

Opioidergic pain relief in humans is mediated by beta and high-gamma modulation in limbic regions

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Abstract

The nature of the neurophysiological effects of opioids, especially those responsible for their analgesic properties, are unknown, hindering efforts to develop non-addictive alternatives. Fentanyl and hydromorphone were administered to patients experiencing semi-chronic, clinically-relevant pain after surgical implantation of electrodes for the localization of seizure onset. Opioids suppressed beta oscillations in lateral amygdala, ventral and dorsolateral prefrontal cortices, and increased beta in medial amygdala and hippocampus. Opioids also suppressed high gamma oscillations in insula and lateral amygdala, and increased high gamma in cingulate cortex and hippocampus. The amplitude of these beta effects in the ventral prefrontal cortex, medial amygdala and hippocampus, and of gamma effects in the insula, were positively correlated with the magnitude of pain relief in response to a constant dose. These findings identify electrophysiological events in a network of limbic structures that may participate in opioidergic pain relief through nociceptive gating and a decreased concerned fixation on pain, providing insights into the neural basis of pain relief and suggesting possible biomarkers for developing non-addictive opioid alternatives.

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Running Title: Clinical pain relief in intracranial EEG

Introduction

Little is known about the neural basis of analgesia for clinically relevant pain in the human forebrain. Studies conducted to date have typically evoked acute pain in their experimental paradigms, which lacks qualities including the persistence, and associated distress, of more representative pain requiring medical management. Aside from its sensory-discriminative properties, human pain requiring clinical treatment is also an affective and interpretive phenomenon¹⁻³, and far less is known about these higher order telencephalic pain substrates. To further examine these substrates, we utilized the precision and sensitivity of bipolar intracranial recordings to measure the effects of opioids in the human brain and isolate signatures of opioidergic analgesia.

The effects of opioids have been extensively studied in afferent nociceptive pathways. This system extends from primary nociceptors in tissue, to peripheral sensory ganglia, spinal cord, brainstem regulatory nuclei including periaqueductal gray and rostral ventromedial medulla, and finally projecting to an array of thalamic nuclei. Opioids inhibit the transmission of nociceptive signals along this pathway, thus reducing the sensation of pain.⁴ In the telencephalon, the μ -opioid receptor, which is most strongly targeted by pain relieving opioid drugs, is highly expressed in amygdala, anterior hippocampus, anterior cingulate cortex, orbitofrontal cortex, and insula.⁵ However, little is known about the neurophysiological mechanisms by which opioids exert their effects, therapeutic or otherwise, in these structures. On a local level, μ -opioid receptors act upon a diverse array of cell types, often exerting its effects as a presynaptic receptor reducing neurotransmitter release, and producing variable circuit-level effects with regional specificity. While a complete picture of the neurophysiological effect of opioids remains elusive, previous studies suggest that cortical regions are, in general, inhibited, whereas in medial temporal lobe, the effect of opioids varies sharply by region and context.^{6,7}

Human neuroimaging studies on the effects of intravenous opioid drugs have produced conflicting results. Studies employing measures sensitive to blood flow (PET rCBF or BOLD fMRI) have generally found increases, most consistently in anterior cingulate, but including other structures such as caudate nucleus, thalamus, and amygdala,⁸⁻¹¹ though one such study employing PET rCBF also found decreased signal in left orbitofrontal cortex.¹² In contrast, the only fluorodeoxyglucose PET study to date found mostly decreases in many of the same structures.¹³ In addition, studies employing measures dependent on blood flow may be confounded by opioid effects on vascular dynamics or cellular metabolism.^{14,15}

Furthermore, some effects of opioids are likely more related to analgesia than others; a few neuroimaging studies have attempted to parse this distinction experimentally, observing drug-acute pain interactions in critical regions including anterior cingulate cortex, insular cortex, amygdala, and inferior frontal cortex, but inconsistent or counterintuitive results (e.g. amplification of pain-related-activation) in some cases..^{9,16,17}

In transient, experimenter-evoked pain paradigms, non-invasive magnetoencephalography (MEG) and EEG studies most commonly report that signals predictive of a “high pain” state have been a decrease in central alpha band power (8-12Hz)^{18–20} and an increase in parietal, prefrontal and thalamic gamma band power (>30Hz).^{21–24} In the prefrontal cortex, tonic experimental noxious stimulation has been associated with enhanced power of gamma oscillations, and suppression of alpha and beta oscillations associated with pain intensity.^{25–27} While acute experimental pain is sometimes associated with beta suppression in scalp EEG, studies on chronic pain have revealed a positive association between frontal beta oscillation power and pain severity in arthritic pain²⁸ as well as enhanced beta coherence in fibromyalgia.²⁹ Cross-sectional studies on neuropathic pain have identified increases in power in alpha-beta range.^{30,31} A systematic review of EEG and MEG studies have concluded that enhanced beta and theta oscillations are characteristic of chronic pain generally.³² Moreover, an EEG study in healthy patients have found decreases in beta and alpha power with moderate doses of an oral opioid.³³

There are, to our knowledge, no peer-reviewed studies investigating the role of opioid administration on intracranial EEG. However, the intracranial evoked potentials and high-frequency activity to acute experimental pain have been reported.^{34–39} Generally, these studies have observed responses in primary somatosensory cortex, in insular cortex, anterior cingulate and other limbic regions, which is consistent with known telencephalic pain pathways.³⁷ Three complementary studies by the same research group characterized the temporal sequence of evoked potentials across the brain, demonstrating a progression from sensory to associative regions, notably involving the medial temporal lobe later than cortical regions.^{34–36} While most evoked pain studies have not dissociated nociception from sensory effects per se, one study tested correlates of gradations in rated thermal pain level while controlling for stimulus temperature, and found that anterior cingulate, hippocampus, frontal regions, striatum, amygdala, and fusiform gyrus predicted pain ratings.³⁷

Notably, a recent study used machine learning to classify endogenous variations in intensity of pain in patients with refractory neuropathic pain.⁴⁰ This was successful in predicting high-pain significantly above chance; however, this model utilized only a limited

set of brain regions and did not attempt to identify LFP signatures which were consistent across subjects. Machine-learning based approaches have also been successful at predicting chronic pain status⁴¹ and endogenous variation in chronic pain state⁴² using scalp EEG. These methods have potential clinical applications in diagnosis or monitoring and demonstrate that EEG contains information relevant to pain state; however, they do not easily generalize to clinically relevant models of pain or pain relief.⁴³

There have been, to date, no peer-reviewed human studies which have uncovered consistent neural signatures of opioid effects on human local field potentials. Furthermore, there are very few studies of any methodology which probe the independent correlates of pain relief in human brain, and none study clinically relevant pain; rather, most studies on pain processing in humans have employed transient (typically seconds) and non-destructive heat or shock stimuli. Patients undergoing epilepsy monitoring with depth electrodes often have a significant degree of post-surgical pain, which is commonly treated with intravenous opioid drugs at various points during the recording period. Sites sampled frequently for the purposes of epilepsy monitoring include regions having well-established associations with pain, including insula, cingulate and orbital cortices, amygdala, hippocampus, and prefrontal cortex. This presents an opportunity to study the forebrain neural signatures of clinically relevant pain relief with the precision of intracranial depth electrode recordings, providing critical insight into distal pathways of pain processing, as well as forebrain mechanisms which function to allay the experience of pain. While this model is not identical to chronic pain, it's clinical relevance furthers our understanding of how opioids relieve post-surgical pain, and may yield insight into how such effects could be replicated consistently and safely to guide the development of much-needed non-opioidergic treatments for intractable chronic pain.

Materials and methods

Patient population

The participants gave written informed consent to participate in this study, and the study was approved by University of California's Institutional Review Board in accordance with the Declaration of Helsinki. The participants were compensated for their participation. Patients were recruited from a population with long-standing drug-resistant complex partial epilepsy who are implanted with depth electrodes in order to determine their seizure origin. While

awaiting spontaneous seizures to occur, the patients may consent to participate in experiments. Regions of the frontal lobe and limbic system are frequently and consistently implanted for the purposes of epilepsy monitoring and are also locations that are implicated in the affective-interpretive aspects of pain. Patients often experience some degree of post-surgical pain as a consequence of the surgical implantation procedure. We identified patients who experienced significant pain and received pain-relieving opioid medication. A subset of four patients (of 26 for which data were available) were given at least 10 doses of intravenous opioids which did not coincide with putative sleep or (post-)seizure periods (defined below); these were chosen for further analysis (see Table 1). All patients had overall cognitive function in the normal range.

Data preprocessing

Archival SEEG recordings were processed and reformatted for analysis using standard methods. A notch filter was applied at 60 Hz line noise frequency and its harmonics up to the Nyquist frequency, before the data was resampled to a uniform 1000 Hz sampling rate, from native sampling rates of 500-1024 Hz, using a delay-compensated FIR antialiasing filter. To ensure that measured signals were focal, all monopolar recordings were bipolar re-referenced, utilizing adjacent depth electrode contacts with 5 mm pitch. Bipolar pairs were excluded if displaying low amplitude (indicating white matter or CSF, approximate threshold of 30 μ V slow-wave amplitude), abnormal LFP (e.g. prominent atypical oscillations), frequent interictal discharges ($>3 \text{ min}^{-1}$ in cortex or 1 min^{-1} in medial temporal lobe), or if located within a seizure focus. All cortical bipolar pairs were selected from non-overlapping contacts; however, due to the limited number of high-quality channels in hippocampus and amygdala, overlap was allowed in these regions. Clock time was reconstructed using file metadata. Electrode location was determined by MRI/CT co-registration, followed by automated parcellation using FreeSurfer^{44,45} and the HCP-MMP1⁴⁶ cortical segmentation. In one patient where surface reconstruction was not possible due to incomplete imaging data, electrode locations were hand-annotated using trajectories drawn from clinical neurosurgery software (ROSA). Electrodes in medial temporal structures were hand-annotated using co-registered MRI and CT scans in 3D Slicer.⁴⁷ Time series analysis was performed using custom MATLAB code and FieldTrip.⁴⁸

Epoch selection and artifact rejection

Interictal epileptiform events (spikes) were detected as described previously.^{49,50} A high frequency score was computed by smoothing the 70-190 Hz analytic amplitude with a 20 ms boxcar function and a spike template score was generated by computing the cross-covariance with a template interictal spike. Both scores were normalized by their channel-wise medians. The high frequency score was weighted by 13 and the spike score was weighted by 25, and an IIS was detected when these weighted sums exceeded 130. In each patient, detected IIS and intervening epochs were visually examined from all channels to confirm high detection sensitivity and specificity. The detected period, 300 ms preceding, and 700 ms following were excluded from further analysis. Putative seizures were marked as unusually sudden and dense periods of epileptiform activity (detected as above), cross-referenced with clinical records, and visually confirmed in the raw LFP. The durations of all seizures, the 5 minutes preceding, and a period of 2 hours following were excluded from analysis. The latter value was chosen to reliably exceed the length of time required for spectral amplitude measures (e.g. high gamma 70-190 Hz envelope, suppressed post-seizure, and slow-wave 0.5-2 Hz envelope, amplified post-seizure) to return to baseline. Epochs containing large artifacts, interruptions in signal, or highly unstable spectral characteristics (assessed by beta, high gamma, slow-wave, 8-12 Hz alpha, and 4-8 Hz theta amplitudes) suggestive of abnormal activity, for example during an extended period of repeated seizures, were similarly identified and excluded.

Sleep rejection

Sleep rejection methodology performed as described previously,⁵⁰ consistent with criteria for N2/N3 identification described by Silber et al.⁵¹ The median slow-wave (0.5-2 Hz) analytic amplitude across channels was used as a slow-wave sleep score to identify and exclude sleep. Slow-wave sleep cycles were detected as periods in which the slow-wave sleep score was greater than threefold times the median of the score over the preceding 18 through the following 18 hours. The use of the local median allowed correction for amplitude drift during the recording. 25 minutes preceding or following slow-wave sleep peaks thus defined were excluded in order to ensure that Stage 1 and REM periods, expected to occur before and after such detections, were also eliminated.

Testing of drug effect

Administration times of intravenous opioid medications were recorded with minute-scale precision as a function of clinical recordkeeping. We compared neurophysiological metrics during the period immediately preceding administration of an opioid to a period of expected peak analgesic effect (10-30 minutes) for both intravenous fentanyl and hydromorphone.^{52,53}

Spectral perturbation plots

Event-related spectral perturbation plots were generated using power spectra computed for each minute of data. Each minute of data was segmented into continuous non-overlapping segments of at least 8 seconds without detected IIS or artifacts, and the average of all such segments taken as the spectrum of the corresponding minute. A multi-taper time-frequency spectral estimation method was employed, as implemented in the Chronux^{54,55} toolbox. Resultant spectra were resampled from linear to log-frequency scales while applying a delay-compensated finite impulse-response antialiasing filter. Drug-evoked changes were normalized to the pre-drug (30-10 minute) baseline period. For event-related spectral perturbation (ERSP) plots (Fig. 1A), the mean spectral response across trials was expressed in dB for each minute interval relative to drug dose. For spectral change plots (Fig. 1B), the average spectral power from 10-30 minutes post-drug was normalized to the pre-drug baseline, and the result expressed as a raw proportion. Results were smoothed for display using a Gaussian filter over time (ERSP) and frequency (ERSP and spectral change plots).

Evoked amplitude-modulation plots

Drug-evoked changes in beta or high gamma amplitude were further analyzed by applying a third-order Butterworth bandpass filter to isolate the corresponding frequency (15-25 Hz or 70-190 Hz, respectively), and computing the exact Hilbert analytic amplitude.⁵⁶ In order to eliminate the potential effects of long-term drifts in spectral characteristics, the amplitude time-course for each trial was baselined and normalized by the mean and variance, respectively, of the baseline period (30 to 10 minutes prior to each dose) for that trial. Trials were rejected if more than 75% of the baseline period was rejected or more than 25% of the effect averaging period was rejected. Channels were pooled within predefined regions of interest, which were chosen on the basis of sampling, known neurophysiology of pain, and conventional neuroanatomical distinctions. The z-score of the drug effect, defined as the

average of the above timecourses within the 10-30 minute window following each drug dose, was computed for each recording site, and used for cross-site analyses.

Testing of pain-relief correlates

To assess the neural correlates of pain relief, change in VAS score pre-to-post opioid was compared to change in measures of interest (beta or high gamma analytic amplitude, computed as above) pre-opioid to the 10 minutes preceding the post-opioid pain measurement. Only trials in which the modal dose of opioid was given were included. Each trial was normalized to its baseline mean and variance, as above, to account for long-term shifts. For each recording site, the R value of the correlation between VAS change and neurophysiological change was computed, and used for cross-site analyses.

Cross-site drug-effects and pain relief models

Separate models were employed for each combination of frequency (beta/HG) and analysis type (drug effect/pain relief) in order to assess overall and regional patterns in drug-effect or pain-relief signatures. A linear mixed model, including a random effect by subject was employed to account for within-subject correlations. Z-scores (drug effect) or R values (pain-relief correlations) were modeled as a function of drug type, hemisphere, and region as categorical covariates. A separate model was used to predict pain-relief R values using drug-effect Z-values in the same frequency (beta or HG), including random effects by subject and an intercept term. No other covariates were included in this second model.

Control analysis of non-opioid nurse check-ins

In order to verify that observed neurophysiological effects were specific to opioid administrations, rather than following interactions with nurses generally, we conducted the same analysis used for testing IV opioid-evoked effects, but applied to non-opioid nurse check-ins. These were events in which the nurse similarly interacted with the patient and the patient reported a VAS score, but opioids were not given. Furthermore, we restricted our analysis to cases where no opioid had been given for 90 minutes, nor was any opioid given for at least 30 minutes (the end of the post-event averaging period) afterward. As with our analysis of IV opioid effects, we conducted an analyses using a linear-mixed effects model to

test for any overall changes in beta or HG amplitude associated with such events, as well as any region-wise or hemispheric variation.

Results

Spectral characterization of opioid-evoked responses

Recordings from bipolar channels were examined in their responses to opioids across time and frequency. Sites from all subjects exhibited pronounced suppression in the 15-25 Hz beta frequency range (Fig. 1A). The period 10-30 minutes post-opioid was chosen to measure drug effect, as this falls within the period of peak pain relief for both drugs;^{52,53} this period captured the distinct suppression of beta-frequency amplitude (Fig. 1B). Nevertheless, we observed that the time-course of the drug effect matched the kinetics of either the short-acting (fentanyl, FENT) or longer-acting (hydromorphone, HMOR) opioid (# of sites significant at 30-50 minutes: 2 of 23 active FENT sites, 14 of 31 active HMOR sites, site-wise effects FDR corrected $p < 0.05$, chi squared test by drug and duration $p = 0.004$).

Regional characteristics of opioid-induced beta suppression

Overall, opioids tended to decrease beta amplitude in both hemispheres. Both FENT and HMOR exhibited this effect (Fig. 2D). In cortex, Prefrontal Cortex (PFC, including Ventral, VPFC; Dorsolateral, DLPFC) showed the greatest suppression (Fig. 2C,D). In medial temporal regions, opioids relatively *increased* beta amplitude in Anterior Hippocampus (AHC), Posterior Hippocampus (PHC), and Medial Amygdala (MAmyg). Interestingly, Lateral Amygdala (Lamyg) exhibited strong beta suppression, similarly to cortical regions (Fig. 3C). A functional division along the medial-lateral axis of the amygdala is demonstrated in a series of bipolar pairs spanning the right amygdala of one subject (Pt. 1): MAmyg exhibits exclusively a transient beta facilitation from 5-10 minutes post-drug, whereas in Lamyg FENT reduced beta amplitude for approximately 30 minutes (Fig. 3A,B).

High gamma amplitude modulation by opioids

In contrast to broad beta suppression, HG amplitude showed no overall trend following administration of opioids. However, region-specific effects were apparent. HG amplitude was generally suppressed in Insula (INS) and in Lamyg. Again, other medial temporal structures

(Mamyg, AHC, PHC) responded oppositely to Lamyg, with many sites in these regions increasing in HG amplitude, with significant overall increases in both AHC and PHC.

Opioid effects are not accounted for by nurse interactions

We conducted analogous testing of nurse interactions which were not associated with opioid doses, to verify that neurophysiological changes were not the result of social interaction, VAS score reporting, changes in arousal, or other covariates of general clinical care. No region-wise or overall effects observed peri-opioid were also evoked by nurse check-ins generally (Table S2). Note that such interactions may have resulted in a pain drug being given (e.g. acetaminophen) or a non-pain-related medication, but did not result in an opioid being given (e.g. oral oxycodone or any IV opioid).

Neural correlates of opioid analgesia

In order to dissociate nontherapeutic effects of opioid medication and probe for neural signatures specific to pain, we tested for correlations between beta or HG amplitude and endogenous variability in reported pain relief, while holding opioid dose constant. Amplitude changes were measured between the 30-10 minute pre-drug baseline and the 10 minute period preceding the post-drug pain evaluation. We modeled site-wise R values using linear mixed-effects models in order to identify regions specifically associated with pain relief. Interestingly, a brain-wide effect for beta amplitude was significant, though of modest effect size; decreases in beta are on average associated with pain relief. Region-wise pain-relief correlates also broadly aligned with the effects of opioids: suppression of beta in VPFC, enhancement of beta in Mamyg and AHC, and suppression of HG in INS are all expected to correlate with pain relief. Cingulate showed opposite effects, with opioids increasing HG but a negative correlation with pain relief; visual inspection of Cingulate sites suggests that this may be due heterogeneity in our relatively sparse sample, as most anterior sites displayed reduced HG amplitude. Finally, beta amplitude in INS and HG in VPFC also correlated negatively with pain relief. Drug type was a significant factor in both beta and HG pain-relief models; this may represent a partial loading of subject-wise variance. In order to test whether the overall pattern of drug-evoked beta or high gamma modulation overall predicted pain relief, we modeled site-wise R values for the correlation of beta or HG change and pain relief, using the drug-effect z-score as an independent variable, and including random effects by

subject to account for heterogeneity. Coefficients for R value conditional on z-score in both the beta (0.13, $p=0.004$) and HG (0.09, $p=0.017$) models were positive and significant (Table S1), indicating that the patterns of opioid effects on beta and HG were overall significantly associated with pain relief.

Discussion

The present study represents, to our knowledge, the first characterization of the neurophysiological effects of opioids using intracranial EEG in humans, and is amongst the first efforts to isolate the neural correlates of pain relief in EEG generally, which has otherwise only been attempted in neuroimaging studies.^{8,9,16} We sought to elucidate consistent changes in critical telencephalic structures following opioidergic treatment in a clinically-relevant pain condition. Though preliminary, these findings provide novel insight into the therapeutic effects of opioids beyond early sensory systems, on substrates of the interpretive and affective processes responsible for the psychological impact of pain.

Opioids induced a widespread reduction in beta-frequency amplitude, most prominent in the prefrontal cortex, as well as suppression of high gamma amplitude in the insula. Effects in medial temporal lobe were mixed, with suppression of beta and HG in lateral amygdala, but relative increases in medial amygdala (beta) and hippocampus (beta and high gamma). We compared changes in beta and HG amplitude between equal opioid doses that resulted in variable levels of pain relief, in order to determine which, if any, opioid effects were specifically predictive of pain relief. Such effects included beta decreases in VPFC, HG suppression in insular cortex, and beta increases in medial amygdala and hippocampus. Critically, there were significant positive correlations between the effects of opioids and the signatures of pain relief in both beta and high gamma amplitudes, lending further support to the interpretation that the neural processes indexed by beta and HG play a broad role in opioidergic pain relief. Our findings are consistent with prior work indicating that the aforementioned regions, as well as the beta frequency band in which we observed the most consistent opioid effects, are implicated in pain processing, particularly in clinically relevant pain models.^{32,57}

Pain-specific responses, dissociated from neutral sensory stimulation and proportional to intensity,⁵⁸ have been observed in both anterior⁵⁹ and posterior insula,^{34,60} in studies utilizing both iEEG and fMRI. Models of cortical pain processing in which the posterior insula is often considered as the central hub for sensory processing of painful stimuli,^{57,61,62} whereas the anterior insula has been associated with pain salience.^{63,64} Consistent with these findings, we observed a tendency for opioids to suppress high gamma amplitude in the insula, as well as a correlation between high gamma suppression and pain relief. These findings consistently demonstrate that opioids may have a direct, blunting effect on the sensation of pain.

Chronic pain which requires clinical treatment is a complex experience dominated by its interpretive and affective components.¹⁻³ Such interpretive ‘top-down’ processing is often associated with beta oscillations in PFC⁶⁵ with ventral PFC supporting more affective evaluations.⁶⁶⁻⁶⁸ Specifically, spontaneous fluctuations in chronic pain are associated with medial and ventral PFC fMRI-BOLD activation.⁶⁹⁻⁷¹ VPFC lesions alter the influence of expectancy on pain perception (medial regions),⁷² and VPFC activation represents the relative valuation of acute painful stimuli.⁷³ Post-surgical pain shares certain features with chronic pain, including residual inflammation of the surgical wound, often some degree of damage to sensory nerves,⁷⁴ and resultant sensitization of CNS nociceptive pathways,^{75,76} which can develop into true chronic pain.⁷⁷ Accordingly, in VPFC, we found that beta oscillations, rather than high gamma, were associated with opioidergic pain relief. Beta oscillations are consistently found in chronic pain conditions, particularly in frontal regions,²⁸⁻³² which may reflect a transition from a naïve bottom-up form of pain-processing to one in which subjective pain is amplified by concerned fixation.²⁶ If so, beta suppression, as observed in the present study, would indicate relief from cognitive fixation and excessive concern regarding pain.

Recent research has implicated hippocampus in the processing of pain and especially in the development of inflammatory or chronic pain-like conditions.^{78,79} Activation of the dorsal hippocampus relieves neuropathic pain in both rats and mice,⁸⁰ whereas a model of inflammatory pain reduces neurogenesis in the rat dentate gyrus.⁸¹ In humans, chronic pain is associated with reduced hippocampal gray matter volume,^{82,83} and increases in total hippocampal volume have been observed in patients undergoing non-pharmaceutical treatment for chronic pain.⁸⁴ In the hippocampus, μ -opioid activation increases the excitability of pyramidal neurons through both reducing GABAergic interneuron activity⁸⁵ and releasing astrocytic glutamate in the vicinity of pyramidal cells.⁸⁶ Consistent with the known effects of opioids on hippocampal excitability, we observed an increase in high gamma amplitude in both anterior and posterior hippocampus. However, it was only beta oscillations, in anterior hippocampus specifically, which were associated with both opioid effects and pain relief. We are not aware of prior literature relating hippocampal beta oscillations to pain. However, beta may be particularly prominent in the human hippocampal LFP.^{87,88} In human intracranial recordings, enhanced beta oscillations have been observed during motor planning,⁸⁹ while increased beta coherence has been observed between hippocampus and amygdala during fear memory retrieval,⁷⁸ and between hippocampus, occipital cortex, and prefrontal cortex when recognizing known but highly distorted images,⁹¹ suggesting that beta rhythms may be involved in internally generated mentation.

Similarly to the anterior hippocampus, we observed that opioids relatively increased beta power in medial amygdala and that this was associated with pain relief. We also observed evoked beta in individual medial amygdala sites (Fig. 3A), distinct from suppression in adjacent lateral sites. In the rodent amygdala, μ -opioid receptors produce inhibitory or disinhibitory effects, depending on the nucleus and cell type in question.^{92–94} Rodent studies also indicate that the amygdala plays a role in pain-related affective responses,⁹⁵ and via reciprocal connections to medial prefrontal cortex and brainstem nuclei, could link nociception to protective behavioral and autonomic responses.⁹⁶ As a central mediator of fear and stress processes, the amygdala is implicated in the analgesia that attends fight-or-flight responses;^{97,98} accordingly, stimulation of noradrenergic receptors in the amygdala of the rat induces analgesia in a thermally-evoked tail-flick test.⁹⁹ Interestingly, in the same model, the analgesic effects of morphine are attenuated with lesion or inactivation of the central amygdala,⁸⁸ suggesting that opioids may act through the amygdala pathway for stress-induced analgesia.

Another highly μ -opioid-receptor-enriched structure, the anterior cingulate cortex has long been recognized as a key hub for the processing of pain-associated affect.^{35,89–91} Lesion of ACC (cingulotomy) reduces pain-associated distress in humans without interfering with the sensation of pain,^{92–94} and stimulation of ACC in rats,¹⁰⁸ and mice¹⁰⁹ is aversive. In this study, we found that, in the cingulate as a whole, high gamma amplitude was generally increased by opioids, whereas *decreases* are associated with pain relief. Inspecting the distribution of these effects, this incongruity is likely due to heterogeneity across the cingulate cortex, as more anterior sites did not tend to show high gamma increases.

Overall, our results suggest a model in which opioidergic pain relief results from: (1) nociceptive gating (driven by amygdala and observed in reduced insular excitation); (2) decreased concerned fixation on pain (carried by reduced beta in VPFC); and (3) a shift toward internally generated experience (driven by the hippocampus). The current findings, though preliminary, provide a launching point for future investigation into the pathophysiology of clinical pain and strategies to combat it.

Data Availability

All data and analysis code underlying the study will be made available upon reasonable request, with the proviso that sharing of raw data in particular is subject to approval by the Institutional Review Board.

Acknowledgements

The authors express their sincere thanks to Syd Cash, Burke Rosen, Ilya Verzhbinsky, and Adam Niese for their contributions to this work.

Funding

This work was supported by National Institutes of Health grants no. T32MH020002 (J.C.G.), K08NS123543 (S.B.H.), and by a seed grant from the T. Denny Sanford Institute for Empathy and Compassion (J.C.G & E.H.).

Competing interests

The authors report no competing interests.

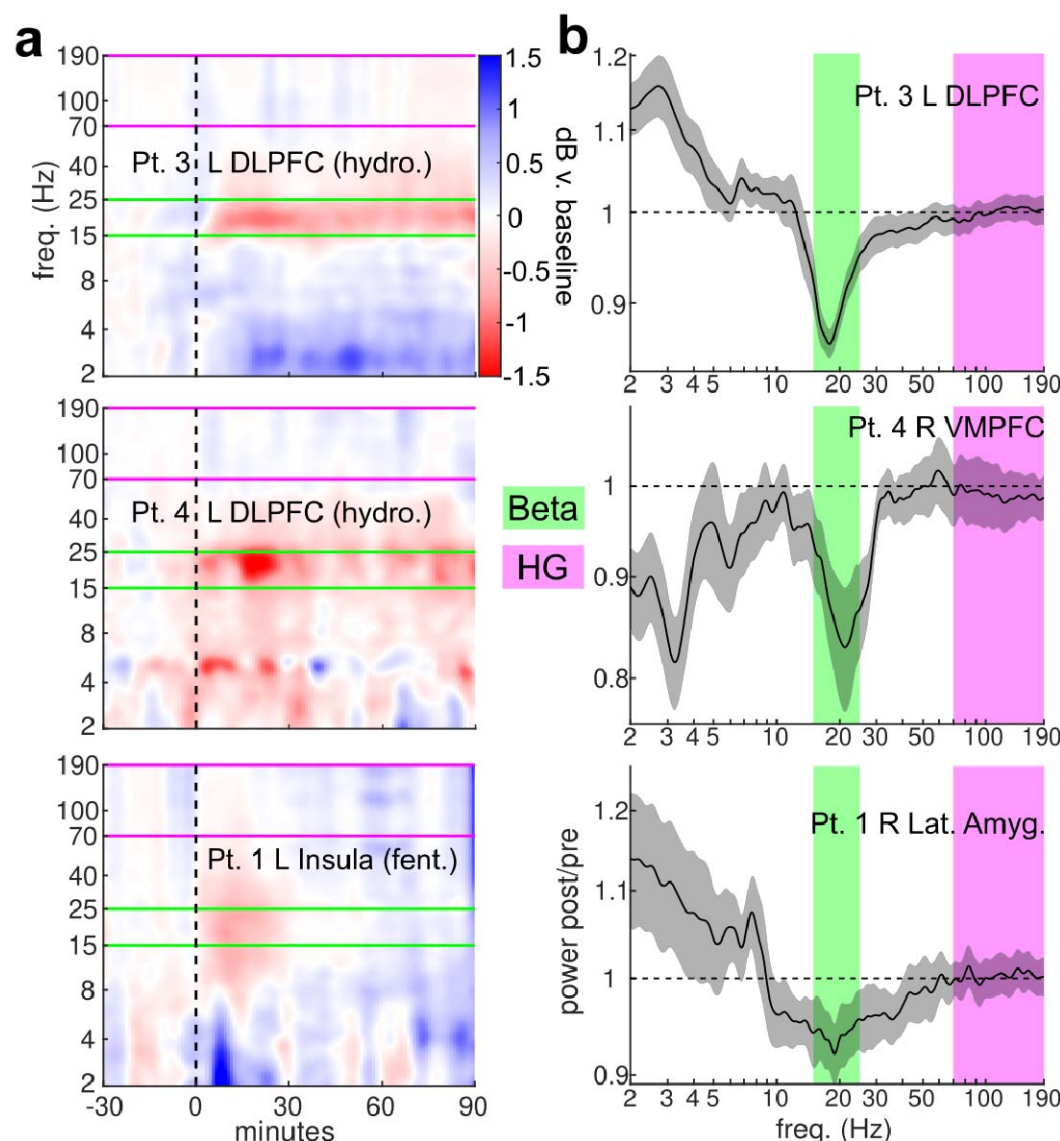


Figure legends

Figure 1. Intravenous opioids produce characteristic amplitude suppression in beta frequencies. **A.** Event-related spectral perturbation (ERSP) relative to recorded drug administration time. Each frequency is normalized to average amplitude during -30 to -10 minute baseline. Hydromorphone (top, middle) produces a longer duration suppression of beta amplitude as compared to fentanyl (bottom), as expected due to the longer kinetics of hydromorphone. **B.** Post-drug (10-30 minute average) power spectra normalized to pre-drug baseline. Shaded area indicates SEM. Each plot shows a different site from the same subject as the adjacent ERSP (A).

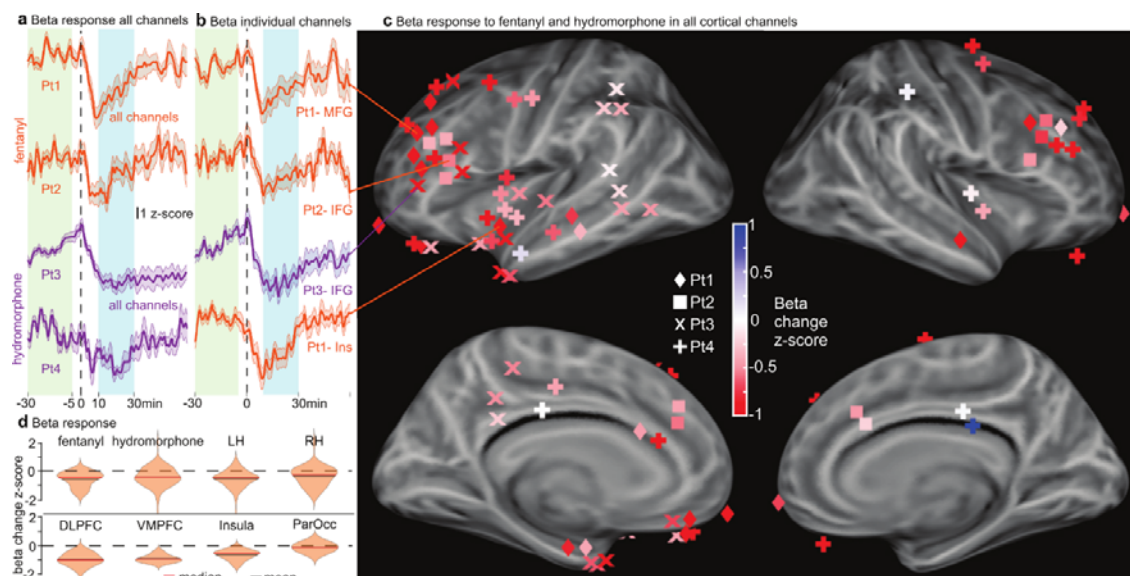


Figure 2. Beta amplitude modulation by intravenous opioids. A. Mean (SEM) beta amplitude over channels and trials, relative to opioid doses. Opioid-evoked beta modulation was calculated as the mean difference between post-drug (cyan) and baseline (green) periods. Orange traces correspond to fentanyl and purple traces correspond to hydromorphone. B. Same as A but for example sites. C. All sites color-coded according to beta amplitude modulation. Overall, opioids tended to reduce beta amplitude. Beta suppression was most profound in prefrontal cortex, including VPFC and DLPFC, and in insular cortex. D. Distributions of modulation values for various categories of site. Fentanyl and hydromorphone similarly suppressed beta amplitude, and left hemisphere sites showed somewhat more beta suppression than right hemisphere sites.

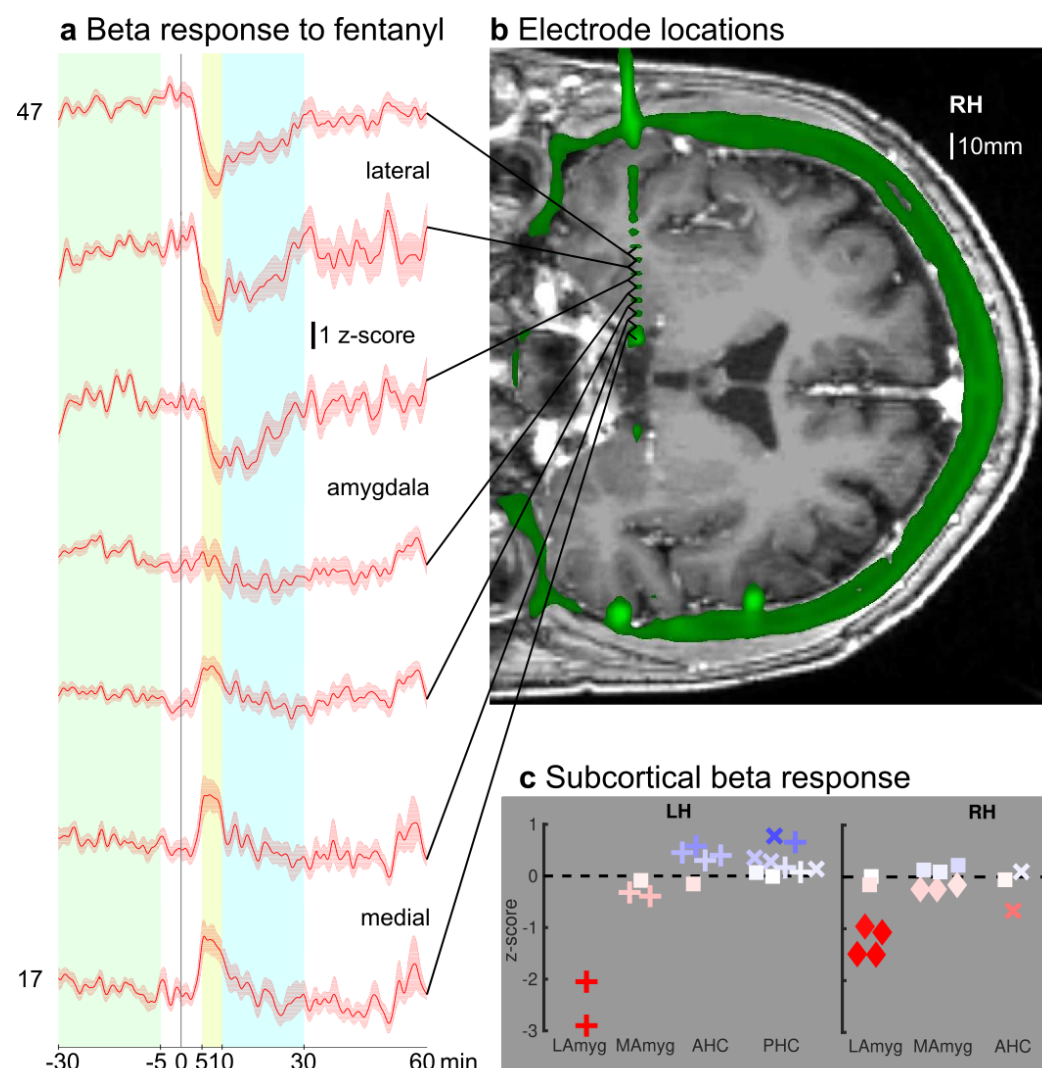


Figure 3. Modulation of beta amplitude in medial temporal lobe by intravenous opioids.

A. Opioid-locked beta amplitude from sites corresponding to a single medial-lateral trajectory through right amygdala, showing a strong gradient in direction and time-course of response. Lateral amygdala exhibits strong suppression of beta amplitude developing by 10 minutes and abating within an hour post-drug, similarly to responses observed in cortical regions. In contrast, medial amygdala shows a transient beta amplification 5-10 minutes post-drug ($p=0.004, 0.005, 0.027$, FDR corrected, post-hoc). **B.** Coregistered MRI/CT showing locations of sites (bipolar pairs) represented in A. **C.** All sites in medial temporal structures. Across all sites, lateral amygdala tended to show cortex-like beta suppression, whereas medial amygdala showed inconsistent responses during the 10-30 minute post-drug epoch. Hippocampus, both anterior and posterior, displayed generally evoked beta amplitude increases or no response, in stark contrast to the suppression seen in cortex and Lateral Amygdala.

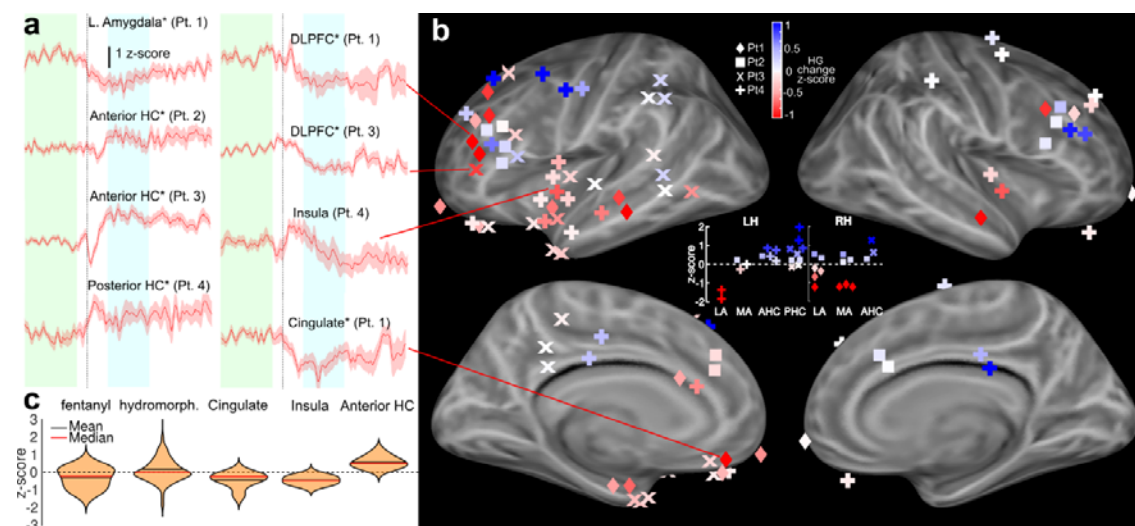


Figure 4. High gamma amplitude modulation by intravenous opioids. A. Mean (SEM) high gamma amplitude relative to opioid doses for example sites. Opioid-evoked HG modulation was calculated as the mean difference between post-drug (cyan) and baseline (green) periods. Evoked changes in high gamma amplitude vary widely in direction and time-course. B. All sites color-coded according to high gamma amplitude modulation. High gamma amplitude decreased in Insula and Lateral Amygdala, while increases were present in Cingulate, AHC, and PHC. C. Distributions of modulation values for various categories of site. Mean increases and decreases were present to similar degrees (no overall average effect). Sites in subjects that received fentanyl tended to exhibit slightly lower post-drug high gamma amplitude than those in hydromorphone subjects.

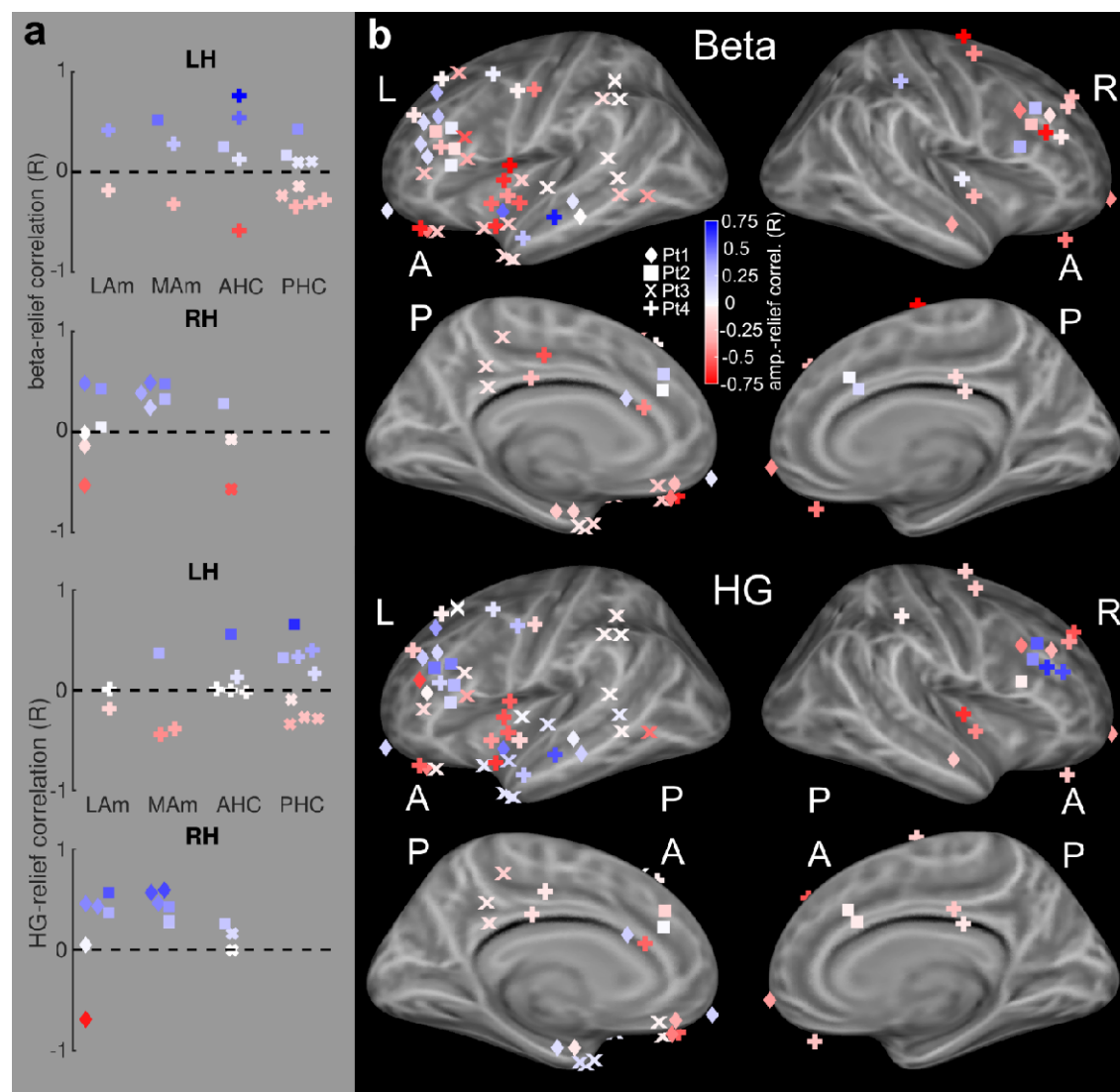


Figure 5. Site-wise correlations between beta and high gamma amplitude and pain relief. A. Correlations (R-values) in medial temporal sites for 15-25 Hz beta (top) and 70-190 Hz high gamma (bottom) versus pre-to-post-drug pain relief. B. Equivalent correlations to A in cortical sites.

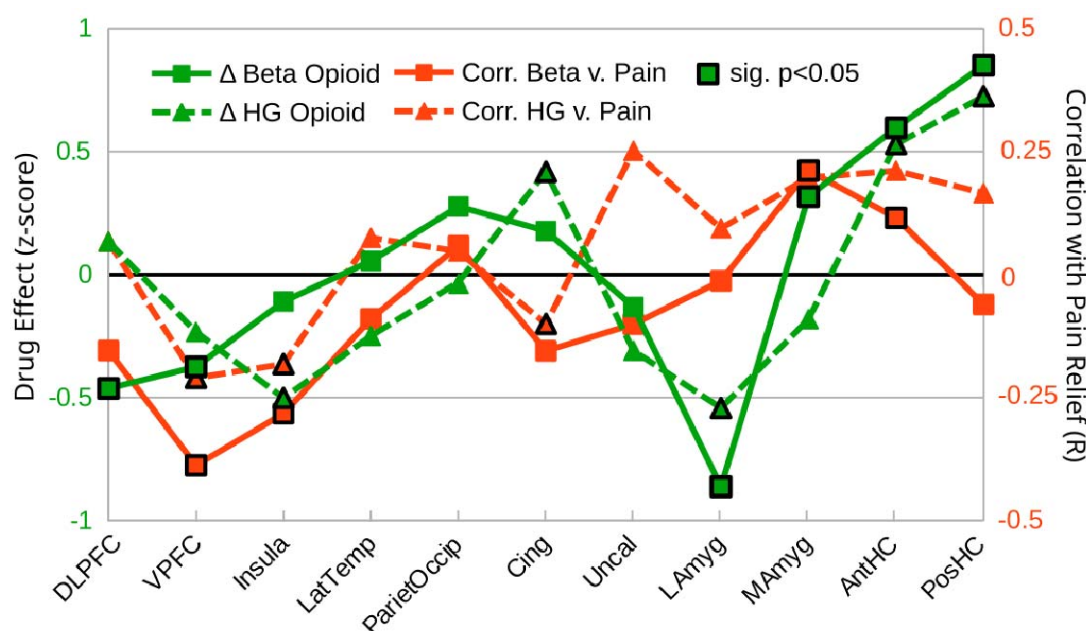


Figure 6. Variation of drug effects and pain-relief correlations in beta and high gamma amplitude, along a cortical-to-limbic axis. Beta and high gamma amplitude (red lines) are strongly suppressed in cortical structures including VPFC, Insula, and (beta only) DLPFC. Limbic structures (Anterior and Posterior HC, Medial Amygdala) show relative increases in beta/high gamma amplitude, with the exception of Lateral Amygdala which shows suppression similarly to cortical areas. Correlations with pain relief (green lines) are seen exclusively in regions associated with pain or affect: VPFC, Insula, Cingulate, Medial Amygdala, Anterior HC. Only one such region (Lateral Amygdala) does not show any relationship between beta or high gamma amplitude and pain relief. In all such regions, either beta or high gamma effects of opioids would be predicted to relieve pain, with the exception of Cingulate, where the effect of the drug (increased high gamma) contrasts with the correlate of pain relief (decreased high gamma).

Subject	Age	Sex	# Days Data	Drug	# Doses	Modal Dose	# Modal doses	# Sites	Median (Range) VAS
S1	36-40	M	10.7	Fentanyl	19	50 mcg	16	25	5 (0-10)
S2	21-25	F	9.0	Fentanyl	31	12.5 mcg	21	22	4 (0-9)
S3	31-35	M	13.0	Hydromorphone	41	1 mg	30	35	7 (0-10)
S4	31-35	M	15.0	Hydromorphone	16	0.5 mg	8	47	5.5 (0-10)

Table 1. Subject characteristics.

Drug Effect	Coefficient (z-score)		p-value	
Model Term	Beta	HG	Beta	HG
Main Effect	-0.45	-0.02	3.1E-13	0.82
DLPFC	-0.46	0.13	9.9E-06	0.39
VPFC	-0.38	-0.23	0.03	0.37
Parietooccipital	0.28	-0.03	0.17	0.92
Insular	-0.11	-0.50	0.42	0.02
Lat. Temporal	0.05	-0.25	0.69	0.24
Cingulate	0.18	0.42	0.16	0.03
Uncus	-0.13	-0.31	0.55	0.37
Lat. Amygdala	-0.86	-0.54	1.1E-06	0.04
Med. Amygdala	0.32	-0.18	0.05	0.47
Anterior HC	0.60	0.53	2.5E-04	0.03
Posterior HC	0.85	0.72	1.8E-07	3.0E-03
Hemisphere (LH)	-0.13	-0.23	0.01	0.01
Drug (Hydro.)	0.03	0.29	0.61	4.4E-04
Pain Relief	Coefficient (R-value)		p-value	
Model Term	Beta	HG	Beta	HG
Main Effect	-0.07	0.04	0.04	0.27
DLPFC	-0.06	0.01	0.33	0.85
VPFC	-0.29	-0.27	3.0E-03	0.01
Parietooccipital	0.16	-0.01	0.17	0.95
Insular	-0.18	-0.24	0.02	0.01
Lat. Temporal	0.01	0.02	0.91	0.81
Cingulate	-0.06	-0.16	0.43	0.04
Uncus	0.00	0.20	0.99	0.15
Lat. Amygdala	0.09	0.04	0.37	0.70
Med. Amygdala	0.31	0.14	8.6E-04	0.15
Anterior HC	0.21	0.16	0.02	0.11
Posterior HC	0.04	0.11	0.66	0.24
Hemisphere (LH)	0.05	0.02	0.13	0.54
Drug (Hydro.)	-0.13	-0.12	1.1E-05	1.8E-04
Key	negative, not sig.		not sig.	
	+, drug or pain sig.		drug or pain sig.	
	-, drug and pain sig.		drug and pain sig.	

Table 2. Linear mixed-effects model coefficients for evoked drug effects and pain-relief correlations in beta and high gamma amplitude. Negative values (red) indicate that decreases were related to drug effect (top) or pain relief (bottom) and significant coefficients are bolded. Opioids decreased beta amplitude overall, but did so particularly in prefrontal cortex (VPFC and DLPFC) and Lateral Amygdala; in VPFC this decrease was concordant with pain relief. In other structures of medial temporal lobe, opioids increased beta in relative (Med. Amyg.) and absolute (Ant. and Pos. HC) terms; this aligns with pain relief in Med. Amyg. and Ant. HC. High gamma was suppressed by opioids in Insular Cortex, matching pain relief in that structure, while in Cingulate Cortex, opioid-evoked high gamma contrasts with expected lower high gamma amplitude during pain relief.

Supplementary Tables

	Pt. 1	Pt. 2	Pt.3	Intercept	Z-Score
Beta/Pain R	0.12	0.2	-0.15	0.24	0.13
p	0.027	<0.001	0.002	0.51	0.004
HG/Pain R	0.07	0.23	-0.1	0.04	0.09
p	0.25	<0.001	0.037	0.11	0.017

Table S1. Specificity of drug effect for pain-relief correlates. Models were used to assess whether opioids tended to produce pain-relief-related effects, when comparing across sites and correcting for subject heterogeneity. The relationship between drug effect and pain relief was positive and significant for both beta amplitude and high gamma.

Evoked Coeff. (z-score)	No opioid		IV opioid	
Model Term	Beta	HG	Beta	HG
Main Effect	0.10	0.20	-0.45	-0.02
DLPFC	-0.01	0.01	-0.46	0.13
VPFC	-0.03	0.04	-0.38	-0.23
Parietooccipital	-0.03	-0.18	0.28	-0.03
Insular	-0.13	-0.13	-0.11	-0.50
Lat. Temporal	-0.03	0.21	0.05	-0.25
Cingulate	-0.25	-0.36	0.18	0.42
Uncus	0.22	-0.20	-0.13	-0.31
Lat. Amygdala	0.27	0.32	-0.86	-0.54
Med. Amygdala	0.14	0.01	0.32	-0.18
Anterior HC	-0.05	0.16	0.60	0.53
Posterior HC	-0.14	0.02	0.85	0.72
Hemisphere (LH)	0.04	-0.03	-0.13	-0.23
p-value	No opioid		IV opioid	
Model Term	Beta	HG	Beta	HG
Main Effect	0.05	0.03	3.1E-13	0.82
DLPFC	0.81	0.93	9.9E-06	0.39
VPFC	0.74	0.74	0.03	0.37
Parietooccipital	0.73	0.21	0.17	0.92
Insular	0.07	0.21	0.42	0.02
Lat. Temporal	0.70	0.04	0.69	0.24
Cingulate	7.3E-05	1.3E-04	0.16	0.03
Uncus	0.05	0.23	0.55	0.37
Lat. Amygdala	1.4E-03	0.01	1.1E-06	0.04
Med. Amygdala	0.07	0.96	0.05	0.47
Anterior HC	0.54	0.16	2.5E-04	0.03
Posterior HC	0.06	0.84	1.8E-07	3.0E-03
Hemisphere (LH)	0.18	0.48	0.01	0.01
Key	negative, not sig.		not significant	
	negative, significant		significant (p<0.05)	

Table S2. Linear mixed-effects model coefficients for control (non-opioid nurse check-in) events as compared to opioid doses, evoked beta and HG amplitudes. Negative values (red) indicate that decreases were related to non-opioid nurse interactions (left) or IV opioids (right) and significant coefficients are bolded. No significant effects occurred in the same direction between the non-opioid control events and IV opioids.

References

1. Machado AG, Baker KB, Plow E, Malone DA. Cerebral Stimulation for the Affective Component of Neuropathic Pain. *Neuromodulation Technol Neural Interface*. 2013;16(6):514-518. doi:10.1111/j.1525-1403.2012.00517.x
2. Hashmi JA, Baliki MN, Huang L, et al. Shape shifting pain: chronification of back pain shifts brain representation from nociceptive to emotional circuits. *Brain*. 2013;136(9):2751-2768. doi:10.1093/brain/awt211
3. Wilkie DJ, Savedra MC, Holzemer WL, Tesler MD, Paul SM. Use of the McGill Pain Questionnaire To Measure Pain: A Meta-Analysis. *Nurs Res*. 1990;39(1):36.
4. Riedel W, Neeck G. Nociception, pain, and antinociception: current concepts. *Z Für Rheumatol*. 2001;60(6):404-415. doi:10.1007/s003930170003
5. Kantonen T, Karjalainen T, Isojärvi J, et al. Interindividual variability and lateralization of μ -opioid receptors in the human brain. *NeuroImage*. 2020;217:116922. doi:10.1016/j.neuroimage.2020.116922
6. Reeves KC, Shah N, Muñoz B, Atwood BK. Opioid Receptor-Mediated Regulation of Neurotransmission in the Brain. *Front Mol Neurosci*. 2022;15. doi:10.3389/fnmol.2022.919773
7. Lau BK, Ambrose BP, Thomas CS, Qiao M, Borgland SL. Mu-Opioids Suppress GABAergic Synaptic Transmission onto Orbitofrontal Cortex Pyramidal Neurons with Subregional Selectivity. *J Neurosci*. 2020;40(31):5894-5907. doi:10.1523/JNEUROSCI.2049-19.2020
8. Jones AKP, Friston KJ, Qi LY, et al. Sites of action of morphine in the brain. *The Lancet*. 1991;338(8770):825. doi:10.1016/0140-6736(91)90717-4
9. Adler LJ, Gyulai FE, Diehl DJ, Mintun MA, Winter PM, Firestone LL. Regional Brain Activity Changes Associated with Fentanyl Analgesia Elucidated by Positron Emission Tomography. *Anesth Analg*. 1997;84(1):120.

10. Schlaepfer TE, Strain EC, Greenberg BD, et al. Site of Opioid Action in the Human Brain: Mu and Kappa Agonists' Subjective and Cerebral Blood Flow Effects. *Am J Psychiatry*. 1998;155(4):470-473. doi:10.1176/ajp.155.4.470
11. Leppä M, Korvenoja A, Carlson S, et al. Acute opioid effects on human brain as revealed by functional magnetic resonance imaging. *NeuroImage*. 2006;31(2):661-669. doi:10.1016/j.neuroimage.2005.12.019
12. Firestone LL, Gyulai F, Mintun M, Adler LJ, Urso K, Winter PM. Human Brain Activity Response to Fentanyl Imaged by Positron Emission Tomography. *Anesth Analg*. 1996;82(6):1247.
13. London ED, Broussolle EP, Links JM, et al. Morphine-induced metabolic changes in human brain. Studies with positron emission tomography and [fluorine 18]fluorodeoxyglucose. *Arch Gen Psychiatry*. 1990;47(1):73-81. doi:10.1001/archpsyc.1990.01810130075010
14. Nagamachi K, Shitara K, Yamashita Y, et al. Role of endogenous opioids and central opioid receptors in cerebral cortical blood flow autoregulation. *Jpn J Physiol*. 1995;45(1):137-149. doi:10.2170/jjphysiol.45.137
15. Shih YYI, Chiang YC, Shyu BC, Jaw FS, Duong TQ, Chang C. Endogenous opioid-dopamine neurotransmission underlie negative CBV fMRI signals. *Exp Neurol*. 2012;234(2):382-388. doi:10.1016/j.expneurol.2011.12.042
16. Oertel B, Preibisch C, Wallenhorst T, et al. Differential Opioid Action on Sensory and Affective Cerebral Pain Processing. *Clin Pharmacol Ther*. 2008;83(4):577-588. doi:10.1038/sj.clpt.6100441
17. Shah YB, Haynes L, Prior MJW, Marsden CA, Morris PG, Chapman V. Functional magnetic resonance imaging studies of opioid receptor-mediated modulation of noxious-evoked BOLD contrast in rats. *Psychopharmacology (Berl)*. 2005;180(4):761-773. doi:10.1007/s00213-005-2214-6
18. Babiloni C, Brancucci A, Del Percio C, et al. Anticipatory electroencephalography alpha rhythm predicts subjective perception of pain intensity. *J Pain*. 2006;7(10):709-717. doi:10.1016/j.jpain.2006.03.005

19. Mouraux A, Guérit JM, Plaghki L. Non-phase locked electroencephalogram (EEG) responses to CO₂ laser skin stimulations may reflect central interactions between A δ - and C-fibre afferent volleys. *Clin Neurophysiol.* 2003;114(4):710-722.
doi:10.1016/S1388-2457(03)00027-0
20. Ploner M, Gross J, Timmermann L, Pollok B, Schnitzler A. Oscillatory activity reflects the excitability of the human somatosensory system. *NeuroImage.* 2006;32(3):1231-1236. doi:10.1016/j.neuroimage.2006.06.004
21. Chien JH, Liu CC, Kim JH, Markman TM, Lenz FA. Painful cutaneous laser stimuli induce event-related oscillatory EEG activities that are different from those induced by nonpainful electrical stimuli. *J Neurophysiol.* 2014;112(4):824-833.
doi:10.1152/jn.00209.2014
22. Kim JH, Chien JH, Liu CC, Lenz FA. Painful cutaneous laser stimuli induce event-related gamma-band activity in the lateral thalamus of humans. *J Neurophysiol.* 2015;113(5):1564-1573. doi:10.1152/jn.00778.2014
23. Shirvalkar P, Sellers KK, Schmitgen A, et al. A Deep Brain Stimulation Trial Period for Treating Chronic Pain. *J Clin Med.* 2020;9(10):3155. doi:10.3390/jcm9103155
24. Zhang ZG, Hu L, Hung YS, Mouraux A, Iannetti GD. Gamma-Band Oscillations in the Primary Somatosensory Cortex—A Direct and Obligatory Correlate of Subjective Pain Intensity. *J Neurosci.* 2012;32(22):7429-7438. doi:10.1523/JNEUROSCI.5877-11.2012
25. Kim JA, Davis KD. Neural Oscillations: Understanding a Neural Code of Pain. *Neurosci Rev J Bringing Neurobiol Neurol Psychiatry.* 2021;27(5):544-570.
doi:10.1177/1073858420958629
26. Ploner M, Sorg C, Gross J. Brain Rhythms of Pain. *Trends Cogn Sci.* 2017;21(2):100-110. doi:10.1016/j.tics.2016.12.001
27. Schulz E, May ES, Postorino M, et al. Prefrontal Gamma Oscillations Encode Tonic Pain in Humans. *Cereb Cortex N Y N 1991.* 2015;25(11):4407-4414.
doi:10.1093/cercor/bhv043

28. Simis M, Imamura M, Pacheco-Barrios K, et al. EEG theta and beta bands as brain oscillations for different knee osteoarthritis phenotypes according to disease severity. *Sci Rep*. 2022;12(1):1480. doi:10.1038/s41598-022-04957-x
29. Alves RL, Zortea M, Serrano PV, et al. High-beta oscillations at EEG resting state and hyperconnectivity of pain circuitry in fibromyalgia: an exploratory cross-sectional study. *Front Neurosci*. 2023;17. doi:10.3389/fnins.2023.1233979
30. Stern J, Jeanmonod D, Sarnthein J. Persistent EEG overactivation in the cortical pain matrix of neurogenic pain patients. *NeuroImage*. 2006;31(2):721-731. doi:10.1016/j.neuroimage.2005.12.042
31. Di Pietro F, Macey PM, Rae CD, et al. The relationship between thalamic GABA content and resting cortical rhythm in neuropathic pain. *Hum Brain Mapp*. 2018;39(5):1945-1956. doi:10.1002/hbm.23973
32. Zebhauser PT, Hohn VD, Ploner M. Resting-state electroencephalography and magnetoencephalography as biomarkers of chronic pain: a systematic review. *Pain*. 2023;164(6):1200-1221. doi:10.1097/j.pain.0000000000002825
33. Bromm B, Meier W, Scharein E. Pre-stimulus/post-stimulus relations in EEG spectra and their modulations by an opioid and an antidepressant. *Electroencephalogr Clin Neurophysiol*. 1989;73(3):188-197. doi:10.1016/0013-4694(89)90119-3
34. Frot M, Faillenot I, Mauguière F. Processing of nociceptive input from posterior to anterior insula in humans. *Hum Brain Mapp*. 2014;35(11):5486-5499. doi:10.1002/hbm.22565
35. Bastuji H, Frot M, Perchet C, Magnin M, Garcia-Larrea L. Pain networks from the inside: Spatiotemporal analysis of brain responses leading from nociception to conscious perception. *Hum Brain Mapp*. 2016;37(12):4301-4315. doi:10.1002/hbm.23310
36. Bastuji H, Frot M, Perchet C, Hagiwara K, Garcia-Larrea L. Convergence of sensory and limbic noxious input into the anterior insula and the emergence of pain from nociception. *Sci Rep*. 2018;8(1):13360. doi:10.1038/s41598-018-31781-z

37. Caston RM, Smith EH, Davis TS, Singh H, Rahimpour S, Rolston JD. Characterization of spatiotemporal dynamics of binary and graded tonic pain in humans using intracranial recordings. *PLOS ONE*. 2023;18(10):e0292808. doi:10.1371/journal.pone.0292808
38. Dowman R, Darcey T, Barkan H, Thadani V, Roberts D. Human intracranially-recorded cortical responses evoked by painful electrical stimulation of the sural nerve. *NeuroImage*. 2007;34(2):743-763. doi:10.1016/j.neuroimage.2006.09.021
39. Liberati G, Mulders D, Algoet M, et al. Insular responses to transient painful and non-painful thermal and mechanical spinothalamic stimuli recorded using intracerebral EEG. *Sci Rep*. 2020;10(1):22319. doi:10.1038/s41598-020-79371-2
40. Shirvalkar P, Prosky J, Chin G, et al. First-in-human prediction of chronic pain state using intracranial neural biomarkers. *Nat Neurosci*. 2023;26(6):1090-1099. doi:10.1038/s41593-023-01338-z
41. Levitt J, Edhi MM, Thorpe RV, et al. Pain phenotypes classified by machine learning using electroencephalography features. *NeuroImage*. 2020;223:117256. doi:10.1016/j.neuroimage.2020.117256
42. Ryu S, Gwon D, Park C, Ha Y, Ahn M. Resting-state frontal electroencephalography (EEG) biomarkers for detecting the severity of chronic neuropathic pain. *Sci Rep*. 2024;14(1):20188. doi:10.1038/s41598-024-71219-3
43. Rosa MJ, Seymour B. Decoding the matrix: benefits and limitations of applying machine learning algorithms to pain neuroimaging. *Pain*. 2014;155(5):864-867. doi:10.1016/j.pain.2014.02.013
44. Fischl B, van der Kouwe A, Destrieux C, et al. Automatically Parcellating the Human Cerebral Cortex. *Cereb Cortex*. 2004;14(1):11-22. doi:10.1093/cercor/bhg087
45. Fischl B. FreeSurfer. *NeuroImage*. 2012;62(2):774-781. doi:10.1016/j.neuroimage.2012.01.021
46. Glasser MF, Coalson TS, Robinson EC, et al. A multi-modal parcellation of human cerebral cortex. *Nature*. 2016;536(7615):171-178. doi:10.1038/nature18933

47. Kikinis R, Pieper SD, Vosburgh KG. 3D Slicer: A Platform for Subject-Specific Image Analysis, Visualization, and Clinical Support. In: Jolesz FA, ed. *Intraoperative Imaging and Image-Guided Therapy*. Springer; 2014:277-289. doi:10.1007/978-1-4614-7657-3_19
48. Oostenveld R, Fries P, Maris E, Schoffelen JM. FieldTrip: Open Source Software for Advanced Analysis of MEG, EEG, and Invasive Electrophysiological Data. *Comput Intell Neurosci*. 2011;2011:1-9. doi:10.1155/2011/156869
49. Dickey CW, Verzhbinsky IA, Jiang X, et al. Cortical Ripples during NREM Sleep and Waking in Humans. *J Neurosci*. 2022;42(42):7931-7946. doi:10.1523/JNEUROSCI.0742-22.2022
50. Dickey CW, Verzhbinsky IA, Kajfez S, et al. Thalamic spindles and Up states coordinate cortical and hippocampal co-ripples in humans. *PLOS Biol*. 2024;22(11):e3002855. doi:10.1371/journal.pbio.3002855
51. Silber MH, Ancoli -Israel Sonia, Bonnet MH, et al. The Visual Scoring of Sleep in Adults. *J Clin Sleep Med*. 2007;03(02):121-131. doi:10.5664/jcsm.26814
52. Dahan A. Opioid chronopharmacology: influence of timing of infusion on fentanyl's analgesic efficacy in healthy human volunteers. *J Pain Res*. Published online September 2010:183. doi:10.2147/JPR.S13616
53. Coda B, Tanaka A, C. Jacobson R, Donaldson G, Chapman CR. Hydromorphone analgesia after intravenous bolus administration. *Pain*. 1997;71(1):41-48. doi:10.1016/S0304-3959(97)03336-8
54. Chronux Home. Accessed November 13, 2024. <http://chronux.org/>
55. Mitra P. *Observed Brain Dynamics*. Oxford University Press; 2007.
56. Marple L. Computing the discrete-time “analytic” signal via FFT. *IEEE Trans Signal Process*. 1999;47(9):2600-2603. doi:10.1109/78.782222
57. Garcia-Larrea L, Peyron R. Pain matrices and neuropathic pain matrices: A review. *PAIN®*. 2013;154:S29-S43. doi:10.1016/j.pain.2013.09.001

58. Coghill RC, Sang CN, Maisog JMa, Iadarola MJ. Pain Intensity Processing Within the Human Brain: A Bilateral, Distributed Mechanism. *J Neurophysiol.* 1999;82(4):1934-1943. doi:10.1152/jn.1999.82.4.1934
59. Hu L, Zhang L, Chen R, Yu H, Li H, Mouraux A. The primary somatosensory cortex and the insula contribute differently to the processing of transient and sustained nociceptive and non-nociceptive somatosensory inputs. *Hum Brain Mapp.* 2015;36(11):4346-4360. doi:10.1002/hbm.22922
60. Mazzola L, Faillenot I, Barral FG, Mauguière F, Peyron R. Spatial segregation of somato-sensory and pain activations in the human operculo-insular cortex. *NeuroImage.* 2012;60(1):409-418. doi:10.1016/j.neuroimage.2011.12.072
61. Segerdahl AR, Mezue M, Okell TW, Farrar JT, Tracey I. The dorsal posterior insula subserves a fundamental role in human pain. *Nat Neurosci.* 2015;18(4):499-500. doi:10.1038/nn.3969
62. Ostrowsky K. Representation of Pain and Somatic Sensation in the Human Insula: a Study of Responses to Direct Electrical Cortical Stimulation. *Cereb Cortex.* 2002;12(4):376-385. doi:10.1093/cercor/12.4.376
63. Wiech K, Lin C shu, Brodersen KH, Bingel U, Ploner M, Tracey I. Anterior Insula Integrates Information about Salience into Perceptual Decisions about Pain. *J Neurosci.* 2010;30(48):16324-16331. doi:10.1523/JNEUROSCI.2087-10.2010
64. Menon V, Uddin LQ. Saliency, switching, attention and control: a network model of insula function. *Brain Struct Funct.* 2010;214(5-6):655-667. doi:10.1007/s00429-010-0262-0
65. Arnal LH, Giraud AL. Cortical oscillations and sensory predictions. *Trends Cogn Sci.* 2012;16(7):390-398. doi:10.1016/j.tics.2012.05.003
66. D'Argembeau A. On the Role of the Ventromedial Prefrontal Cortex in Self-Processing: The Valuation Hypothesis. *Front Hum Neurosci.* 2013;7:372. doi:10.3389/fnhum.2013.00372

67. Schneider B, Koenigs M. Human lesion studies of ventromedial prefrontal cortex. *Neuropsychologia*. 2017;107:84-93. doi:10.1016/j.neuropsychologia.2017.09.035
68. Sakagami M, Pan X. Functional role of the ventrolateral prefrontal cortex in decision making. *Curr Opin Neurobiol*. 2007;17(2):228-233. doi:10.1016/j.conb.2007.02.008
69. Baliki MN, Chialvo DR, Geha PY, et al. Chronic Pain and the Emotional Brain: Specific Brain Activity Associated with Spontaneous Fluctuations of Intensity of Chronic Back Pain. *J Neurosci*. 2006;26(47):12165-12173. doi:10.1523/JNEUROSCI.3576-06.2006
70. Geha PY, Baliki MN, Chialvo DR, Harden RN, Paice JA, Apkarian AV. Brain activity for spontaneous pain of postherpetic neuralgia and its modulation by lidocaine patch therapy. *Pain*. 2007;128(1):88-100. doi:10.1016/j.pain.2006.09.014
71. Baliki MN, Geha PY, Jabakhanji R, Harden N, Schnitzer TJ, Apkarian AV. A preliminary fMRI study of analgesic treatment in chronic back pain and knee osteoarthritis. *Mol Pain*. 2008;4:47. doi:10.1186/1744-8069-4-47
72. Motzkin JC, Hiser J, Carroll I, et al. Human ventromedial prefrontal cortex lesions enhance the effect of expectations on pain perception. *Cortex*. 2023;166:188-206. doi:10.1016/j.cortex.2023.04.017
73. Winston JS, Vlaev I, Seymour B, Chater N, Dolan RJ. Relative Valuation of Pain in Human Orbitofrontal Cortex. *J Neurosci*. 2014;34(44):14526-14535. doi:10.1523/JNEUROSCI.1706-14.2014
74. Haroutiunian S, Nikolajsen L, Finnerup NB, Jensen TS. The neuropathic component in persistent postsurgical pain: A systematic literature review. *PAIN®*. 2013;154(1):95-102. doi:10.1016/j.pain.2012.09.010
75. Chaparro LE, Smith SA, Moore RA, Wiffen PJ, Gilron I. Pharmacotherapy for the prevention of chronic pain after surgery in adults. *Cochrane Database Syst Rev*. 2013;(7). doi:10.1002/14651858.CD008307.pub2
76. Buvanendran A, Kroin JS, Della Valle CJ, Kari M, Moric M, Tuman KJ. Perioperative Oral Pregabalin Reduces Chronic Pain After Total Knee Arthroplasty: A Prospective,

- Randomized, Controlled Trial. *Anesth Analg*. 2010;110(1):199.
doi:10.1213/ANE.0b013e3181c4273a
77. Chapman CR, Vierck CJ. The Transition of Acute Postoperative Pain to Chronic Pain: An Integrative Overview of Research on Mechanisms. *J Pain*. 2017;18(4):359.e1-359.e38. doi:10.1016/j.jpain.2016.11.004
78. Vachon-Preseu E, Roy M, Martel MO, et al. The stress model of chronic pain: evidence from basal cortisol and hippocampal structure and function in humans. *Brain*. 2013;136(3):815-827. doi:10.1093/brain/aws371
79. Grilli M. Chronic pain and adult hippocampal neurogenesis: translational implications from preclinical studies. *J Pain Res*. 2017;Volume 10:2281-2286.
doi:10.2147/JPR.S146399
80. Wei X, Centeno MV, Ren W, et al. Activation of the dorsal, but not the ventral, hippocampus relieves neuropathic pain in rodents. *Pain*. 2021;162(12):2865-2880.
81. Duric V, McCarron KE. Persistent Pain Produces Stress-like Alterations in Hippocampal Neurogenesis and Gene Expression. *J Pain*. 2006;7(8):544-555.
doi:10.1016/j.jpain.2006.01.458
82. Mutso AA, Radzicki D, Baliki MN, et al. Abnormalities in Hippocampal Functioning with Persistent Pain. *J Neurosci*. 2012;32(17):5747-5756.
doi:10.1523/JNEUROSCI.0587-12.2012
83. Neumann N, Domin M, Schmidt CO, Lotze M. Chronic pain is associated with less grey matter volume in the anterior cingulum, anterior and posterior insula and hippocampus across three different chronic pain conditions. *Eur J Pain*. 2023;27(10):1239-1248.
doi:10.1002/ejp.2153
84. Gagnon CM, Scholten P, Atchison J, Jabakhanji R, Wakaizumi K, Baliki M. Structural MRI Analysis of Chronic Pain Patients Following Interdisciplinary Treatment Shows Changes in Brain Volume and Opiate-Dependent Reorganization of the Amygdala and Hippocampus. *Pain Med*. 2020;21(11):2765-2776. doi:10.1093/pm/pnaa129

85. McQuiston AR, Saggau P. Mu-opioid Receptors Facilitate the Propagation of Excitatory Activity in Rat Hippocampal Area CA1 by Disinhibition of all Anatomical Layers. *J Neurophysiol.* 2003;90(3):1936-1948. doi:10.1152/jn.01150.2002
86. Nam MH, Won W, Han KS, Lee CJ. Signaling mechanisms of μ -opioid receptor (MOR) in the hippocampus: disinhibition versus astrocytic glutamate regulation. *Cell Mol Life Sci.* 2021;78(2):415-426. doi:10.1007/s00018-020-03595-8
87. Hirai N, Uchida S, Maehara T, Okubo Y, Shimizu H. Beta-1 (10–20 Hz) cortical oscillations observed in the human medial temporal lobe. *NeuroReport.* 1999;10(14):3055.
88. Moroni F, Nobili L, De Carli F, et al. Slow EEG rhythms and inter-hemispheric synchronization across sleep and wakefulness in the human hippocampus. *NeuroImage.* 2012;60(1):497-504. doi:10.1016/j.neuroimage.2011.11.093
89. Caplan JB, Madsen JR, Schulze-Bonhage A, Aschenbrenner-Scheibe R, Newman EL, Kahana MJ. Human θ Oscillations Related to Sensorimotor Integration and Spatial Learning. *J Neurosci.* 2003;23(11):4726-4736. doi:10.1523/JNEUROSCI.23-11-04726.2003
90. Wang D, Huang Z, Ren L, et al. Amygdalar and hippocampal beta rhythm synchrony during human fear memory retrieval. *Acta Neurochir (Wien).* 2020;162(10):2499-2507. doi:10.1007/s00701-020-04276-y
91. Sehatpour P, Molholm S, Schwartz TH, et al. A human intracranial study of long-range oscillatory coherence across a frontal–occipital–hippocampal brain network during visual object processing. *Proc Natl Acad Sci.* 2008;105(11):4399-4404. doi:10.1073/pnas.0708418105
92. Faber ESL, Sah P. Opioids Inhibit Lateral Amygdala Pyramidal Neurons by Enhancing a Dendritic Potassium Current. *J Neurosci.* 2004;24(12):3031-3039. doi:10.1523/JNEUROSCI.4496-03.2004
93. Blaesse P, Goedecke L, Bazet M, Capogna M, Pape HC, Jüngling K. μ -Opioid Receptor-Mediated Inhibition of Intercalated Neurons and Effect on Synaptic

- Transmission to the Central Amygdala. *J Neurosci*. 2015;35(19):7317-7325.
doi:10.1523/JNEUROSCI.0204-15.2015
94. Finnegan TF, Chen SR, Pan HL. Effect of the μ Opioid on Excitatory and Inhibitory Synaptic Inputs to Periaqueductal Gray-Projecting Neurons in the Amygdala. *J Pharmacol Exp Ther*. 2005;312(2):441-448. doi:10.1124/jpet.104.074633
95. Veinante P, Yalcin I, Barrot M. The amygdala between sensation and affect: a role in pain. *J Mol Psychiatry*. 2013;1(1):9. doi:10.1186/2049-9256-1-9
96. Neugebauer V. Amygdala Pain Mechanisms. In: Schaible HG, ed. *Pain Control*. Springer; 2015:261-284. doi:10.1007/978-3-662-46450-2_13
97. Helmstetter FJ, Bellgowan PS. Lesions of the amygdala block conditional hypoalgesia on the tail flick test. *Brain Res*. 1993;612(1-2):253-257. doi:10.1016/0006-8993(93)91669-j
98. Bellgowan PS, Helmstetter FJ. Neural systems for the expression of hypoalgesia during nonassociative fear. *Behav Neurosci*. 1996;110(4):727-736. doi:10.1037//0735-7044.110.4.727
99. Ortiz JP, Heinricher MM, Selden NR. Noradrenergic agonist administration into the central nucleus of the amygdala increases the tail-flick latency in lightly anesthetized rats. *Neuroscience*. 2007;148(3):737-743. doi:10.1016/j.neuroscience.2007.07.003
100. Manning BH, Mayer DJ. The central nucleus of the amygdala contributes to the production of morphine antinociception in the rat tail-flick test. *J Neurosci Off J Soc Neurosci*. 1995;15(12):8199-8213. doi:10.1523/JNEUROSCI.15-12-08199.1995
101. Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MC. Pain Affect Encoded in Human Anterior Cingulate But Not Somatosensory Cortex. *Science*. 1997;277(5328):968-971. doi:10.1126/science.277.5328.968
102. Barthas F, Sellmeijer J, Hugel S, Waltisperger E, Barrot M, Yalcin I. The Anterior Cingulate Cortex Is a Critical Hub for Pain-Induced Depression. *Biol Psychiatry*. 2015;77(3):236-245. doi:10.1016/j.biopsych.2014.08.004

103. Bliss TVP, Collingridge GL, Kaang BK, Zhuo M. Synaptic plasticity in the anterior cingulate cortex in acute and chronic pain. *Nat Rev Neurosci.* 2016;17(8):485-496. doi:10.1038/nrn.2016.68
104. Xiao X, Zhang YQ. A new perspective on the anterior cingulate cortex and affective pain. *Neurosci Biobehav Rev.* 2018;90:200-211. doi:10.1016/j.neubiorev.2018.03.022
105. Ballantine HT, Cassidy WL, Flanagan NB, Marino R. Stereotaxic Anterior Cingulotomy for Neuropsychiatric Illness and Intractable Pain. *J Neurosurg.* 1967;26(5):488-495. doi:10.3171/jns.1967.26.5.0488
106. Hurt WR, Ballantine THJ. Stereotactic Anterior Cingulate Lesions for Persistent Pain: A Report on 68 Cases. *Neurosurgery.* 1974;21:334. doi:10.1093/neurosurgery/21.CN_suppl_1.334
107. Wang GC, Harnod T, Chiu TL, Chen KP. Effect of an Anterior Cingulotomy on Pain, Cognition, and Sensory Pathways. *World Neurosurg.* 2017;102:593-597. doi:10.1016/j.wneu.2017.03.053
108. Johansen JP, Fields HL. Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal. *Nat Neurosci.* 2004;7(4):398-403. doi:10.1038/nn1207
109. Tan LL, Pelzer P, Heintz C, et al. A pathway from midcingulate cortex to posterior insula gates nociceptive hypersensitivity. *Nat Neurosci.* 2017;20(11):1591-1601. doi:10.1038/nn.4645