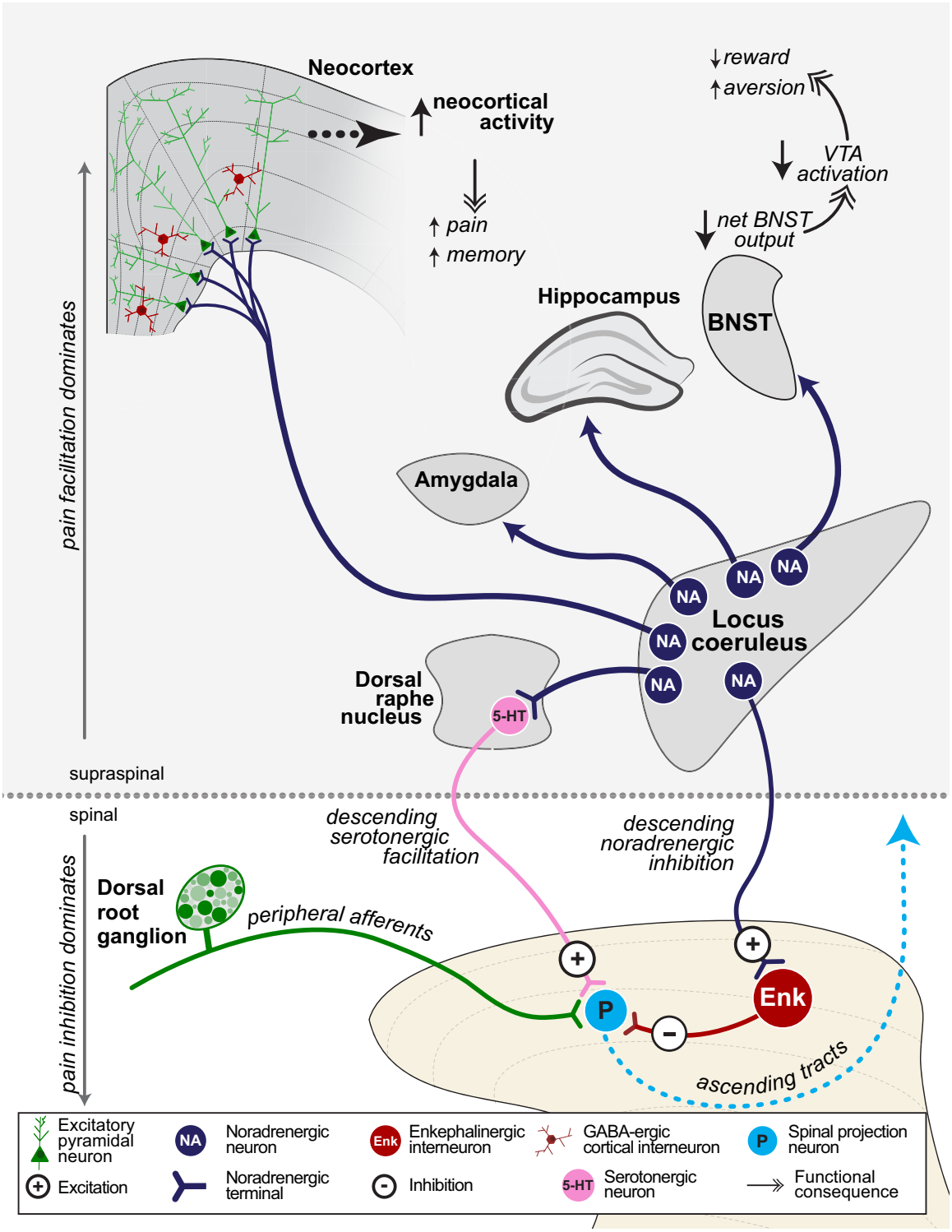

FIGURE 5. Current knowledge of noradrenergic projections modulating brain circuits of pain. The schematic shows noradrenergic neurons in the locus coeruleus and their ascending projections, which largely facilitate pain perception, and descending projections to the spinal cord, which largely inhibit pain. BNST, bed nucleus of the stria terminalis; Enk, enkephalin; 5-HT, 5-hydroxytryptamine/serotonin; NA, noradrenaline; VTA, ventral tegmental area.

connect with GABAergic neurons in target areas, thereby leading to disinhibition rather than the classical inhibitory modulation associated with local GABAergic circuits (49). Indeed, GABAergic neurons in the RVM have been known for a long time to traverse long distance to the spinal cord. A recent study by Francois et al. (95) elegantly utilized opto/chemogenetic manipulations and trans-synaptic tracing to elucidate the cellular identity and function of these

circuits, demonstrating that GABAergic neurons of the RVM connect to enkephalinergic/GABAergic interneurons in the spinal dorsal horn. These interneurons gate synaptic transmission between primary afferents and spinal neurons, resulting in presynaptic inhibition. Thus activation of the GABAergic neurons of the RVM can facilitate spinal nociception **(FIGURE 4)**. Taken together with the studies from Zhang et al. (327) and Tan et al. (275), these findings suggest that local GABAergic circuits in higher brain centers, such as the S1 and the prefrontal cortex, can impact on activity of the RVM and that in addition to descending noradrenergic and serotonergic pathways, descending GABAergic pathways can also be employed to fine tune the processing and relay of incoming nociceptive information in the spinal cord.

Second, an intriguing property of GABAergic neurons, particularly of the PV-type fast-spiking interneurons, is that they are highly interconnected via gap junctions, i.e., the so-called electrical synapses. In the neocortex, gap junctional transmission between individual pairs of interneurons results in a net increase in excitatory drive as well as enhanced timing of action potential generation. Connexins and pannexins constitute major families of neuronal gap junction-forming proteins, and particularly connexin36 has been determined to be primary molecular mediator of electrical coupling between fast-spiking interneurons in the brain (4, 49). Several studies suggest a role for connexin36 in modulation of nociception and pain; for example, disruption of its expression or function in the dorsal root ganglia, spinal dorsal horn, or the ACC has been demonstrated to attenuate neuropathic pain (59, 215). However, because connexin36 is not specific to GABAergic neurons, and also forms a key component of gap junctions connecting satellite glia and peripheral sensory neurons (224), the relevance of these findings to gap junction connectivity between GABAergic neurons in pain remains ambiguous.

IX. NORADRENERGIC MODULATION OF BRAIN CIRCUITS IN PAIN

There is more than four decades of work on noradrenergic control of pain, with both peripheral and central mechanisms described (36, 280). Yet this remains an area of dynamic research, particularly with respect to underlying circuitry, with ever-increasing new insights matched by a growing number of open questions. Centrally, the locus coeruleus (LC; A6 group) is the largest source of noradrenaline in the brain, provided by a key hub of noradrenergic neurons that control activity of diverse cortical regions via corticofugal projections as well as descending projections that markedly modulate spinal nociceptive processing **(FIGURE 5)**.

Both facilitatory as well as inhibitory modes of modulation of nociception and pain are described (280). An emerging concept is that in states of chronic pain, the balance between adrenergic facilitation and inhibition switches to a net facilitatory tone, thereby contributing to chronic pain (225, 280). Remarkably, however, this notion of a pronociceptive role for noradrenergic neurons post-injury is contradicted by the fact that several antidepressants, which elevate monoamine levels at synapses, comprise first line therapy for several chronic pain disorders, particularly neuropathic pain, despite reports on variable efficacy. This includes drugs that target noradrenaline and serotonin reuptake, such as duloxetine.

One explanation for this conundrum is given by the divergence of the circuits involved. Classically, descending noradrenergic pathways that directly affect the activity of spinothalamic neurons in the spinal dorsal horn are known to exert inhibitory modulation of nociceptive processing **(FIGURE 5)**. Accordingly, enhanced noradrenergic axonal sprouting as well as receptor activity have been suggested to underlie the efficacy of duloxetine (280). In contrast, evidence for a net facilitatory role for noradrenergic modulation after injury is fueled by a number of recent studies demonstrating pro-nociceptive roles for ascending noradrenergic projections to diverse brain regions, including diverse neocortical regions, thalamus, hypothalamus, hippocampus, amygdala, as well as to other centers linked to descending facilitation, such as the dorsal reticular nucleus and the spinal trigeminal nucleus (36, 296) **(FIGURE 5)**. The most direct evidence is given by studies employing the neurotoxin anti-dopamine-β-hydroxylase saporin (148). While application of the toxin to the spinal dorsal horn to ablate descending noradrenergic LC-spinal projections enhances nociceptive sensitivity, direct ablation of noradrenergic neuronal somata in the LC and their brain projections via intracerebroventricular injection of the toxin reduces neuropathic hypersensitivity, supporting a dominance of pronociceptive activity of LC neurons upon nerve injury. This shift has been suggested to be mediated by altered balance between excitation and inhibition within the local microcircuits of the LC. Moreover, recent optogenetic studies have uncovered a functional heterogeneity of LC neurons, suggesting an antinociceptive role for spinally-projecting neurons in the ventral LC and a pronociceptive role for noradrenergic neurons of the dorsal LC (122).

Among brain regions prominently involved in pain, the PFC has been best studied as a target of dense noradrenergic innervation. In the PFC, noradrenaline has been reported to induce persistent firing of pyramidal (excitatory) neurons (225, 280); this mechanism, which is thought to be operational in enhancement of working memory by optimal concentrations of noradrenaline, may also be operational in enhancing pro-nociceptive processing in the PFC. In support, a noteworthy chemogenetic study demonstrated that LC neurons projecting to the PFC are functionally linked to aversion, enhanced neuropathic pain, and anxiety, in a

manner that is functionally dichotomous from descending LC projections, which were found to mediate antinociception (124). The LC has also been recently suggested to be a target of the PFC in empathy states, and nociceptive hypersensitivity associated with empathy has been linked to enhanced levels of circulating noradrenaline and its pronociceptive effects on peripheral targets (179).

Other targets of ascending noradrenergic circuits have also been implicated in affective dimensions of pain. The stria terminalis (BNST) has been suggested to play important roles in pain-induced conditioned place aversion (CPA), which has been studied as a putative parameter for the affective component of pain. Noradrenergic afferents to the ventral domain of the BNST activate neuronal circuits with the BNST, ultimately resulting in reduced BNST output to the VTA (83) **(FIGURE 5)**. It has been suggested that reduced activity of dopaminergic neurons in the VTA underlies place aversion downstream of noradrenergic signaling (83).

A second factor that may contribute to the conflicting roles of noradrenergic modulation in facilitation and inhibition of pain is given by relative involvement of distinct subtypes of the GPCRs that are activated by noradrenaline in distinct circuits. In the PFC, selective inhibition of excitatory, $G_{q/11}$-coupled α1 receptors reduces neuropathic hypersensitivity (148). Conversely, antinociceptive effects of systemically administered agonists of inhibitory G_i-coupled α2 receptors as well as the SNRI duloxetine have been found to be associated with increased spontaneous firing and responsivity of prefrontal cortical neurons responding to nociceptive stimuli (61). A study employing electrophysiology and optogenetics has suggested synergistic roles for α1 and α2 receptors, reporting that activation of presynaptic α1 adrenoceptors facilitates glutamate release, while activation of postsynaptic α2 receptors inhibits HCN channel activity, resulting in persistent firing of PFC neurons (326). This is in direct contrast to spinal circuits, where activation of α2 receptors has been linked to the inhibition of nociceptive transmission via descending noradrenergic projections (280).

In the ventral BNST, inhibition of excitatory G_s-coupled β2 receptors has been shown to block pain-induced CPA, while activating them pharmacologically has been linked to CPA in the absence of any external nociceptive stimulus (83), suggesting that noradrenaline-induced enhanced drive of the BNST-VTA pathway is mediated by β2 receptors.

Taken together, distinct supraspinal and spinal noradrenergic circuits may affect pain in opposing ways, leading to limited efficacy of NSRIs. It will be therefore important to elucidate contributions of individual circuits and accordingly design approaches to silence supraspinal noradrenergic modulation in neuropathic pain patients, e.g., via neurostimulation approaches.

Finally, it is noteworthy that apart from modulating the processing of pain itself, noradrenergic circuits may play a pivotal role in mediating the impact of psychosocial factors, such as stress and anxiety on pain. Notably, responses of locus coeruleus to noxious stimuli are potentiated in social stress paradigms in rodents (35). Moreover, noradrenergic contributions are also implicated in anxio-depressive behaviors associated with chronic pain (3). A striking impairment of noradrenergic system has been described to mark the onset of anxio-depressive behaviors in neuropathic models, including bursting activity of locus coeruleus neurons, accompanied by complementary molecular changes in the noradrenaline synthesis and transport machinery and exaggerated sensitivity of α2 receptors (3) as well as enhanced desensitization of μ-opioid receptors (178). Furthermore, projections from the LC to the BLA and activation of β-adrenergic receptors in the BLA were recently functionally associated with pain-induced anxiety and fear learning in chemogenetic and pharmacological studies (178).

Multiple interactions between the LC-noradrenaline system and drugs affecting pain and analgesia, such as opioids and gabapentinoids, have been described at multiple avenues, including the PAG and the RVM (36). An elegant study recently uncovered that PAG neurons projecting to the LC are distinct from PAG neurons projecting to the RVM in the classical descending inhibitory pathway (152). Interestingly, while the PAG-LC pathway is modulated by opioids to suppress descending inhibition, the PAG-RVM pathway is activated by opioids to potentiate descending inhibition of spinal nociception (152). Recent data in zebrafish suggest that diverse anesthetics suppress the activity of noradrenergic LC neurons by enhancing GABAergic inhibition and their lesion prolongs anesthesia (85). A better understanding of the cellular circuitry will facilitate targeting noradrenaline in pain states.

X. SEROTONERGIC MODULATION OF BRAIN CIRCUITS IN PAIN

Serotonin released from descending projections from the brain stem in the spinal dorsal horn is vital in the endogenous control of nociception. In contrast, little is known about whether and how supraspinal serotonergic pathways modulate pain via actions in the brain. Serotonergic mechanisms in the brain were initially believed to be intimately linked to antinociception, since antidepressants that enhance synaptic concentrations of noradrenaline and serotonin via transporter blockade were found to exert strong pain-relieving effects in neuropathic pain models and patients. However, a large body of literature on clinical studies as well as detailed experimental analyses implicate norad-

renergic mechanisms in the locus coeruleus and spinal cord in antidepressant analgesia (161, 212). Serotonin and dopamine modulation are now believed to only contribute in an auxiliary manner.

Two major nuclei constitute the chief sources of serotonin in the brain, namely, the nucleus raphe magnus (NRM) and the dorsal raphe nucleus (DRN) **(FIGURE 6)**. The NRM in the RVM sends dense serotonergic fibers to the spinal dorsal horn, which comprise both descending inhibitory and facilitatory pathways. Spinal facilitation mediated by 5-HT_3 receptors is widely believed to dominate in neuropathic pain conditions, demonstrated not only pharmacologically, but also via ablation and silencing experiments (212). In

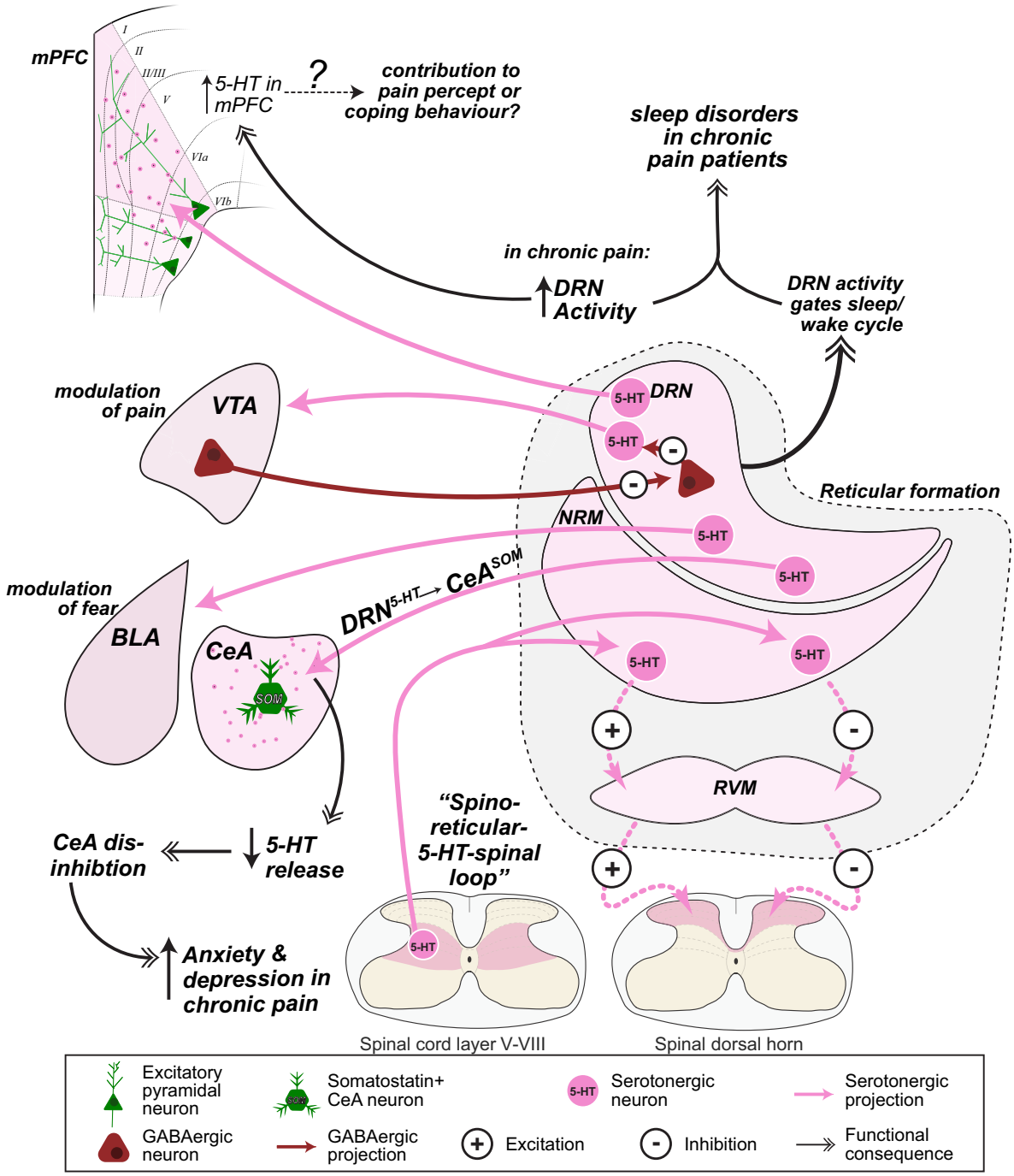

FIGURE 6. Overview on status of knowledge on the role of projections of serotonergic neurons in the reticular formation in chronic pain. While descending projections to the spinal cord govern context-dependent descending inhibition and facilitation (dominant in chronic pain), the ascending projections largely determine anxio-depressive behaviors and sleep deficits in chronic pain. CeA, central amygdala; DRN, dorsal raphe nucleus; 5-HT, 5-hydroxytryptamine/serotonin; mPFC, medial prefrontal cortex; NRM, nucleus raphe magnus; RVM, rostral-ventral medulla oblongata; SOM, somatostatin.

support, depleting serotonin in the RVM by RNA interference-mediated knockdown of the synthesizing enzyme did not alter basal nociceptive sensitivity but decreased nocifensive responses upon prolonged activation of nociceptors by formalin or neuropathic nociceptive hypersensitivity (308). Conversely, directly stimulating serotonergic neurons in the RVM via selective optogenetic manipulations induced nociceptive hypersensitivity in the absence of injury, which persisted up to 2 wk upon repetitive stimulation, suggesting a role for increased serotonergic facilitatory tone in chronic pain (46). In viral tracing experiments, serotonergic neurons in the RVM were recently shown to receive direct excitatory inputs from the S1 cortex, and serotonergic descending facilitation to the spinal cord plays a critical role in nociceptive hypersensitivity induced by gamma oscillatory activity in the S1 (275). Retrograde tracing analyses have also shown that nociceptive neurons located in spinal laminae V-VIII project back to the NRM, but not the DRN, thus establishing a spino-NRM-spino loop for regulating the strength of nociceptive processing (38) **(FIGURE 6)**.

Moreover, in contrast to the NRM, the DRN sends axonal connections to large parts of the forebrain (65). There are two main roles that have been ascribed to the DRN serotonergic neurons in the context of pain modulation. *1*) Electrical or optogenetic stimulation of DRN neurons induces wakefulness and decreases the duration of non-rapid-eye-movement sleep (134). In neuropathic pain states, following nerve injury, the activity of DRN neurons was observed to be enhanced, which was consistent with elevated levels of 5-HT measured in microdialysis analyses in the medial prefrontal cortex (134). These findings have been linked to sleep disturbances in chronic pain disorders, which exacerbate pain **(FIGURE 6)**.

A second link to chronic pain is given by the importance of serotonergic projections from the DRN to the CeA. A seminal, recent study has demonstrated that optogenetic inhibition of DRN serotonergic neurons projecting to somatostatin-expressing cells (SOM+) in the CeA specifically induces depression-related behaviors in the context of chronic neuropathic pain, but not in generalized depression in the absence of pain (331). One interesting quirk of this neuronal population is that although most SOM+ neurons in the brain are GABAergic, these neurons are excitatory and activate their targets in the lateral habenula (LHb) (331) **(FIGURE 6)**. The experimental design also included human fMRI analyses, which revealed that functional connectivity between the DRN and amygdala is specifically reduced in depressive patients with chronic pain, but not in chronic pain patients without depression (331). Moreover, in addition to the DRN-CeA-LHb pathway, serotonin can also directly alter the activity of LHb neurons (334). The LHb receives serotoninergic afferents from the NRM, and serotonin can enhance the spontaneous firing of a large majority of LHb neurons via both pre- and postsynaptic receptors (334). These insights offer hope for further optimizing human neurostimulation-based approaches towards treating pain and depression together as comorbidities.

A recent study has demonstrated that DRN serotonergic neurons projecting to the forebrain and subcortical regions, such as the CeA, represent distinct cell groups with distinct functions (236). While the DRN neurons projecting to the CeA were found to promote anxiety in response to aversive stimuli, DRN neurons projecting to the forebrain were observed to promote coping behavior (236). It remains to be addressed whether these distinct populations are differentially regulated in chronic pain states might explain differences between coping versus anxiety and catastrophizing in chronic pain states. Moreover, not only the CeA, but also the BLA receives serotonergic inputs from the DRN. Anatomical mapping revealed that this DRN-BLA serotonergic pathway is anatomically distinct from other serotonergic projections and is also further connected over the BLA to other brain regions that are involved in fear (254). Direct stimulation of DRN-BLA serotonergic pathway amplifies fear by enhancing the activity of BLA neurons via $5\text{-HT}_{1A/2A}$ receptors (254). Taken together, these new insights demonstrate that a large neuronal network of the diverse serotonergic projections of the DRN to the forebrain, the CeA, the BLA, and the LHb orchestrates emotional responsivity to aversive stimuli, which provide new grounds for testing in chronic pain states.

Conversely, retrograde tracing analyses have also shown that both NRM and DRN are the targets of regulation via projections from several nuclei in the brain stem and the midbrain, including several catecholaminergic and cholinergic cell groups (38). Recently, prostaglandin E_2 (PGE_2) signaling in the RVM area has been associated with inhibition of serotonergic neurons in the RVM and aversive behaviors (264).

XI. CHOLINERGIC MODULATION OF BRAIN CIRCUITS IN PAIN

While local cholinergic neurons form dense networks in a few regions, such as the striatum, the remaining parts of the brain are widely modulated by cholinergic projections originating from key nuclei in the basal forebrain (Ch1-Ch4) (141). Nearly all cortical regions, including those implicated in pain processing, receive inputs from Ch4, also called the nucleus basalis of Meynert (NBM) (141). Both excitatory (pyramidal) as well as diverse classes of GABAergic neurons receive cholinergic afferents, and a broad activation of the basal forebrain cholinergic centers leads to a large overall increase in cortical excitation (41).

While there is a large body of literature on pharmacological studies supporting a key role for cholinergic modu-

lation in pain and analgesia, very little is known about underlying circuitry (202). Pharmacological studies involving systemic administration of cholinergic drugs as well as targeted manipulations in the periphery largely implicate cholinergic modulation in analgesia (202). There is also some pharmacological evidence for central cholinergic contributions to opioid-induced analgesia as well as to endogenous control of pain (102, 333). Particularly, studies on local microinjection of cholinergic agonists and antagonists implicate cholinergic modulation in the amygdala circuits as well as the vlPAG and the RVM in recruitment of descending inhibitory pathways, supporting a role in antinociception (102, 333). A large proportion of neurons that project from the vlPAG to the RVM, which are involved in descending modulation of spinal nociception, express α7 subunit-containing nAChRs and are distinct from vlPAG neurons that are targeted by opioids via μ-opioid receptors (290). Activation of presynaptic α7 subunit-containing nAChRs enhances stimulation of the vlPAG-RVM pathway and elicits antinociception in a manner nonoverlapping with morphine-induced analgesia (290). This suggests considerable scope for additive analgesic effects via combination therapies.

However, a number of previous and new findings highlight an additional pro-nociceptive side to cholinergic modulation. Intracerebroventricular injections of cholinergic antagonists or conjugated-saporins ablating cortical and forebrain cholinergic neurons was reported to suppress nociceptive sensitivity as well as operant responses to painful stimuli (295). Another type of pro-nociceptive modulation may come about from cholinergic neurons in laterodorsal tegmental area, which were recently described to project to the dopaminergic neurons of the VTA (73). These cholinergic inputs enhance the responsivity of VTA dopaminergic neurons to aversive stimuli (73). One recent study bidirectionally modulated the activity of medial septal cholinergic neurons (140), showing that their inhibition suppressed pain affect in a model of inflammatory pain, whereas chemogenetic activation of medial septal cholinergic neurons elicited antinociceptive effects. These recent findings are suggestive of tonic modulation of pain by diverse cholinergic nuclei across the brain, although the precise pathways are largely still ambiguous.

One aspect thereof is likely to be unspecific for pain and given by the broad enhancement of sensory processing in nearly all sensory cortices upon cholinergic stimulation, whereby "signal-to-noise" in cortical circuits is enhanced via diverse nicotinic and muscarinergic mechanisms involving both pyramidal neurons and GABAergic interneurons (55, 202). These would not only directly facilitate processing of nociceptive sensory stimuli and promote saliency detection in the primary S1 cortex, but also contribute indirectly by enhancing attention via modulation of prefrontal cortical circuits (320). Because exaggerated attention has been implicated in certain chronic pain states, particularly in the context of catastrophizing, it will be interesting to determine whether elevation of cholinergic tone comes about in chronic pain patients (89, 294). Furthermore, these considerations are also relevant for dysfunction in pain processing in patients with Alzheimer's disease and dementia, who show a significant loss of forebrain cholinergic neurons (211). This may also be relevant to pain in the aging population, since recent studies show that the heterogeneous phenomena of pain, aging, and dementia are interrelated and ~50% of older patients with dementia develop pain (97).

One key mechanism for cholinergic modulation of sensory processing in the S1 and attentional networks in the prefrontal cortex is given by the ability of cholinergic signaling to facilitate or even directly elicit gamma band oscillatory activity via modulation of local PV-type GABAergic interneurons (218). Furthermore, as discussed in section V*D*, cholinergic afferents also modulate theta oscillations in the hippocampus by modulating the activity of CCK-type GABAergic interneurons (200). It remains to be determined whether this contributes to cortical processing in pain states. Moreover, facilitation of GABAergic inhibition by nicotinic modulation as well as direct suppression of layer 5 excitability have also been implicated as mechanisms of prefrontal deactivation in neuropathic pain (see sect. VII). Furthermore, loss of cholinergic stimulation of prefrontal cortical neurons in neuropathic pain has also been proposed to contribute to mPFC deactivation, leading to enhanced pain and cognitive decline (233), as discussed in section VII.

Overall, there is a broad scope and need for studying the mechanistic basis of cholinergic modulation of pain, not least because a number of cholinergic drugs exist on the market that can be harnessed towards pain relief. This is supported by clinical efficacy of cholinergic modulators, including inhibitors of the acetylcholine esterase which breaks down acetylcholine, and by the observation that a number of analgesic drugs as well as neuromodulatory manipulations may be exerting their analgesic effects at least partially via modulating cholinergic transmission (202). Peripheral and central side effects are main deterrents; therefore, it is imperative to elucidate the cellular and circuitry basis of cholinergic modulation in specific brain centers to determine specific mechanisms. In this regard, modern optogenetic approaches targeted to specific cell populations with temporal precision will aid unravelling the underlying circuitry. Moreover, it will be of value to determine via state-of-the-art in vivo imaging and electrophysiological approaches whether specific brain regions demonstrate changes in endogenous cholinergic circuitry, both structurally and functionally, in the context of pain chronicity.

XII. PEPTIDERGIC MODULATION OF BRAIN CIRCUITS IN PAIN

In most areas of the nervous system, neuropeptides are typically described as cotransmitters or modulators by virtue of their coexistence and release with classical fast-acting neurotransmitters, such as glutamate, GABA, and ATP, among others. However, peptides are primary messengers in the hypothalamus, where they are generated in the neurosecretory cells. Neuropeptides differ from classical neurotransmitters by virtue of their expression in large dense-core vesicles, primarily nonsynaptic release (volume transmission along axons), and slower signaling processes. Hypothalamic and midbrain nuclei express a rich diversity of neuromodulatory peptides, which have been reported to broadly play functional roles in feeding, sleep-wake cycle, energy homeostasis, reproductive and maternal behavior, reward, and pain, among others. Modulation of pain by neuropeptides can come about either via direct actions on processing of noxious inputs at diverse loci along the pathways discussed above or indirectly via virtue of effects in networks involved in reward processing and fear as well as interactions with cannabinoidergic pathways. Opioidergic peptides and calcitonin gene-related protein (CGRP) are the most prominent neuropeptides involved in pain modulation. Several others, including oxytocin, orexin, galanin, neurotensin, CCK, and CRF, have also been described and are gaining prominence in emerging studies. There is a massive wealth of literature on endogenous control of nociception and pain by endogenously expressed opioidergic peptides, namely, endorphin, enkephalins, and dynorphin, which have been a subject of seminal reviews regularly over the years. It would be impossible to reiterate all of the known data which we refer readers to (but please see sects. V and VI for new insights on dynorphin circuits). We would therefore like to give precedence to those peptides and mechanisms on which novel insights have emerged more recently.

A. CGRP

Together with substance P, CGRP is the defining neuropeptide for the eponymous class of peptidergic nociceptors and is released at both central and peripheral terminals of nociceptors. In addition to its well-studied, classical role in nociceptor sensitization, CGRP is now emerging as a key modulator of pain via its involvement in brain circuits.

The parabrachial nucleus (PBN) is the most prominent locus of CGRP-expressing neurons in the brain. The PBN receives direct noxious inputs from pain-specific lamina 1 spinal dorsal horn neurons over the spino-parabrachial pathways and projects in turn to the thalamus, hypothalamus, BSNT, amygdala, and diverse cortical regions **(FIGURE 7)**. Recent studies have brought to light novel and functionally compelling insights into the role of CGRP-expressing neurons in the PBN, showing that they are activated by all types and modalities of noxious stimuli tested, originating from the DRG-spinal cord relay, trigeminal sensory neurons, as well as the vagus nerve (219). An important consequence thereof is that the body learns to avoid harmful situations, which requires learning processes to be established which associate a particular threat with harm. Using mouse genetics, a study by Palmiter and colleagues (116) revealed that the connectivity of CGRP neurons of the PBN with the latero-capsular division of the CeA is critical for the establishment of a threat memory, resulting ultimately in pain avoidance behavior **(FIGURE 7)**. Remarkably, optogenetic activation of CGRP neurons in the PBN was sufficient to induce a threat memory and elicit pain-associated behaviors in the absence of an external noxious stimuli. This raises the critical question as to whether responsivity of these neurons is altered in states of chronic pain, and particularly whether aberrations in their activity could lead to generation of a false alarm in the absence of injury to propagate chronic pain.

Other studies demonstrated that hindering the actions of CGRP in the amygdala by pharmacological blockade of CGRP1 receptors exerted antinociceptive effects in various pain models. Conversely, intra-amygdaloid injection of CGRP led to pain-related behaviors, such as vocalizations and paw withdrawal in the absence of exogenous noxious stimuli and potentiated excitatory synaptic transmission at PBN-amygdaloid synapses (114). In support, it was reported that CGRP enhances NMDA receptor-mediated excitatory potentials via activation of protein kinase A downstream of CGRP1 receptors (213). Importantly, the pathophysiological relevance of these findings is highlighted by studies demonstrating that excitatory transmission at PBN-amygdaloid synapses is indeed potentiated in rodent models of inflammatory pain (115, 262) as well as neuropathic pain (172) **(FIGURE 7)**. Very recently, similar results were found in the rACC, where CGRP enhances NMDA receptor-dependent long-term potentiation (173) **(FIGURE 7)**. These findings collectively suggest a fundamental role for the PBN-amygdala/ACC pathways in mediating emotional components of pain.

Moreover, by performing calcium imaging in vivo, a recent study (48) reported CGRP neurons of the PBN not only respond broadly to all types of cutaneous and visceral pain- and itch-inducing stimuli, but also to satiety, aversive tastes, novel foods, and fear conditioning, suggesting that the PBN-CeA pathway constitutes a main channel of transmission of information on all actual and perceived threats to the forebrain **(FIGURE 7)**. Because the brain is generally capable of distinguishing pain from other threats, it remains to be determined how specificity for pain modulation is generated and merits analyses of downstream pathways.

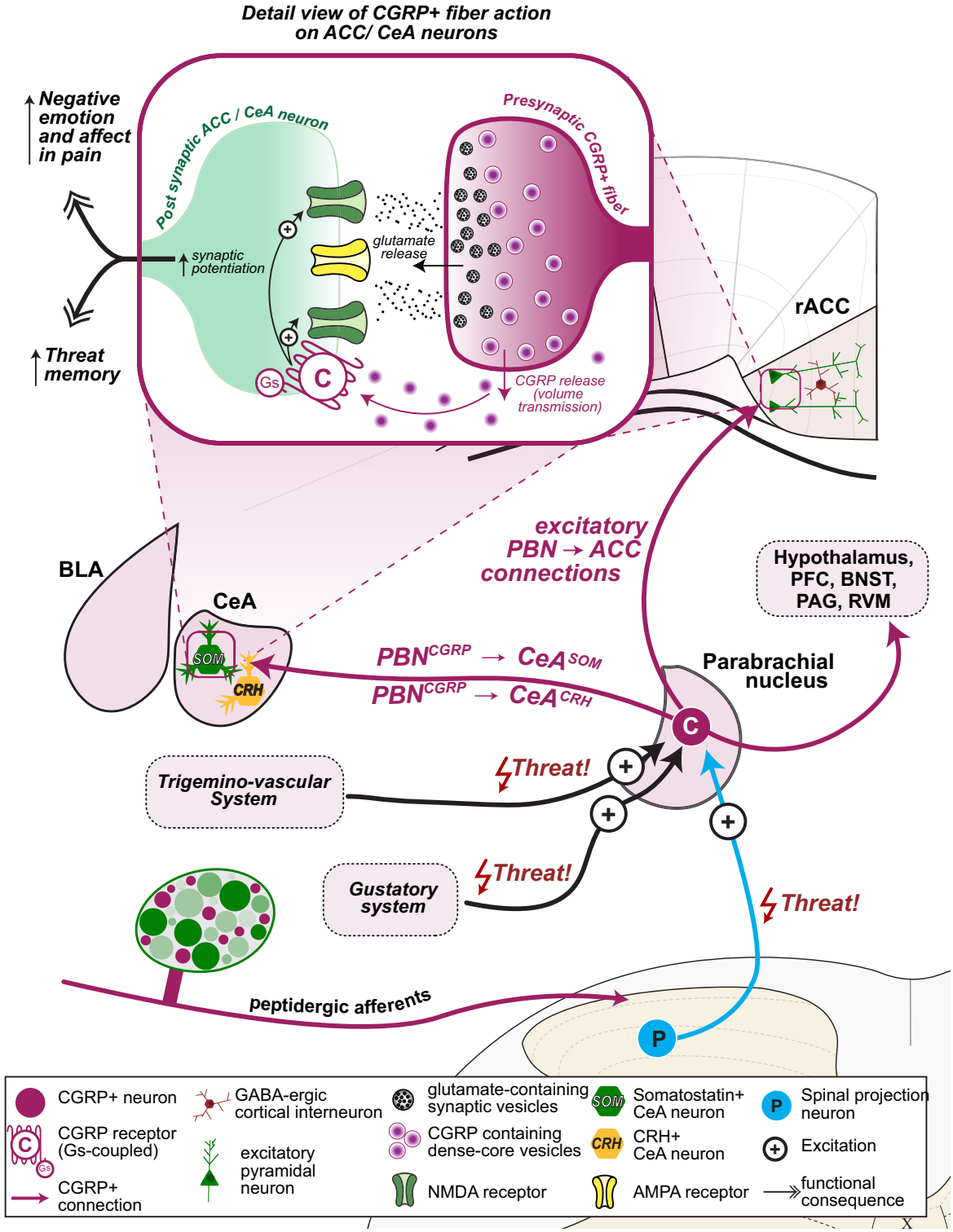

FIGURE 7. Peptidergic circuits arising from parabrachial neurons expressing calcitonin gene-related peptide (CGRP) in the brain regulate synaptic potentiation at amygdala and cingulate synapses and mediate threat memory and aversive responses in response to noxious stimuli. AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; CRH, corticotropin releasing hormone; DRG, dorsal root ganglion; NMDA, *N*-methyl-D-aspartate receptor; PAG, periaqueductal grey; PBN, parabrachial nucleus; rACC, rostral anterior cingulate cortex; RVM, rostral-ventral medulla oblongata; SOM, somatostatin.

B. Orexin

The orexin peptides, orexin-A and -B, also called "hypocretin 1" and "hypocretin 2" acting via orexin receptors 1 and 2, have been reported to exert antinociceptive effects in a number of rodent models, via modulation at spinal and supraspinal levels (125, 239). The orexin peptides are selectively expressed by neurons in the lateral and perifornical areas of the hypothalamus, which project widely to a large number of brain areas involved in arousal, cardiovascular control, and endogenous antinociceptive control (235). Consistent with orexinergic modulation of the locus coeruleus and VTA in the control of arousal, recent studies demonstrate that chemogenetic activation of orexin neurons in the hypothalamus facilitates recovery from anesthesia **(FIGURE 8)**. Moreover, in line with a role in endogenous antinociception, their activation enhances response thresholds to noxious heat and depresses responses to inflammatory and noxious stimuli, such as formalin (330). Interestingly, orexinergic neurons are activated by nociceptive mechanical and heat stimuli, as revealed by calcium imaging via in vivo fiber photometry in the study by Inutsuka et al. (133). In the same study, chemogenetic activation of orexinergic neurons was found to reduce nociceptive sensitivity (133). This suggests that orexinergic neurons are activated as a part of

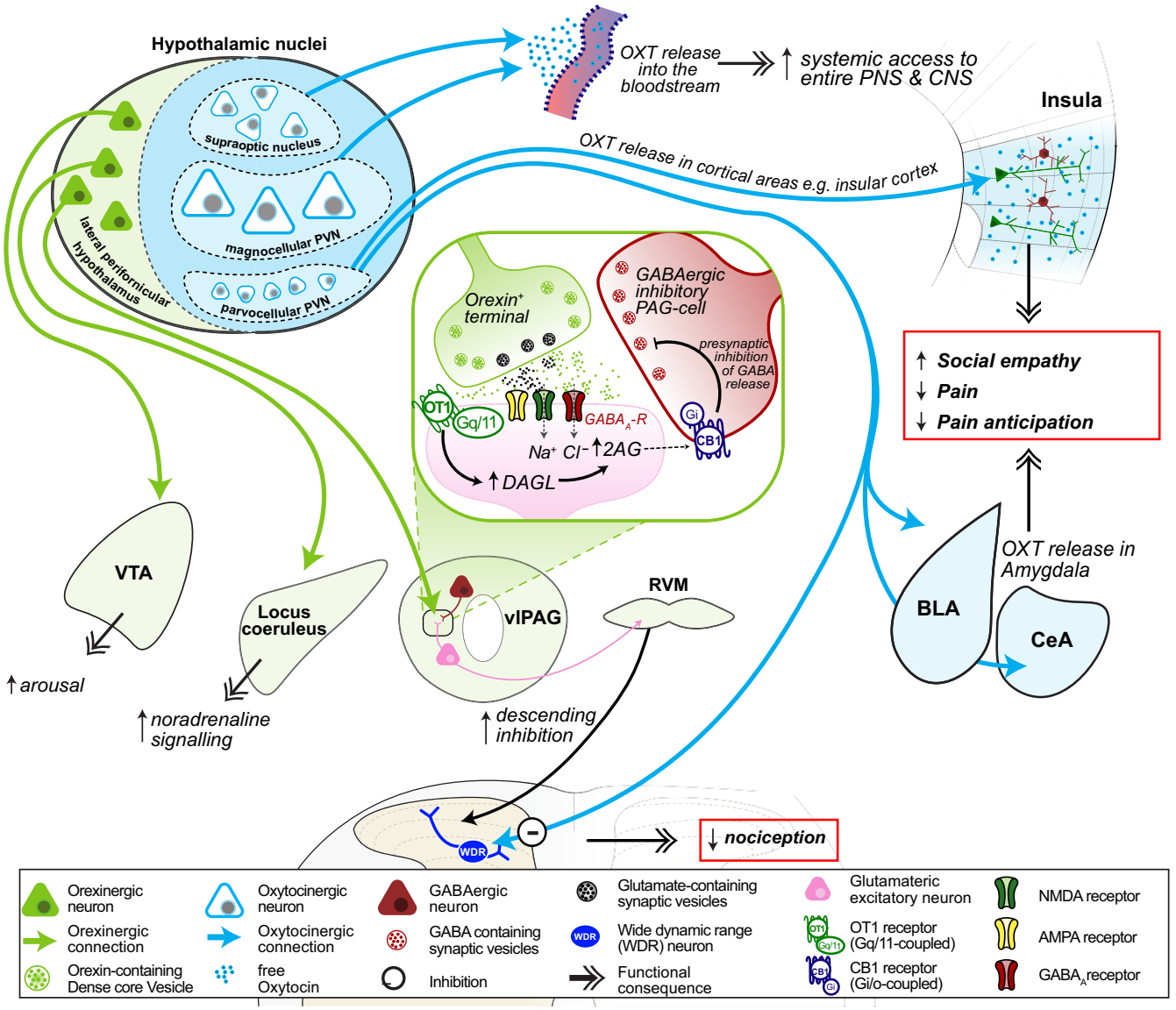

FIGURE 8. Modulation of brain nociceptive networks and regions involved in pain affect and social empathy by circuits harboring the neuropeptides oxytocin and orexin. 2-AG, 2-arachidonyl glycerol; CB1, cannabinoid receptor 1; CNS, central nervous system; LDT, laterodorsal tegmentum; LH, lateral habenula; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; OT1, oxytocin receptor 1; OXT, oxytocin; PNS, peripheral nervous system; PVN, hypothalamic paraventricular nucleus; RVM, rostroventral medulla; vlPAG, ventrolateral periaqueductal grey; VTA, ventral tegmental area.

the endogenous pain control system. Inutsuka et al. (133) discuss in the manuscript that the antinociceptive effects of orexin neuron activation may be attributed not only to the actions of orexin, but could also result from modulation of release of other co-transmitters, such as dynorphin and neurotensin (133).

There is, however, a considerable body of pharmacological literature suggesting potent antinociceptive effects of orexin A, while orexin B is less or not effective (239). Supraspinally, antinociceptive actions of orexin A have been suggested to be mediated via the ventrolateral PAG (vlPAG) and the posterior hypothalamus has also been implicated in the control of the trigeminovascular nociception by orexin (14, 111). Along these lines, together with several other neuropeptides, orexin has been recently reported to be localized in axons of hypothalamic neurons that regulate the functions of the autonomic nervous system in the brain stem and spinal cord, which play a role in aversion to light during migraine (210). Although multiple interactions have been reported between orexin effects and opioidergic mechanisms as well as other neuropeptides, such as nociceptin/orphanin Q, the emerging belief is that orexin-A-mediated antinociception is opioid-independent. Instead, the analgesic effects of orexin-A acting at orexin 1 receptors (OX1) in the vlPAG are postulated to involve endocannabinoid signaling (125). Anatomical and electrophysiological analyses have revealed that vlPAG neurons, which project to the RVM and activate descending inhibitory control of spinal nociception, receive orexinergic inputs from the hypothalamus and express OX1 receptors as well as diacylglycerol lipase (DAGL), an enzyme involved in endocannabinoid synthesis (69) **(FIGURE 8)**. Accordingly, orexin 1 elicits synthesis and release of 2-arachidonoylglycerol (2-AG), which inhibits release of GABA in the vlPAG via presynaptic cannabinoid 1 receptors, resulting in net dominance of excitatory vlPAG-RVM projections. This mechanism of orexin release in the vlPAG from incoming hypothalamic projections has also been postulated to contribute to stress-induced analgesia (168).

Recently, an elegant study by Chen et al. (57) demonstrated that this orexinergic hypothalamus-vlPAG-RVM-spinal dorsal horn pathway is recruited during median nerve stimulation, a therapeutic measure that is long in practice for intractable pain, and mediates analgesia induced by median nerve stimulation in neuropathic mice.

C. Oxytocin

The hypothalamic polypeptide oxytocin is now recognized as an important modulator of emotional processing, particularly in a social context, and pain (33, 162). Oxytocin receptors are broadly expressed in central nervous system and were recently also found on peripheral sensory neurons (33, 228), where they have been recently suggested to bind and desensitize the transient receptor potential channel TRPV1, a prominent sensor of heat, protons, and diverse algogens, thereby indicating analgesic actions for oxytocin (206). It has been long appreciated that exogenously administered oxytocin augments stress-induced analgesia and attenuates inflammatory nociceptive hypersensitivity when administered systemically, spinally, or in the brain. Opioidergic as well as cannabinoidergic mechanisms are implicated in these actions. Conversely, studies with antagonists of oxytocin receptors have also implicated systemically circulating levels of oxytocin in endogenous control of pain in particular situations. A seminal study in zebrafish demonstrated that hypothalamic oxytocinergic neurons are acutely activated by noxious stimuli, including activation of the transient receptor potential channel TRPA1, which is conserved across species as a sensor for noxious and environmentally toxic stimuli (307). It was further demonstrated that activation of oxytocin-expressing hypothalamic neurons is sufficient to elicit defensive behavior via activation of premotor nuclei in the brain stem (307).

An exciting turn in our understanding on oxytocinergic modulation of pain was given by recent studies that have directly addressed underlying circuits. Oxytocin produced in the supraoptic nucleus and the magnocellular part of the paraventricular nucleus (mPVN) of the hypothalamus is secreted and circulated in the systemic vasculature following transport to and release from the posterior pituitary **(FIGURE 8)**. In contrast, neurosecretory cells in the parvocellular part of the PVN were discovered to synthesize oxytocin and transport it via axons to remote parts of the central nervous system, suggesting existence of intricate oxytocinergic circuits. One of the most remarkable recent findings was reported by Eliava et al. (88), who found the existence of a novel subset of parvocellular neurons which project directly to the spinal dorsal horn and inhibit activity of wide dynamic dorsal horn neurons, as well as to the magnocellular part of the PVN **(FIGURE 8)**. Optogenetic activation of these specific oxytocin neurons dampened nociception and inflammatory pain, both via direct suppression of nociceptive processing in the spinal cord and via release of oxytocin in the bloodstream.

Three additional avenues particularly stand out in relation to modulation of pain by oxytocin. First, oxytocin released in the bloodstream from the supraoptic nucleus is reported to act in the PAG following noxious stimulation to release β-endorphin and L- and M-enkephalins, but not dynorphin, in the PAG, thereby activating opioid-dependent descending inhibition (316). Second, the caudate nucleus of the striatum has also been implicated in the antinociceptive role of endogenous oxytocin. Direct activation of the supraoptic nucleus increases the concentration of oxytocin in the caudate nucleus (221). Similar results are observed after noxious stimulation, which can be blocked by lesioning the supraoptic nu-

cleus. Finally, ascending oxytocinergic axons arising from parvocellular part of the PVN have been reported to innervate limbic and cortical brain regions, particularly the central amygdala, and thereby mediate effects of oxytocin on emotional processing, including the emotional components of pain (33).

In contrast to inflammatory pain, much less is known about actions of endogenous or therapeutically administered oxytocin in neuropathic pain. A recent study employing transgenic reporter mice for oxytocin expression suggested upon nerve injury, hypothalamic synthesis of oxytocin and its axonal transportation to the posterior pituitary and the spinal cord is enhanced (209).

Human studies have implicated oxytocinergic signaling in effects of early life stress and social empathy on pain. Preterm infants are very susceptible to pain and stress, and it has been suggested that diverse protective interventions during this sensitive period, including parental exposure and maternal vocalization, stimulate the oxytocin system in the infant and serve to reduce pain and improve outcomes (93). Indeed, diverse types of early life stress, including maternal separation, are known to lead to exaggerated pain sensitivity and a lack of stress-induced analgesia later in life, which can be reproduced in animal models (5, 93, 187). While one recent study found that increased pain sensitivity to noxious stimulus can be reduced upon intracerebroventricular administration of oxytocin (5), another study reported a failure to rescue hypersensitivity caused by neonatal maternal separation by acutely administered oxytocin (187). Instead, the study showed that supplementation of oxytocin daily over the period of maternal separation partially prevented pro-algesic effects of maternal separation (187). They identified epigenetic modifications as an underlying mechanism (187).

Finally, given the proposed importance of oxytocin in promoting social behavior and empathy (34, 162), there is major interest in addressing how effects of socio-emotional components of oxytocin action contribute to its role in pain reduction (33). It will be important in future studies to dissect underlying circuits and address their significance in chronic pain and analgesia. Along these lines, a recent study reported that oxytocin effects on acute pain were associated with increased activity in the ventral striatum and decreased activation of the anterior insula. Moreover, oxytocin modulated the accuracy of pain anticipation, which correlated with decreased activation of the posterior insula (120) **(FIGURE 8)**.

D. Other Neuropeptides

Several other neuropeptides are emerging as important and potentially therapeutically relevant modulators. They share in common their ability to act on the hypothalamus-PAG-brain stem axis of pain and exert predominantly analgesic actions in animal models (208). For example, neurons of the paraventricular nucleus of the hypothalamus release CRF, which modulates stress response by acting on various brain regions (208). Anatomically, CRF has been reported to elicit strong antinociceptive effects at the peripheral, spinal, and brain levels (197, 208). In the brain stem, recruitment of descending opioidergic pathways is implicated. In addition, CRF acts on the locus coeruleus to influence noradrenergic modulation of pain (see sect. IX). Galanin peptides activate galanin type 1 and type 3 receptors, which are inhibitory $G_{i/o}$-coupled GPCRs and type 2 receptors, which can couple to $G_{i/o}$ as well as $G_{q/11}$ and $G_{12/13}$ receptors (167). A large body of literature suggests that galanin peptides and agonists exert analgesic actions by acting on multiple peripheral and central avenues (167). In the brain, inhibitory actions have been reported in the amygdala, nucleus accumbens, and recently also in the anterior cingulate cortex (323). However, recent circuit analyses also suggest pro-nociceptive actions of galanin signaling in the dorsomedial nucleus of the hypothalamus, which projects to the medullary dorsal reticular nucleus (DRt) as well as the serotonergic raphe nucleus (NRM) located within the RVM, both of which can enhance spinal nociceptive processing via descending serotonergic facilitation (see sect. X) (6). In contrast, neurotensin peptides largely exert antinociceptive actions by recruiting descending noradrenergic pathways by acting on type 1 and type 2 neurotensin receptors in the RVM (42). Recent tracing analyses show that RVM neurons expressing the type 2 neurotensin receptor get direct neurotensin-expressing projections from the PAG (304). Although CRF and neurotensin both act on the PAG-RVM-spinal cord axis, the analgesic actions of neurotensin are believed to be largely opioid-independent, which is important from the therapeutic perspective.

XIII. SUMMARY, CHALLENGES, AND NEW DIRECTIONS

Taken together, this wealth of scientific documentation on structural dissection and functional elucidation of regional and cellular circuits in the brain has brought to light important new insights into how pain is encoded in brain networks. In a concise manner, the review emphasizes the importance of interconnectivity between different functional domains of the brain in determining the multidimensionality of the percept of pain. Along these lines, it becomes evident that models and predictions made in human macroscopic imaging experiments can be tested and largely validated in rodent models. Thus, although individual networks emerge that divide the sensory from the affective dimensions of pain, there exists a considerable level of crosstalk. The studies we discussed pointed to an augmentation of networks that contribute to pain affect over the course of pain chronicity, particularly in the context of neuropathic pain, suggesting a progressive shift from noci-

ceptive to affective networks in the transition to chronic pain. Moreover, new unprecedented insights have emerged that show that although there is a major regional commonality across networks mediating diverse pain-related and unrelated functions, there is remarkable specificity at the level of cellular connectivity and circuit function.

The analyses on decoding brain circuits are by no means complete; this riveting field is now in a dynamic growth phase, with novel insights pouring in continuously. A large number of brain areas remain unexplored at the cellular level, and it is expected that the application of circuit dissection techniques discussed here will deliver more precise knowledge on circuits that are amenable to interventional modulation. Nevertheless, one of the key challenges remains to rigorously establish causality from ensembles of neurons and their activity patterns to behavior and generation of internal states and subjective percepts. Understanding the role of individual components in the highly divergent stream of nociceptive information in generating the multifaceted state of pain seems another big challenge. Translation and reverse translation between rodents, monkeys, and humans will require more optogenetics in monkeys and in general more systematic anatomical circuit mapping. The mouse as a model system makes systematic circuit mapping on the whole brain level with cellular resolution feasible and could be combined with systematic single cell sequencing to integrate molecular identity of neurons with their placement within the circuit and computational functions. The latter will also require a much better understanding of the functional properties of defined synaptic inputs, information that is rarely available. Upon all modern circuit interrogation, we need to be reminded that key aspects of these findings need to be validated with "old-fashioned" electrophysiology approaches.

This reverse translation strategy of breaking down and testing networks implicated in human pain disorders to the cellular and circuit level in rodent models will ultimately provide the impetus for forward translation back to human therapies. Thus newly identified circuits can be exploited for therapeutic benefits, and herein we envisage not only conventional pharmacological treatments, but particularly therapies involving neurostimulation and neuromodulation. A major new direction therefore will be to harness the deep insights gained from recent and future studies on imaging and optogenetic/chemogenetic manipulation of specific circuits towards improving efficacy and reliability of neurostimulation and neuromodulation therapies. The "power of the mind" is seemingly endless, and harnessing it holds promise for solving the riddle of chronic pain.

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DISCLOSURES

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