Pain is an unpleasant experience. How the brain’s affective neural circuits attribute this aversive quality to nociceptive information remains unknown. By means of time-lapse in vivo calcium imaging and neural activity manipulation in freely behaving mice encountering noxious stimuli, we identified a distinct neural ensemble in the basolateral amygdala that encodes the negative affective valence of pain. Silencing this nociceptive ensemble alleviated pain affective-motivational behaviors without altering the detection of noxious stimuli, withdrawal reflexes, anxiety, or reward. Following peripheral nerve injury, innocuous stimuli activated this nociceptive ensemble to drive dysfunctional perceptual changes associated with neuropathic pain, including pain aversion to light touch (allodynia). These results identify the amygdalar representations of noxious stimuli that are functionally required for the negative affective qualities of acute and chronic pain perception.
expressed the negative valence marker gene \textit{Rspo2} but not the positive valence marker gene \textit{Pprrr1b} \cite{24} (fig. SI, D and E).

We next determined if the nociceptive ensemble was engaged during aversive experiences other than pain by presenting a panel of sensory, but nonsomatosensory or nonnaturalistic, aversive stimuli, including repulsive odor, bitter taste, loud tone, facial air puff, and electric shock. We found that while there was overlap between the neural ensembles that encode nociceptive, aversive, and electric shock stimuli (~10% of all imaged neurons), there remained a subset of BLA neurons (~6% of imaged neurons) that responded only to naturalistic nociceptive stimuli (Fig. 1K and fig. S8).

By analyzing the neural ensemble dynamics with pattern classification methods, we were able to classify and distinguish with high accuracy noxious stimuli from other aversive stimuli (fig. S8E), supporting the finding that noxious stimuli induce a distinct mode of BLA activation (supplementary text SI). Moreover, sensory stimuli of different valences, intensities, and modalities are represented by unique activity codes. Noxious stimuli were encoded distinctively from one another and could be distinguished

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**Fig. 1.** A distinct nociceptive neural ensemble in the BLA represents diverse painful stimuli. (A) BLA neural activity was imaged in freely behaving mice with a microendoscope and the virally expressed fluorescent Ca\textsuperscript{2+} indicator GCaMP6m. Noxious mechanical (pin prick) and thermal (55°C H\textsubscript{2}O and 5°C H\textsubscript{2}O or acetone) stimuli were delivered to the left hindpaw, while reflexive and affective-motivational behavior were monitored via a scope-mounted accelerometer. (B) Microendoscope placement and GCaMP6m expression in the right, contralateral BLA. The red line marks the focal plane and is also a 1.0-mm scale bar. (C and D) Map of active BLA neurons (n = 131 neurons) with numbers in (C) matching independent component analysis–derived neuron activity traces in (D). Scale bar, 100 μm. (E) Spearman’s correlation between reflexive withdrawal and affective-motivational escape acceleration. (F) Mean Ca\textsuperscript{2+} response (Z-scored ΔF/F per trial) across all trials for all BLA neurons imaged during a single session (n = 215 neurons) from the same animal. Neurons are aligned from high to low Ca\textsuperscript{2+} responses in the noxious heat trials. Individual neuron identifications between different stimuli are consistent across the trial rows. (G) Stimulus-locked mean Ca\textsuperscript{2+} activity within the nociceptive ensemble (cyan) and mean affective-motivational escape acceleration (red). Shaded region, ±SEM. Pie charts indicate the percentages of significantly responding neurons. (H) Venn diagram of neural populations encoding nociceptive information in response to noxious heat, cold, and pin stimuli. Numbers show means ± SEM of percentages of significantly responding neurons across imaging sessions (see fig. S5E). (I) Neural populations within the nociceptive ensemble that encode innocuous light touch (0.07-g filament) and mild touch (a 1.4- or 2.0-g filament). (J) Divergent neural populations (versus the nociceptive ensemble) encoding appetitive stimuli (10% sucrose consumption). (K) Overlapping BLA populations between the nociceptive ensemble, electric footshock, and aversive stimuli (isopentylamine odor, facial air puff, 85-dB noise, and quinine consumption). A subset of nociceptive ensemble neurons were pain specific (~6% of the BLA neurons). (L) Accuracies of a nine-way Naïve Bayes decoder that distinguishes the ensemble activities for noxious, innocuous, aversive, anticipatory, and appetitive stimuli. The percentage of decoder accuracy to output for the actual stimuli (diagonal) was compared to that for the incorrect stimuli (off the diagonal) and normalized so that each actual stimuli column added up to 100%. Stars on the diagonal indicate the correct prediction of said stimulus was significantly greater than all off-diagonal stimuli within the same column (Wilcoxon sign-rank, Benjamini-Hochberg corrected). (M) Spearman’s correlation (ρ) between per trial pain behavioral responses and nociceptive ensemble activation. Error bars, ±SEM per session animal responses; n = 9 mice, 3 to 4 sessions each.
with even higher fidelity from innocuous, non-noxious aversive, and appetitive stimuli (Fig. 1L and fig. S9, A and B), indicating that there is a core set of BLA neurons that encodes nociceptive stimuli via specific dynamic neural codes. One crucial finding was that greater activation of this BLA nociceptive ensemble was predictive of increased pain behaviors, suggesting that BLA nociceptive processing influences the magnitude of pain behaviors (Fig. 1M and fig. S7, H and I).

To test the causal role of the BLA nociceptive ensemble for pain behaviors, we expressed a Cre-dependent inhibitory DREADD neuromodulator (hM4-mCherry) in mutant TRAP mice (FoxCreERT2) by applying noxious pin pricks that induced activity-dependent, spatially, and temporally controlled DNA recombination and hM4-mCherry expression (noci-TRAPhM4 mice) (Fig. 2, A to C, and fig. S10) (25, 26). Since the BLA encodes multiple modalities of nociceptive stimuli within a core ensemble (Fig. 1H), we hypothesized that silencing the neurons activated by noxious pin prick would alter behavioral responses to all types of noxious stimuli. Indeed, the hM4 agonist clozapine-N-oxide (CNO; 10 mg/kg) significantly reduced both attending and escape behaviors, but not stimulus detection and withdrawal, for both mechanical and thermal noxious stimuli (Fig. 2, D to G, and fig. S11, A and B). CNO alone had no effect on pain behaviors in control mice (fig. S11C). To test operant pain behavior, we next allowed noci-TRAPhM4 mice to explore a thermal gradient track in which the polar ends were set at noxious cold (5 to 15°C) and hot (42 to 48°C) temperatures (Fig. 2H). The noci-TRAPhM4 mice injected with control saline rapidly acquired an adaptive avoidance strategy of the noxious zones. In contrast, noci-TRAPhM4 mice treated with CNO visited the noxious zones more frequently and for prolonged periods (Fig. 2, H to J, and fig. S12). Similarly, inhibition of the BLA nociceptive ensemble eliminated pain affective-motivational behaviors induced by the optogenetic activation of peripheral primary afferent nociceptors (fig. S13).

Whether pain and anxiety rely on common or distinct BLA ensembles is unknown; therefore, we placed noci-TRAPhM4 mice within an elevated plus maze, in which anxiety drives avoidance of the open arms (Fig. 2K). The noci-TRAPhM4 mice given either saline or CNO displayed equivalent visits to and occupancy of the open arms (fig. S14, A and B). Since nociceptive and
A hallmark of chronic neuropathic pain is the appearance of allodynia and hyperalgesia, both pathological perceptual states in which aversion is ascribed to innocuous somatosensory stimuli and exacerbated in response to noxious stimuli, respectively (Fig. 3A) (29). We hypothesized that this pathological perceptual switch might result from maladaptive transformations in BLA coding. We tracked the longitudinal dynamics of BLA ensembles before and after the development of neuropathic pain induced by sciatic nerve injury (17,396 neurons, n = 17 mice) (Fig. 3). Throughout the development of chronic neuropathic pain, a subset of neurons stably encoded the nociceptive ensemble for both noxious mechanical and cold stimuli (fig. S6). Nerve injury did not significantly increase the spontaneous activity of the nociceptive ensemble and overall BLA population (fig. S15, A and B). However, BLA neural activity elicited in response to light touch displayed a significant expansion within the nociceptive ensemble in neuropathic (291 ± 88% increase) but not in uninjured mice (38 ± 14% decrease) (Fig. 3, D to G, and fig. S15, C to E). The emergence of this neuropathic coding schema was accompanied by the development of reflexive paw withdrawal hypersensitivity and by enhanced affective-motivational pain behaviors (Fig. 3, B and C, and fig. S4, C to F). The magnitudes of

**Fig. 3. Convergence of BLA neural ensemble representations of innocuous and noxious information during chronic pain.** (A) Long-term tracking of BLA neural activity with microendoscopes throughout the development of chronic neuropathic pain. Peripheral nerve injury results in an increased sensitivity and perceived aversion to innocuous (allodynia) and noxious (hyperalgesia) stimuli. (B) Affective-motivational escape acceleration for neuropathic (top row; n = 5 mice) and uninjured (bottom row; n = 4 mice) animals in response to noxious pin or light touch stimuli before and after nerve injury. Dark lines, means; shaded regions, ±SEM. (C) Hyperalgesic and allodynic behavioral responses in neuropathic (n = 13 mice for paw withdrawal, n = 5 mice for escape acceleration) or uninjured (n = 4 mice for both measures) animals after application of light touch (0.07-g filament), noxious pin, or noxious cold (acetone or 5°C H2O drop) stimuli, respectively. Data were quantified by reflexive hypersensitivity (left axis) and affective-motivational escape acceleration (right axis). (D) Mean Ca2+ activity (Z-scored ΔF/ΔF per trial) of all neurons from the same animal for that imaging session, before and after nerve injury, in response to noxious pin prick, noxious cold, and light touch stimuli. Neuron identifications were consistent between stimuli within a day, but not across days (n = 157 and 156 neurons, for days −7 and 42, respectively). (E) Mean Ca2+ response within the nociceptive ensemble for neuropathic (top row; n = 13 mice, 12,026 total neurons imaged) and uninjured (bottom row; n = 4 mice, 5370 total neurons imaged) animals in response to noxious pin or light touch stimuli. (F) Venn diagrams of percentages of significantly responding neurons to noxious pin, noxious cold, and light touch before and after nerve injury. (G) Overlapping neural populations responsive to light touch within the nociceptive ensemble (pin prick and 5°C water or acetone responsive neurons) after nerve injury (n = 13 mice) or in uninjured animals (n = 4 mice). Numbers indicate means ± SEM. (H) Percentages of nociceptive ensemble activated and escape acceleration per imaging session (light-colored points) and across animal groups and conditions (dark, larger points) show significant correlations [Spearman’s ρ = 0.54 (normal), 0.33 (Neuropathic), and 0.58 (Uninjured) groups]. All tests results in the figure were analyzed via Wilcoxon rank-sum with Benjamini-Hochberg correction unless otherwise noted. Stars, P < 0.01.
Fig. 4. Inhibition of neuropathic BLA ensemble activity reduces the aversive quality of chronic pain. (A) Utilization of light touch to gain genetic access to, and manipulate, the neuropathic nociceptive ensemble. (B) Quantification of light touch TRAP neurons in the BLA of neuropathic mice compared to uninjured mice; n = 7 per group. (C) Behavioral raster plots from neuropathic mice showing the effects of inhibiting the BLA nociceptive ensemble on reflexive and affective-motivational pain behaviors associated with cold allodynia. (D and E) Summary of the effects of ensemble inhibition against reflexive (D) and affective-motivational (E) pain behaviors in response to noxious pin prick, noxious cold (acetone drop), or formerly innocuous touch stimuli (0.07-g filament). Behavior was assessed before and 42 days after nerve injury and again at 60 min after CNO or saline administration on day 42; n = 14 per group. (F and G) Effects of neuropathic ensemble inhibition on adaptive avoidance during a cold place aversion assay. (F) Group mean exploration paths, color coded for the relative occupancy time, and lines represent individual subjects. Error bars, ±SEM. For (B), Student’s t test; (D and E), two-way ANOVA with Bonferroni correction; (G) three-way ANOVA with Bonferroni correction.

The behavioral responses and the BLA nociceptive ensemble Ca²⁺ activity were significantly correlated before and after injury (Fig. 3H and fig. S13F). These results suggest a role for the BLA in the emergence of allodynia in chronic pain states.

We next asked if we could prevent the neural transformation of light touch sensory information into an aversive signal and eliminate chronic pain unpleasantness by gaining genetic access to the nociceptive ensemble with innocuous stimuli in neuropathic TRAP mice. At 21 days post–nerve injury, when allodynia had fully developed (fig. S16, B to E), we delivered a light touch TRAP protocol to express hM4-mCherry in the BLA nociceptive ensemble (neuropathic TRAPhM4 mice) (Fig. 4, A and B, and fig. S16). At day 42 postinjury, neuropathic TRAPhM4 mice displayed significant allodynia and hyperalgesia, for both reflexive and affective-motivational pain responses, relative to uninjured mice (Fig. 4, C to E). While the injection of CNO in neuropathic TRAPhM4 mice did not alter reflexive hyper-sensitivity (Fig. 4D), we observed a profound decrease in neuropathic affective-motivational behaviors, regardless of stimulus intensity or modality (Fig. 4E and fig. S17, A and B). Uninjured TRAPhM4 mice given the light touch TRAP protocol expressed levels of hM4-mCherry in the BLA that were similar to those of nonstimulated control mice (Fig. 4B and fig. 2C), presumably because the nociceptive ensemble does not strongly encode innocuous information under normal conditions (Fig. 1I). We observed neither CNO-mediated changes in affective-motivational pain behaviors in these uninjured mice nor CNO effects on neuropathic reflexive or affective-motivational behaviors in these uninjured mice compared to uninjured mice; n = 7 per group; open star, P < 0.05; closed star, P < 0.01; open square, P < 0.01; closed square, P < 0.001. Overlaid dots and lines represent individual subjects. Error bars, ±SEM. For (B), Student’s t test; (D and E), two-way ANOVA with Bonferroni correction; (G) three-way ANOVA with Bonferroni correction.

Thus, disrupting neural activity in a nociceptive ensemble in the BLA is sufficient to reduce the affective dimension of pain experiences, without altering their sensory component. The unconditioned nociceptive ensemble described here is a stable network of amygdalar principal neurons that is responsive to a diverse array of noxious stimuli. Within this ensemble, combinatorial neural ensemble codes distinguish the
various thermal and mechanical nociceptive stimuli. These codes likely represent stimulus modality, intensity, salience, and valence to provide additional qualitative information that enriches individual pain affect percepts (30). The presence of a purely nociceptive-specific subpopulation of neurons within the larger BLA nociceptive ensemble, distinct from general aversion-encoding populations, suggests the capacity for computing and assigning an accompanying “pain tag” to valence information. This categorical signal could prioritize the negative valence of intense noxious stimuli and scale the selection of conative pain protective behaviors. It is thought that hierarchical pathways transform low-level sensory inputs into higher-order affective responses (5, 37). Our chemogenetic manipulations suggest that this critical node in the nociceptive brain circuitry plays a critical role in shaping pain experiences, by providing an evaluation of nociceptive information that, in turn, intrinsically motivates protective behaviors associated with pain (32).

The phenomenological description of a pain experience is normally that of a complex but unified sensory and emotional perception that can neither exist alone as an unanchored aversive state nor stand merely on its emotionally inert sensory qualities (33, 34). Though activity within the BLA nociceptive ensemble cannot account for the instantiation of the entire pain experience, we propose that the BLA nociceptive ensemble transmits abstracted valence information to the central amygdala, striatal, and cortical networks (35–37). For example, BLA neurons projecting to the CeA may send a “pain tag” that helps select for appropriate defensive responses to impending or immediate threats (23) (supplementary text S2). In parallel, connected cortical regions might coalesce BLA affective signals with experience, we propose that the BLA nociceptive ensemble might coalesce BLA affective signals with amygdalar input, or intra-amygdala plasticity (II) remains an open question. Chronic pain is not simply a sensory disorder but a neurological disease with affective dysfunction that profoundly impacts the mental state of millions of pain patients (40). Clinical management of chronic pain remains a staggering challenge, given the heterogeneity of underlying causes, and the overreliance on opioid analgesics has contributed to the opioid epidemic (41, 42). Comprehensive strategies that provide substantive relief across pain types are urgently needed (43). To make progress along this translational path, we have identified in the BLA a critical neural ensemble target that mediates chronic pain unpleasantness. This finding may enable the development of chronic pain therapies that could selectively diminish pain unpleasantness, regardless of etiology, without influencing reward, and importantly, preserving reflexes and sensory-discriminative processes necessary for the detection and localization of noxious stimuli (+4, 45). Collectively, our findings begin to refine the neural basis and coding principles underlying the multiple dimensions and complexity of the pain experience for developing more effective analgesic therapies.

REFERENCES AND NOTES

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SUPPLEMENTARY MATERIALS
www.sciencemag.org/content/363/6424/276/suppl/DC1
Supplementary Text
Materials and Methods
Figs. S1 to S17
Table S1
References (47–94)
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An amygdalar neural ensemble that encodes the unpleasantness of pain
Gregory Corder, Biafra Ahanonu, Benjamin F. Grewe, Dong Wang, Mark J. Schnitzer and Grégory Scherrer

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The emotional dimension of pain
The unpleasantness of pain is an emotional phenomenon distinct from pain's sensory qualities. To study how the brain processes pain-related emotions, Corder et al. used in vivo neural calcium imaging in freely behaving mice. They identified brain circuits that respond to pain and directly tested their causal role in motivational behaviors associated with acute and chronic pain. Science, this issue p. 276