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The association between endogenous opioid function and morphine responsiveness: a moderating role for endocannabinoids?

Stephen Bruehl¹, John W. Burns², Amanda Morgan³, Kelli Koltyn⁴, Rajnish Gupta¹, Asokumar Buvanendran⁵, David Edwards¹, Melissa Chont¹, Philip J. Kingsley^{6,7}, Larry Marnett⁶, Amanda Stone¹, and Sachin Patel³

¹Department of Anesthesiology, Vanderbilt University Medical Center, Nashville, TN, USA

²Department of Behavioral Science, Rush University, Chicago, IL, USA

³Department of Psychiatry, Vanderbilt University Medical Center, Nashville, TN, USA

⁴Department of Kinesiology, University of Wisconsin, Madison, WI, USA

⁵Department of Anesthesiology, Rush University, Chicago, IL, USA

⁶Department of Biochemistry, Vanderbilt University, Nashville, TN, USA

⁷A.B. Hancock Memorial Laboratory for Cancer Research, Vanderbilt University, Nashville, TN

Abstract

We sought to replicate prior findings that low endogenous opioid (EO) function predicts greater morphine analgesia, and extended these findings by examining whether circulating endocannabinoids and related lipids moderate EO-related predictive effects. Individuals with chronic low back pain (n=46) provided blood samples for endocannabinoid analyses, then underwent separate identical laboratory sessions under three drug conditions: saline placebo, i.v. naloxone (opioid antagonist; 12mg total), and i.v. morphine (0.09 mg/kg total). During each session, participants rated low back pain intensity, evoked heat pain intensity, and non-pain subjective effects four times in sequence following incremental drug dosing. Mean morphine effects (morphine-placebo difference) and opioid blockade effects (naloxone-placebo difference; to index EO function) for each primary outcome (low back pain intensity, evoked heat pain intensity, non-pain subjective effects) were derived by averaging across the four incremental doses. The association between EO function and morphine-induced back pain relief was significantly moderated by endocannabinoids [2-Arachidonoylglycerol (2-AG) and N-arachidonylethanolamide (AEA)]. Lower EO function predicted greater morphine analgesia only for those with relatively lower endocannabinoids. Endocannabinoids also significantly moderated EO effects on morphine-related changes in VAS evoked pain intensity (2-AG), drug liking (AEA and 2-AG), and desire to take again (AEA and 2-AG). Absent significant interactions, lower EO function predicted significantly greater morphine analgesia (as in past work) and euphoria. Results indicate that EO effects on analgesic and subjective responses to opioid medications are greatest

when endocannabinoid levels are low. These findings may help guide development of mechanism-based predictors for personalized pain medicine algorithms.

Brief Summary:

Individual differences in endogenous opioid function predict morphine analgesic and subjective responses, and these effects are moderated by circulating endocannabinoids.

Keywords

pain; opioid; analgesia; endocannabinoid; endogenous opioid; morphine; naloxone

As the negative consequences of increased reliance on opioid analgesics for chronic pain management over the past two decades have become apparent [29,33], interest has increased in targeting opioid therapy to those individuals most likely to experience strong analgesic benefits with low side effects and misuse risk [5,6]. For this personalized pain medicine approach to be feasible, reliable predictors of opioid analgesic responses must be identified [5,6].

Existing work documents that both genotype status (e.g., A118G single nucleotide polymorphism of the *OPRM1* gene) and phenotypic factors, such as gender, negative affect, pain sensitivity, and “fibromyalgia-ness,” each appear to be associated with differential opioid analgesic responsiveness [7,13–15,22,24,26,32,41–43]. Our prior work found that low endogenous opioid (EO) function, quantified by changes in pain responses following pharmacological opioid receptor blockade, predicted greater analgesic effects of morphine in terms of both evoked pain responses and low back pain intensity [7,8]. EO function was further found to mediate the ability of pain sensitivity and both negative affect and catastrophizing to predict morphine responses [7,14]. The degree to which EO systems may interact with other endogenous antinociceptive systems to influence responses to analgesic medications remains largely untested.

Interest in the role of endocannabinoids as modulators of pain has been increasing [4,36]. N-arachidonylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG) are two endocannabinoids known to exert antinociceptive effects via activation of CB1 and CB2 receptors [37]. Anatomical studies indicate that cannabinoid receptors are present in the same nociceptive spinal and brain regions as are opioid receptors [38]. It has been suggested that endocannabinoid and EO systems may interact [44,45], with a number of animal studies suggesting that endocannabinoids can influence responses to exogenous opioid analgesics [2,3,28,34,35,46,53]. However, human studies evaluating endocannabinoid-opioid interactions are rare. One recent study indicated that exogenous opioid administration reduced endocannabinoid levels in the perioperative context [30]. Another recent study found that AEA, and the endocannabinoid-related lipid oleylethanolamine (OEA), increased in response to acute exercise under a placebo condition, but did not do so under opioid blockade with oral naltrexone [19]. Results of both studies support opioid and endocannabinoid system interactions in humans, with the latter suggesting more specifically that EOs and endocannabinoids may interact.

The current study tested whether circulating endocannabinoids and related lipid markers directly influence opioid analgesic responses or interact with EO function to influence opioid analgesic responses. We hypothesized that if endocannabinoids directly influenced opioid analgesic responses, there would be significant main effects of endocannabinoid levels on placebo-controlled morphine responses. To evaluate alternative moderation models, we also examined whether interactions between EO function and endocannabinoids predicted placebo-controlled morphine responses.

Method

Design

This study was part of a separate, larger, ongoing study evaluating the effects of a structured aerobic exercise training program on chronic low back pain and opioid analgesic responsiveness, and the role of EO mechanisms in observed effects (NCT02469077). The study used a double-blinded within-subject design, with study drugs (placebo, morphine, and naloxone) administered in randomized, counterbalanced order across 3 separate identical sessions (conducted over a 10-day period), with this protocol carried out both before and again after an 18 session (6 week) aerobic exercise training program. Data presented herein are based on the pre-exercise sessions only, to avoid confounding with intervention effects. The study was conducted at two separate study locations using identical procedures in parallel in a closely coordinated fashion. All procedures were approved by the Institutional Review Boards at the respective institutions.

Sample

The sample included 46 individuals with chronic low back pain who were not using any opioid analgesics on a daily basis and who had valid endocannabinoid assay data available. Participants using as-needed opioid analgesics were asked to abstain from any opioid use within the 3 days prior to each laboratory session (confirmed via urine opioid screen), and all participants were instructed not to use any non-steroidal anti-inflammatory drugs or over-the-counter analgesics for at least 12 hours prior to each laboratory session. Participants were recruited through an informatics-based targeted recruiting system mining electronic medical records to identify potentially eligible patients previously indicating a willingness to participate in research studies ("My Research at Vanderbilt"), on-line advertisements on the Vanderbilt employee e-mail recruitment system, the Rush Pain Clinic, advertisements in local print media, and posted flyers. General criteria for participation included age between 18–55; no self-reported history of liver or kidney disorders, posttraumatic stress disorder, bipolar disorder, psychotic disorder, diabetes, seizure disorder, or alcohol or drug dependence; no daily use of opioid analgesics; and engaged in moderate or vigorous exercise < 2 days/week and < 60 min/week (due to requirements of the primary study). Chronic low back pain was defined as daily low back pain of at least 3 months duration, with an average past month severity of at least 3/10 on a 0–10 verbal numeric pain intensity scale. All participants were required to be able to provide documentation of a previous medical provider diagnosis consistent with chronic back pain. Individuals self-reporting chronic pain related to malignancy or autoimmune disorders were excluded. Potential participants who

were pregnant (determined by urine pregnancy screens) were excluded. All participants were compensated for their time (\$75 for initial screening and \$100 for each lab visit).

Study Drugs

The opioid analgesic examined in this study was morphine sulfate, the prototypic mu opioid receptor agonist. Because of the hypotheses of the primary study, morphine was infused intravenously over a 2-minute period through an intravenous cannula placed in the non-dominant arm in four incremental doses: 0.03 mg/kg initially, followed by $0.02 \text{ mg/kg} \times 3$. After each dose, there was a 10-minute seated rest to allow onset of drug effects followed by assessment of back pain, evoked pain responsiveness, and subjective drug effects, with incremental drug doses provided at 25-minute intervals. The total morphine dosage used was the equivalent of 6.75mg for a 165 lb. individual. The opioid antagonist naloxone was administered in a second drug condition to permit derivation of a quantitative index of EO system function (the difference between naloxone and placebo condition responses)[7,8]. Naloxone was infused incrementally as in the morphine condition with an initial 8mg dose, followed by saline placebo, a 4mg maintenance dose (to maintain full opioid blockade during the extended procedures), and a final saline placebo dose. Normal saline was infused in the same incremental manner across all four doses during the placebo condition. Drug order randomization was determined using the Proc Plan procedure in SAS version 9.2 (SAS Institute, Cary, NC).

Measures

Endocannabinoids

Materials.: HPLC-grade methanol, acetonitrile and water for LC-MS/MS analysis were JT Baker-brand (ThermoFisher Scientific, Waltham, MA). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO). Acquity columns were purchased from Waters Corp. (Milford, MA). All LC MS/MS analysis was performed on a Shimadzu Nexera system in-line with a SCIEX 6500 QTrap. The QTrap was equipped with a TurboV Ionspray source and operated in both positive and negative ion mode. SCIEX Analyst software (ver 1.6.2) was used to control the instruments and acquire and process the data.

The deuterated internal standards AEA d4, 2-AG d5, OEA d4 and AA d8 were purchased from Cayman Chemicals (Ann Arbor, MI). OG-d5 was purchased from TRC (Toronto, ON, Canada). Authentic standards of these analytes were purchased from Cayman.

Sample Collection.: A 4mL sample of whole blood was drawn into a tube with EDTA from an indwelling venous cannula prior to beginning lab procedures at the first laboratory session. Within 10 minutes of collection, each sample was centrifuged for 10 minutes at 3500rpm and 4°C. Plasma was then pipetted into microcentrifuge tubes (0.5mL aliquots), and stored at -80°C until assayed.

Endocannabinoid Assays.: For the current work, we assayed two ECs known to have antinociceptive effects (AEA and 2AG), as well as arachidonic acid (AA; a metabolic product of AEA and 2AG degradation). Additionally, we assayed the oleic acid derivatives

N-oleoyl ethanolamine (OEA) and oleoyl glycerol (OG). These compounds are in the same chemical class as AEA and 2-AG, respectively.

The analytes were extracted from plasma via a liquid-liquid extraction scheme modified from that reported by Zoerner et al [52]. Briefly, plasma samples were thawed, vortexed and 1 mL was transferred to new glass vials. Deuterated internal standards (AEA-d4, 2-AG-d5, OEA-d4, OG-d5, AA-d8) were added to each sample, as well as a 20µl aliquot of methanolic butylated hydroxytoluene (BHT) at a concentration of approximately 100 mg/mL. 2 mL of 10% acetic acid in de-ionized H2O was added to each sample, followed by 0.2 mL of saturated NaCl solution. The samples were vortexed and 3.5 mL of 20:1 toluene:cyclohexane (v:v) was added to each sample. The samples were capped, mixed well and then centrifuged at ~3,000rpm for 15 minutes at 40°C. The upper layer of each sample was transferred into a new vial, and dried under nitrogen. Samples were then capped and stored at -200°C. Immediately prior to LC-MS analysis, the samples were reconstituted in 70µL MeOH and 40µL H2O. The reconstituted samples were centrifuged at ~3,000rpm for 15 minutes at 40°C as some particulate was generated upon the addition of H2O, and the clear portion of the sample was transferred to a 96-well plate.

The samples were analyzed on the above-referenced LC/MS system in a scheme similar to that described by Morgan et al [31]. In summary, the analytes were chromatographed on an Acquity UPLC BEH C18 reversed phase column (5.0 × 0.21 cm; 1.7 µm) which was held at 40°C. A gradient elution profile was applied to each sample; after an initial hold for 0.25 minutes, %B was increased from 25% (initial conditions) to 99% over 3.5 minutes, and held at 99% for 1.3 minutes. Then the column was returned to initial conditions for 1.3 minutes prior to the next injection. The flow rate was 330 µL/ minute and component A was water with 0.1% formic acid while component B was 2:1 acetonitrile:methanol (v:v) with 0.1% formic acid.

All analytes were detected by the SCIEX 6500 QTrap via selected reaction monitoring (SRM). The SRM transition (Da) and collision energy (volts) are given for each analyte: AEA (348.2 – 62.0, 24); AEA-d4 (352.2 – 66.0, 24); 2-AG (379.2 – 287.2, 24); 2-AG-d5 (384.3 – 287.2, 24); AA (303.1 – 259.0, 17); AA-d8 (311.1 – 267.0, 17); OEA (326.2 – 62.0, 30); OEA-d4 (330.2 – 66.0, 30) OG (357.2 – 265.1, 17) and OG-d5 (362.2 – 265.1, 17). All analyses were done in positive ion mode except for AA, which was detected in negative ion mode.

Analytes were quantitated by stable isotope dilution against their deuterated internal standard. Data were normalized to plasma volume.

Pain Measures.

Short Form McGill Pain Questionnaire-2.: Clinical back pain was assessed pre-drug and repeatedly 10-min after each incremental drug dose during each laboratory session using the Short Form-McGill Pain Questionnaire-2 (MPQ-2) [20,21] to rate the low back pain being experienced “at this moment.” The MPQ-2 was also used to rate the intensity of thermal evoked pain stimuli applied following each incremental drug dose. The MPQ-2 is a validated measure containing 22 items rated using a numeric rating scale format (0 = *none* and 10 =

worst possible) that allows assessment of the intensity of multiple qualitative features of pain [20]. It contains 4 subscales (Continuous, Intermittent, Neuropathic, and Affective) as well as a total score (only the total score is reported here).

Visual Analog Pain Intensity Scale: A 10cm visual analog pain intensity scale (VAS), anchored with “*no pain*” and “*worst possible pain*” was also used to rate overall clinical back pain and evoked pain intensity at each assessment time point. At baseline screening (prior to the first laboratory session), a similar 10cm VAS pain intensity scale was used to describe the average level of chronic back pain experienced in the prior week. This measure was obtained to characterize the level of chronic pain experienced outside of the laboratory.

Pain Detect Questionnaire: Participants completed the Pain Detect Questionnaire at baseline to characterize the relative contributions of nociceptive versus neuropathic mechanisms to the chronic back pain [25]. The Pain Detect Questionnaire is a 9-item questionnaire specifically designed to assess for neuropathic back pain characteristics, and has been shown to be both reliable and valid [25]. Scores ≤ 12 indicate that a neuropathic pain component is unlikely, whereas a score ≥ 19 indicates that a neuropathic component is likely.

Subjective Opioid Effect Measures.—Non-analgesic subjective effects of acute opioid administration were assessed as in several prior opioid studies by Zacny and colleagues using the Drug Effect Liking/Take Again (DELTA) questionnaire and a separate 26-item VAS opioid effects rating scale [49–51]. This assessment approach has been used in our prior work as well [9,12,14].

DELTA Questionnaire: The DELTA consists of 3 items asking about overall perceived effect of the drug (1–5 scale anchored with *no effect* and *very strong effect*), degree to which the effects of the drug were liked (10cm VAS anchored with *dislike a lot* and *like a lot*), and the level of desire to take the drug again (10cm VAS anchored with *definitely would not* and *definitely would*).

Visual Analog Opioid Effects Scale: Items on the VAS opioid effects scale (anchored with *not at all* and *extremely*) tap into both the positive and negative subjective (e.g., cognitive, emotional) effects of opioids. To reduce the number of items examined on the VAS opioid effect measure (mitigate against elevated Type I error rate) and target misuse-relevant subjective effects, we focused on the Euphoria factor identified in our previous work based on principal components analysis in a large sample receiving morphine [14]. As in our recent work [12], the Euphoria factor reflected the sum of observed values for the top 3 items loading on this factor in prior work, specifically “stimulated,” “elated,” and “having pleasant thoughts” [14]. Positive subjective opioid effects like those assessed by the Euphoria factor of the VAS opioid effects scale as well as the DELTA are believed to contribute to abuse liability of opioids [40,47].

Evoked Pain Stimulus

The evoked pain stimulus in the current study was a heat pain task using a computerized Medoc TSAII NeuroSensory Analyzer (Medoc US., Minneapolis, MN) as in our prior work [7,10,11]. After administration of each incremental drug dose and completion of low back pain ratings 10 minutes after each infusion, three trials were conducted for heat pain tolerance, with the thermode starting at an adaptation temperature of 40°C and increasing at a ramp rate of 0.5°C/second until tolerance was reached. Immediately upon completion of the final heat pain tolerance trial at each drug dosage, participants were asked to rate the pain just experienced using the MPQ-2 and VAS intensity scales described above. These two pain ratings were used as the evoked pain outcomes for the current study to provide parallelism with the clinical pain measures. Participants underwent standardized training to familiarize them with the thermal stimulus procedures prior to undergoing the laboratory tasks for the first time.

Procedure

All procedures were conducted at the Vanderbilt General Clinical Research Center or a dedicated research room at the Rush University Pain Center. After providing informed consent, participants completed a packet of questionnaires, including chronic pain-related measures and information regarding demographics. Individuals then participated in identical experimental procedures across all three drug conditions (morphine, naloxone, and saline placebo), with all sessions scheduled at the same time of day within individuals to control for variance due to circadian rhythms. The laboratory protocol for the three laboratory sessions is summarized in Figure 1.

Participants remained seated upright in a chair throughout all laboratory procedures. The investigational pharmacy at each institution prepared and provided the study drugs in blinded fashion to the study nurses. At the beginning of each session, an indwelling venous cannula was inserted into the non-dominant arm by a trained research nurse under physician supervision. In the first session only, a 4mL blood sample was then drawn for endocannabinoid assays. Participants next completed the MPQ-2 to describe their current low back pain intensity. Participants then received (via the cannula) their first dose of the study drug as assigned per the randomization protocol. After a 10-min rest period to allow drug activity to peak, participants again described their current (post-drug) level of low back pain using the MPQ-2 and VAS, followed by the evoked thermal pain tasks (and MPQ-2 and VAS pain ratings of evoked pain), and then completion of the DELTA and the VAS opioid effects measure. Fifteen minutes later, the second assigned drug dose was given, followed again by ratings of current chronic pain, the evoked thermal pain tasks, and completion of the DELTA and VAS opioid effects measure, with the same procedure followed through the fourth and final drug dose. All participants remained in the lab under observation for 1 hour after the final drug dose to allow drug effects to remit, after which they were released to a responsible adult.

Statistical Analysis

All analyses were conducted using IBM SPSS for Windows Version 24 (SPSS Inc., Chicago, IL). In preparation for conducting analyses, changes in back pain intensity from pre-drug

baseline to the assessment following each drug administration were derived (as pre-drug minus post-drug value) separately within the morphine, naloxone, and placebo conditions (similar to [8]). The four resulting change values within each drug condition were then separately averaged as an overall index of within-session changes in back pain, a strategy potentially increasing the accuracy of these change measures by averaging out random measurement error associated with the individual measures. Next, morphine effects (as a placebo-controlled index of opioid analgesia) were derived for changes in low back pain intensity based on the MPQ-2 and VAS. These morphine effects were derived by subtracting pre- to post-drug changes under placebo from comparable changes after morphine had been administered, such that more positive morphine effect values indicated greater morphine-induced reductions in back pain intensity. For example, if VAS intensity decreased from 50 to 45 pre- to post-drug in the placebo condition (change of 5), but decreased from 50 to 30 after morphine administration (change of 20), the morphine effect value would be +15, indicating that morphine produced analgesia relative to the placebo condition. Similar average placebo-controlled morphine effect variables (across the four drug doses) were derived for evoked pain ratings, with positive morphine effect values indicating greater morphine analgesia. The same approach was used to derive placebo-controlled morphine effects for the DELTA and the VAS opioid effects Euphoria scale, with positive values indicating greater morphine-related overall drug effects, drug liking, desire to take the drug again, and euphoria.

In order to provide a quantitative index of the impact of EO function on pain outcomes and subjective responses, placebo-controlled blockade effect measures were derived in a manner similar to that used for deriving morphine effects, such that positive blockade effect values indicated greater EO modulation of pain or subjective state. That is, greater increases in pain responses under naloxone relative to placebo indicate greater EO inhibition of pain in the placebo condition, with blockade effects for the DELTA and Euphoria factor measures similarly indexing the impact of EO function on the participant's subjective state.

Preliminary analyses used Pearson correlations (r) to examine zero order correlations between endocannabinoid-related measures, EO function (blockade effect measures), and morphine effects for pain and subjective outcomes. Primary analyses were a series of hierarchical multiple regressions, conducted using a publicly-available SPSS cross-product regression macro to permit ease of deriving mean-centered interactions and testing the simple slopes contributing to interactions (<http://my.ilstu.edu/~wjschne/CrossProductRegressionMacro.sps>). Each regression focused on a different morphine effect outcome as the dependent measure. All hierarchical regressions entered control variables in step one. Age and sex were included as control variables because of associations noted with either endocannabinoid levels (age) or blockade and morphine effect measures (sex). Next, the main effect of the relevant EO function variable was entered in step two (e.g., the EO measure for VAS back pain intensity was used for analyses with VAS back pain intensity morphine effects as the outcome), and then the targeted endocannabinoid measure was entered in step three, followed by entry of the mean-centered multiplicative interaction term ($EO \times \text{Endocannabinoid}$) in step four. Main effects were only interpreted in the absence of a significant $EO \times \text{Endocannabinoid}$ interaction in the model. The source of significant interactions is displayed graphically by solving regressions computed for low and high

endocannabinoid levels (based on median splits) for hypothetical low and high EO values (-1 SD and $+1$ SD from the mean EO level for the variable of interest) as described by Aiken and West [1]. All analyses used the maximum number of available cases and a two-tailed probability value of $p < .05$ as the criterion for significance.

Results

Preliminary Analyses

Table 1 displays the study sample characteristics. The majority of participants were female, white, and non-Hispanic. Chronic pain was characterized as moderate in intensity and of long duration. The mean Pain Detect Questionnaire score indicates that patients overall displayed chronic back pain that was primarily nociceptive in character (total score = 12; [25]). Less than 18% of the sample was using as-needed opioids, and none had used opioids in the 3 days prior to each laboratory session (confirmed by urine screen). The endocannabinoid-related measures were all correlated positively as expected ($r = 0.28 - 0.90$, all $p < .05$ except for the association between AEA and 2-OG).

Low Back Pain Outcomes

Zero order correlations between endocannabinoid measures and back pain-related opioid blockade and morphine effects are summarized in Table 2. Endocannabinoid measures were not significantly associated with any blockade effects or morphine effects for clinical back pain outcomes. As in our past work [8], lower EO function (i.e., indexed by blockade effect values) was associated with greater morphine analgesia on the corresponding measure for both MPQ-2 Total and VAS intensity ratings of back pain.

Regression Results for MPQ-2 Total Back Pain Measure—Hierarchical linear regressions for the MPQ-2 Total measure of back pain indicated that the impact of EO function on morphine analgesic responses was moderated by AEA ($p = 0.016$), AA ($p < .001$), OEA ($p = .001$), and 2-OG ($p = .037$). The source of these moderation effects was evaluated through simple effects analyses. Results indicated that for individuals with high AEA values, EO function was not significantly associated with morphine responses ($\beta = -0.162$, $p = .427$), whereas when AEA values were low, reduced EO function was associated with significantly greater morphine responses ($\beta = -0.838$, $p < .001$). A similar pattern was observed for AA (High AA: $\beta = -0.064$, $p = 0.698$; Low AA: $\beta = -1.086$, $p < .001$), OEA (High OEA: $\beta = -0.108$, $p = 0.539$; Low OEA: $\beta = -1.016$, $p < .001$), and 2-OG (High 2-OG: $\beta = -0.147$, $p = 0.523$; Low 2-OG: $\beta = -0.967$, $p < .001$). Figures 2 and 3 provide a graphical display of the source of the moderation effects for AEA and 2-OG, respectively. Inspection of these figures indicates that for both, the greatest level of morphine analgesia (i.e., positive morphine effects) was observed in those having both low EO function and low endocannabinoid levels.

For analyses of 2-AG, there was no significant interaction with EO function ($p > .35$). However, the main effect of EO function on MPQ-2 Total morphine effects was significant in Step 2 of this analysis ($\beta = -0.562$, $p < .001$), with lower EO function associated with greater morphine analgesia. Addition of the main effect of endocannabinoids in Step 3 of

models predicting the effects of morphine on MPQ-2 Total ratings of low back pain did not result in significantly increased variance accounted for in the 2-AG model in which there was no significant EO $\times$ Endocannabinoid interaction ($p = .36$).

Regression Results for VAS Intensity Back Pain Measure—Regarding results for the VAS intensity measure of low back pain, the influence of EO function on morphine analgesia was significantly moderated by 2-AG ($p=.007$) and AA ($p=.022$), with a nonsignificant trend for 2-OG ($p = 0.09$). Simple effects analyses indicated that for individuals with high 2-AG values, EO function was not significantly associated with morphine responses ($\beta = -0.036$, $p = .883$), however when 2-AG values were low, reduced EO function was associated with significantly greater morphine responses ($\beta = -0.767$, $p<.001$). As displayed in Figure 4, the greatest morphine analgesia was displayed by those individuals having both low EO function and low 2-AG levels. Simple effects analyses indicated that the interaction between AA and EO function derived from a similar source. For individuals with high AA values, EO was not significantly associated with morphine responses ($\beta = -0.25$, $p = .212$), however for those with low AA values, lower EO function was associated with significantly greater morphine responses ($\beta = -0.758$, $p<.001$). The trend for 2-OG also resulted from a lack of association between EO function and morphine responses in those with higher 2-OG levels ($\beta = -0.326$, $p = .112$), but a significant association between low EO function and greater morphine responses in those with lower 2-OG levels ($\beta = -0.786$, $p < .001$).

EO effects on morphine analgesia as indexed by the low back pain VAS intensity outcome were not moderated by AEA or OEA (p 's $>.15$). However, the main effect of EO function on VAS intensity morphine effects (Step 2) was significant in these analyses ($\beta = -0.561$, $p<.001$), with lower EO function associated with greater morphine analgesia. Addition of the main effect of endocannabinoids in Step 3 of models predicting the effects of morphine on VAS ratings of low back pain intensity did not result in significantly increased variance accounted for in models without significant EO $\times$ Endocannabinoid interactions (all p 's $>.12$).

Possible Confounding by Placebo Condition Values—The same placebo condition pain rating was used to derive both morphine effects and opioid blockade effect values (the measure of EO function) for the clinical pain measures. We considered it possible that this procedure could confound (inflate) tests of associations between EO measures and corresponding morphine effect measures. For example, extremely low placebo condition pain ratings might permit expression of large opioid blockade effects (increased pain under naloxone relative to the placebo condition response) but produce correspondingly small morphine effects (difference between placebo and morphine conditions) simply due to floor effects related to the already low placebo condition ratings. As in our past work [7], partial correlations (controlling for age and sex as in primary analyses) were used to test this possibility. Results indicated that the partial correlations between opioid blockade effects and their corresponding morphine effects (i.e., main effect associations) were significant both before and after controlling for the relevant placebo measure: low back pain MPQ-2 Total partial $r = -0.50$, $p<.001$ (vs. partial $r = -0.46$, $p = .001$ when controlling for placebo

pain levels) and low back pain VAS Intensity partial $r = -0.54$, $p < .001$ (vs. partial $r = -0.43$, $p = 0.003$ when controlling for placebo pain levels).

Evoked Pain Outcomes

Zero order correlations between endocannabinoid measures and evoked thermal pain blockade and morphine effects are summarized in Table 2. Greater 2-OG levels were associated with significantly greater EO inhibition of thermal task MPQ-2 Total responses, with a similar nonsignificant trend noted on this outcome for 2-AG levels. All other endocannabinoid measures were not significantly associated with blockade effects or morphine effects on either evoked pain index. As observed in our past work [7], lower EO function was associated with significantly greater morphine analgesia on the MPQ-2 Total measure of evoked pain responsiveness, with a similar trend approaching significance for VAS intensity ratings of evoked pain.

Regression Results for MPQ-2 Total Evoked Pain Measure—Hierarchical regressions for the evoked pain MPQ-2 Total outcome did not reveal any significant interactions between EO function (blockade effects) and any endocannabinoids (all p 's $> .20$). However, the main effect of EO function on morphine analgesic effects for this evoked pain outcome was significant in Step 2 of all models ($\beta = -0.593$, $p < .001$). This main effect was due to an association between lower EO function and greater morphine analgesia. Addition of the main effect of endocannabinoids in Step 3 of all models did not result in significantly increased variance accounted for (all p 's $> .23$).

Similar to above, we tested whether the observed main effect association between EO function and morphine effects for evoked pain measures was due to confounding related to the common placebo value used in calculating both morphine effects and blockade effects. Partial correlations (controlling for age and sex) were significant both before and after controlling for the relevant placebo measure: evoked thermal pain MPQ-2 Total $r = -0.67$, $p < .001$ (vs. partial $r = -0.58$, $p < .001$ when controlling for placebo pain levels).

Regression Results for VAS Intensity Evoked Pain Measure—Hierarchical regression results for the evoked pain VAS Intensity outcome revealed significant moderation of EO effects on morphine analgesia only for 2-AG ($p = .003$). Simple effect analyses indicated that the source of this interaction differed notably from that observed for the clinical back pain outcomes. For individuals with *high* 2-AG levels, low EO function was associated with significantly greater morphine analgesia ($\beta = -0.471$, $p = .029$). In contrast, for those with low 2-AG levels, lower EO function was associated with significantly less morphine analgesia ($\beta = 0.551$, $p = .015$). For the evoked pain VAS intensity outcome, tests of interactions between EO and all other endocannabinoid measures were nonsignificant (all p 's $> .10$). The main effect of EO function on morphine analgesia in these analyses was also not significant ($p = 0.815$). Addition of the main effect of endocannabinoids in Step 3 of all models did not result in significantly increased variance accounted for (all p 's $> .23$).

Non-Pain Subjective Responses

Zero order correlations between endocannabinoid measures and blockade and morphine effects for non-pain subjective effects are summarized in Table 3. Higher levels on several endocannabinoid measures were associated with nonsignificant trends towards greater EO modulation of drug liking (AEA, AA, OEA), desire to take the drug again (AEA, OEA), and euphoria (OEA). No endocannabinoids were significantly correlated with morphine-related subjective effects. Lower EO-related modulation of drug liking, desire to take the drug again, and euphoria was significantly associated with greater morphine effect on the corresponding measures, a pattern similar to that observed for pain outcomes.

Regression Results for DELTA Measure of Subjective Responses—Results of hierarchical regression analyses for the DELTA Drug Effect measure indicated that the impact of EO function indexed by this measure on overall perceived morphine effects was moderated significantly by 2-OG ($p = .048$), with nonsignificant trends for 2-AG ($p = 0.055$) and AA ($p = .052$). Results of simple effects analyses indicated that for individuals with higher 2-OG values, EO was not significantly associated with perceived overall morphine effects ($\beta = 0.150$, $p = .459$), whereas for individuals with lower AA values, reduced EO function was associated with significantly greater overall morphine effects ($\beta = -0.448$, $p = .045$). Results for 2-AG showed a similar pattern. For individuals with high 2-AG values, EO was not significantly associated with perceived overall morphine effects ($\beta = 0.197$, $p = .375$), but when 2-AG was low, reduced EO function was associated with marginally greater perceived morphine effects ($\beta = -0.397$, $p = .061$). For AA, EO was also not significantly associated with perceived overall morphine effects in individuals with high AA ($\beta = 0.201$, $p = .381$), however reduced EO function was associated with significantly greater overall perceived morphine effects when AA was low ($\beta = -0.496$, $p = .028$). This pattern was similar to that observed for morphine analgesic outcomes. Interaction effects were not significant for other endocannabinoids, and in these models, neither the main effects of EO ($p = 0.336$) nor endocannabinoid levels (0.902) were significant.

Results for the DELTA Drug Liking measure were stronger and more consistent. These analyses indicated that the impact of EO function on morphine-related drug liking was moderated by AEA ($p = 0.003$), 2-AG ($p < 0.001$), AA ($p = .004$), OEA ($p = .007$), and 2-OG ($p < 0.001$). Simple effects analyses indicated that for individuals with higher AEA values, EO was not significantly associated with morphine responses ($\beta = -0.033$, $p = .892$), however when AEA values were low, reduced EO function (i.e., less effect of naloxone on drug liking) was associated with significantly greater morphine-related drug liking ($\beta = -0.934$, $p < .001$). This moderation effect is portrayed in Figure 5, which indicates that the only participants displaying morphine-related drug liking (i.e., positive morphine effects) were those with low AEA and low EO function. A similar pattern was observed for 2-AG (High 2-AG: $\beta = 0.560$, $p = 0.086$; Low 2-AG: $\beta = -1.048$, $p < .001$), AA (High AA: $\beta = -0.266$, $p = 0.098$; Low AA: $\beta = -0.972$, $p < .001$), OEA (High OEA: $\beta = -0.272$, $p = 0.119$; Low OEA: $\beta = -0.907$, $p < .001$), and 2-OG (High 2-OG: $\beta = 0.167$, $p = 0.474$; Low 2-OG: $\beta = -1.206$, $p < .001$).

Results for the DELTA Desire to Take Again measure also indicated that the impact of EO function on morphine-related drug liking was moderated by AEA ($p=0.021$), 2-AG ($p=0.008$), AA ($p=.016$), OEA ($p=.031$), and 2-OG ($p = 0.001$). Simple effects analyses revealed that in all cases, EO was not significantly associated with desire to take morphine again when endocannabinoid levels were high, but when endocannabinoid values were low, reduced EO function was associated with significantly greater desire to take morphine again: AEA (High AEA: $\beta = -0.09$, $p = 0.72$; Low AEA: $\beta = -0.876$, $p = .003$), 2-AG (High 2-AG: $\beta = 0.481$, $p = 0.224$; Low 2-AG: $\beta = -0.928$, $p < .001$), AA (High AA: $\beta = -0.202$, $p = 0.263$; Low AA: $\beta = -0.86$, $p < .001$), OEA (High OEA: $\beta = -0.242$, $p = 0.211$; Low OEA: $\beta = -0.856$, $p = .002$), and 2-OG (High 2-OG: $\beta = 0.208$, $p = 0.421$; Low 2-OG: $\beta = -1.038$, $p < .001$).

Regression Results for VAS Opioid Effects Measure of Euphoria—Results for the VAS opioid effects Euphoria measure indicated that the impact of EO modulation of euphoria on subsequent morphine-related euphoria was not moderated significantly by any of the endocannabinoids assessed (p 's > 0.16). However, main effects of EO levels (in step 2) were significant for all models ($\beta = -0.352$, $p = 0.013$), indicating that lower EO function was associated with greater morphine-related euphoria. Main effects for all endocannabinoids in step 3 of these models were nonsignificant (p 's > 0.239).

Discussion

Numerous studies have been conducted evaluating potential predictors of opioid analgesic responses, with the ultimate clinical goal of developing an accurate predictive algorithm for identifying patients likely to obtain good relief with opioid medications while at the same time experiencing low side effects and risk of misuse [6]. Our prior work found that individuals with lower levels of EO function displayed greater morphine-induced reductions in low back pain and evoked pain responsiveness than those with higher levels of EO function [7,8]. Zero-order correlations and main effect models in the present sample independently replicated these prior findings for both low back pain and evoked pain, indicating an inverse association between EO function and morphine analgesic responsiveness. Partial correlation analyses indicated that these main effect associations were not confounded by participants' levels of placebo condition pain responses that were used in deriving both morphine effects values and opioid blockade effect values, a finding consistent with our past work [7].

The current study built upon these previous findings by addressing the question of whether non-opioid antinociceptive systems, specifically the endocannabinoid system, independently predicts morphine responses, or alternatively, interacts with EO function to predict morphine analgesic effects (i.e., moderate morphine analgesic effects). Emerging work indicates that endocannabinoids can have significant antinociceptive effects [37], with recent work hinting that endocannabinoid and EO systems may interact [19]. Unlike some prior animal studies [2,28,34,35], we did not find that any of the endocannabinoid-related measures examined were directly associated with morphine analgesic responses. Our findings might also be viewed as inconsistent with recent human work suggesting that variation in the gene that codes for fatty acid amide hydrolase (FAAH), which degrades AEA and can thus influence

endocannabinoid function, was associated with vomiting and respiratory effects of morphine [17], and with both evoked pain responses and postoperative opioid requirements [16]. Nonetheless, we found evidence that endocannabinoids moderate observed associations between EO function and morphine's analgesic effects. For clinical back pain outcomes, the pattern was consistently one in which the previously reported inverse association between EO function and morphine analgesia was only apparent in individuals having relatively lower levels of endocannabinoids. This moderation effect was apparent not only for AEA and 2-AG, but also for AA, a metabolite of AEA and 2-AG degradation, as well as other lipids (OEA and 2-OG) deriving from the same metabolic processes regulating AEA and 2-AG levels. Significant moderation by 2-AG of EO effects on evoked pain morphine effects was also noted, although in this case, was due to an inverse association between EO function and morphine analgesia (as for clinical pain outcomes) in those with *high* 2-AG levels, but an opposite pattern in those with low 2-AG levels. Reasons for this discrepancy across clinical and evoked pain outcomes are not clear.

One interpretation of the current findings regarding clinical pain outcomes is that analgesic medications like morphine may work best in individuals having low endogenous pain inhibitory capacity, both opioid and non-opioid (i.e., endocannabinoid). Such an interpretation might appear to be common sense, suggesting that analgesics such as morphine can be used to offset deficient endogenous pain inhibitory systems. This interpretation might be consistent with prior controlled trials suggesting that opioid medications provide better analgesia in individuals showing greater central pain sensitization [24] or elevated pain sensitivity [7], both of which might be expected to reflect in part lower endogenous pain inhibitory function. Also relevant may be parallel findings indicating that the selective norepinephrine reuptake inhibitor duloxetine provides better pain relief in individuals displaying low endogenous pain inhibitory capacity, as indexed by a conditioned pain modulation protocol [48]. Finally, recent work [19] reporting that blockade of EO activity with naltrexone prevented exercise-induced increases in AEA and OEA suggests that functional mechanistic links between EO and endocannabinoid systems may contribute to differential morphine responses. This interpretation is consistent with the positive associations (nonsignificant trends) observed in the current work between endocannabinoid levels and several EO indexes (evoked pain, morphine liking, desire to take morphine again).

The present study also builds on past work by extending findings of associations between EO and morphine response to non-pain subjective effects. As for analgesic outcomes, these associations were for the most part moderated by circulating endocannabinoid levels. The pattern of findings was such that lower EO modulation of subjective effects (e.g., drug liking) predicted greater morphine effects on these measures only for individuals with lower circulating endocannabinoid levels. One exception was for the morphine-related euphoria factor, which was significantly predicted only by the main effect of EO function (inverse association). The latter finding suggests that low EO activity may predict not only better pain relief with opioid analgesics, but also greater opioid-induced positive subjective responses. Greater positive subjective responses to opioids, whether related to low EO function or the combination of low EO function with low endocannabinoid levels, may be related to risk for opioid misuse [18,27,40,47]. In this context, it may be noteworthy that chronic pain patients at elevated risk for opioid misuse (based on a psychometric risk

measure), regardless of current opioid use status, displayed elevated evoked pain sensitivity [23], a response pattern that our prior work suggests is a marker for low EO function [7].

Overall, results of the current study suggest that circulating levels of endocannabinoids may alter the impact of EO function on opioid analgesic responsiveness. This finding provides further evidence in humans supporting interactions between endocannabinoid and EO systems [19], and prior work in animals supporting such interactions [28,46]. A recent review suggests that endocannabinoid and EO systems may interact specifically in brain regions underlying reward and reinforcement [46], highlighting potential relevance of the present work to opioid misuse risk.

If confirmed, the current findings may have clinical implications for development of personalized pain medicine algorithms. While quantitative assessment of EO function in the clinical context would be challenging (i.e., placebo-naloxone pain assessment protocols), assessment of circulating endocannabinoids would be more feasible. It is possible that combining endocannabinoid levels with surrogate markers of EO function suggested by prior work (evoked pain responsiveness, negative affect; [7,14]) might enhance ability to predict opioid analgesic responses. However, this possibility remains to be proven. From a broader perspective, results of this study highlight the importance of considering multiple endogenous pain inhibitory systems jointly (both opioid and nonopioid) in efforts to identify mechanistically-relevant predictors of opioid analgesic responses.

The current work has several limitations. First, unlike some prior animal work [2,35], the current study did not find evidence that endocannabinoid function was directly associated with morphine analgesic responses in models not exhibiting EO $\times$ Endocannabinoid interactions. This may in part have been related to inadequate statistical power given the relatively small sample size. Absence of other human experimental studies directly examining endocannabinoid effects on opioid analgesia makes it difficult to determine whether or not such endocannabinoid predictive effects would be expected in humans, although recent genetic work suggests such associations might be expected [16,17]. Second, because it is unknown to what extent circulating endocannabinoid levels assessed in this study corresponded with central nervous system endocannabinoid activity, the current findings are most accurately viewed as demonstrating that endocannabinoid levels are a biomarker empirically predictive of opioid analgesic responses (in tandem with EO function) rather than directly indicating involvement of central endocannabinoid mechanisms. Third, as in our past work, the current findings examining opioid analgesic responses within a single laboratory session cannot necessarily be generalized to prediction of responses to sustained daily opioid use, as is often the case in chronic pain management settings. Due to use of naloxone in the study protocol, individuals using daily opioids were excluded, and thus these findings may only apply to the general population living with chronic pain rather than tertiary care pain clinic populations characterized by more severe dysfunction and chronic opioid use. Fourth, the study used a relatively low dose of morphine to limit possible analgesic floor effects that might obscure individual differences in morphine responding. It is possible that this low dosage may have contributed to instances in which morphine appeared to somewhat increase rather than decrease pain responses. Finally, initiating assessment of pain outcomes at 10 minutes after morphine administration might not have

provided sufficient time to reach peak effects, although pharmacokinetic data suggest morphine reaches 80% of peak CNS concentration within 6 minutes [39].

In summary, the current findings independently replicate our prior work showing associations between low EO function and greater morphine responsiveness. It also adds nuance to these prior findings, indicating that these associations are most prominent in individuals displaying low circulating endocannabinoid levels. It appears that opioid analgesic medications may provide the best relief in individuals with inadequate endogenous pain inhibitory systems. Additional work is required to determine whether these findings may have clinical utility in developing personalized pain medicine algorithms.

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References

- [1]. Aiken LS, West SG. Multiple regression: Testing and interpreting interactions. Thousand Oaks, CA: Sage, 1991.
- [2]. Altun A, Ozdemir E, Yildirim K, Gursoy S, Durmus N, Bagcivan I. The effects of endocannabinoid receptor agonist anandamide and antagonist rimonabant on opioid analgesia and tolerance in rats. *Gen Physiol Biophys* 2015;34:433–440. [PubMed: 26374993]
- [3]. Altun A, Yildirim K, Ozdemir E, Bagcivan I, Gursoy S, Durmus N. Attenuation of morphine antinociceptive tolerance by cannabinoid CB1 and CB2 receptor antagonists. *J Physiol Sci* 2015;65(5):407–415. [PubMed: 25894754]
- [4]. Anand P, Whiteside G, Fowler CJ, Hohmann AG. Targeting CB2 receptors and the endocannabinoid system for the treatment of pain. *Brain research reviews* 2009;60(1):255–266. [PubMed: 19150370]
- [5]. Bruehl S Personalized pain medicine: Pipe dream or reality? *Anesthesiology* 2015;122(5):967–968. [PubMed: 25751235]
- [6]. Bruehl S, Apkarian AV, Ballantyne JC, Berger A, Borsook D, Chen WG, Farrar JT, Haythornthwaite JA, Horn SD, Iadarola MJ. Personalized medicine and opioid analgesic prescribing for chronic pain: opportunities and challenges. *J Pain* 2013;14(2):103–113. [PubMed: 23374939]
- [7]. Bruehl S, Burns JW, Gupta R, Buvanendran A, Chont M, Kinner E, Schuster E, Passik S, France CR. Endogenous opioid function mediates the association between laboratory-evoked pain sensitivity and morphine analgesic responses. *Pain* 2013;154(9):1856–1864. [PubMed: 23748117]
- [8]. Bruehl S, Burns JW, Gupta R, Buvanendran A, Chont M, Schuster E, France CR. Endogenous opioid inhibition of chronic low back pain influences degree of back pain relief following morphine administration. *Reg Anesth Pain Med* 2014;39(2):120–125. [PubMed: 24553304]
- [9]. Bruehl S, Burns JW, Passik SD, Gupta R, Buvanendran A, Chont M, Schuster E, Orłowska D, France CR. The contribution of differential opioid responsiveness to identification of opioid risk in chronic pain patients. *J Pain* 2015;16(7):666–675. [PubMed: 25892658]
- [10]. Bruehl S, Chung OY. Parental history of chronic pain may be associated with impairments in endogenous opioid analgesic systems. *Pain* 2006;124(3):287–294. [PubMed: 16725261]
- [11]. Bruehl S, Dengler-Crish CM, Smith CA, Walker LS. Hypoalgesia related to elevated resting blood pressure is absent in adolescents and young adults with a history of functional abdominal pain. *Pain* 2010;149(1):57–63. [PubMed: 20122805]

- [12]. Bruehl S, Stone AL, Palmer C, Edwards DA, Buvanendran A, Gupta R, Chont M, Kennedy M, Burns JW. Self-reported cumulative medical opioid exposure and subjective responses on first use of opioids predict analgesic and subjective responses to placebo-controlled opioid administration. *Reg Anesth Pain Med* In Press.
- [13]. Brummett CM, Janda AM, Schueller CM, Tsodikov A, Morris M, Williams DA, Clauw DJ. Survey criteria for fibromyalgia independently predict increased postoperative opioid consumption after lower-extremity joint arthroplasty prospective, observational cohort study. *Anesthesiology* 2013;119(6):1434–1443. [PubMed: 24343289]
- [14]. Burns JW, Bruehl S, France CR, Schuster E, Orlowska D, Buvanendran A, Chont M, Gupta RK. Psychosocial factors predict opioid analgesia through endogenous opioid function. *Pain* 2017;158(3):391–399. [PubMed: 27898491]
- [15]. Burns JW, Bruehl S, France CR, Schuster E, Orlowska D, Chont M, Gupta RK, Buvanendran A. Endogenous opioid function and responses to morphine: the moderating effects of anger expressiveness. *J Pain* 2017;18(8):923–932. [PubMed: 28365372]
- [16]. Cajanus K, Holmstrom EJ, Wessman M, Anttila V, Kaunisto MA, Kalso E. Effect of endocannabinoid degradation on pain: role of FAAH polymorphisms in experimental and postoperative pain in women treated for breast cancer. *Pain* 2016;157(2):361–369. [PubMed: 26808012]
- [17]. Chidambaram V, Pilipenko V, Spruance K, Venkatasubramanian R, Niu J, Fukuda T, Mizuno T, Zhang K, Kaufman K, Vinks AA, Martin LJ, Sadhasivam S. Fatty acid amide hydrolase-morphine interaction influences ventilatory response to hypercapnia and postoperative opioid outcomes in children. *Pharmacogenomics* 2017;18(2):143–156. [PubMed: 27977335]
- [18]. Comer SD, Sullivan MA, Vosburg SK, Kowalczyk WJ, Houser J. Abuse liability of oxycodone as a function of pain and drug use history. *Drug Alcohol Depend* 2010;109(1–3):130–138. [PubMed: 20079977]
- [19]. Crombie KM, Brellenthin AG, Hillard CJ, Koltyn KF. Endocannabinoid and opioid system interactions in exercise-induced hypoalgesia. *Pain Med* 2017;19(1):118–123.
- [20]. Dworkin RH, Turk DC, Revicki DA, Harding G, Coyne KS, Peirce-Sandner S, Bhagwat D, Everton D, Burke LB, Cowan P. Development and initial validation of an expanded and revised version of the Short-form McGill Pain Questionnaire (SF-MPQ-2). *Pain* 2009;144(1–2):35–42. [PubMed: 19356853]
- [21]. Dworkin RH, Turk DC, Trudeau JJ, Benson C, Biondi DM, Katz NP, Kim M. Validation of the Short-form McGill Pain Questionnaire-2 (SF-MPQ-2) in acute low back pain. *J Pain* 2015;16(4):357–366. [PubMed: 25640290]
- [22]. Edwards RR, Haythornthwaite JA, Tella P, Max MB, Raja S. Basal heat pain thresholds predict opioid analgesia in patients with postherpetic neuralgia. *Anesthesiology* 2006;104(6):1243–1248. [PubMed: 16732096]
- [23]. Edwards RR, Wasan AD, Michna E, Greenbaum S, Ross E, Jamison RN. Elevated pain sensitivity in chronic pain patients at risk for opioid misuse. *J Pain* 2011;12(9):953–963. [PubMed: 21680252]
- [24]. Eisenberg E, Midbari A, Haddad M, Pud D. Predicting the analgesic effect to oxycodone by ‘static’ and ‘dynamic’ quantitative sensory testing in healthy subjects. *Pain* 2010;151(1):104–109. [PubMed: 20621419]
- [25]. Freynhagen R, Baron R, Gockel U, Tölle TR. Pain DETECT: a new screening questionnaire to identify neuropathic components in patients with back pain. *Curr Med Res Opin* 2006;22(10):1911–1920. [PubMed: 17022849]
- [26]. Geha H, Nimeskern N, Beziat J. Patient-controlled analgesia in orthognathic surgery: evaluation of the relationship to anxiety and anxiolytics. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009;108(3):e33–e36. [PubMed: 19716489]
- [27]. Griffiths RR, Bigelow GE, Ator NA. Principles of initial experimental drug abuse liability assessment in humans. *Drug Alcohol Depend* 2003;70(3):S41–S54. [PubMed: 12759196]
- [28]. Haller VL, Stevens DL, Welch SP. Modulation of opioids via protection of anandamide degradation by fatty acid amide hydrolase. *Eur J Pharmacol* 2008;600(1–3):50–58. [PubMed: 18762181]

- [29]. Han B, Compton WM, Blanco C, Crane E, Lee J, Jones CM. Prescription opioid use, misuse, and use disorders in US adults: 2015 National Survey on Drug Use and Health. *Ann Intern Med* 2017;167(5):293–301. [PubMed: 28761945]
- [30]. Kaczocha M, Azim S, Nicholson J, Rebecchi MJ, Lu Y, Feng T, Romeiser JL, Reinsel R, Rizwan S, Shodhan S. Intrathecal morphine administration reduces postoperative pain and peripheral endocannabinoid levels in total knee arthroplasty patients: a randomized clinical trial. *BMC Anesthesiol* 2018;18(1):27. [PubMed: 29486720]
- [31]. Morgan AJ, Kingsley PJ, Mitchener MM, Altemus M, Patrick TA, Gaulden AD, Marnett LJ, Patel S. Detection of cyclooxygenase-2-derived oxygenation products of the endogenous cannabinoid 2-arachidonoylglycerol in mouse brain. *ACS Chem Neurosci* 2018;9(7):1552–1559. [PubMed: 29722963]
- [32]. Niesters M, Dahan A, Kest B, Zacny J, Stijnen T, Aarts L, Sarton E. Do sex differences exist in opioid analgesia? A systematic review and meta-analysis of human experimental and clinical studies. *Pain* 2010;151(1):61–68. [PubMed: 20692097]
- [33]. Okie S A flood of opioids, a rising tide of deaths. *N Engl J Med* 2010;363(21):1981–1985. [PubMed: 21083382]
- [34]. Pacheco DDF, Klein A, Perez ADC, Pacheco CDF, De Francischi J, Duarte I. The μ -opioid receptor agonist morphine, but not agonists at δ -or κ -opioid receptors, induces peripheral antinociception mediated by cannabinoid receptors. *Br J Pharmacol* 2008;154(5):1143–1149. [PubMed: 18469844]
- [35]. Pacheco FD, Klein A, Perez AC, Pacheco C, Reis G, Duarte I. Central antinociception induced by mu-opioid receptor agonist morphine, but not delta-or kappa-, is mediated by cannabinoid CB1 receptor. *Br J Pharmacol* 2009;158(1):225–231. [PubMed: 19594755]
- [36]. Pertwee RG. Cannabinoid receptors and pain. *Prog Neurobiol* 2001;63(5):569–611. [PubMed: 11164622]
- [37]. Sagar DR, Gaw AG, Okine BN, Woodhams SG, Wong A, Kendall DA, Chapman V. Dynamic regulation of the endocannabinoid system: implications for analgesia. *Mol Pain* 2009;5(1):59. [PubMed: 19814807]
- [38]. Salio C, Fischer J, Franzoni MF, Mackie K, Kaneko T, Conrath M. CB1-cannabinoid and μ -opioid receptor co-localization on postsynaptic target in the rat dorsal horn. *Neuroreport* 2001;12(17):3689–3692. [PubMed: 11726775]
- [39]. Upton RN, Semple TJ, Macintyre PE. Pharmacokinetic optimisation of opioid treatment in acute pain therapy. *Clin Pharmacokinet* 1997;33(3):225–244. [PubMed: 9314613]
- [40]. Walsh SL, Nuzzo PA, Lofwall MR, Holtman JR, Jr. The relative abuse liability of oral oxycodone, hydrocodone and hydromorphone assessed in prescription opioid abusers. *Drug Alcohol Depend* 2008;98(3):191–202. [PubMed: 18606504]
- [41]. Walter C, Lötsch J. Meta-analysis of the relevance of the OPRM1 118A> G genetic variant for pain treatment. *Pain* 2009;146(3):270–275. [PubMed: 19683391]
- [42]. Wasan AD, Davar G, Jamison R. The association between negative affect and opioid analgesia in patients with discogenic low back pain. *Pain* 2005;117(3):450–461. [PubMed: 16154274]
- [43]. Wasan AD, Michna E, Edwards RR, Katz JN, Nedeljkovic SS, Dolman AJ, Janfaza D, Isaac Z, Jamison RN. Psychiatric comorbidity is associated prospectively with diminished opioid analgesia and increased opioid misuse in patients with chronic low back pain. *Anesthesiology* 2015;123(4):861–872. [PubMed: 26375824]
- [44]. Welch SP. Interaction of the cannabinoid and opioid systems in the modulation of nociception. *Int Rev Psychiatry* 2009;21(2):143–151. [PubMed: 19367508]
- [45]. Welch SP, Eads M. Synergistic interactions of endogenous opioids and cannabinoid systems. *Brain Res* 1999;848(1–2):183–190. [PubMed: 10612710]
- [46]. Wenzel J, Cheer J. Endocannabinoid regulation of reward and reinforcement through interaction with dopamine and endogenous opioid signaling. *Neuropsychopharmacology* 2018;43(1):103. [PubMed: 28653666]
- [47]. Wightman R, Perrone J, Portelli I, Nelson L. Likeability and abuse liability of commonly prescribed opioids. *J Med Toxicol* 2012;8(4):335–340. [PubMed: 22992943]

- [48]. Yarnitsky D, Granot M, Nahman-Averbuch H, Khamaisi M, Granovsky Y. Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy. *Pain* 2012;153(6):1193–1198. [PubMed: 22480803]
- [49]. Zacny JP. A possible link between sensation-seeking status and positive subjective effects of oxycodone in healthy volunteers. *Pharmacology Biochemistry and Behavior* 2010;95(1):113–120.
- [50]. Zacny JP, Gutierrez S. Subjective, psychomotor, and physiological effects of oxycodone alone and in combination with ethanol in healthy volunteers. *Psychopharmacology (Berl)* 2011;218(3):471–481. [PubMed: 21603891]
- [51]. Zacny JP, Paice JA, Coalson DW. Separate and combined psychopharmacological effects of alprazolam and oxycodone in healthy volunteers. *Drug Alcohol Depend* 2012;124(3):274–282. [PubMed: 22365897]
- [52]. Zoerner AA, Batkai S, Suchy MT, Gutzki FM, Engeli S, Jordan J, Tsikas D. Simultaneous UPLC-MS/MS quantification of the endocannabinoids 2-arachidonoyl glycerol (2AG), 1-arachidonoyl glycerol (1AG), and anandamide in human plasma: minimization of matrix-effects, 2AG/1AG isomerization and degradation by toluene solvent extraction. *J Chromatogr B Analyt Technol Biomed Life Sci* 2012;883–884:161–171.
- [53]. Zubrzycki M, Janecka A, Liebold A, Ziegler M, Zubrzycka M. Effects of centrally administered endocannabinoids and opioids on orofacial pain perception in rats. *Br J Pharmacol* 2017;174(21):3780–3789. [PubMed: 28771697]

Figure 1.
 Laboratory protocol for sessions 1–3.

Figure 2. Moderation of association between endogenous opioid (EO) function and morphine effects on MPQ2-Total back pain intensity by AEA levels. Larger positive values indicate greater morphine analgesia.

Figure 3. Moderation of association between endogenous opioid (EO) function and morphine effects on MPQ2-Total back pain intensity by 2-OG levels. Larger positive values indicate greater morphine analgesia.

Figure 4. Moderation of association between endogenous opioid (EO) function and morphine effects on VAS back pain intensity by 2-AG levels. Larger positive values indicate greater morphine analgesia.

Figure 5. Moderation of association between endogenous opioid (EO) inhibition and morphine effects on DELTA Drug Liking by AEA levels. Larger positive values indicate greater morphine-related drug liking.

Table 1.

Sample characteristics (n=46).

Characteristic	Summary Statistic
Sex (% Female)	63.0
Race (%):	
White	56.5
African-American	30.4
Ethnicity (% Non-Hispanic)	95.6
Age (Mean $\pm$ SD, years)	39.7 $\pm$ 10.41
Body Mass Index (Mean $\pm$ SD)	31.2 $\pm$ 6.97
Pain Duration (median, months)	75.9
Pain Detect Score (Mean $\pm$ SD)	9.6 $\pm$ 7.48
Past week VAS pain intensity (Mean $\pm$ SD)	57.2 $\pm$ 18.38
AEA (Mean $\pm$ SD, pmol/g)	0.90 $\pm$ 0.75
2-AG (Mean $\pm$ SD, nmol/g)	10.80 $\pm$ 9.86
AA (Mean $\pm$ SD, nmol/g)	1.88 $\pm$ 1.41
OEA (Mean $\pm$ SD, pmol/g)	5.39 $\pm$ 4.41
2-OG (Mean $\pm$ SD, nmol/g)	91.37 $\pm$ 90.24
Postmenopausal (%)	15.6
Birth control pill use (%)	18.2
As needed opioid use (%)	17.6
NSAID use (%)	39.2
Neuroleptic use (%)	9.8
Antidepressant use (%)	21.6

Table 2.
Zero order correlations between endocannabinoids and EO and morphine analgesic responses.

Measure	EO Thermal MPQ-Tot	EO Thermal VAS	EO LBP MPQ-Tot	EO LBP VAS	MOR Thermal MPQ-Tot	MOR Thermal VAS	MOR LBP MPQ-Tot	MOR LBP VAS
Endocannabinoids								
AEA	0.01	0.09	0.05	0.16	-0.02	-0.03	0.17	0.01
2-AG	0.25 [†]	0.15	-0.02	0.13	-0.24	0.09	0.14	0.06
AA	-0.06	0.04	0.13	0.20	-0.10	-0.04	0.18	-0.09
OEA	0.04	0.16	0.02	0.12	-0.04	-0.14	0.23	0.09
2-OG	0.31 [*]	0.10	0.01	0.04	-0.14	0.04	0.16	0.19
Endogenous Opioid Blockade Effect (EO)								
EO Thermal MPQ-Tot	--	0.21	0.04	0.12	-0.68 ^{***}	-0.11	-0.17	-0.20
EO Thermal VAS	--	--	-0.01	-0.06	-0.16	-0.26 [†]	-0.32 [*]	-0.05
EO LBP MPQ-Tot	--	--	--	0.43 ^{**}	-0.01	-0.17	-0.49 ^{***}	-0.36 [*]
EO LBP VAS	--	--	--	--	-0.03	0.15	-0.30 [*]	-0.54 ^{***}
Morphine Analgesic Effect (MOR)								
MOR Thermal MPQ-Tot	--	--	--	--	--	0.33 [*]	0.12	0.15
MOR Thermal VAS	--	--	--	--	--	--	0.15	0.18
MOR LBP MPQ-Tot	--	--	--	--	--	--	--	0.58 ^{***}

[†]
p<.10

^{*}
p<.05

^{**}
p<.01

^{***}
p<.001

Note: MPQ-Tot = Short Form McGill Pain Questionnaire-2 total score, VAS = Visual analog pain intensity scale

Table 3.
Zero order correlations between endocannabinoids and EO and morphine-related subjective effects.

Measure	EO Drug Effect	EO Drug Liking	EO Take Again	EO Euphoria	MOR Drug Effect	MOR Drug Liking	MOR Take Again	MOR Euphoria
Endocannabinoids								
AEA	-0.11	0.26 [‡]	0.26 [‡]	0.10	0.06	-0.13	-0.11	0.15
2-AG	0.20	0.14	0.06	0.05	-0.05	-0.04	0.04	0.14
AA	-0.02	0.28 [‡]	0.23	-0.07	-0.04	-0.12	-0.07	0.13
OEA	-0.13	0.27 [‡]	0.26 [‡]	0.26 [‡]	0.02	-0.10	-0.05	0.07
2-OG	0.11	0.20	0.12	0.23	-0.06	0.05	0.20	0.12
Endogenous Opioid Blockade Effect (EO)								
EO Drug Effect	--	-0.12	-0.22	-0.21	-0.14	0.04	0.02	0.18
EO Drug Liking		--	0.93 ^{***}	0.35 [*]	-0.24 [‡]	-0.44 ^{**}	-0.38 ^{**}	-0.10
EO Take Again			--	0.28 [*]	-0.21	-0.42 ^{**}	-0.42 ^{**}	-0.09
EO Euphoria				--	0.04	-0.18	-0.17	-0.39 ^{**}
Morphine Effect (MOR)								
MOR Drug Effect					--	0.07	0.07	-0.10
MOR Drug Liking						--	0.90 ^{***}	0.54 ^{***}
MOR Take Again							--	0.51 ^{***}

[‡] p<.10
* p<.05
** p<.01
*** p<.001