Although single high-dose injections of dopamine are known to be acutely toxic[3], surprisingly, the gradual increase of dopamine in the TH-RREE model had no effect on substantia nigra neurons in WT mice. However, when the authors injected the TH-RREE construct into mice expressing mutant A53T α-synuclein, an autosomal dominant mutation seen in familial Parkinson's disease, there was a substantial loss of neuronal cell bodies in the substantia nigra, along with degeneration of projecting presynaptic terminals in the striatum. This pathology was most prominent 5 months after injection, with degeneration of more than half of the synapses at this timepoint. Moreover, these mice had locomotor impairment, likely related to the nigrostriatal damage. By contrast, 2.5 months after injection there was mild synaptic damage but no degeneration of cell bodies and no locomotor impairment (**Fig. 1**), suggesting that the synaptic defects might precede the other pathologic changes in these mice. Collectively, the data suggest that the vulnerability of substantia nigra neurons is related to the collective pathologic interplay of dopamine and α-synuclein.

Previous studies have suggested that α-synuclein oligomers promote the degeneration of substantia nigra neurons. To explore mechanisms by which α-synuclein and dopamine might conspire to kill these neurons, the authors looked at α-synuclein oligomers in the A53T TH-RREE mice. Studies suggest that oxidized dopamine helps stabilize toxic α-synuclein oligomers[4], though the pathophysiological roles of these oligomers remain unclear. The authors found that tissue from the substantia nigra of A53T TH-RREE mice contained several oligomeric α-synuclein species, suggesting that the initiation and progression of pathology in these mice might be related to oligomeric α-synuclein. Finally, the authors recapitulated the cooperative effect of α-synuclein and dopamine in a *Caenorhabditis elegans* model of dopaminergic neuronal degeneration, showing that the physical interaction of dopamine and α-synuclein is critical to pathogenesis. Specifically, expression of CAT-2, a *C. elegans* TH homolog expected to increase dopamine levels, also enhanced the degeneration of dopaminergic neurons expressing A53T. Furthermore, mutating the A53T synuclein at residues known to mediate the interaction of α-synuclein with dopamine-products ($_{125}$YEMPS$_{129}$) abrogated the A53T-induced degeneration, highlighting the importance of this association in disease pathogenesis.

The importance of α-synuclein to the pathogenesis of Parkinson's disease is unquestionable, yet until now there has been no α-synuclein mouse model that can robustly recapitulate the loss of substantia nigra neurons—a cardinal feature of the human disease. The authors have now generated such a model, which should be a useful tool for the community. A noteworthy feature of this model is that the α-synuclein-induced synaptic degeneration precedes the loss of neuronal cell bodies and locomotor impairment. In the physiologic state, α-synuclein is highly enriched at presynapses, maintaining synaptic homeostasis[5–9]. Thus, several groups, including ours, have suggested that the pathology of α-synuclein is initiated at synapses, perhaps starting with the aggregation of α-synuclein here[10–13]. Since oligomerization of α-synuclein seems to be a key feature of the A53T TH-RREE model as well, the early synaptic degeneration in these mice may be induced by α-synuclein oligomers, with eventual degeneration of nigral cell bodies (a 'dying back' consistent with the human neuropathology). However, the spatial and temporal distribution of α-synuclein oligomers in the A53T TH-RREE model is as yet unknown.

The mechanism by which α-synuclein oligomers lead to synaptic toxicity and neuronal loss in this mouse model are unclear. Previous studies suggest that α-synuclein oligomers can directly impair neurotransmitter release[14] and chaperone-mediated autophagy[15], but the underlying cell biology is largely unexplored. Most Parkinson's disease is sporadic rather than familial, and it's unclear whether the mechanisms proposed by Mor *et al.*[1] using the aggregation-prone A53T mutant are generally applicable. Mouse models expressing wild-type α-synuclein in the setting of dysregulated dopamine may shed further light. It is also unclear whether genetic elevation of dopamine levels is truly representative of the pathology seen in human disease. Animal and cellular models that better recapitulate the sporadic pathology are badly needed, not just for Parkinson's but for all neurodegenerative diseases.

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The cingulate cortex: divided in pain

Thomas Nevian

The discovery of a circuit from the midcingulate cortex to the posterior insula that is essential for cortical sensitization sheds light on the plasticity mechanisms responsible for the transition from acute to chronic pain.

Sensitization of the pain processing system is a hallmark of its response to injury or inflammation. It is physiologically highly relevant for protecting the body from further harm, as it results in adaptive behavioral responses to promote healing. For example, a sprained ankle results in a painful perception that reminds one not to walk on and stress the injured area. There are a multitude of underlying cellular plasticity mechanisms that cause sensitization, ranging from changes in cellular excitability and synaptic plasticity to network rearrangements and altered functional connectivity[1]. These alterations can be found in the peripheral nociceptors, spinal cord and supraspinal brain areas. Seminal breakthroughs in this respect were the discoveries of

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peripheral sensitization[2] and the concept of central sensitization resulting from long-term plasticity mechanisms in the spinal cord[3]. These basic findings can explain, to some extent, the development of phenomena such as allodynia (in which a normally non-noxious stimulus becomes painful) and hyperalgesia (increased pain sensitivity) on the cellular level.

In this issue of *Nature Neuroscience*, Tan *et al.*[4] add a new perspective on sensitization in the pain processing system. This study places the midcingulate cortex (MCC) in the spotlight, attributing to it a cortical mechanism of pain sensitization. Furthermore, the study presents striking evidence that different aspects of pain processing are associated with distinct parts of the cingulate cortex and that pain-related memory processes can be found in the MCC that might be instrumental for the development of chronic pain. It has been long speculated that the similarities between the mechanisms causing pain sensitization and memory formation suggest the existence of a 'pain memory' that develops concomitantly with long-lasting pain states[5]. Accordingly, a multitude of cortical and subcortical cellular changes have been reported that contribute to the sensitization of the nociceptive system after injury[6]. As healing progresses, the hypersensitivity recedes in most cases, but for unknown reasons this process can also go astray, resulting in the development of chronic pain[1,6].

In general, the cingulate cortex is consistently activated in nociception in human and animal studies[7]. It also has been shown that neuronal plasticity in the cingulate cortex is correlated with the development of chronic pain[8,9]. Furthermore, imaging studies suggest that activity in the frontal cortex can predict the transition to chronic back pain[10]. In addition to its importance in pain processing, cingulate cortex serves many functions: it is involved in tasks or states ranging from anxiety, depression, disgust, negativity mismatch, fear, stress, sadness and anger to reward processing[11]. How these different functions are represented in the cingulate cortex is still elusive. The cingulate gyrus consists of four major subdivisions that are commonly identified on the basis of cytoarchitecture, neurochemistry and connectivity. They consist of the anterior cingulate cortex (ACC), MCC, posterior cingulate and retrosplenial cortex[11]. The different parts of cingulate cortex have been associated with different aspects of pain, but so far the precise functional mapping of these aspects has remained largely unknown. Most commonly, the rostral ACC (rACC) has been associated with the emotional and affective processing of pain. Patients who underwent cingulotomy for intractable pain reported that they could still feel the pain but that it did not bother them anymore[12]. Accordingly, animals in which the rACC is lesioned do not show affective pain behavior such as conditioned place aversion[13]. In contrast, the MCC has been associated with sensory processing of pain, particularly with pain intensity and motor responses directed toward the stimulus[14]. As a whole, the cingulate cortex is a central hub in the pain matrix and is highly connected with most other brain areas involved in the processing of pain. One major unsolved question is what functional influence increased activity in the cingulate cortex has on downstream target brain areas. Tan *et al.*[4] elegantly show, with a combination of optogenetics, anatomical quantification of staining for c-Fos—an immediate early gene that is used as a marker for neuronal activity—and a battery of tests for pain behavior, that activation of MCC is necessary and sufficient for pain sensitization. The authors identify a connection from the MCC to the posterior insula and from there to the raphe magnus nucleus mediating descending facilitation as a key pathway for this process (**Fig. 1**).

Tan *et al.*[4] used the optogenetic silencer archaerhodopsin T (ArchT) in excitatory neurons in the MCC and in the hind limb representation in the somatosensory cortex (S1HL) to show that, despite blockade of activity in the MCC or S1HL, the initial nocifensive behavior (for example, licking or lifting of the injected paw), as well as mechanical sensitization that can be observed upon capsaicin injection, is still present. This suggests that acute pain perception is independent of both MCC and S1HL activity. Next, they used a model for central sensitization to test for the acquisition and maintenance of a pain memory. Capsaicin was injected into the leg of the mouse. This causes an intense activation of nociceptive c-fibers, leading to the development of secondary hypersensitivity characterized by a long-lasting sensitization of neighboring body areas such as the paw to non-noxious touch, as well as to noxious stimuli. Blocking activity in MCC or S1HL during mechanical testing of the paw with a von Frey filament early (15–30 min) after capsaicin injection also blocked the development of mechanical hypersensitivity. Strikingly, mechanical testing at a later timepoint (45–60 min) revealed that the S1HL-silenced animals did develop mechanical hypersensitivity, but MCC silenced animals did not. This important finding suggests that persistent activity in MCC, but not S1HL, is required for the maintenance of nociceptive hypersensitivity. Subsequent activation of MCC with channelrhodopsin-2 (ChR2) was sufficient to reinstate the sensitization. Furthermore, pairing ChR2 activation of MCC with non-noxious mechanical stimulation alone resulted in sensitization. Thus, the peripherally driven increase in activity in the MCC triggers plastic changes that promote and maintain cortical sensitization.

These results give important insight into the role of MCC in the acute phase of sensitization. But is MCC activity also instrumental in the maintenance of chronic pain states? Acute silencing of MCC in a chronic inflammatory pain model (injection of complete Freund's adjuvant) resulted in alleviation of mechanical hypersensitivity, but it had no effect on mechanical allodynia in a neuropathic pain model (spared nerve injury). This finding highlights the distinction between inflammatory and neuropathic pain, which employ different afferent pain pathways that activate the MCC in distinct manners. Tan *et al.*[4] identified the target areas downstream of the MCC that were involved in the central sensitization by c-Fos mapping of the brain after activating or silencing of the MCC. A number of projection regions were identified that are commonly associated with pain processing, but only two of them, the nucleus accumbens and posterior insula, showed activity patterns (as quantified by increases

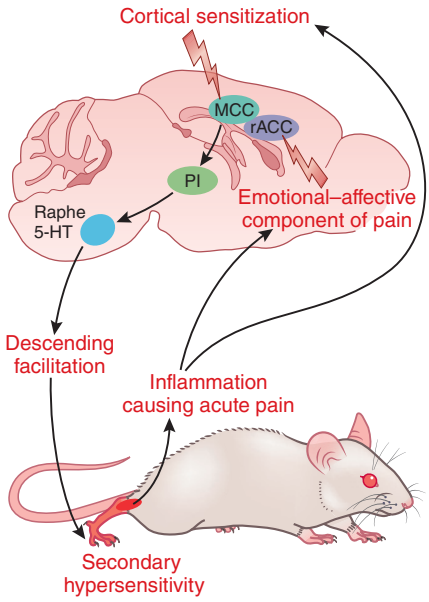

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Figure 1 Cortical sensitization gates sensory hypersensitivity. The cingulate cortex can be divided in distinct subregions. While the rostral ACC (rACC) is involved in the emotional and affective components of pain, a new function for the MCC has been discovered. Increased noxious drive (for example, during inflammation, produced here by nearby injection of capsaicin) results in long-lasting cortical sensitization in the MCC that controls a network involving the posterior insular cortex (PI) and the serotoninergic (5-HT) raphe nuclei. This pathway induces and maintains secondary hypersensitivity.

or decreases in c-Fos expression) correlated with those in the MCC. To determine which projection target contributes to the long-term sensitization, they used target-region-specific silencing with the ArchT variant eArchT and activation with the optogenetic activator ChR2. The results clearly showed that only inhibiting the projection to the posterior insula blocked the development of hypersensitivity, whereas activation promoted it, paralleling the results from the MCC. Finally, blocking descending serotoninergic facilitation of nociception, which emanates from the raphe magnus nucleus, prevented the hypersensitization induced by the pathway from MCC to posterior insula. This finding completes the definition of the pathway that is required for central sensitization and pinpoints the importance of the MCC in influencing descending neuromodulatory systems to control nociception.

In summary, this comprehensive study reveals a pathway from the MCC to the posterior insula that is sufficient to induce and maintain nociceptive hypersensitivity even in the absence of nociceptive drive. This pathway interacts with descending modulatory systems located in the raphe nuclei. Functionally, the MCC modulates pain independently of the emotional or affective dimension of pain that is controlled by the rACC[13].

This newly identified afferent pathway from the MCC to the posterior insula that induces and maintains nociceptive hypersensitivity needs more attention in future research. It will be of great interest to unravel the underlying circuit and cellular plasticity mechanisms that cause the sensitized state. This insight may suggest ways to prevent the induction of hypersensitivity or reverse this chronic state once it is induced. Since this seminal finding yields insight into the transition from acute to chronic pain, the revelation of the underlying mechanisms will have high translational potential. But beyond that, this study shows that cortical brain regions strongly influence spinal pain processing via the descending neuromodulatory system[15]: pain is indeed in the brain.

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Polymorphic computation in locus coeruleus networks

Dong-oh Seo & Michael R Bruchas

Physiological and optogenetic dissection of discrete locus coeruleus neuronal populations reveals a functional disassociation, with heterogeneous engagement of locus coeruleus neurons in either fear learning or extinction models.

Mammals are awash in sensory stimuli, received through many different modalities. Our brains must be equipped to efficiently process this wealth of sensory information to generate an appropriate behavioral response to a constantly changing environment. This process can be optimized by continuously adjusting arousal and attention states. A failure in this optimization not only results in poor performance in daily cognitive tasks but is also associated with psychopathology, including stress-related disorders such as anxiety and depression[1]. The modulation of arousal and attention states is often regulated by noradrenaline, which is released from a pontine nucleus, the locus coeruleus (LC)—one of the smallest neuronal structures in the brain, consisting of ~1,500 neurons in rodents. In this issue of *Nature Neuroscience*, Uematsu *et al.*[2] demonstrate that a heterogeneous population of LC neurons coordinates with a behaviorally specific neural code for adaptive tuning of emotional responding and behavioral flexibility.

LC noradrenergic neurons project extensively to diverse regions throughout the brain[3]. The classical view of the LC noradrenaline system is that the homogenous neuronal populations in the LC broadly influence neural activity throughout the brain by simultaneously releasing noradrenaline to target neuronal structures[1,3]. This view has been generally well accepted, given that it has been difficult to disprove with traditional neuroscience tools, such as lesioning or pharmacological microinjection, and given the technical limitations of characterizing a small subset of the LC cell population within such a small nucleus *in vivo*.

The longstanding global operation hypothesis for the LC noradrenaline system has been challenged by recent studies showing that subpopulations of LC efferent neurons are connected to separate anatomical targets and that each different target area has a distinct molecular phenotype and electrophysiological properties[4]. Furthermore, a heterogeneous network hypothesis of the LC noradrenaline system has been supported by rabies-based input–output mapping at the anatomical level in a few recent studies[5,6]. However, while these viral tracing studies show some limited heterogeneity[5] or specific cortical specificity[6] in some LC neurons, there has been a general lack of evidence for functional dissociation among the heterogeneous population of LC neurons. The concept of functional heterogeneity in the LC network has been debated. Now Uematsu *et al.*[2] demonstrate functional dissociation with their report of heterogeneous engagement of LC neurons using a Pavlovian fear conditioning model that they combined with optogenetic approaches, trans-synaptic tracing and electrophysiological techniques (i.e., photo-tagging) to selectively monitor

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