A novel role for the cholinergic basal forebrain nucleus of Meynert in chronic pain via modulation of the prelimbic cortex

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A novel role for the cholinergic basal forebrain nucleus of Meynert in chronic pain via modulation of the prelimbic cortex

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Abstract

The basal nucleus of Meynert (NBM) subserves critically important functions in attention, arousal and cognition via its profound modulation of neocortical activity and is emerging as a key target in Alzheimer’s and Parkinson’s dementias. Despite the crucial role of neocortical domains in pain perception, however, the NBM has not been studied in chronic pain. Here, using in vivo tetrode recordings in behaving mice, we report that beta and gamma oscillatory activity is evoked in the NBM by noxious stimuli and is facilitated at peak inflammatory pain. Optogenetic and chemogenetic cell-specific, reversible manipulations of NBM cholinergic-GABAergic neurons reveal their role in endogenous control of nociceptive hypersensitivity, which are manifest via projections to the prelimbic cortex, resulting in layer 5-mediated antinociception. Our data unravel the importance of the NBM in top-down control of neocortical processing of pain and suggest a potential for its therapeutic modulation via neurostimulation strategies in chronic pain disorders.

Introduction:

A major hindrance to adequate therapy of chronic pain disorders is given by incomplete knowledge on brain circuits underlying the perception of pain and their modulation over transition from acute to chronic pain. Their elucidation is therefore important for yielding mechanistic insights as well as for therapeutic advance. Recent studies on functional interrogation of brain circuits have led to breakthroughs on structure-function properties of some brain networks involved in pain, and particularly revealed key roles for neocortical domains. Pain perception is subject to profound modulation by contextual, environmental and psychosocial factors. As contributing neural mechanisms, insights are now emerging on modulation of neocortical processing by afferent input from GABAergic, dopaminergic and serotonergic pathways.
In comparison, very little is known about the scope and functions of cholinergic pathways in the brain in modulating pain perception. This is in contrast to extensive pharmacological studies from the past two decades, which report effects of cholinergic signaling via both ionotropic nicotinic receptors as well as metabotropic muscarinic receptors on pain and analgesia. Systemic, peripheral, as well as spinal administration of cholinergic ligands modulates nociception and studies with central administration have implicated cholinergic signaling in opioidergic analgesia and descending modulatory systems. However, there has been very little progress in exploiting cholinergic modulation towards pain relief, owing primarily to major gaps in understanding the underlying circuitry, particularly with respect to the delineation of the origin of cholinergic inputs. This is particularly important, because both facilitatory and inhibitory effects are associated with pharmacological modulation of cholinergic receptors, which can be attributed not only to diversity of receptor-mediated signaling but also to the locus of cholinergic modulation in the nervous system.

In the brain, cholinergic neurons are abundant either in form of local interneurons in specific areas, such as the caudate putamen, or organized in the cholinergic nuclei Ch1-Ch6 of the basal forebrain and brainstem to function as projection neurons with distant targets. Amongst these, the basal forebrain system comprises discrete groups of cholinergic cells (Ch1-Ch4), with neurons in the medial septum (MS) and the vertical limb of the diagonal band of Broca (vDB) primarily targeting the hippocampus, while the neurons in Ch4 largely account for the cholinergic input into the neocortical mantle and also project to the amygdala. In the rodent brain, the structure most analogous to the Ch4 is given by the nucleus basalis magnocellularis (NBM; basal nucleus of Meynert), also extending into a band ventral to the anterior commissure called the substantia innominata, which are collectively referred to under the term
NBM in this study, consistent with several other published studies (e.g.,\(^5\); schematic view in Fig. 1a). This sector contains the largest component of corticopetal projections from the basal forebrain and is overwhelmingly cholinergic in nature. The NBM has been ascribed a modulatory role in specific key functions, such as arousal, attention, fear and social interactions, including social recognition memory\(^5\). Furthermore, the NBM has been implicated in sharpening the acuity of sensory processing by enhancing the ‘signal-to-noise’ ratio in cortical circuits via nicotinic and muscarinic mechanisms involving both pyramidal neurons and GABAergic interneurons\(^6,7\). These properties potentially place the NBM in a critical position to modulate pain perception and its plasticity, given the importance of neocortical processing in pain\(^1\). Surprisingly, however, the NBM has hardly been studied in the context of pain, barring a few studies with excitotoxic lesions and broad toxin-mediated ablation of cholinergic groups. Importantly, there have been no studies functionally delineating the underlying native circuitry. Moreover, it remains unknown whether and how activity patterns in the NBM change in association with pain and the NBM undergoes plasticity during the transition to chronic pain \textit{in vivo}.

Here, we performed \textit{in vivo} recordings using tetrodes to dynamically capture changes in activity of single neurons as well as oscillatory field rhythms in the NBM in freely-moving, behaving mice during nociception and the transition to inflammatory hypersensitivity. We report specific responses of the NBM to pain-inducing (noxious) stimuli, which demonstrate a switch in responsivity to low intensity stimuli in inflammatory pain, thus mirroring behavioral hypersensitivity. Simultaneously, gamma and beta oscillatory rhythms undergo potentiation of spectral power. Using reversible, cell type-specific chemogenetic and optogenetic manipulations in conjunction with behavior, we demonstrate that this potentiation of cholinergic activity in the NBM and its projections to the prefrontal cortex suppresses
nociceptive hypersensitivity in both inflammatory and neuropathic pain conditions, thus paving the way for novel therapeutic strategies specifically targeting these cholinergic cell groups.

Results:

Oscillatory rhythmic activity in the NBM in nociception and inflammatory pain

Intraplantar hindlimb injection of capsaicin in wild-type mice, which acutely induces strong, tonic pain, led to a robust increase in expression of the activity-dependent immediate early gene product, Fos, in the NBM (schematically shown in Fig. 1a), including cholinergic neurons as seen via co-labelling for the marker choline acetyltransferase (ChAT; Fig. 1b, d). We next targeted this area in electrophysiology experiments in awake, behaving mice to directly study changes in NBM activity, both at the level of field potentials and single cells using tetrodes. Application of mechanical force via von Frey filaments was associated with an increase in activity across all frequency bands over baseline (pre-stimulus) activity levels (Fig. 1d, e; also see Suppl. Fig. 1a). Across multiple trials and animals, the increase was statistically significant in the power of beta oscillations (14-30 Hz) when stimuli at and above the nociceptive thresholds were applied (von Frey force of 0.6-1.0 g) as well as with low intensity, non-noxious tactile stimuli (0.07-0.16 g), whereas power of gamma oscillations (30-100 Hz) selectively increased with nociceptive strength stimulation (Fig. 1f). This finding is particularly interesting because gamma oscillations in cortical areas have been functionally linked with nociception in both human and rodent studies [8,9,10], and are known to be associated with synchronization of activity via GABAergic interneurons [11]. Noxious mechanical stimulation-induced increase in gamma activity was seen to reach statistically significant levels prior to the behavioral nocifensive response and was maintained for 2 seconds after application of the stimulus (Fig. 1g).
We next sought to test the potential significance of the NBM in the progression of nociception to hypersensitivity that is characteristic to persistent inflammatory pain. Indeed, mice with unilateral hindpaw inflammation induced by injection of Complete Freund’s Adjuvant (CFA) demonstrated enhanced Fos expression in cholinergic neurons in the NBM (Fig. 2a, b). In a longitudinal study design, we then compared oscillatory activity between naïve conditions and after CFA-induced hypersensitivity was established (Suppl. Fig. 1b). At the time of peak mechanical hypersensitivity, paw stimulation elicited a significantly larger increase in the power of beta and gamma rhythms (Fig. 2c, d), but not of alpha and theta activity (Suppl. Fig. 1c) in the NBM. An interesting finding was that the inflammatory pain-associated increase in gamma and beta power was seen with low intensities of mechanical stimulation, which are typically non-noxious in physiological conditions but are perceived as noxious in inflammatory pain (Fig. 2e). Taken together, these findings indicate that the NBM is recruited during nociception and shows facilitation of its responsivity over the transition to hypersensitivity in inflammatory pain.

**Single cell analysis of NBM activity in nociception and inflammatory pain**

Analyzing activity at the single cell level via spike sorting led to interesting insights into the cellular nature of NBM responsivity and plasticity in pain. Amongst the 221 units recorded under naïve conditions, 14 % showed a consistent increase in firing rate in withdrawal trials upon applying 20 mechanical paw stimulations with either the weak or strong filament pair, and 11 % of units were consistently inhibited in activity by paw stimulation (Fig. 3a, b); Example traces and average Z-scores (denoting number of standard deviations for data points above or below mean) are shown in Fig. 3a and unit proportions in Fig. 3b. In mice with inflammatory pain, the proportion of units responding to mechanical stimulation did not change.
significantly during strong hypersensitivity over the first 4 days after CFA injection (Fig. 3b). Over this period however, maximal z-score values increased significantly in neurons excited by noxious intensities of mechanical stimulation (Fig. 3c), but not in neurons inhibited by mechanical stimulation (Suppl. Fig. 2a), thus corresponding to the overall increase in the power of oscillatory activity which we observed at the LFP level. Furthermore, by analyzing the shape of the spike wave-form, we then classified units into Class 1 and Class 2 with broad or narrow spike wave-forms, respectively; fast-spiking classes of GABAergic projection neurons and interneurons are represented within class 2 units. Interestingly, although maximal z-score values appeared to be elevated in both classes post-CFA, the change was only robust and statistically significant for Class 2 neurons (Fig. 3d). These data suggest that NBM neurons, which are excited by mechanical stimuli, undergo facilitation over the manifestation of inflammatory nociceptive hypersensitivity and further that fast-spiking, Class 2 GABAergic neurons in the NBM particularly contribute to these changes. This finding is noteworthy, since in the mouse NBM, 92% of ChAT-expressing cholinergic neurons are known to be GABAergic. At late time points after CFA injection (7-14 days), after normal nociceptive sensitivity is recovered, we observed that patterns of oscillatory and single cell activity in the NBM not only normalize, but partly even fall below baseline values (Suppl. Fig. 2; Suppl. Fig. 3).

**Optogenetic stimulation of NBM acutely suppresses hypersensitivity**

To directly uncover the significance of our findings, we employed a cell-specific optogenetic approach by targeting the blue light-gated cation channel Channelrhodopsin to ChAT-expressing neurons. Recombinant adenoassociated virions (AAV) were stereotactically injected to express yellow fluorescent protein-tagged Channelrhodopsin in a Cre-dependent manner (rAAV-Dio-ChR2-YFP) unilaterally in the NBM of ChAT-Cre transgenic mice (Fig. 4a).
4a, b). Cre-negative mice subjected to the same treatments served as controls. Delivery of blue light to the NBM via chronically implanted optic fibers significantly increased Fos expression in cholinergic neurons, thereby establishing in vivo validation of the approach (Fig. 4c, d). Upon blue light stimulation, baseline sensitivity to mechanical stimuli remained unchanged (Fig. 4e, baseline); however, the left-ward and up-ward shift in the von Frey stimulus-response function, representing the manifestation of inflammatory hypersensitivity, was significantly lowered in Cre+ mice as compared to Cre- controls when tested at peak sensitization on day 2 post-CFA (Fig. 4e, middle panel). Likewise, the mechanical withdrawal threshold was significantly increased in mice with CFA upon blue light stimulation in Cre+, but not in control Cre- mice (Fig. 4f). Overall, the magnitude of mechanical hypersensitivity over baseline values was decreased and the return to baseline sensitivity was faster upon optogenetic stimulation of NBM cholinergic neurons (Fig. 4f). However, CFA-induced heat hyperalgesia was not significantly altered in magnitude or duration (Fig. 4g).

Dissecting the contribution of NBM cholinergic-GABAergic projections to the medial prefrontal cortex

Because the NBM projects to a large number of neocortical targets, many of which affect pain and hypersensitivity in multiple ways, we then sought to dissect the significance of NBM neuronal projections to the medial prefrontal cortex (mPFC), which represents a key hub in brain circuits underlying pain. The mPFC undergoes marked plasticity in several human clinical chronic pain conditions and particularly, a major focus has emerged on its deactivation observed in chronic pain patients 15, a finding that is also reported in animal models 16, 17, 18, 19. We therefore expressed ChR2-YFP in ChAT neurons of the NBM and placed the optic fiber for blue light illumination in the prelimbic cortex (PL), the mouse counterpart of the human mPFC (Fig. 5a). Selectively activating NBM cholinergic projections to the PL did not influence
baseline mechanical sensitivity, but had an even stronger analgesic effect than direct activation of NBM neurons on CFA-induced mechanical hypersensitivity, which was completely reversed to baseline values (Fig. 5b). Furthermore, thermal hypersensitivity was robustly suppressed over a long period post-CFA in mice with optogenetic stimulation of the NBM-PL projections (Fig. 5c).

Electrophysiological and modelling studies indicate that cholinergic inputs from the basal forebrain exert direct excitatory effects via receptor-mediated signaling on cortical pyramidal neurons and also have the ability to evoke either inhibition or dis-inhibition of pyramidal neurons via signaling on different classes of local neocortical GABAergic interneurons or via signaling through different types of nicotinic and muscarinic receptors \(^\text{14, 20}\). Interestingly, recent studies also indicate that GABA is co-released from cholinergic projections originating from the basal forebrain nuclei and can thus disinhibit neocortical pyramidal neurons by suppressing local inhibitory interneurons \(^\text{14, 21}\) (schematic in Fig. 5d). Both mechanisms have been suggested to act towards enhancing cortical signal-to-noise processing of sensory inputs, e.g., to visual inputs in the visual cortex and tactile inputs in the somatosensory cortex \(^\text{21}\). To address how NBM-PL cholinergic-GABAergic projections affect the PL, we performed viral tracing and c-Fos mapping across layers in conjunction with optogenetics. Interestingly, while NBM projections to most of the neocortical mantle diffusely span all cortical layers, our tracing analyses revealed that projections from the NBM to PL terminate in layer 5 in a particularly abundant manner as compared to other layers (Fig. 5e; compare with neighboring motor cortex M2 and cingulate cortical domains). In both baseline conditions (naïve mice) and mice with inflammatory pain, optogenetic stimulation of NBM-PL projections led to a significant increase in Fos levels throughout the PL (Fig. 5f). Recent studies have shown that layer 5 pyramidal neurons in the PL project to the periaqueductal grey and thereby link to descending
We observed that optogenetically activating NBM-PL connections enhances the activity in layer 5 neurons, beyond the increase induced by inflammatory pain (Fig. 5g). We therefore selectively stimulated layer 5 neurons chemogenetically by virally directing hM3D(Gq) expression in the layer 5-specific Rbp4-Cre line and observed that selectively enhancing layer 5 outputs in the PL mimics the anti-hyperalgesic action of NBM-PL stimulation on mechanical allodynia in mice with CFA, while baseline sensitivity was not altered (Fig. 5h left panel; mCherry expression was employed as control, Fig. 5h right panel).

**Targeting the NBM in chronic pain**

From the viewpoint of translational relevance, it is important to address whether targeting the NBM cholinergic system is also beneficial in other forms of pain, particularly neuropathic pain. Hence, we addressed whether the cholinergic NBM system is recruited in the neuropathic pain state and is related to mechanical allodynia by studying Fos expression in the absence of or upon plantar application of low intensity mechanical von Frey force, which is innocuous under baseline conditions. Fos expression in the NBM was significantly elevated in neuropathic mice as compared to sham-injured mice and showed a further increase upon paw stimulation associated with mechanical allodynia (Fig. 6a, b). Importantly, this was also reflected in ChAT-expressing cholinergic neurons (Fig. 6a, b), which suggests increased recruitment by stimuli that are innocuous in baseline conditions but perceived as noxious in neuropathic pain conditions, thus showing parallels to our findings in electrophysiological experiments in the inflammatory pain model described above.

To test the functional significance of these findings in the context of neuropathic pain conditions, we employed a chemogenetic approach to activate cholinergic neurons of the basal nociceptive modulatory systems. We observed that optogenetically activating NBM-PL connections enhances the activity in layer 5 neurons, beyond the increase induced by inflammatory pain (Fig. 5g). We therefore selectively stimulated layer 5 neurons chemogenetically by virally directing hM3D(Gq) expression in the layer 5-specific Rbp4-Cre line and observed that selectively enhancing layer 5 outputs in the PL mimics the anti-hyperalgesic action of NBM-PL stimulation on mechanical allodynia in mice with CFA, while baseline sensitivity was not altered (Fig. 5h left panel; mCherry expression was employed as control, Fig. 5h right panel).

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forebrain, which provided two advantages: one, it enabled targeting a larger area than optogenetic stimulation (which is limited owing to the maximum area that can be sufficiently illuminated), and second, it permitted achieving more long-lasting activation of cholinergic neurons. ChAT-Cre transgenics were injected with rAAV expressing either the excitatory chemogenetic actuator (mcherry-tagged hM3D(Gq)) or control (mCherry) protein in a Cre-dependent manner and treated with Clozapine-N-Oxide, which enables inducible activation of hM3D(Gq) \(^{23}\) (Fig 6c). Dual immunohistochemistry for ChAT and Fos demonstrated that 77% of cholinergic neurons in the NBM expressed hM3D(Gq) and 74% demonstrated Fos expression upon CNO treatment, while less than 5% showed Fos expression in the absence of CNO (Fig. 6d), thereby validating the efficacy and specificity of chemogenetic activation of NBM cholinergic neurons. Consistent with data from optogenetics experiments, we observed that baseline nociceptive sensitivity to mechanical pressure and heat was not altered; however, tonic pain induced by plantar injection of capsaicin was reduced significantly upon chemogenetic activation of cholinergic neurons (Fig. 6e, f, g).

We then employed the chronic constriction injury (CCI) model involving unilateral loose ligation of the sciatic nerve, leading to local inflammation and swelling of the nerve, neuropathy and nociceptive hypersensitivity lasting up to a month \(^{24}\). CCI-induced mechanical hypersensitivity was markedly reduced in hM3D(Gq)-expressing mice in comparison to mCherry-expressing mice starting from day 4 after CCI surgery (Fig. 6f). At day 11, mechanical responses in hM3D(Gq)-expressing mice were indistinguishable from baseline sensitivity, while mCherry-expressing mice continued to show mechanical hypersensitivity and regained baseline values only on day 28 (Fig. 6f). Similarly, heat hypersensitivity was robustly reduced in hM3D(Gq)-expressing mice as compared to mCherry-expressing mice until day 14.
post-CCI (Fig. 6g). These data show that activation of the NBM robustly suppresses neuropathic hypersensitivity over a long duration.

**Potential contributions of modulation of anxiety, attention and motor function**

Activity of NBM neurons has the potential to affect pain processing directly via cholinergic signaling in neocortical targets that are important in pain networks, as indicated by our observations above on prefrontal layer 5 neurons. However, because the NBM is known to be a key modulator of circuits underlying arousal and attention, there is also a possibility that the observed antihyperalgesic effects are related to attention and expectancy. We therefore also addressed whether optogenetic stimulation of NBM cholinergic neurons affects attention behavior using the widely accepted five choice serial reaction task test (5-CSRT; Fig. 7a) under the same conditions that were implemented in mice used for nociceptive testing in the experiments described in Figures 4-6. Over 3 weeks, water-restrained mice were trained to learn in operant conditioning tasks to make correct decisions to receive a water reward (Fig. 7a). Chemogenetic stimulation of NBM cholinergic neurons significantly enhanced the accuracy of reactions and reduced the rate of omissions, indicating a higher attention level (Fig. 7b). However, when we optogenetically stimulated NBM-PL projections under the same conditions that were employed in the pain analyses, there was no significant impact on accuracy of reactions and the rate of omissions (Fig. 7c; Suppl. Fig. 4), suggesting that the manifestation of anti-hyperalgesic behavior was not *per se* related to attentional alterations.

Secondly, the NBM receives direct inputs from the CeA and is linked to neural circuits of anxiety and fear. We observed that neither direct chemogenetic (Fig. 7d) nor optogenetic stimulation of NBM neurons (Fig. 7e) induced fear-associated behaviors in the open field test (center-to-margin ratio was unchanged). In contrast, optogenetically stimulating NBM-PL
projections reduced the center-to-margin ratio in naïve mice, suggesting anxiolytic effects (Fig. 7f). Finally, locomotion was unchanged in all of the groups involving optogenetic or chemogenetic modulation of the NBM or NBM-PL circuits, suggesting a lack of confounding effects on motor function in behavioral analyses (Fig. 7d-f).

**Discussion**

Literature on the basal forebrain cholinergic nucleus and pain perception is surprisingly scarce. To date, fewer than a handful of studies have tested the activity of the basal forebrain cholinergic nucleus upon noxious stimulation \(^{26, 27}\). In this study, we now report the precise nature of oscillatory rhythms in the NBM as well as a detailed analysis at a single cell level *in vivo*, showing that the NBM not only responds to noxious stimuli, but also undergoes dynamic changes during the transition to chronic pain. The most interesting observation was that the power of gamma oscillatory activity in the NBM is specifically enhanced in conjunction with noxious stimulation prior to the behavioral response. Gamma oscillations in the S1 have been functionally linked to nociceptive modulation in both human and rodent systems *in vivo* \(^{28, 29}\), and these pain-related alterations in gamma activity have only been recently extended to other neocortices, such as the prefrontal and insular cortices \(^9, 30, 31\). This study, to the best of our knowledge, represents the first report linking gamma rhythms in a sub-cortical structure to nociceptive sensitivity. These have been likely missed in human studies owing to technical limitations from scalp recordings.

Importantly, our observation that in inflammatory pain, the power of gamma activity is potentiated in response to non-noxious tactile stimulation correlates with the manifestation of mechanical allodynia and mimics similar observations made in the S1 cortex \(^{29}\). Taken together with current knowledge, a tantalizing implication of our findings is that gamma activity in the
NBM is functionally linked via cholinergic pathways to neocortical gamma oscillations during nociceptive processing. This is supported by several conceptual points and experimental observations. First, a recent study in rats reported hemodynamic blood flow changes in the NBM following noxious stimulation and demonstrated that cerebral blood flow changes in the ipsilateral S1 cortex evoked by noxious stimulation were significantly reduced upon lesioning the NBM, thus suggesting importance of the NBM in the full manifestation of pain-related responses in the somatosensory cortex. Second, in both S1 and the prefrontal cortex, cholinergic signaling facilitates or even directly elicits gamma band oscillatory activity via modulation of local GABAergic interneurons, thereby enhancing acuity of stimulus processing in sensory and attentional networks, although these phenomena have not been addressed in the context of pain so far. Moreover, gamma oscillatory activity has been proposed to coordinate and link activity states across distant sites in the brain, which is a particularly noteworthy concept in the context of pain, since pain is essentially a network function.

A salient role in the emergence of gamma oscillatory activity is attributed to fast-spiking GABAergic interneurons, which are extensively interconnected via gap junctions; they not only streamline and synchronize excitatory output within a region, but are also capable of doing so at distant sites via long range GABAergic projections, which typically synapse on GABAergic neurons thereby leading to disinhibition. Importantly, here, we observed that while different sets of NBM neurons showed excitation or inhibition upon nociceptive stimulation, the NBM neurons undergoing significant changes during the transition to nociceptive hypersensitivity were derived from waveform analysis to be fast-spiking GABAergic neurons. In the NBM, an overwhelmingly large majority of cholinergic neurons are GABAergic and these comprise long range projections to the neocortical mantle, thus providing further credence to the association between the origins of gamma oscillatory activity...
in the NBM and cortical modulation of pain. We also observed changes in the beta frequency range of oscillatory activity; however, much less is known about its cellular origins and functional significance to pain. Studies in healthy subjects have reported that activity in the low frequency bands, particularly in the alpha and beta ranges, is suppressed in the S1, prefrontal and insular cortices in correlation to subjective pain ratings \(^9,37\). More work will be needed to unravel the significance of beta rhythm changes in the NBM in pain states and whether and how this contributes to changes in beta oscillations in the neocortex.

Our analyses with the activity-induced immediate early gene product, Fos, suggest that cholinergic neurons of the NBM are increasingly recruited in inflammatory and neuropathic pain states, particularly in conjunction with sensory stimulation. This finding as well as the overall functional profile of NBM cholinergic neurons discussed above could equally well argue for a role for the NBM in either pro-nociceptive or anti-nociceptive modulation. Here, two independent modes of activation of NBM cholinergic neurons revealed that the net result of their activation is to suppress nociceptive hypersensitivity. A previous study on large scale damage to cholinergic neurons using conjugated saporin administered via intracerebroventricular injections reported reduced voluntary escape from both noxious heat as well as stressful sound without changes in spinal nocifensive behaviors, concluding that affect, but not sensory behaviors, are modulated by forebrain cholinergic neurons \(^38\); however, those conclusions were based on widespread ablation across the brain, parenchymal toxicity and loss of connectivity to a large number of areas, including the hippocampus, amygdala and the cortex. Here, cell-specific and reversible manipulations of activity, rather than neuronal integrity, that were limited to the NBM suggest that recruitment of NBM cholinergic neurons subserves an overall protective role in limiting perceived pain. Because NBM neurons project to diverse neocortical domains with differing functions as well as to the amygdala, it cannot be
ruled out that individual connections may play different roles. Along these lines, a recent study addressing a different cholinergic nucleus, namely the medial septal nucleus, reported that both inhibition as well as activation paradoxically led to suppression of pain by opposing effects on the rostral anterior cingulate cortex and the ventral hippocampal CA1 region \(^39\). Here, specifically activating NBM projections to the PL cortex made an unequivocal case for an antinociceptive function, which was accompanied by the observations of denser targeting of afferent projections to layer 5 and enhanced Fos expression in layer 5 pyramidal neurons of the PL. As further support, we provide evidence that enhancing output of layer 5 PL neurons via optogenetic stimulation has a similar antinociceptive outcome as stimulation of NBM-PL connections. This is a particularly important finding since several types of chronic pain have been associated with deactivation of the PL in chronic pain patients \(^15\), \(^19\) as well as rodent models \(^16, 17, 18, 19\). In neuropathic pain models, enhancing PL output, either optogenetically \(^22\), \(^40\), pharmacologically \(^41\) or via non-invasive transcranial brain stimulation \(^42\) has been demonstrated to elicit analgesia. Indeed, the mechanisms underlying deactivation of the prefrontal cortex in chronic pain are a topic of intense current interest, with studies demonstrating enhanced feedforward inhibition via potentiation of amygdalar inputs onto fast spiking GABAergic neurons \(^17, 22\). In contrast, incoming cholinergic afferents from the NBM, which are known to co-release GABA, have the ability to both inhibit or disinhibit neocortical pyramidal neurons via direct modulation vs. modulation of local GABAergic neurons, respectively. The results of this study show that cholinergic-GABAergic projections of the NBM to the PL serve to enhance PL activity, and could thus counteract deactivation of the PL in chronic pain states. In support, there is evidence from an ex-vivo study showing reduction in synaptic expression of excitatory M1 muscarinic receptors in layer 5 neurons of the PL in neuropathic mice \(^43\) and a study reporting suppression of neuropathic allodynia upon application of a M1-M4 agonist in the anterior cingulate cortex \(^44\).
Because the NBM subserves various different functions, it is important to temper our
inferences with alternative interpretations. Modulation of attention is one of the best studied
functions of the NBM, which was indeed confirmed in this study in mice receiving direct
optogenetic stimulation of NBM cholinergic neuronal somata. Attention has been suggested to
profoundly modulate pain perception, e.g., by altering descending modulation or in terms of
enhancing pain perception by hypervigilanz, rendering it possible that attentional factors
play a role in modulation of pain by the NBM. However, selective activation of NBM
cholinergic projections to the PL was insufficient to modulate attention and motivation, and is
thus unlikely to fully account for changes in nocifensive behavior. Instead, the enhanced
activation of layer 5 PL neurons by cholinergic-GABAergic NBM afferent input would render
it more plausible that the known direct connectivity of layer 5 pyramidal neurons to the PAG
leads to descending modulation of nociception. Motor activity or overall activity were
unchanged, suggesting that the observed analgesic effects were independent of arousal and
motor dysfunction. The potential anxiolytic effects observed upon stimulating NBM-PL
projections are interesting, since they may particularly hold promise in addressing fear as a
comorbidity of pathological pain.

In total, the results of this study support further investigation of employing cholinergic
modulation in pain treatment. While drugs targeting cholinergic receptors have been
demonstrated to show efficacy in preclinical models, the large range of side effects impede
clinical application. Inhibitors of the acetylcholine esterase, such as Rivastigmine and
Neostigmine, which enhance the bioavailability of this neurotransmitter at the sites where it is
physiologically released, have shown clinical efficacy in a number of studies in pain. It is
therefore imperative to delineate the loci of action as well as alterations in the circuitry in
chronic pain that show highest promise in terms of beneficial effects whilst producing fewer side effects. Using cutting-edge in vivo electrophysiology and specific circuit manipulations, this study demonstrates that the NBM is an important modulator of circuitry involved in pain perception and that directly enhancing NBM activity or its projections to the PFC, e.g., via novel designs in neurostimulation techniques, holds promise in the treatment of inflammatory and neuropathic pain disorders. Along these lines, it is noteworthy that the NBM have already been implicated as a potential site of action of general anesthetics 49.

Targeting the NBM may also hold therapeutic promise in the context of other aspects of chronic pain that were not addressed in this study. For example, chronic pain conditions are frequently accompanied by sleep disorders, which are pathogenic in worsening their prognosis and treatment response 50, 51. Sleep deprivation has been shown to lead to reduced connectivity of the NBM to the prefrontal cortex 52. Furthermore, neuronal loss in the NBM has been reported in disorders such as Alzheimer’s disease and Parkinson’s disease, and during aging 53, 54, 55, 56. The results of this study suggest that neuronal depletion in the NBM will likely lead to a loss of central antinociceptive modulatory effects, thereby contributing to pain disorders that are frequently associated with these states. Our observation of reduction in NBM activity over very late stages after paw inflammation are also interesting in this regard. The ongoing development and testing of deep brain stimulation of the NBM 54, 56 thus holds promise in not only reducing cognitive decline, but also suppressing pain and restoring normal sleep in these disorders.

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Author contributions:

MO, YH, ZG, HL, DU, BO, SM and PVN performed all wet experiments under supervision from RK. MO assisted with the planning and execution of electrophysiology protocols. RK conceptualised the project and all authors provided regular conceptual inputs. MO, HL and ZG prepared the figures. RK wrote the manuscript and all authors provided comments and methodical information in writing the manuscript and presenting the data.

Competing interests

The authors declare no competing interests.

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Methods:
Animals

Experiments were performed in male and female two to eight-month old heterozygous Chat-IRES-Cre mice (B6;129S6-Chat\textsuperscript{tm2(cre)Low/\textsc{Uhg}}, \textsuperscript{57}) with a C57BL/6 background, and referred to here as ChAT-Cre mice. Cre-ve littermates were used for control experiments. Two- to four-month-old male and female Rbp4-Cre animals (B6.FVB/CD1-Tg(Rbp4cre)KL.100Gsat/\textsc{Uhg}) with a C57BL/6 background were used for chemogenetic targeting layer 5 pyramidal neurons in the prelimbic cortex. C57BL/6J male and female animals aged 8 – 20 weeks were purchased from Janvier Labs. Animals were housed with food and water \textit{ad libitum} on a 12 hr light/12 hr dark cycle. All experimental procedures were performed according to the ethical guidelines set by the local governing body (Regierungspräsidium Karlsruhe, Germany; approval numbers 35-9185.81/G44/17 and 35-9185.81/G184/18).

Surgical procedures

Mice were deeply anaesthetized by intraperitoneal injection of fentanyl (0.01 mg/kg), medetomidine hydrochloride (0.3 mg/kg), midazolam (4 mg/kg). Lidocaine (10 \%) was applied to the surface of the skin and a small hole was drilled above the region of interest. \textit{In vivo} delivery of recombinant adeno associated virus (rAAVs) was performed by stereotactic injections. The NBM coordinates used relative to bregma were posterior 0.35 mm, lateral 1.6 mm, at a depth of 4.55 mm from the pia. rAAV2-EF1a-DIO-hChR2(H134R)-EYFP (University of North Carolina Vector Core, USA) virus (250 nl) was delivered over 20 min undiluted whereas rAAV5-Syn-DIO-hM3D(Gq)-mCherry and rAAV5-Syn-DIO-mCherry (Addgene Inc., USA) viral solutions were diluted 1:1 in PBS and 400 nl injected over 20 min. Animals were kept for at least 3 weeks to achieve optimal \textit{in vivo} viral expression prior to behavioral and electrophysiological experiments.
For optogenetic experiments, a chronic optical fiber implant (200 µm core diameter, numerical aperture (NA) of 0.5) was inserted 100 µm above the site of the viral injection in the NBM, or bilaterally in the PL cortex using a lateral rotation of 15° (1.94 mm anterior from bregma, 0.9 mm lateral, at a depth of 1.5 mm from the pia), and secured on the skull with dental cement and a screw.

For electrophysiological experiments, two stainless steel screws were implanted on the skull above the cerebellum and right sensory cortex to serve as ground and reference electrodes, respectively. A cranial window was then prepared above the left basal forebrain (AP = -0.35 mm, ML = 0.9 mm). The dura was removed and a versadrive-4 (Neuralynx), composed of 4 independently drivable tetrodes (Tungsten, 12 µm in diameter, California Fine Wire), was implanted into the left basal forebrain at an initial depth of 4.3 mm. The cranial window was covered with bone wax and the versadrive-4 setup fixed to the skull with dental cement.

For the chronic constriction injury (CCI, 24) mice were placed under isoflurane anesthesia (2%) and the fur of the right thigh was shaved. An incision was made to the lateral skin surface of the thigh and through the biceps femoris muscle to expose the sciatic nerve just above it branches into sural, common peroneal and tibial nerves. Four loose ligatures were placed around the sciatic nerve using cat gut surgical sutures, the muscle and skin subsequently sutured close, and animals left to recover in a heated cage for 24 h. Behavioral testing commenced from day 2 after the operation. The same surgery was performed without placing the sciatic nerve ligatures on sham control animals.

**Optical stimulation**

Mice were restrained securely in a soft cotton cloth in order to attach the optical patch cables (0.5 NA dual fibers connected to a 1x2 wavelength division fiber optic rotary joint, Doric Lenses Inc., Canada) to the optical fiber implants. The fiber optic rotary joint in turn was
coupled via an optical patch cord (200 µm core diameter, Thorlabs GmbH) to a 473 nm laser (Shanghai Laser & Optics Century Co. Ltd, China). The laser intensity was set to 4 mW as measured at the fiber tip with a light meter (PM100D, Thorlabs). Pulsed laser light (20 Hz, 10 ms pulse duration) was typically applied for a duration of 30 s, starting 15 - 20 s before mechanical or thermal stimulation events, or with each trial initiation event in the 5-CSRT test, using a pulse generator (cat. no. 33220A, Meilhaus Electronic GmbH, Germany).

**Behavioral tests**

Behavioral tests were carried out during the light cycle of the animals. Animals underwent two acclimatization sessions in the setup chambers used for testing mechanical or thermal sensitivity. Baseline sensitivity was assessed over several days in three test sessions before conducting CCI surgeries or inducing an inflammation of the paw via a subcutaneous plantar injection of 20 µl Complete Freund’s Adjuvant (CFA, Sigma-Aldrich) under brief isoflurane anesthesia. Behavioral tests involving animals expressing hM3D(Gq) and corresponding mCherry controls were conducted 1 h after injecting either saline or clozapine N-oxide (CNO, 2 mg/kg intraperitoneal injection; Biomol, Germany). Experimenters were always blinded to the identity of the treatment groups.

**Capsaicin-induced nocifensive behavior**

Capsaicin (Sigma) was diluted from a frozen dimethyl sulfoxide (DMSO, Thermo Fisher Scientific) stock solution (50X) with phosphate buffered saline (PBS, Thermo Fisher Scientific) to obtain a capsaicin concentration of 0.02 % (weight/vol) in 2 % DMSO. Animals were briefly anesthetized with 2 % isoflurane (Baxter, Germany) and 20 µL of the capsaicin solution was injected with a 30G needle subcutaneously into the plantar surface of the hind
paw. Animals were then placed in a transparent box (20 x 20 cm) on an acrylic glass plate, and the total time the animal displayed nocifensive behavior (paw lifting, licking, flinching, writhing) was assessed over a period of 5 min by an experimenter blinded to the treatment condition.

**Mechanical sensitivity**

After acclimatization to the von Frey setup (Ugo Basile Inc., Italy), a set of monofilaments that bend at forces of 0.04 g, 0.07 g, 0.16 g, 0.4 g, 0.6 g, 1.0 g and 1.4 g were applied perpendicular on the plantar surface of the hind paw. Five applications per filament were applied with a minimum interval of 1 min between each application. In optogenetic stimulation experiments the laser was turned on 20 s before applying the filament and turned off 10 s after applying the mechanical stimulus. In all, 5 applications per filament and per laser state were applied during a test session for each animal. Trials were scored positive if the animal exhibited nocifensive response behaviors, including rapid paw withdrawal, licking, or shaking of the paw, either during the mechanical stimulation or immediately after the filament was removed. The paw withdrawal threshold was determined using Dixon’s up-down method 58.

**Thermal sensitivity**

The Hargreaves plantar test setup (Ugo Basile Inc., Italy) with an infra-red heat source (Model 37370-001, Ugo Basile) was used to test thermal withdrawal thresholds by applying radiant heat to the plantar surface of the hind paw. The intensity level was set to 25 and the cut-off time to 30 s. A heat stimulus was applied only during the quite wake phase and the withdrawal latency from stimulus onset was recorded. Six trials were performed per treatment condition and animal on a test day, using a minimum inter-trial interval of 2 min. Laser ON trials were randomly interspersed with laser OFF trials during an optogenetic Hargreaves test session. The
laser was turned on 20 s before initiating the thermal stimulus and switched off 5 s after a paw withdrawal response.

*Open field test*

The open field test was performed in a square box (40 x 40 cm, 38 cm in height) with a USB camera fixed above the box in order to track animal movement patterns and record experimental parameters using ANY-maze software (Stoelting Co., Ireland). The animals were not acclimatized to this setup so that they encountered a novel arena to explore. The box was divided into three zones for analysis. A 3-cm-wide border along the walls of the box was defined as the thigmotaxic zone. A square zone of 20 x 20 cm in the geometric center of the box was defined as the center zone. The remaining area was defined as the marginal zone. Each mouse was placed in the center of the box and allowed to explore the entire field freely for an 8 min period. The 8 min test was divided into 30 s periods with the laser turned ON and OFF alternately in a random tact so that each mouse received 4 min illumination overall. During the test, the locomotion parameters (distance and mean speed) within each segment were recorded. The ratio of the time spent in the center versus the thigmotaxic zones was used to assess anxiety-like behavior. Motor function was assessed from the total distance moved in all three zones.

*5-Choice serial reaction time (CSRT) test*

Two cohorts of ChAT-Cre mice either expressing the hM3(Gq) DRADD (n = 10) or the ChR2(H134R) opsin (n = 8) in cholinergic NBM neurons as well as 6 Cre-ve control animals for the optogenetic test group were trained in the 5-choice serial reaction time (5-CSRT) task using automated Bussey-Saksida Mouse Touch Screen operant chambers (Campden Instruments, Loughborough, UK) and ABET II TOUCH software (Lafayette Instrument, IN, USA). Throughout training and testing stages, animals had limited access to drinking water (30
min per day) and hence water could be used as a reward to reinforce correct choice behavior in individual trials during the task. For habituation and training, procedures outlined in Humby, and the ABET II TOUCH 5-CSRT task module (version 3) were followed. Briefly, a light cue was presented in one of five windows for a given time period and a trial was counted as correct if the animal touched the monitor of the cued window within an extended time of 5 s after the cue disappeared. If the mouse interacted with another window (incorrect trial) or no touch screen interaction was detected (omission trial) following cue presentation a punishing time out period was signaled by the house light turning on for 5 s. A session consisted of 60 trials and mice performed one session per day. The cue duration was successively reduced from 30 s to 1.4 s until performance at each stage reached a criterion of > 80 % accuracy [number of correct trials / total number responded trials (correct + incorrect)] and < 20 % omissions [number of missed trials / number of trials presented] for two consecutive days. DRADD animals were tested three times over a 5-day period using a cue duration of 1.2 s after having received a saline or CNO injection. The cue presentation period was increased by 0.2 s in maintenance sessions between test days.

Optical patch cords were connected daily already during the later training phase of the optogenetic cohort without turning the laser on. Upon reaching the performance criterion with cues displayed for 1.8 s, animals were tested using a cue presentation period of 1.6 s with the laser turned ON at the start of each trial and OFF after collection of the water reward, or immediately after the 5 s extended time window if no correct touch response was detected.

**Histology and immunohistochemistry**

At the end of the experiment, mice were killed with an overdose of carbon dioxide and transcardially perfused with phosphate-buffered saline (PBS) followed by 10 % formalin (Merck, Germany). Brains were collected and post-fixed additionally for 24 h at 4 °C. Brain
sections were cut with a vibratome at 50 µm thickness, mounted with Mowiol and imaged with a fluorescent microscope to confirm the location of the electrode sites or the AAV injection in Cre+ animals.

To assess cholinergic neuron activity changes in acute and inflammatory pain conditions, animals were perfused 90 min following a capsaicin injection into the hind paw, repetitive mechanical stimulation (0.16 g filament, 20 s interval over a 10 min period) of the inflamed paw on CFA day 2, and on day 4 to the ipsilateral paw of CCI and sham animals. To assess neuronal activity induced by the optogenetic stimulation, the pulsed Laser light was turned ON five times for 30 s during a 10 min period in Cre+ and Cre- animals that were then perfused 90 min later. Similarly, CNO or saline was injected in DREADD animals 2 h before perfusion.

Dual immunolabelling with anti-Fos (rabbit; ab190289, Abcam, UK) and anti-ChAT (goat; AB144P, Merck) were used at 1:1000 and 1:250, respectively. Briefly, sections were incubated in PBS / 50 mM glycine for 10 min, followed by a blocking step of 60 min in 4 % horse serum with 0.2 % Triton in PBS. Sections were incubated with both primary antibodies in the blocking solution for 24 h at 4 °C. The sections were subsequently washed in blocking solution (three 10 min washes) and incubated with a secondary antibody mixture of donkey anti-rabbit-Alexa-488 and donkey anti-goat-Alexa-633 (Invitrogen, USA; 1:700 each) in blocking solution for 2 h at room temperature. Donkey anti-rabbit-Alexa-594 was used for brains with AAV-induced EYFP expression. To enhance EYFP fluorescence of AAV-transduced NBM terminals a subset of frontal brain sections was immunolabelled with a cocktail of rabbit anti-Fos (1:1000) and chicken anti-GFP (ab13970, Abcam; 1:1000, pre-incubated first for 72 h at 4 °C at a 1:100 dilution with brain sections from naïve C57bl6 mice) primary antibodies (48 h incubation at 4 °C), using donkey anti-rabbit-Alexa-594 and goat anti-chicken-Alexa-488 (Invitrogen, USA; 1:700 each) secondary antibodies (as above, incubated for 2 h at room temperature). Tissues were washed in PBS twice, incubated in Hoechst 33342 (diluted 1:10,000 in PBS from 10
mg/ml stock solution, Invitrogen) for 10 min, washed again in PBS and further incubated for 10 min in 10mM TRIS-HCl before mounting.

**Imaging and counting**

Sections were imaged using a laser-scanning confocal microscope (Leica TCS SP8, Germany) with a pixel resolution of 1024 x 1024. The illumination parameters were kept identically for an image series across all animals. A dry air objective (Leica, 10x/0.40, HC PL APO) was used for imaging Fos-labelled sections and an immersion objective with correction collar (Leica, 20x/0.75, HC PL APO) for imaging triple-labelled sections. A montage of confocal image stacks was acquired over a depth of 25 µm centered over the region of interest at the mid-level of each section and the maximum z-projection of images were applied for counting in ImageJ software (version 1.50b, National Institutes of Health, USA). The mouse brain stereotaxic atlas and the reference atlas from the Allan Institut (2011) were respectively used to define region of interest and cortical layer outlines according to corresponding reference sections. Fos-labelled, double and triple-labelled cells within each region of interest were counted manually using the same contrast and threshold settings for all sections within an experimental group. Positive cells lying on the boundary were excluded. Cell counts were converted to indicate the number of positive cells in the imaged stack volume within the region of interest (cells/mm$^3$).

**Cell counting in the basal forebrain**

A region of interest (1.5 mm mediolateral x 1.0 mm dorsoventral in size) was used as a counting frame above the NBM region with highest expression of ChAT+ or EYFP+ neurons. The lower edge of the globus pallidus was used as an upper boundary. Fos+ double and triple-labelled cells within the counting frame in both hemispheres were counted manually using the same contrast and threshold settings for all sections within an experimental group. Counts of double-
labelled Fos+ neurons of the capsaicin treatment group are averages of three brain sections expressed as % of ChAT+ neurons in each hemisphere. As the number of double- and triple-labelled Fos+ neurons did not differ significantly between hemispheres for all other treatment groups, data from the two hemispheres were averaged for each brain slice, and converted to indicate the number of positive cells in the imaged stack volume within the region of interest (cells/mm$^3$).

**Electrophysiology**

After one week of recovery from the implantation surgery, tetrodes were lowered down by 0.5 mm on average into the region of interest and remained unchanged until the end of the experiment. Mice were allowed 2 days to habituate to the elevated grid of the von Frey test recording setup. Naïve mechanical sensitivity tests were performed with weak (0.07 g and 0.6 g) and strong (0.6 g and 1.0 g) filaments for 4 days. Each filament was applied 10 times on the planter surface of the right hind paw with a minimal 60 s interval between stimulation trials. Chronic inflammatory pain was induced by injecting CFA solution (25µl, Complete Freund’s Adjuvant, Sigma) subcutaneously on the plantar side of right hind paw. Mechanical nociception tests were performed on days 1, 2, 3, 4, 7, 9, 12, 14 after CFA injection with the same filaments used for the baseline tests. At the end of the behavioral experiments, mice were deeply anesthetized with 2 % isoflurane, the location of each tetrode tip labelled by applying electrical current to induce a small lesion, and the animals perfused transcardially to fix the brain tissue.

Neural signals were acquired via a HS-18-MM headstage using Digital Lynx 4SX system and cheetah data acquisition software (Neuralynx). The raw data was acquired at 32 kHz with a bandpass filter (1-6000 Hz). The von Frey stimulation was recorded by a custom-made piezo transducer (Piezo ceramic element, part #717770, Conrad), which transduced the pressure of
von Frey stimulation into an analog signal bandpass filtered at 1-2000 Hz. In addition, videos of mechanical stimulation events were recorded by a USB camera (20 frames/second), synchronized via a keyboard-generated event signal to the piezo signal. Stimulation onset was defined as the time of contact of the von Frey filament with the hind paw corresponding with an initial deflection of the piezo signal by visually inspecting the video and piezo recordings, respectively.

Analysis of electrophysiology data

Local field potential (LFP) and single unit activity were analyzed with custom-written scripts using MATLAB (The Mathworks Inc, Version R2014a). Statistical analysis and post-hoc tests were performed in Graphpad Prism (version 9).

Power spectrogram analysis

For the spectrogram analysis of the LFP activity, 3s before and 3s after the onset of the von Frey filament application for withdrawal trials was extracted from the raw data. One channel of a tetrode was analyzed per animal. Raw data episodes were filtered with 3rd-order lowpass Chebyshev type I filter with 0.5 dB ripples in the passband and a passband edge frequency of 200 Hz and down sampled to 1000 Hz. Power spectrograms were generated with the Morlet wavelets function, setting the central frequency to 0.8125 Hz, frequency accuracy at 0.5 Hz, and the time resolution to 1 ms. The 1 s-baseline period before the stimulation onset was used to normalize each 0.5 Hz frequency segment by the respective mean, and expressed as % deviations from the pre-stimulation baseline. The normalized power spectrograms of individual trials were then averaged for weak and strong filaments for each mouse and day. Grand mean
averages of these normalized spectrograms for all animals are shown in Fig. 1e, 2c, and Suppl. Fig. 3a.

For the quantitative analysis, averages of four frequency bands, including theta (4-8 Hz), alpha (8-14 Hz), beta (14-30 Hz), and gamma (30-100 Hz), in the power spectrograms of each animal were calculated over the entire 2 s post-stimulation period. For the time course analysis normalized spectrograms of each animal were binned into 100 ms bins and averages calculated for each frequency band. The median withdrawal time of the corresponding trials for a spectrogram was calculated as a reference. To correlate individual filament force with the increase in the spectrogram power, the normalized power over the 2 s post-stimulation was averaged for each filament and frequency band for each animal.

*Single unit analysis:*

Spike sorting was performed with Kilosort2 \(^{61}\) to isolate single units. Raw data was pre-processed with a bandpass filter from 300 to 6000 Hz. Drift correction, unit clustering, and template matching was automatically performed based on the template matching method. Automatically clustered units were manually curated in Phy (version 2.0; https://github.com/cortex-lab/phy) using waveform similarity and cluster features, firing rate, as well as cross-correlation and auto-correlation features.

* Detecting stimulation responsive units:

For the analysis of evoked activity changes in the single unit data, firing activity of each withdrawal trial was aligned to the stimulation onset for the withdrawal trials of either all filaments, or separately for weak and strong filament groups. The firing rate across trials was calculated for 250 ms bins and z-scores computed based on the mean and standard deviation
of the 3 s pre-stimulation baseline activities. Units showing significantly increased or
decreased activity were identified if at least one of the normalized bins in the 3 s post-
stimulation period exceeded 3.09 or -3.09, respectively, corresponding to a significance level
of \( p < 0.001 \). Otherwise, the unit was classified as an unresponsive unit. Units were excluded
from this analysis if the mean firing rate was smaller than 1 Hz or the number of withdrawal
trials less than 3. In order to compare the magnitude of stimulation-evoked responses, the
maximal and minimal z-score was extracted for all units with significantly increased or
decreased firing rates within the 3 s post-stimulation periods, respectively.

Unit type classification:

For the single unit classification, various parameters were calculated, including: firing rate,
coefficient of variation of the inter-spike intervals, peak-peak amplitude of the waveform, time
between early and late waveform peaks, time from waveform trough to the return to baseline,
and waveform asymmetry (the quotient of the difference between the baseline to early peak,
and late peak to return of baseline times, to the sum of these two times). These multi-
dimensional parameters were projected into two dimensions using the t-SNE (t-distributed
stochastic neighbor embedding) Matlab function. Then k-means algorithm was applied to
cluster these units into two clusters. Based on cluster separation, the best unit classification was
achieved using just two waveform parameters: the asymmetry parameter and the time from
trough to the return to baseline. We did not attempt to distinguish if the units we classified were
excitatory or inhibitory neurons, nor can we distinguish between projection neurons and
interneurons.
Statistical analysis
All data are expressed as mean ± S.E.M. unless stated otherwise. Prism (version 9) was used for the statistical analysis of all behavioral data and for performing post-hoc comparison tests of electrophysiological data sets. A one-sample t-test was performed to detect if specific frequency bands of the LFP power spectrogram of withdrawal trials deviated significantly from the pre-stimulation baseline. A repeated measures one-way ANOVA with Fischer’s LSD test was used for the time-course analysis in Fig. 1g. All grouped data sets were analyzed with a two-way ANOVA using Sidak’s test for multiple comparisons for relevant treatment combinations that had significant main group effects. The unpaired Student’s t-test was used to test for treatment effects compared to a control group. The Chi-square contingency test for the unit response types (Suppl. Fig. 2b) was applied for all time periods, as well as for pairwise time period combinations to detect the deviating data set. In all tests, a p value of < 0.05 was considered significant.

Figure legends:

Fig. 1: Recruitment of the Nucleus Basalis of Meynert (NBM), a basal forebrain cholinergic nucleus, by noxious stimuli eliciting pain. (a) Schematic of the main cholinergic nuclei in mouse brain and their predominant projection paths; the substantia innominata is included under the NBM. (b, c) Immunohistochemical detection of the activity marker, Fos, in cholinergic neurons (ChAT+ve; arrows indicate co-labelled cells) in the NBM following unilateral intraplantar injection of the strong noxious stimulus, capsaicin as compared to naïve mice (sham). Shown are typical examples (a) and quantification (b); n = 3 mice/group; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test. (d) Schematic representation of in vivo electrophysiological recordings in awake, behaving mice using tetrodes implanted in the NBM (white arrow showing electrode tip lesion in brain section). Mechanical stimuli
were delivered to the plantar surface of the contralateral hindpaw in mice acclimatized to a grid via triggered von Frey filaments. (e) Time frequency representation of spectral modulation in the NBM for all trials with paw withdrawal response to either low intensity forces (0.07 g and 0.16 g; weak filaments) or forces close to or at nociceptive threshold (0.6 g and 1 g; strong filaments). Power is coded as event related perturbation (ERP) representing the deviation from the mean over a 1000 ms baseline period immediately preceding stimulus onset. Shown are mean values from 5 animals after 6-10 repetitions of von Frey application. (f) Quantification of power of oscillatory activity in frequency ranges theta (4 – 8 Hz), alpha (8 – 14 Hz), beta (14 – 30 Hz) and gamma (30 – 100 Hz) derived from time frequency representations shown in (e), represented as % change in 2 s post-application period over 1 s baseline activity prior to stimulus application, in response to non-noxious (top) and noxious (bottom) mechanical stimuli; (g) Time course of change in gamma band oscillatory power calculated as % increase over the mean of baseline (1s prior to stimulus application); time of paw withdrawal is indicated by vertical blue line. In panels e-g, n = 5 mice; *p<0.05, one-sample t-test (f), and one-way repeated measures ANOVA with Fisher’s LSD multi-comparison to baseline (g). Scale bars represent 0.5 mm and 50 µm (image on the right) in b and 250 µm in panel c. Abbreviations: MS: medial septal nucleus; vDB: diagonal band of Broca; LDT: laterodorsal tegmental nucleus; PPT: pedunculopontine tegmental nucleus; mPFC: medial prefrontal cortex AA, anterior amygdaloid area; ac, anterior commissure; CPu, caudate putamen; GP, glopus pallidus; LOT, nucleus of the lateral olfactory tract; mfb, medial forebrain bundle; ic: internal capsule.

Fig. 2. Enhanced responsivity in cholinergic neurons and increased power of beta and gamma oscillatory activity in the NBM at peak of inflammatory pain. (a, b) Comparison of activity of cholinergic neurons of the NBM in the presence or absence of application of mechanical stimulation with 0.16 g force to the contralateral plantar hindpaw under baseline
conditions or 1 day after CFA injection. Shown are typical examples (a) and quantification (b); n = 4 mice/group; *p<0.05, two-way ANOVA with Sidak’s multiple comparisons test. (c) Time frequency representation of spectral power in the NBM in mice at day 1 after CFA injection (n = 5 mice/treatment). (d, e) Comparison of the power of oscillatory activity in beta and gamma frequency ranges between naïve (sham) conditions and CFA day 1, calculated as % increase in 2 s post-application period over 1 s baseline activity prior to stimulus application. Shown are stimulus-response curves (d) or analysis of % change in spectral power (e) in response to innocuous filaments (0.07 and 0.16 g) and noxious mechanical pressure (0.6-1.0 g); n = 3 mice/group; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test.

**Fig. 3. Resolving changes in NBM activity during acute nociception and inflammatory at the single cell level.** (a) Typical examples (upper panels) and average z-scores, which represent number of standard deviations for data points above or below mean, demonstrating NBM units that are excited by noxious stimulation of the paw (left-most panel), units that are suppressed in activity by paw stimulation (middle panel) and units that are not altered significantly in activity following paw stimulation. (b) Distribution of NBM units responding to mechanical stimulation in naïve (sham) conditions and during hindpaw CFA-induced peak inflammatory pain (day 1-4). (c, d) The maximum enhancement of activity over average baseline values for units excited by mechanical stimulation is demonstrated in naïve mice and post-CFA. In panel d, units are subdivided into class 1 and class 2 (fast spiking) types of neurons based on waveform. n = 5 mice/group; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test (c), and unpaired t-test (d).

**Fig. 4. Optogenetic activation of the NBM cholinergic neurons attenuates inflammatory mechanical, but not thermal, hypersensitivity.** (a) Scheme for optogenetically manipulating
NBM cholinergic neurons with blue laser light. (b, c, d) Expression of the excitatory opsin, Channelrhodopsin-EYFP in the NBM (a), typical examples (c) and quantification (d) of enhanced Fos expression in EYFP+ and ChAT+ neurons (arrows in c) with blue light, thus validating the efficacy of optogenetic activation in vivo. n = 3 mice/group; unpaired t-test. Scale bar = 200 µm in b and 50 µm in c. (e, f) Significant attenuation of peak mechanical hypersensitivity (day 2) induced by intraplantar CFA injection, shown as stimulus-response curves (e) and withdrawal thresholds (f) in response to von Frey stimulation; p values in inset represent ANOVA-based comparison of the two entire stimulus-response curves. (g) Lack of modulation of hypersensitivity to a heat ramp. In panels e and f, n = 7 ChAT-Cre- and 8 ChAT-Cre+ mice; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test.

**Fig. 5. Strong attenuation of inflammatory mechanical and thermal hypersensitivity by optogenetic stimulation of NBM cholinergic-GABAergic projections to the prelimbic cortex (PL) and the role of layer 5 PL neurons.** (a) Scheme for optogenetically manipulating NBM-PL cholinergic-GABAergic projections with blue laser light. (b, c) Significant attenuation of peak mechanical hypersensitivity (day 2; b) and heat hypersensitivity (c) induced by intraplantar CFA injection; p values in inset (b) represent ANOVA-based comparison of the two entire stimulus-response curves. n = 6 ChAT-Cre- and 11 ChAT-Cre+ mice; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test. (d) Different schemes of connectivity between NBM cholinergic-GABAergic projections, excitatory afferents and diverse PL neurons, leading to different types of impact on firing of PL pyramidal neurons via nicotinic and muscarinic cholinergic signaling. IN: GABAergic interneuron; PN: pyramidal neuron; PV: parvalbumin-type fast-spiking GABAergic interneuron; SOM: somatostatin-type GABAergic interneuron; VIP: vasoactive intestinal peptide-type GABAergic interneuron. (e) Example images showing NBM-PL projections, in comparison to diffuse NBM connectivity.
to adjoining cortices (top: different high resolution confocal fields stitched together) and high magnification view of terminations in PL across diverse neocortical layers (below). Images represent anti-YFP immunohistochemistry. Scale bars = 250 µm and 100 µm in images on the top and below, respectively. (f, g) Quantification of activity marker Fos across all layers (f) and specifically in layer 5 of the PL (g) in response to optogenetic activation of NBM-PL cholinergic-GABAergic projections. n = 5 mice/group; student’s unpaired t-test. (h) In Rbp4-Cre mice expressing the excitatory DREADD, hm3D(Gq) in a Cre-dependent, activation of layer 5 of PL by Clozapine N-oxide (CNO) attenuates peak mechanical hypersensitivity. n = 8 mice/group; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test.

**Fig. 6: Enhanced recruitment of NBM cholinergic neurons in neuropathic pain and attenuation of neuropathic allodynia by chemogenetic activation of the NBM.** (a, b) Typical examples and quantitative summary of expression of the activity marker Fos in cholinergic neurons (ChAT-expressing) of the NBM in naïve mice and mice with chronic constriction nerve injury (CCI) in the presence or absence of a low intensity (0.16 g) mechanical stimulus at the contralateral hindpaw. Scale bar = 50 µm; n = 4 mice/group; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons tests. (c, d) Scheme for chemogenetically activating NBM cholinergic neurons expressing hm3D(Gq) with clozapine N-oxide (CNO; c) and validation of its efficacy in increasing Fos expression in comparison to mice expressing mCherry in cholinergic neurons (control; d). In panel d, n = 3 mice/group. (e, f, g) Comparison of capsaicin-induced nocifensive responses (e) and the development of CCI-induced mechanical hypersensitivity (f) or thermal hypersensitivity (g) between mice with chemogenetic activation of NBM cholinergic neurons and control (mCherry) mice. p values in inset (f) represent ANOVA-based comparison of the two entire stimulus-response curves. n =
5 sham & 6 hmD(Gq) mice; * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test.

Fig. 7. Analysis of attention, anxiety and motor function in mice with diverse manipulations of NBM cholinergic neurons. (a) Scheme of training mice in attention-related tasks in the 5-Choice Serial Reaction Task test (5-CSRT). (b, c) Increased attention-related parameters in mice with chemogenetic activation of cholinergic neurons in the NBM (b), but not in mice with optogenetic activation of the NBM cholinergic projections to the PL (c). n = 10 mice/group (b); n = 6 ChAT-Cre- and 8 ChAT-Cre+ mice (c); * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test. (d, e, f) Analysis of anxiety-related behavior (upper panels in d, e and f) and locomotion (lower panels in d, e and f) in the open field test in mice with chemogenetic (d) or optogenetic (e) activation of cholinergic neurons in the NBM or mice with optogenetic activation of the NBM cholinergic projections to the PL (f), as compared to their respective control groups. n = 6 mCherry and 8 hm3D(Gq) mice (e); n = 7 ChAT-Cre- and 8 ChAT-Cre+ mice (f); n = 6 mice/group (g); * p < 0.05, two-way ANOVA with Sidak’s multiple comparisons test.

Data availability: All raw data from electrophysiology experiments will be uploaded to a public repository at the time of publication.

References

1. Tan LL, Kuner R. Neocortical circuits in pain and pain relief. Nature Reviews Neuroscience 22, 458-471 (2021).
2. Kuner R, Kuner T. Cellular Circuits in the Brain and Their Modulation in Acute and Chronic Pain. *Physiological reviews* **101**, 213-258 (2021).

3. Naser PV, Kuner R. Molecular, Cellular and Circuit Basis of Cholinergic Modulation of Pain. *Neuroscience* **387**, 135-148 (2018).

4. Mesulam MM, Mufson EJ, Wainer BH, Levey AI. Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience* **10**, 1185-1201 (1983).

5. Okada K, Nishizawa K, Kobayashi T, Sakata S, Hashimoto K, Kobayashi K. Different cholinergic cell groups in the basal forebrain regulate social interaction and social recognition memory. *Scientific reports* **11**, 13589 (2021).

6. Chaves-Coira I, Rodrigo-Angulo ML, Nunez A. Bilateral Pathways from the Basal Forebrain to Sensory Cortices May Contribute to Synchronous Sensory Processing. *Frontiers in neuroanatomy* **12**, 5 (2018).

7. Fang Y-Y, Yamaguchi T, Song SC, Tritsch NX, Lin D. A Hypothalamic Midbrain Pathway Essential for Driving Maternal Behaviors. *Neuron* **98**, 192-207.e110 (2018).

8. Tan LL, Oswald MJ, Kuner R. Neurobiology of brain oscillations in acute and chronic pain. *Trends in neurosciences* **44**, 629-642 (2021).

9. Ploner M, Sorg C, Gross J. Brain Rhythms of Pain. *Trends Cogn Sci* **21**, 100-110 (2017).
10. Yue L, Iannetti GD, Hu L. The Neural Origin of Nociceptive-Induced Gamma-Band Oscillations. *The Journal of Neuroscience: the official journal of the Society for Neuroscience* **40**, 3478-3490 (2020).

11. Melzer S, Monyer H. Diversity and function of corticopetal and corticofugal GABAergic projection neurons. *Nature Reviews Neuroscience*, (2020).

12. Bartho P, Hirase H, Monconduit L, Zugaro M, Harris KD, Buzsaki G. Characterization of neocortical principal cells and interneurons by network interactions and extracellular features. *Journal of neurophysiology* **92**, 600-608 (2004).

13. Yague JG, Tsunematsu T, Sakata S. Distinct Temporal Coordination of Spontaneous Population Activity between Basal Forebrain and Auditory Cortex. *Frontiers in neural circuits* **11**, 64 (2017).

14. Saunders A, Granger AJ, Sabatini BL. Corelease of acetylcholine and GABA from cholinergic forebrain neurons. *eLife* **4**, (2015).

15. Apkarian AV, *et al.* Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *The Journal of Neuroscience: the official journal of the Society for Neuroscience* **24**, 10410-10415 (2004).

16. Ji G, *et al.* Cognitive impairment in pain through amygdala-driven prefrontal cortical deactivation. *The Journal of Neuroscience: the official journal of the Society for Neuroscience* **30**, 5451-5464 (2010).

17. Zhang Z, Gadotti VM, Chen L, Souza IA, Stemkowski PL, Zamponi GW. Role of Prelimbic GABAergic Circuits in Sensory and Emotional Aspects of Neuropathic Pain. *Cell Rep* **12**, 752-759 (2015).
18. Cheriyan J, Sheets PL. Altered Excitability and Local Connectivity of mPFC-PAG Neurons in a Mouse Model of Neuropathic Pain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **38**, 4829-4839 (2018).

19. Jefferson T, Kelly CJ, Martina M. Differential Rearrangement of Excitatory Inputs to the Medial Prefrontal Cortex in Chronic Pain Models. *Frontiers in neural circuits* **15**, 791043 (2021).

20. Zaborszky L, *et al.* Specific Basal Forebrain-Cortical Cholinergic Circuits Coordinate Cognitive Operations. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **38**, 9446-9458 (2018).

21. Tritsch NX, Granger AJ, Sabatini BL. Mechanisms and functions of GABA co-release. *Nature reviews Neuroscience* **17**, 139-145 (2016).

22. Huang J, *et al.* A neuronal circuit for activating descending modulation of neuropathic pain. *Nature neuroscience* **22**, 1659-1668 (2019).

23. Roth Bryan L. DREADDs for Neuroscientists. *Neuron* **89**, 683-694 (2016).

24. Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33**, (1988).

25. Bourgeais L, Gauriau C, Bernard JF. Projections from the nociceptive area of the central nucleus of the amygdala to the forebrain: a PHA-L study in the rat. *The European journal of neuroscience* **14**, 229-255 (2001).
26. Detari L, Semba K, Rasmusson DD. Responses of cortical EEG-related basal forebrain neurons to brainstem and sensory stimulation in urethane-anaesthetized rats. *The European journal of neuroscience* 9, 1153-1161 (1997).

27. Zhang YQ, Mei J, Lu SG, Zhao ZQ. Age-related alterations in responses of nucleus basalis magnocellularis neurons to peripheral nociceptive stimuli. *Brain research* 948, 47-55 (2002).

28. Gross J, Schnitzler A, Timmermann L, Ploner M. Gamma oscillations in human primary somatosensory cortex reflect pain perception. *PLoS biology* 5, e133 (2007).

29. Tan LL, *et al.* Gamma oscillations in somatosensory cortex recruit prefrontal and descending serotonergic pathways in aversion and nociception. *Nature communications* 10, 983-999 (2019).

30. Schulz E, *et al.* Prefrontal Gamma Oscillations Encode Tonic Pain in Humans. *Cerebral cortex (New York, NY : 1991)* 25, 4407-4414 (2015).

31. Ploner M, Gross J. Gamma Oscillations Shape Pain in Animals and Humans. *Trends Cogn Sci* 23, 1086 (2019).

32. Paquette T, Tokunaga R, Touj S, Leblond H, Piche M. Regulation of cortical blood flow responses by the nucleus basalis of Meynert during nociceptive processing. *Neuroscience research* 149, 22-28 (2019).

33. Pafundo DE, Miyamae T, Lewis DA, Gonzalez-Burgos G. Cholinergic modulation of neuronal excitability and recurrent excitation-inhibition in prefrontal cortex circuits: implications for gamma oscillations. *The Journal of physiology* 591, 4725-4748 (2013).
34. Howe WM, et al. Acetylcholine Release in Prefrontal Cortex Promotes Gamma Oscillations and Theta-Gamma Coupling during Cue Detection. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 37, 3215-3230 (2017).

35. Schweinhardt P, Bushnell MC. Pain imaging in health and disease--how far have we come? *The Journal of clinical investigation* 120, 3788-3797 (2010).

36. Tracey I. Neuroimaging mechanisms in pain: from discovery to translation. *Pain* 158 Suppl 1, S115-S122 (2017).

37. Kim JA, Davis KD. Neural Oscillations: Understanding a Neural Code of Pain. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry*, 1073858420958629 (2020).

38. Vierck CJ, Yezierski RP, Wiley RG. Pain sensitivity following loss of cholinergic basal forebrain (CBF) neurons in the rat. *Neuroscience* 319, 23-34 (2016).

39. Jiang YY, et al. Neural pathways in medial septal cholinergic modulation of chronic pain: distinct contribution of the anterior cingulate cortex and ventral hippocampus. *Pain* 159, 1550-1561 (2018).

40. Dale J, et al. Scaling Up Cortical Control Inhibits Pain. *Cell Reports* 23, 1301-1313 (2018).

41. Talay RS, et al. Pharmacological restoration of anti-nociceptive functions in the prefrontal cortex relieves chronic pain. *Progress in neurobiology* 201, 102001 (2021).
42. Gan Z, et al. Repetitive non-invasive prefrontal stimulation reverses neuropathic pain via neural remodelling in mice. *Progress in neurobiology* **201**, 102009 (2021).

43. Radzicki D, Pollema-Mays SL, Sanz-Clemente A, Martina M. Loss of M1 Receptor Dependent Cholinergic Excitation Contributes to mPFC Deactivation in Neuropathic Pain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **37**, 2292-2304 (2017).

44. Koga K, et al. Stimulating muscarinic M1 receptors in the anterior cingulate cortex reduces mechanical hypersensitivity via GABAergic transmission in nerve injury rats. *Brain research* **1704**, 187-195 (2019).

45. Dunckley P, Aziz Q, Wise RG, Brooks J, Tracey I, Chang L. Attentional modulation of visceral and somatic pain. *Neuropsychopharmacology* **19**, 569-577 (2007).

46. Ploner M, Lee MC, Wiech K, Bingel U, Tracey I. Prestimulus functional connectivity determines pain perception in humans. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 355-360 (2010).

47. Baliki MN, Apkarian AV. Nociception, Pain, Negative Moods, and Behavior Selection. *Neuron* **87**, 474-491 (2015).

48. Eldufani J, Blaise G. The role of acetylcholinesterase inhibitors such as neostigmine and rivastigmine on chronic pain and cognitive function in aging: A review of recent clinical applications. *Alzheimers Dement (N Y)* **5**, 175-183 (2019).

49. Laalou FZ, de Vasconcelos AP, Oberling P, Jeltsch H, Cassel JC, Pain L. Involvement of the basal cholinergic forebrain in the mediation of general (propofol) anesthesia. *Anesthesiology* **108**, 888-896 (2008).
50. Mathias JL, Cant ML, Burke ALJ. Sleep disturbances and sleep disorders in adults living with chronic pain: a meta-analysis. *Sleep Med* **52**, 198-210 (2018).

51. Choy EH. The role of sleep in pain and fibromyalgia. *Nat Rev Rheumatol* **11**, 513-520 (2015).

52. Qi J, *et al.* Altered functional connectivity between the nucleus basalis of Meynert and anterior cingulate cortex is associated with declined attentional performance after total sleep deprivation. *Behavioural brain research* **409**, 113321 (2021).

53. Liu AKL, Chang RC-C, Pearce RKB, Gentleman SM. Nucleus basalis of Meynert revisited: anatomy, history and differential involvement in Alzheimer's and Parkinson's disease. *Acta neuropathologica* **129**, 527-540 (2015).

54. Lv Q, Du A, Wei W, Li Y, Liu G, Wang XP. Deep Brain Stimulation: A Potential Treatment for Dementia in Alzheimer's Disease (AD) and Parkinson's Disease Dementia (PDD). *Frontiers in neuroscience* **12**, 360 (2018).

55. Gratwicke J, *et al.* Resting state activity and connectivity of the nucleus basalis of Meynert and globus pallidus in Lewy body dementia and Parkinson's disease dementia. *Neuroimage* **221**, 117184 (2020).

56. Oswal A, *et al.* Cortical connectivity of the nucleus basalis of Meynert in Parkinson's disease and Lewy body dementias. *Brain : a journal of neurology* **144**, 781-788 (2021).

57. Lowell BB, Olson D, Yu J. Development and phenotype of ChAT-IRES-Cre mice.). [MGI Ref ID J:114556] (2006).
58. Dixon WJ. Efficient analysis of experimental observations. *Annual review of pharmacology and toxicology* **20**, 441-462 (1980).

59. Humby T, Laird FM, Davies W, Wilkinson LS. Visuospatial attentional functioning in mice: interactions between cholinergic manipulations and genotype. *The European journal of neuroscience* **11**, 2813-2823 (1999).

60. Paxinos G, Franklin KBJ. *The mouse brain in stereotaxic coordinates*, 2nd edn. London, Academic Press (2001).

61. Pachitariu M, Steinmetz NA, Kadir SN, Carandini M, Harris KD. Fast and accurate spike sorting of high-channel count probes with KiloSort. In: *Advances In Neural Information Processing Systems* (eds Lee DD, Sugiyama M, Luxburg UV, Guyon I, Garnett R). Curran Associates, Inc. (2016).

62. Van der Maaten L, Hinton G. Visualizing data using t-SNE. *Journal of machine learning research* **9**, (2008).
Figure 1

Recruitment of the Nucleus Basalis of Meynert (NBM), a basal forebrain cholinergic nucleus, by noxious stimuli eliciting pain.
Enhanced responsivity in cholinergic neurons and increased power of beta and gamma oscillatory activity in the NBM at peak of inflammatory pain.
Figure 3

Resolving changes in NBM activity during acute nociception and inflammatory at the single cell level.

Figure 4

Optogenetic activation of the NBM cholinergic neurons attenuates inflammatory mechanical, but not thermal, hypersensitivity.

Figure 5

Strong attenuation of inflammatory mechanical and thermal hypersensitivity by optogenetic stimulation of NBM cholinergic-GABAergic projections to the prelimbic cortex (PL) and the role of layer 5 PL neurons.

Figure 6

Enhanced recruitment of NBM cholinergic neurons in neuropathic pain and attenuation of neuropathic allodynia by chemogenetic activation of the NBM.

Figure 7

Analysis of attention, anxiety and motor function in mice with diverse manipulations of NBM cholinergic neurons.

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