Neuropsychopharmacology (2015) 40, 554–565

www.neuropsychopharmacology.org

Dynamic Interactions Between Plasma IL-1 Family Cytokines and Central Endogenous Opioid Neurotransmitter Function in Humans

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Evidence in animal models suggests IL-1 family cytokines interact with central endogenous opioid neurotransmitter systems, inducing or perpetuating pathological states such as persistent pain syndromes, depression, substance use disorders, and their comorbidity. Understanding these interactions in humans is particularly relevant to understanding pathological states wherein this neurotransmitter system is implicated (ie, persistent pain, mood disorders, substance use disorders, *etc*). Here, we examined relationships between IL-1β, IL-1ra, and functional measures of the endogenous opioid system in 34 healthy volunteers, in the absence and presence of a standardized sustained muscular pain challenge, a psychophysical challenge with emotionally and physically stressful components. Mu-opioid receptor availability *in* vivo was examined with [^{11}C]carfentanil positron emission tomography (PET) scanning. Sex and neuroticism impacted IL-1 family cytokines; higher baseline IL-1β and IL-1ra was identified in females with lower neuroticism. Higher baseline IL-1β was also associated with reduced μ-opioid receptor availability (amygdala) and greater pain sensitivity. The pain challenge increased IL-1β in females with high neuroticism. Strong associations between IL-1ra (an anti-nociceptive cytokine) and μ-opioid receptor activation (VP/NAcc) were identified during the pain challenge and the resulting analgesic effect of μ-opioid receptor activation was moderated by changes in IL-1β whereby volunteers with greater pain induced increase in IL-1β experienced less endogenous opioid analgesia. This study demonstrates the presence of relationships between inflammatory factors and a specific central neurotransmitter system and circuitry, of relevance to understanding interindividual variations in regulation of responses to pain and other physical and emotional stressors.

Neuropsychopharmacology (2015) **40,** 554–565; doi:10.1038/npp.2014.202; published online 24 September 2014

INTRODUCTION

It is well known that peripheral inflammatory factors influence central nervous system functioning, effects that contribute to behavioral correlates of systemic inflammation. Increasing evidence suggests these effects are bi-directional, whereby central mechanisms, including altered immunological, neural, and neuroendocrine activity modulate peripheral immune function, potentially creating positive feedback loops that influence risk or perpetuate pathological states (Anisman *et al*, 1993; Dantzer *et al*, 2008). Pro-inflammatory cytokine elevations have been described in pathological states that include depressive illness (Miller *et al*, 2009), persistent pain states (Watkins *et al*, 1994; Hutchinson *et al*, 2008), and their comorbidity (Zautra *et al*, 2007). However, substantial inter-individual inflammatory variability has been noted across studies. For example, although individual studies in fibromyalgia (FM) identified elevations in certain peripheral inflammatory cytokines (eg, IL-1 receptor antagonist (IL-1ra), IL-6, IL-8) in FM volunteers (Salemi *et al*, 2003; Uceyler *et al*, 2006; Gur and Oktayoglu, 2008), meta-analyses did not show significance for many of these effects, likely reflecting heterogeneity in this syndromic presentation and methodological variation (Uceyler *et al*, 2011). Beyond variations in pathology, a fundamental question not yet addressed is how inflammatory variability impacts brain mechanisms associated with risk or resiliency for pathological states.

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Received 20 February 2014; revised 2 June 2014; accepted 28 June 2014; accepted article preview online 20 August 2014

The central endogenous opioid neurotransmitter system (including μ-opioid receptors) is implicated in inflammatory-central nervous system interactions (Day and Akil, 1996; Peterson *et al*, 1998; Prossin *et al*, 2011), emotion dysregulation (Prossin *et al*, 2010), and chronic pain (Jones *et al*, 1994; Harris *et al*, 2007). Early *in vitro* evidence showed that interleukin-1β (IL-1β) reduced μ-opioid receptor binding in brain membrane preparations (Ahmed *et al*, 1985) and acutely increased μ-opioid receptor and pro-enkephalin mRNA expression in astrocyte-enriched cultures (Ruzicka and Akil, 1997). Peripheral IL-1β potently reduces opioid analgesia (Hutchinson *et al*, 2008) and facilitates morphine tolerance (Shavit *et al*, 2005), effects antagonized by IL-1ra (Raghavendra *et al*, 2002; Shavit *et al*, 2005; Hutchinson *et al*, 2008). These opposing IL-1β and IL-1ra moderated effects are thought to take place through interactions with both non-classic opioid receptors on glial cells and traditional μ-opioid receptors involved in endogenous opioid neurotransmission (Hutchinson *et al*, 2011).

In animal models, *in vivo* systemic administration of IL-1β has been shown to increase early gene (c-fos) expression in cells within the central nucleus of the amygdala and bed nucleus of the striae terminalis, two brain regions critical in regulating pain and stress responses (Day and Akil, 1996). Follow-up work showed the majority of these cells expressed primarily enkephalin, an endogenous ligand for μ-opioid receptors. These findings suggest IL-1β effects are selective for endogenous opioid neurotransmitter systems, particularly within these brain regions (Day and Akil, 1999a; Day *et al*, 1999b). Interactions between IL-1β and central opioid neurotransmission have also been demonstrated through enhanced fos-immunoreactivity in the amygdala and bed nucleus of the striae terminalis in rats pre-treated with intra-arterial IL-1β and naloxone, an opioid receptor antagonist (Buller *et al*, 2005). Mu-opioid-receptor-mediated neurotransmission in the amygdala and interconnected brain regions including the ventral basal ganglia, thalamus, and anterior cingulate cortex can regulate emotional states (Prossin *et al*, 2010), reward mechanisms and behavior associated with substance abuse (Ray *et al*, 2011), the pain experience (Zubieta *et al*, 2001, 2002), the pathophysiology of persistent pain states (Jones *et al*, 1994; Harris *et al*, 2007), and sex differences in these phenomena (Zubieta *et al*, 2002).

In addition to immune to central effects, central neurotransmission also appears to influence innate immune responses to stress. Mu-opioid receptor agonists can regulate peripheral immune response, either directly or indirectly via modulation of both the hypothalamic-pituitary-adrenal axis and the autonomic nervous system (Vallejo *et al*, 2004). Psychological stress challenges known to activate the hypothalamic-pituitary-adrenal axis have also been shown to increase plasma IL-1β concentrations (Yamakawa *et al*, 2009). Similar psychological stress challenges have been shown to induce plasma IL-1ra, an antagonist to IL-1β, but with a temporal delay (Rohleder *et al*, 2006).

In the present study, we examined the relationship between peripheral cytokines, specifically IL-1β and IL-1ra, and central μ-opioid receptor availability in healthy volunteers using molecular imaging techniques with PET and the selective μ-opioid receptor radiotracer [^{11}C]carfentanil. These effects were examined at baseline and during a psychophysical challenge with both emotionally and physically stressful components, moderate levels of deep muscular pain, known to activate endogenous, μ-opioid-receptor-mediated neurotransmission (Zubieta *et al*, 2001). The potential effects of sex and neuroticism scores (NEO-PI, (Costa and McCrae, 1992), known to influence innate immune responses, risk for idiopathic pain syndromes and psychopathology (Angst and Clayton, 1986; Berkley *et al*, 2006), were also explored in these analyses. On the basis of animal data, it was hypothesized that in healthy controls, baseline plasma IL-1 family cytokines (including both IL-1β and IL-1ra) and their changes in response to the pain challenge would be linearly proportional to baseline μ-opioid receptor availability and endogenous μ-opioid receptor activation during the pain challenge, considering the contribution of other influencing factors (sex, neuroticism).

MATERIALS AND METHODS

Subjects

Participants included 34 healthy volunteers (22 female, 12 male) recruited through local advertising. Absence of current or past psychiatric diagnoses including substance abuse or dependence was confirmed with the Structured Clinical Interview for DSM-IV non-patient version (First and Spitzer 1995). All volunteers were right-handed, non-smokers without active medical illness and free from psychotropic medications, including sleep aids for the prior 6 months. Females had regular menstrual cycles (28–32 days) and were studied during their follicular phase (days 2–10 post menses), as determined by menstrual diaries and confirmed by plasma levels of estradiol and progesterone at the time of scanning. All volunteers had negative pregnancy and drug screens. All PET scans were conducted at the same time of day, with radiotracer administration at 13 : 30 h to account for circadian variations. Calculated body mass index (BMI = weight(kg)/height(m)^2) was normally distributed across our study population. Volunteers completed the NEO-PI; their scores were compared with median scores of comparable age population samples, using a median split to define relatively lower and higher neuroticism in between group statistical analyses.

The Institutional Review Board and the Radioactive Drug Research Committee approved the study. All subjects provided informed written consent at study entry.

Experimental Pain Challenge

We employed a physical and emotional stressor, moderate levels of sustained pain of experientially adjusted intensity as the challenge paradigm. In short, a steady state of moderate muscle pain was maintained 45–65 min after radiotracer administration via computer-controlled infusion of medication-grade hypertonic saline (5%) into the relaxed left masseter muscle. The intensity of painful stimulus was standardized across subjects, as previously described (Zubieta *et al*, 2002). A non-painful control condition, 0.9% saline, was introduced in the right masseter muscle 5 min after radiotracer administration and also

maintained for 20 min. To avoid carry-over effects of the pain challenge, conditions were not randomized. Pain intensity was rated for both conditions every 15 s with a visual analogue scale (VAS) from 0 (no pain) to 100 (most intense pain imaginable) and were recorded in the computer controller and averaged for statistical analyses. The ratio of pain intensity to the infusion volume required for pain maintenance provided a measure of volunteer sensitivity to sustained pain (VAS/ml). Integrative measures of the pain experience were obtained using the McGill pain questionnaire (MPQ: Total, Sensory and Affective Pain scores) and VAS pain intensity scale, administered upon completion of the pain challenge (Melzack, 1975). The Positive and Negative Affect Schedule (Watson *et al*, 1988), assessing the internal affective state of the volunteers, was obtained before and after the challenge.

Neuroimaging Measures

One [^{11}C]carfentanil PET scan per subject was acquired with a Siemens HR$^+$ scanner (Knoxville, TN) in 3-D mode (reconstructed FWHM resolution, 5.5 mm in-plane and 5.0 mm axially) over 90 min, comprising baseline (0–45 min) and experimental pain challenge conditions (45–90 min). Radiotracer synthesis, image acquisition, co-registration, and reconstruction protocols were identical to those previously described (Zubieta *et al*, 2002). Image data were then transformed on a voxel-by-voxel basis into two sets of parametric maps, a tracer transport measure (K_1 ratio), and a receptor-related measure (non-displaceable binding potential, BP_{ND}, or receptor availability *in vivo* (Yasuno *et al*, 2007). To avoid the need for arterial blood sampling, these measures were calculated using a modified Logan graphical analysis (Logan *et al*, 1996), and the occipital cortex (an area devoid of μ-opioid receptors) as reference region. Using a bolus-continuous radiotracer infusion, the slope of the Logan plot becomes linear 5–7 min post tracer administration and is proportional to the receptor concentration divided by its affinity for the radiotracer ($BP_{ND}+1$, or $(f_2Bmax/K_d)+1$). Bmax is the receptor concentration and K_d, the receptor-ligand dissociation constant. The term f_2 refers to the concentration of free radiotracer in the extracellular fluid and is considered to represent a constant and very small value. Reductions in the *in vivo* availability of receptors, the BP_{ND} measure, after the pain stress challenge are thought to reflect processes associated with neurotransmitter release, such as competition between radiotracer and endogenous ligand and changes in receptor affinity induced by endogenous ligand–receptor interactions, the latter relevant for agonist radiotracers, which preferentially label high affinity, functional receptors (Narendran and Martinez, 2008). Anatomical MRI studies were acquired on a 3-T scanner (General Electric, Milwaukee, WI). Acquisition sequences were axial spoiled gradient recall inverse recovery prepared magnetic resonance (echo time, 3.4 ms; repetition time, 10.5 ms; inversion time, 200 ms; flip angle, 25°; number of excitations, 1; using 124 contiguous images, 1.5-mm thickness). The K_1 and BP_{ND} images for each experimental period and the anatomical MRI were co-registered to each other and to the Montreal Neurological Institute (MNI) stereotactic atlas orientation (Meyer *et al*, 1997).

Inflammatory Measures

IL-1β and IL-1ra plasma levels were obtained after the control condition and pain challenge, 40 and 90 min after radiotracer administration, respectively. Plasma was aliquotted and stored at $-80\,^{\circ}$C until assayed. All plasma samples were run in duplicate using multiplex ELISA kits (Millipore, MA) with minimal detectable levels of 15 pg/ml (IL-1β) and 30 pg/ml (IL-1ra). This was calculated by adding two standard deviations to the mean fluorescence index obtained when the zero standard was assayed 30 times. The highest standard was 12 810 pg/ml for IL-1β, and 30 900 pg/ml for IL-1ra. Both IL-1β and IL-1ra were $\log_{10}$ normalized for statistical analyses.

Data Analysis

Analyses included baseline and pain challenge states. Using MANCOVA, we evaluated effects of factors of interest (sex, neuroticism) on inflammatory variables (IL-1β and IL-1ra) at baseline. Subsequently, using hierarchical, linear regression, we evaluated the contributions of categorical (sex, neuroticism) and continuous predictors (baseline IL-1β, IL-1ra) to pain sensitivity (VAS/ml). Non-neuroimaging analyses involving inflammatory variables were not corrected for multiple comparisons.

Using repeated measures MANOVA, we evaluated effects of challenge condition (pain, control) on inflammatory variables (IL-1β and IL-1ra), and interactions with sex and neuroticism. Hierarchical, linear regression evaluated the contributions of categorical (sex, neuroticism) and continuous predictors (IL-1β, IL-1ra) on measures of pain sensitivity during the pain challenge.

Using the general linear model in SPM8 (Wellcome Trust, London, England), we tested for the presence of linear relationships between baseline μ-opioid receptor availability and inflammatory measures (eg, IL-1β, IL-1ra) at baseline and following the pain challenge on a voxel-by-voxel basis with sex and neuroticism (low *vs* high) as covariates. *t*-Statistic values were calculated with pooled variance across voxels (Worsley *et al*, 1995) with a statistical threshold of $p<0.001$ for the amygdala, an *a priori* hypothesized region). A threshold controlling for a type-I error rate at $p<0.05$ after correcting for focal extent was employed for all other regions (Friston *et al*, 1994).

Given IL-1β's known hyperalgesic effects (Watkins *et al*, 1994; Shavit *et al*, 2005; Hutchinson *et al*, 2008), we hypothesized that variations in IL-1β levels during the pain stressor would moderate endogenous opioid system responses and their pain regulatory effects. Using the Process function in SPSS (Hayes, 2012), we explored the moderating effects of plasma IL-1β (moderator) following the pain challenge on the magnitude of μ-opioid system activation (independent variable) with MPQ Pain Scores as the outcome variable, accounting for the contribution of BMI and baseline IL-1 cytokine concentrations.

RESULTS

Volunteer demographic and anthropometric data and baseline measures are shown in Table 1. No sex differences were observed for age, BMI, or neuroticism (two-tailed

Table 1 Volunteer Demographic, Psychophysical, and Anthropometric Information and Baseline Measures

Measures ($n=34$) **Females = 22** **Males = 12**	**Baseline (Mean +/− SD)**			
	Total ($n=34$)	**Females ($n=22$)**	**Males ($n=12$)**	**T-Test**
Age (years)	22 ± 11	21 ± 13	24 ± 8	$T=-0.8$; $p=0.4$
Body Mass Index (Kg/m^2)	25 ± 4	24 ± 4	26 ± 4	$T=-1.3$; $p=0.2$
Neuroticism Factor	84 ± 4	84 ± 4	83 ± 5	$T=0.7$; $p=0.5$
Females ($n=22$)				
Cycle Day		8 ± 6 days		
Testosterone		56 ± 21 ng/dl		
Estrogen		104 ± 74 pg/ml		
Progesterone		3 ± 5 ng/ml		
Males ($n=12$)				
Testosterone		617 ± 297 ng/dl		

Table 1 provides demographic, psychophysical, and anthropometric information on our study volunteers. Measures of age, weight, height, and NEO PI-R were obtained at study entry for all volunteers. Body Mass Index (BMI) was calculated using the ratio (weight in kg)/(height in m^2). N-Factor scores were calculated as described in the NEO PI-R manual. Results for all measures are reported as mean +/− standard deviation. Independent sample T-testing showed that no significant sex difference was found with regards to either age, BMI, or NEO-PI neuroticism ($p>0.05$ for each). Also described in the table are sex hormone levels for all study volunteers.

t-tests, $p>0.05$). Psychophysical and inflammatory data during control and pain challenges are described in Table 2.

Baseline Measures: Effects of Sex and Neuroticism on Baseline IL-1β and IL-1ra

At baseline, females showed higher IL-1β ($F_{1,30}=32.2$, $p<0.001$) and IL-1ra ($F_{1,30}=14.4$, $p=0.001$) concentration compared with males (Figure 1a). Volunteers with high neuroticism had lower baseline IL-1β ($F_{1,30}=6.1$, $p=0.02$) than those with low neuroticism, but effects of neuroticism on baseline IL-1ra were not significant ($F_{1,30}=2.5$, $p=0.13$) (Figure 1b). Significant sex by neuroticism interactions were noted for baseline IL-1β ($F_{1,30}=6.1$, $p=0.02$), but not IL-1ra ($F_{1,30}=1.2$, $p=0.28$). Low neuroticism scores were associated with greater baseline plasma IL-1β in females, but not in males (Figure 1c).

Baseline Measures: Relationships between IL-1 Family Cytokines at Baseline and Pain Sensitivity

Results from hierarchical linear regression were: *Step 1:* Without considering effects of sex and neuroticism, the model significantly predicted pain sensitivity (VAS/ml) with $\Delta R^2=0.26$, $\Delta F_{2,31}=5.4$, $p=0.01$ and acceptable collinearity (Tolerance = 0.4; VIF = 2.7). Both baseline IL-1β ($\beta=0.62$, $T=2.5$, $p=0.02$) and IL-1ra ($\beta=-0.83$, $T=-3.3$, $p=0.003$) were associated with sustained pain sensitivity during the challenge, but with opposing effects (ie, higher concentration of IL-1β was associated with greater pain sensitivity; higher concentration of IL-1ra was associated with less pain sensitivity). Sex and neuroticism were included in *Step 2:* IL-1β ($\beta=0.62$, $T=2.2$, $p=0.01$) and IL-1ra ($\beta=-0.83$, $T=-3.2$, $p=0.004$) predicted pain sensitivity, but sex and neuroticism did not have a significant effect on this model ($p>0.10$ for each).

Baseline Measures: Relationships between IL-1β, IL-1ra, and Baseline μ-Opioid Receptor Availability

IL-1β was negatively associated with baseline μ-opioid receptor BP_{ND} exclusively in the amygdala bilaterally (Right: $Z_{25}=-4.7$, $p<0.001$, $r=-0.44$ for extracted data; Left: $Z_{25}=-4.8$, $p<0.001$, $r=-0.36$ for extracted data) (Table 3, Figure 2a). No associations were identified between baseline IL-1ra and μ-opioid receptor availability. Pain sensitivity correlated with baseline μ-opioid receptor availability bilaterally at trend, non-statistically significant levels (Right: $r=-0.29$, $p=0.08$; Left: $r=-0.29$, $p=0.09$).

Sustained Pain Challenge: Effects of Sex and Neuroticism on Changes in IL-1β and IL-1ra During the Pain Stressor

Overall, the pain challenge had a nearly significant effect on IL-1β plasma levels ($F_{1,30}=3.7$, $p=0.06$; mean $\log_{10}$ IL-1β reduction, 0.15), but not on IL-1ra ($p>0.10$). Interactions between challenge condition (pain/control) and sex had nearly significant effects on IL-1β ($F_{1,30}=3.7$, $p=0.06$) but not on IL-1ra ($p>0.10$). Pain induced an IL-1β increase in females (mean $\log_{10}$ IL-1β increase of 0.30) but no noticeable IL-1β changes (mean $\log_{10}$ IL-1β increase of 0.002) in males (Figure 3a). Significant effects of neuroticism on pain associated cytokine changes were observed for IL-1β ($F_{1,30}=9.6$, $p=0.004$) but not IL-1ra ($p>0.10$). Pain induced an increase in IL-1β (mean $\log_{10}$ IL-1β increase of 0.10) in volunteers with high neuroticism and an IL-1β reduction (mean $\log_{10}$ IL-1β reduction of 0.40) in those with low neuroticism (Figure 3b). Three-way interactions

Table 2 Psychophysical and Inflammatory Response to Pain

Measures ($n=34$) **Females = 22** **Males = 12**	**Control condition (Mean +/− SD)**	**Following pain challenge (Mean +/− SD)**	**Paired t-test**
IL-1β (Log_{10})	1.5 ± 0.4	1.5 ± 0.5	T = 1.0; $p=0.34$
IL-1ra (Log_{10})	2.1 ± 0.5	2.2 ± 0.6	T = 0.8; $p=0.40$
Negative Affect (PANAS)	2.3 ± 2.8	2.4 ± 4.8	T = 0.1; $p=0.91$
Positive Affect (PANAS)	9.1 ± 6.5	9.4 ± 5.6	T = 0.6; $p=0.55$

Measures ($n=34$)	**Results from Pain Challenge (Mean +/− SD)**
VAS Average (0–20 min)	28 ± 13
Volume Infused (ml)	2.9 ± 1.2
(VAS Average)/(Volume Infused)	1.5 ± 1.2
Pain Intensity (VAS)	40 ± 14
MPQ Sensory (1–10)	15 ± 7
MPQ Affect (11 − 16 + 20)	5.3 ± 4.7
MPQ Total (1–20)	24 ± 12

Table 2 provides information regarding measures assessed during the study and includes data obtained on IL-1 family cytokines, measures of affective state (PANAS; both Positive and Negative Affect scores), as well as measures of both pain sensitivity (VAS average/volume of pain stimuli infused) and the pain experience (eg, MPQ Sensory, Affect, and Total Pain scores). Cytokine concentrations are reported in their Log10 normalized format. Raw cytokine concentrations were detected via standard ELISA techniques in units of pg/dl. Independent sample T-testing showed that when looking at the entire study sample, without accounting for factors associated with inflammatory and pain variance, the pain challenge did not induce a significant change in either our IL-1 family cytokines measures or our measures of affective state (eg, PANAS). The McGill Pain Questionnaire (MPQ) was obtained following the pain induction. Results for all measures are reported as mean +/− standard deviation.

between neuroticism, sex, and challenge condition (pain/control) were observed for IL-1β ($F_{1,30}=9.5$, $p=0.004$) but not IL-1ra ($p>0.05$). No change in IL-1β was observed in males (irrespective of neuroticism group), but pain induced an increase in IL-1β (mean $\log_{10}$ IL-1β increase of 0.19) in females with higher neuroticism, and induced a reduction in IL-1β (mean $\log_{10}$ IL-1β reduction of 0.80) in females with lower neuroticism (Figure 3c).

Sustained Pain Challenge: Relationships between IL-1 Family Cytokines During the Pain Challenge and Pain Sensitivity

Results from the hierarchical linear regression were: *Step 1:* Without considering sex and neuroticism, the model was significant with $\Delta R^2=0.19$, $\Delta F_{2,31}=3.7$, $p=0.04$ and acceptable collinearity (Tolerance = 0.5; VIF = 1.8). The impact of pain on both IL-1β ($\beta=-0.56$, $T=-12.6$, $p=0.01$) and IL-1ra ($\beta=0.52$, $T=2.4$, $p=0.02$) significantly predicted pain sensitivity, but with opposing effects for IL-1β and IL-1ra. Including sex and neuroticism in the model in *Step 2* rendered the model non-significant ($\Delta R^2<0.01$, $\Delta F_{2,29}<0.01$, $p=0.99$

Sustained Pain Challenge: Relationships between IL-1β, IL-1ra, and Pain-induced μ-Opioid System Activation

No significant relationships between endogenous opioid system activation (measured as reductions in BP_{ND} during the challenge, compared to the control condition) and changes in IL-1β concentrations were observed. Conversely, challenge-induced changes in IL-1ra were associated with μ-opioid system activation bilaterally in the dorsomedial nucleus accumbens/ventral caudate (Right: $Z_{25}=5.5$, $p<0.001$; Left: $Z_{25}=5.4$, $p<0.001$) (Table 3, Figure 2b).

Moderating Effects of Pain-induced Changes in IL-1β on Endogenous Opioid Analgesia following the Pain Challenge

Results from moderation analyses showed that changes in IL-1β associated with the pain challenge moderated the relationship between challenge-induced endogenous opioid system activation in the left dorsomedial nucleus accumbens/ventral caudate region and the subjective experience of pain as measured by MPQ Pain Sensory, and MPQ pain Affect scores following the pain challenge (Figure 4). *Model 1:* the model was significant ($\Delta R^2=0.23$, $F_{6,26}=2.8$, $p=0.03$), whereby pain induced changes in IL-1β moderated the relationship between endogenous opioid system activation in the dorsomedial nucleus accumbens/ventral caudate and reductions in MPQ Sensory scores ($B=-36.3$, $T=-3.6$, $p=0.001$) (Figure 4a). *Model 2:* the model was significant ($\Delta R^2=0.20$, $F_{6,26}=5.8$, $p<0.001$) whereby pain-associated changes in IL-1β moderated the relationship between endogenous opioid system activation in the dorsomedial nucleus accumbens/ventral caudate and reductions in MPQ Affect scores ($B=-18.2$, $T=-5.0$, $p<0.0001$) (Figure 4b). Two additional models were tested to determine the moderating effect of IL-1β on the

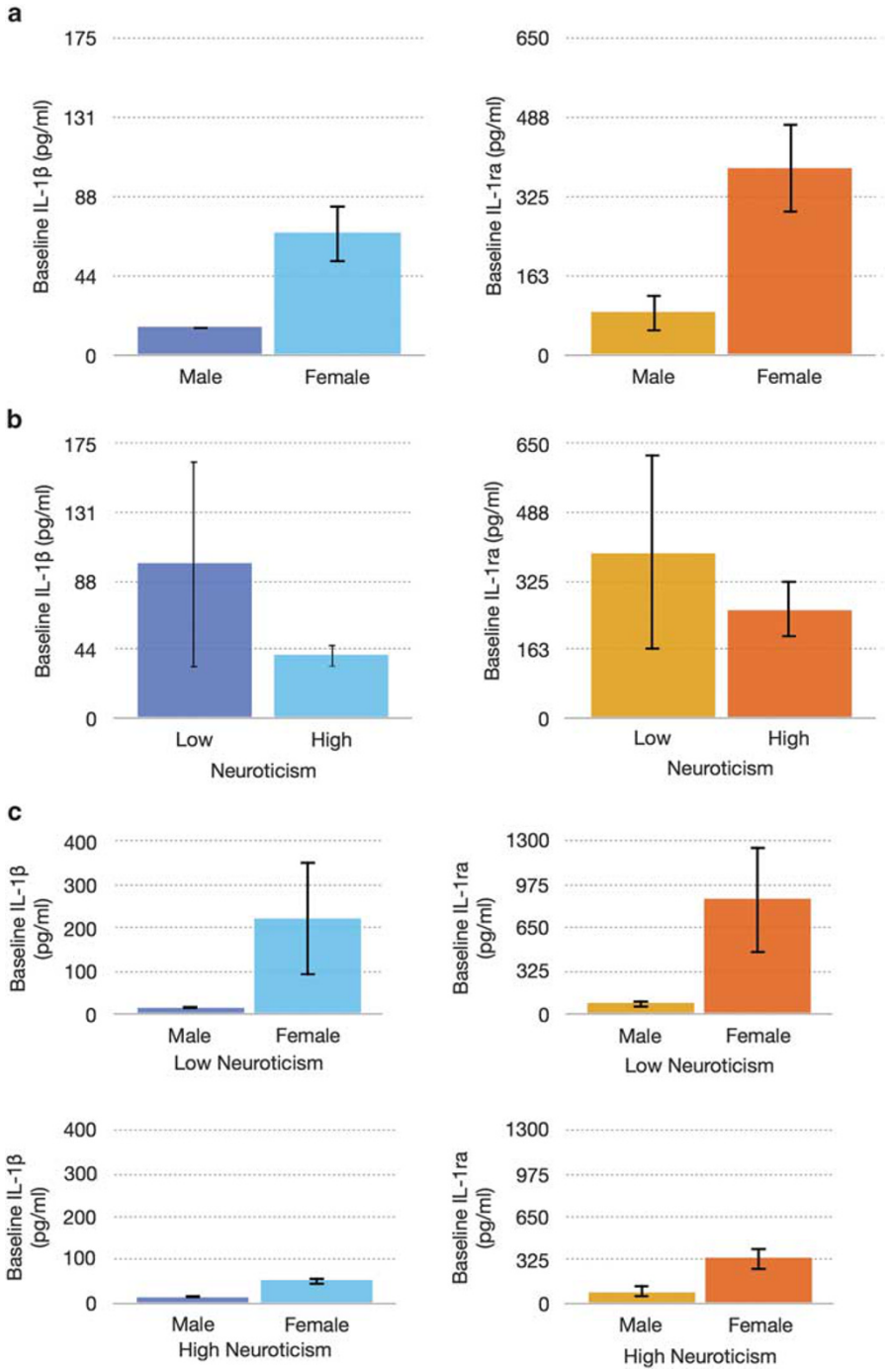

Figure 1 Impact of sex and neuroticism on baseline plasma IL-1β and IL-1ra. Many factors are believed to be associated with variability in plasma concentration of inflammatory cytokines. Here, we present graphical comparisons of plasma concentration of IL-1β (depicted here in blue) and IL-1ra (depicted here in Maize) across two biobehavioral factors, sex, and neuroticism. For clarity in presentation, here we present the raw cytokine concentrations. (a) Graphs here illustrate the effect of sex (eg, male *vs* female) (X-axes) on baseline plasma IL-1β (depicted here in blue) and IL-1ra (depicted here in Maize) (Y-axes) in our sample. (b) Graphs here illustrate the effect of neuroticism (eg, lower: $<$mean - 1SD; higher: $>$ mean - 1SD) (X-axes) on baseline plasma IL-1β (depicted here in blue) and IL-1ra (depicted here in Maize) (Y-axes) in our sample. (c) Graphs here illustrate the sex by neuroticism interaction effect on baseline plasma IL-1β (depicted here in blue) and IL-1ra (depicted here in Maize) (Y-axes) in our sample. Sex is shown on the X-axes and neuroticism is depicted on the right side of the graphs. Significant sex by neuroticism interaction effects on IL-1β were detected in our sample. The sex by neuroticism interaction significantly impacted baseline IL-1ra.

relationship between endogenous opioid system activation in the right dorsomedial nucleus accumbens/ventral caudate and the extent of pain experienced. One of these models related to MPQ sensory pain and one model related to MPQ affective pain. The results from each of these additional analyses were found non-significant ($p > 0.05$).

Table 3 Pain-induced Changes in Peripheral IL-1β and IL-1ra is Associated with Concurrent Pain-induced Central Regional μ-Opioid Receptor Activation

Cytokine	Brain Regional μ-OR BP_{ND}	MNI Coordinates (x, y, z)	Statistics
Control Condition			
IL-1β	Right Amygdala	−24, −2, −21	Size = 319; $Z_{25} = -4.7$, $p < 0.001$
	Left Amygdala	21, −1, −23	Size = 429; $Z_{25} = -4.8$, $p < 0.001$
IL-1ra	None	None	None
Cytokine Change	**Brain Regional μ-OR Activation**	**MNI Coordinates (x, y, z)**	**Statistics**
Response to Pain Challenge			
IL-1β	None	None	None
IL-1ra	Right VP/NAcc	−12, 13, 1	Size = 579; $Z_{25} = 5.5$, $p < 0.001$
	Left VP/NAcc	7, 9 −1	Size = 844; $Z_{25} = 5.4$, $p < 0.001$

Table 3 outlines the results of correlational analyses between IL-1 family cytokines and measures of μ-opioid receptor (MOR) availability (and pain-induced MOR activation). MOR availability is reported in units of BP_{ND} (eg, non-displaceable binding potential at central μ-opioid receptors). Pain-induced μ-opioid receptor activation is defined as the pain-induced reduction in BP_{ND}. MNI: Montreal Neurological institute. Here, we identify brain regions by name, associated MNI coordinates, as well as the size and statistics of the brain regions where MOR measures correlate with measures of peripheral IL-1 family cytokines.

DISCUSSION

This study examined IL-1*β* and IL-1ra plasma levels at baseline and during a sustained pain challenge, the latter a model of physical and emotional stress, including sex and neuroticism as potential covariates, and their relationship with functional measures of a neurotransmitter system, the endogenous opioid and μ-opioid receptors, involved in pain and stress regulation (Zubieta *et al*, 2002), known to be influenced by peripheral inflammatory changes in animal models (Day and Akil, 1999a). We observed significant relationships between peripheral IL-1 family cytokines and baseline μ-opioid receptor availability *in vivo,* as well as pain-activated central endogenous opioid neurotransmission, one of the principal anti-nociceptive mechanisms, and which is implicated both in regulating the pain experience and stress responses (Zubieta *et al*, 2001) and sex differences in these phenomena (Zubieta *et al*, 2002).

At baseline, female sex was associated with higher plasma concentrations of IL-1*β* and IL-1ra, whereas neuroticism was linked to lower levels of IL-1*β*. These inflammatory relationships with both female sex and neuroticism, factors generally attributed to increased risk for stress-induced emotional reactivity, negative affective states (Renner *et al*, 2013), and higher pain sensitivity (Pauli *et al*, 1999), suggest that activation of inflammatory pathways involving IL-1 family cytokines may contribute to biological risk for such pathologies. Further, lower μ-opioid receptor availability has been associated with higher clinical pain in FM (Harris *et al*, 2007) and with elevated cortisol, ACTH, and treatment refractoriness in Major Depression (Kennedy *et al*, 2006). In our data, higher plasma IL-1*β* was associated with lower μ-opioid receptor availability in the amygdala, a region where μ-opioid receptors are implicated in the regulation of pain (Zubieta *et al*, 2002) and affective states (Liberzon *et al*, 2002; Prossin *et al*, 2011). Day and colleagues(Day *et al*, 1999b) reported similar findings in animal models wherein peripheral IL-1*β* administration selectively increased enkephalin expression and activated enkephalinergic cells within the central nucleus of the amygdala. Additional work also suggests that activation of extracellular signal-regulated kinases within the central nucleus of the amygdala can also modulate peripheral, inflammatory pain through descending pathways (Neugebauer *et al*, 2004; Carrasquillo and Gereau, 2007) suggesting a potential mechanism for bi-directional relationships between central systems and peripheral inflammatory responses. Additionally, contrary to IL-1*β*, IL-1ra can penetrate the blood brain barrier (Cawthorne *et al*, 2011), and therefore the IL-1*β*–central opioid relationships observed could also be modulated by IL-1ra, a potential effect that could be explored with larger sample sizes. Exactly why lower μ-opioid receptor availability in the amygdala was only marginally associated with pain sensitivity is unclear. However, our findings from the pain challenge imply that pain sensitivity early in the pain challenge is more dependent on the extent of concurrent activation of central μ-opioid receptors in response to pain rather than baseline endogenous μ-opioid receptor availability. Findings in chronic pain patients are expectedly different, with lower baseline endogenous μ-opioid receptor availability (within specific brain regions involved in regulating response to pain). In these individuals, the chronicity of pain likely serves to reduce central μ-opioid receptor availability through either increased baseline endogenous opioid tone, or μ-opioid receptor downregulation, both reducing effective analgesic capacity through their regionally specific effects.

During the pain stressor, an interaction between female sex and neuroticism was observed, whereby increases in IL-1*β* were observed in females in the high neuroticism group, but reductions in the female low neuroticism sample. In the neuroimaging data, activation of μ-opioid receptor mediated neurotransmission by the pain stressor was positively associated with plasma IL-1ra, but not IL-1*β* in the dorsomedial nucleus accumbens, a brain region implicated in hedonic, social, and affiliative behavior (Resendez *et al*, 2013). The inflammatory sex differences identified are consistent with previous findings in animal

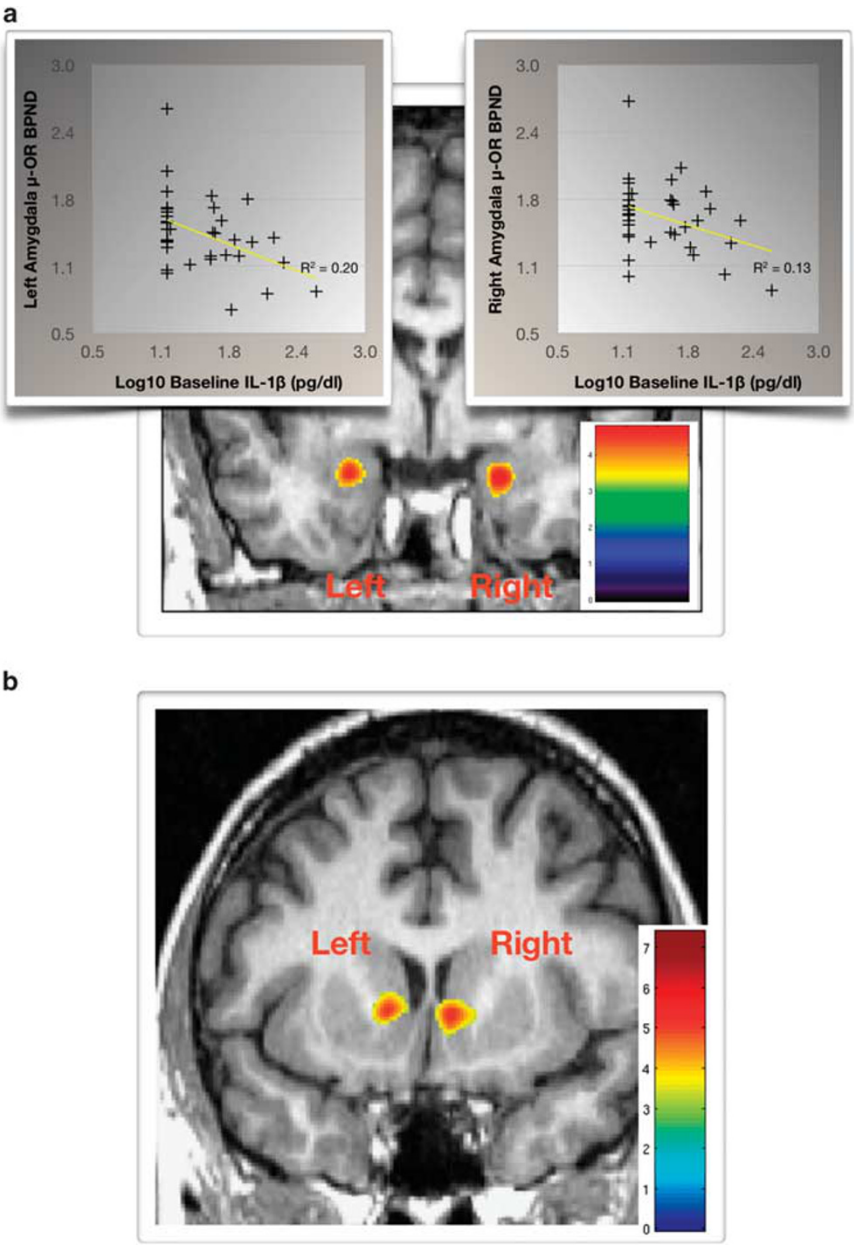

Figure 2 Correlations between concentrations of plasma IL-1 family cytokines and measures of central μ-opioid neurotransmission during a pain challenge. Z scores of statistical significance are represented by a pseudo color scale (inset) and are superimposed over an anatomically standardized magnetic resonance image in a coronal view. Image data are displayed according to standard radiological convention so that the left side of the image corresponds to the right side of the brain. (a) Shows the relationship between amygdala μ-opioid receptor availability at baseline and plasma IL-1β, on a voxel-by-voxel basis and on the extracted data from those regions (Right: $r = -0.44$, $p = 0.004$; Left: $r = -0.36$, $p = 0.015$). (b) Shows significant linear relationships between pain-induced activation of μ-opioid neurotransmission and plasma IL-1ra concentrations in the dorsomedial NAcc/Ventral caudate (Right: $Z_{25} = 5.5$, $p < 0.001$; Left: $Z_{25} = 5.4$, $p < 0.001$).

models where sex differences in immune functioning have been reported (see review by Berkley and Zalcman) (Berkley *et al*, 2006). Here, we show that the pain challenge increased IL-1β but not IL-1ra (ie, increase in IL-1ra not statistically significant) in females. In the low neuroticism group, we observed higher IL-1β at baseline and a reduction in IL-1β following the sustained pain challenge. In contrast, high neuroticism, a trait often associated with risk for stress-induced pathological states, including idiopathic forms of pain (Angst and Clayton, 1986; Pauli *et al*, 1999; Zubieta *et al*, 2002; Paine *et al*, 2009; Vassend *et al*, 2013), was associated with lower baseline IL-1β but sustained pain-induced increases in that proinflammatory cytokine. Both sex and neuroticism accounted for substantial inter-individual inflammatory heterogeneity within healthy volunteers both at baseline and following the sustained pain stressor.

Isolated elevations in IL-1β, in relation to IL-1ra have been shown in animal models to precipitate a state of hyperalgesia (Raghavendra *et al*, 2002; Shavit *et al*, 2005). Here, we identified negative linear relationships between

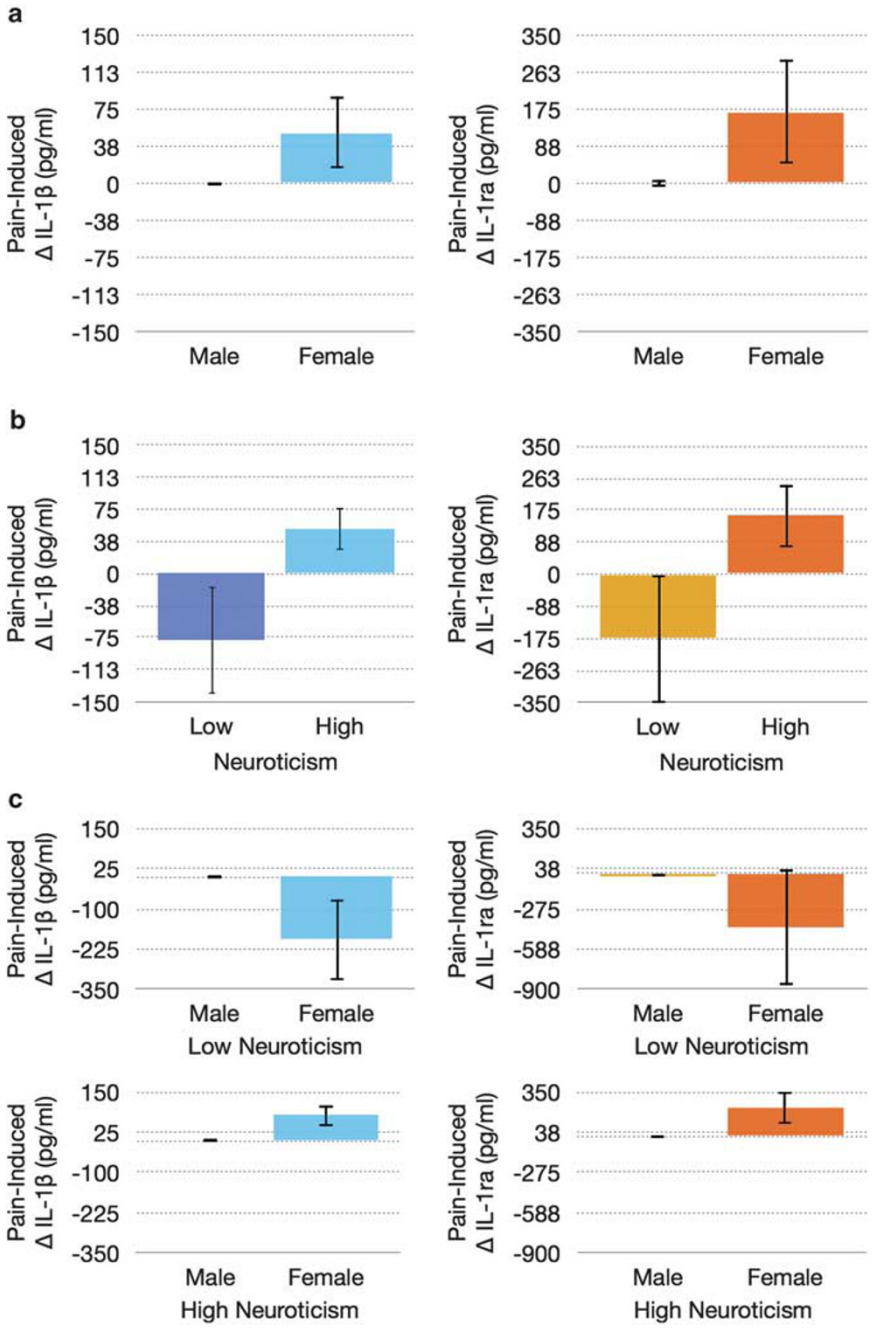

Figure 3 Impact of neuroticism and sex on pain induction of plasma IL-1β and IL-1ra. Many bio-behavioral factors are believed to be associated with variability in the pain experience. Here, we provide graphical illustrations of two such factors (eg, sex, neuroticism) and their impact on pain-induced changes in plasma concentration of two inflammatory cytokines, IL-1β and IL-1ra, implicated for their involvement in the pain experience. The Y-axes of the graphs represent the pain-induced change in cytokine concentrations; IL-1β (blue bar graphs) and IL-1ra (maize bar graphs). Positive values represent a pain-induced increase (and negative values a pain-induced decrease) in the particular cytokine concentration. The effect of sex is depicted in (a), the effect of neuroticism is depicted in (b), and their interaction (eg, sex by neuroticism) is depicted with sex on the X-axes and neuroticism on the right-side axes in (c). (a) Here, we illustrate the effect of sex (X-axes) on both the pain-induced change in IL-1β (Y-axis, blue bars) and the pain-induced change in IL-1ra (Y-axis, maize bars). (b) Here, we illustrate the effect of neuroticism (X-axes) on both the pain-induced change in IL-1β (Y-axis, blue bars) and the pain-induced change in IL-1ra (Y-axis, maize bars). (c) Graphs here illustrate the sex by neuroticism interaction effect on both the pain-induced change in IL-1β (Y-axis, blue bars) and the pain-induced change in IL-1ra (Y-axis, maize bars). Sex is shown on the X-axes and neuroticism is depicted on the right side of the graphs.

IL-1β and μ-opioid receptor availability, hence reducing the functional capacity of this endogenous anti-nociceptive mechanism. Baseline IL-1β may therefore represent a marker of risk for the development of persistent pain states and other stress-associated pathologies, reducing μ-opioid receptor availability and therefore the capacity to engage this neurotransmitter system to regulate pain and other forms of stress.

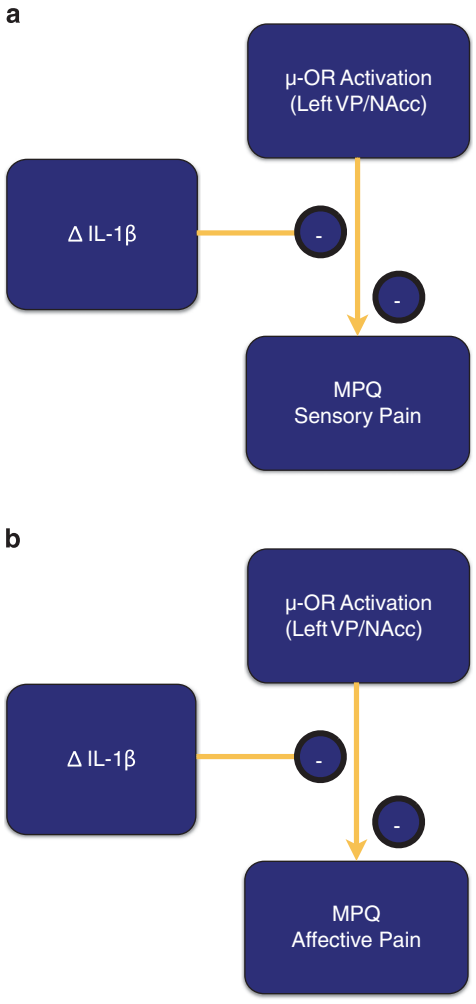

Figure 4 Moderating effect of the pain induced change in IL-1β on the relationship between endogenous opioid activation in the left dorsomedial nucleus accumbens/ventral caudate and the extent of pain experienced in healthy volunteers during a standardized pain challenge. Two models are represented in this figure, one depicting ΔIL-1β moderating effects on endogenous opioid activation in the left dorsomedial nucleus accumbens/ventral caudate where MPQ Sensory Pain score is the outcome (a) and another where MPQ Affective Pain score is the outcome variable (b). Two additional models were tested to determine the moderating effect of ΔIL-1β on the relationship between endogenous opioid activation in the right dorsomedial nucleus accumbens/ventral caudate and the extent of pain experienced, one model with MPQ sensory pain as the dependent and one model with MPQ affective pain as the dependent variable. The results from each of these additional analyses were found non-significant ($p > 0.05$) and are not represented in the figures.

We did not observe significant effects of the sustained pain challenge on IL-1ra plasma levels. However, temporal delays in stress-induced changes of IL-1ra have previously been reported that may account for this lack of findings. In one such study, Rohleder and colleagues (Rohleder *et al*, 2006) observed a 60-min delay following induction of psychosocial stress before significant stress-induced elevation in plasma IL-1ra was detected. Sampling of IL-1ra at periods longer than the 45 min employed in this study may therefore help to clarify the effects of IL-1ra on pain measures and of the effects of the pain challenge on IL-1ra. However, such changes to the research paradigm would likely require an extension of the study time from 90 min to at least 120 min, posing other limitations related to the decay of short-lived radiotracers (^{11}C half-life = 20 min), or the use of two separate radiotracer administrations. In spite of these potential limitations, IL-1ra, with its IL-1β antagonizing capacity, was found associated with greater pain-induced endogenous opioid system responses in the dorsomedial nucleus accumbens, potentially reducing the impact of the stressor on hedonic and motivational functions modulated by this brain region.

Endogenous opioid neurotransmission has been previously been shown to integrate systemic IL-1β input to the CNS (central amygdala) (Day *et al*, 1999b), modulate CNS outflow to the peripheral immune system in response to stressful events (amygdala, hypothalamus) (Buller *et al*, 2005), and regulate behavioral expectation and reward in anticipation of future events (nucleus accumbens) (Zubieta and Stohler, 2009), as well as regulating emotional responses to pain and other stressors (Zubieta *et al*, 2002; Prossin *et al*, 2011), as part of ascending pain-responsive pathways (Gear and Levine, 1995). The opposing effects of IL-1β (associated here with lower baseline receptor availability) and IL-1ra (associated with increases in endogenous opioid release) are potentially consistent with animal models of IL-1ra-induced analgesia, opposing the hyperalgesic effect of IL-1β (Raghavendra *et al*, 2002; Shavit *et al*, 2005). The observed linear relationships between measures of regional μ-opioid receptor mediated neurotransmission and peripheral IL-1β (and IL-1ra), localized to brain regions in the mesolimbic reward and saliency response pathways could then be interpreted as participating in homeostatic responses to peripheral nociceptive stress as previously suggested in animal models (Gear and Levine, 1995; Gear *et al*, 1999). That IL-1β moderates the antinociceptive μ-opioid receptor activation in the dorsomedial nucleus accumbens, an area in which endogenous opioid system activation was proportional to IL-1ra, increasing sensory and affective pain report in our data, also provides preliminary evidence to explain the role of these interdependent neuroimmune interactions in the host response to pain stress. That this effect was lateralized to the left, the same side as the algesic stimulus, may implicate the modulatory sensory matrix, previously reported to lateralize to the side of the nociceptive challenge as opposed to contra-lateral thalamic projections (Goadsby, 2005).

The data reported in the present manuscript translate initial observations of IL-1 family cytokine–central opioid interactions in animal models into human samples. Both IL-1β and IL-1ra appear associated with the function of endogenous opioid antinociceptive and stress regulatory mechanisms and to a lesser extent with sustained pain sensitivity measures. Stress-induced elevation in IL-1β levels, by moderating pain-induced μ-opioid receptor activation in the nucleus accumbens, may represent a marker of vulnerability to persistent pain states. On the contrary, IL1-ra was associated with more efficient activation of potentially homeostatic endogenous opioid neurotransmission in the nucleus accumbens, regulating both responses to pain and motivational mechanisms. Future work in this area should consider the modulation of inflammatory cytokines as a potential mechanism to regulate

dysfunctional central neurotransmission in pathological states, opening new therapeutic avenues for a number of disease processes, such as idiopathic pain syndromes, mood and substance use disorders, where endogenous opioid neurotransmission is implicated.

FUNDING AND DISCLOSURE

Dr Koch has recently been employed by Eli Lilly companies and has consulted for Metastatix, Pennside Partners, Cypress Bioscience, Takeda Pharmaceuticals, NiCox SA, Celtaxsys, Gerson Lehrman Group of Healthcare and Biomedical Advisors, Guidepoint Global, UCB Pharmaceuticals, and the Fund for Autoimmune Research and has been an expert for Kirkland and Ellis. Dr Koch has received investigator-initiated research grants for Bristol-Myers Squibb, Roche, and Takeda Pharmaceuticals. All other authors report no biomedical financial or potential conflicts of interest. K99 DA 033454 (ARP); University of Michigan Comprehensive Depression Center Rachel Upjohn Clinical Scholars Award (ARP); R01 DA 022520, R01 DA 027492 and the Phil F. Jenkins Foundation (JKZ).

ACKNOWLEDGEMENTS

Virginia Murphy-Weinberg, Tiffany Love, Heng Wang, Marta Pecina, MBNI staff, Jill Rothley, Edward McKenna, Andrew Weeden, Paul Kison, Shayna Huber. Presented in part at the annual meeting of the American College of Neuropsychopharmacology, (2010, Miami, FL) and the Psychoneuroimmunology Research Society (2010, Dublin, Ireland) (ARP).

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