



ARTICLE

Lowered endogenous mu-opioid receptor availability in subclinical depression and anxiety

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Major depressive disorder is associated with lowered mood, anxiety, anhedonia, sleep problems, and cognitive impairments. Many of these functions are regulated by μ -opioid receptor (MOR) system. Preclinical, in vivo, and post-mortem studies have however yielded inconclusive results regarding the role of the MOR in depression and anxiety. Moreover, it is not known whether alterations in MOR are already present in subclinical depression and anxiety. In a large-scale retrospective cross-sectional study we pooled data from 135 (113 males and 22 females) healthy subjects whose brain's MOR availability was measured with positron emission tomography (PET) using an agonist radioligand [¹¹C]carfentanil that has high affinity for MORs. Depressive and anxious symptomatology was addressed with BDI-II and STAI-X questionnaires, respectively. Both anxiety and depression scores in the subclinical range were negatively associated with MOR availability in cortical and subcortical areas, notably in amygdala, hippocampus, ventral striatum, and orbitofrontal and cingulate cortices. We conclude that dysregulated MOR availability is involved in altered mood and pathophysiology of depression and anxiety disorders.

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INTRODUCTION

Major depressive disorder (MDD) is associated with lowered mood, diminished hedonia, sleep problems, and executive function impairments. Many of these functions are centrally regulated by opioidergic neurotransmission [1–3]. MDD has high comorbidity with anxiety disorders [4], and several studies also point towards the role of opioid system in modulating anxiety [5–7]. Among opioid receptor (OR) classes (μ , δ , and κ receptors), the μ -opioid receptors (MORs) mediate the effects of endogenous β -endorphins, endomorphins, enkephalins, and various exogenous opioid agonists [8]. Preclinical studies suggest that MOR agonists have both antidepressant and anxiolytic effects [3]. MOR activation with buprenorphine has antidepressant-like effects which are mediated via μ -receptor partial agonism and κ -receptor antagonism [9, 10]. Clinical studies also suggest that low-dose buprenorphine treatment might be effective for depression (e.g. [11–13]), and low-dose buprenorphine therapy also leads to significant reduction in suicidality in acutely suicidal patients [14]. Uncontrolled cohort studies have further found that morphine administration following an acute trauma decreases the likelihood of subsequent post-traumatic stress disorder (PTSD), presumably via inhibition of fear conditioning following the traumatic event thus suggesting anxiolytic action of the MOR system [15–17].

In vivo imaging and post-mortem studies have yielded mixed evidence on the role of MOR in depression and anxiety. One positron emission tomography (PET) imaging study found that experimentally induced sadness decreases opioidergic neurotransmission in rostral anterior cingulate cortex (ACC) in healthy volunteers [18]. However, a more recent study reported opposite

findings, with negative emotions induced by social rejection increasing opioidergic processing in amygdala and thalamus [19]. One clinical study also found increased MOR availability in the thalamus in major depression [20]; this finding was however not replicated in a subsequent study [21]. Personality trait harm avoidance (which is also a risk factor for developing affective disorders) has also been reported to correlate positively with cerebral MOR availability [22]. In PTSD, μ -receptors are upregulated in the OFC and downregulated in the amygdala and ACC [23]. Data from post-mortem studies with suicide victims are mixed, with some reporting higher OR binding [24, 25], some lower binding [26], and some no differences [27] compared with controls subjects.

The current study

Altogether preclinical experiments and human pharmacological and clinical studies point towards depression and anxiety related alterations in the MOR system, whereas human in vivo imaging and post-mortem studies have yielded mixed results, possibly due to compromised statistical power. Furthermore, it is known that subthreshold depressive and anxious symptomatology predict future onset of mood disorders [28], yet the contribution of MOR to depressive or anxious symptoms that occur prior to the first onset of a full depressive/anxious episode remain unknown. Here we tested for the association between subclinical depression and anxiety, as measured with standardized self-reports, and MOR availability in the brain in a large cross-sectional register-based study. We compiled data from 135 individuals scanned with radioligand [¹¹C]carfentanil who had also completed Beck

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Depression Inventory II (BDI-II; [29]) and trait anxiety scale from the state-trait anxiety inventory ((STAI-X; [30])). We show that worse subclinical symptoms of depression (i.e. BDI-II scores in the range of 0–12) and anxiety are associated with lowered MOR availability, most clearly in the brain circuits governing mood and emotion regulation.

MATERIALS AND METHODS

Subjects

The sample consisted of 135 historical, healthy individuals (22 females, 113 males, mean age 29, *SD* age 10.35 years; Tables 1 and S-1) studied with high-affinity agonist radioligand [¹¹C]carfentanil [31], retrieved from the AIVO (<http://aivo.utu.fi>) database of in vivo PET images hosted at the Turku PET Centre. All subjects signed informed consent forms. Data were pooled from four scanners (GE Healthcare Discovery: 23 subjects, Siemens ECAT Siemens High Resolution Research Tool (HRRRT) scans: 17 subjects, GE Healthcare Discovery 690 PET/CT scanner: 43 subjects and Philips Ingenuity PET/MR scanner: 52 subjects). All subjects had given informed consent and completed the BDI-II questionnaires as a part of the corresponding experimental protocol; subsample of 105 subjects (84 males, 21 females) had also completed the STAI-X form. Four subjects with BDI-II scores exceeding the clinical cut-off (12 points) were excluded from the sample. Because the STAI-X is not a clinical screening instrument, no cut-off could be specified; however none of the subjects had clinically diagnosed anxiety disorders. No subjects had neurologic or psychiatric diseases or currently abused alcohol or illicit drugs; subjects with medications affecting the CNS were also excluded. Seven female subjects smoked. The data were acquired between 2003 and 2018. The study protocol was approved by the Turku University Hospital Clinical Research Services Board, and the study was conducted in accordance with the declaration of Helsinki. Power analysis on prior molecular imaging studies on personality and [¹¹C]carfentanil binding [22, 32, 33] suggested that an expected effect size of $r = 0.4$, a sample size of 71 subjects would be sufficient for establishing the predicted effects at power of 0.95.

PET and MR image preprocessing

PET images were preprocessed using the automated PET data processing pipeline Magia [34, 35] (<https://github.com/tkjarjal/magia>) running on MATLAB (The MathWorks, Inc., Natick, MA, United States). Radiotracer binding was quantified using non-displaceable binding potential (BP_{ND}), which is the ratio of specific binding to non-displaceable binding in the tissue [36]. This outcome measure is not confounded by differences in peripheral distribution or radiotracer metabolism. BP_{ND} is traditionally interpreted by target molecule density (B_{max}), even though [¹¹C]carfentanil is also sensitive to endogenous neurotransmitter activation [37]. Accordingly, the BP_{ND} for the tracer should be interpreted as density of the receptors unoccupied by

endogenous ligands, i.e. receptor availability. Binding potential was calculated by applying basis function method [38] for each voxel using the simplified reference tissue model [39], with occipital cortex serving as the reference region [40]. The parametric images were spatially normalized to MNI-space via segmentation and normalization of T1-weighted anatomical images, and finally smoothed with an 8-mm FWHM Gaussian kernel.

Previous studies have established that depression [41] and subclinical depressive symptoms are linked with compromised cerebral integrity [42]. Cerebral integrity in turn is associated with neuroreceptor-specific radiotracer uptake [43–45]. To rule out the contribution of cerebral atrophy in our results, we performed a complementary voxel-based morphometry (VBM) analysis of the T1 images of the subjects to control for the effect of grey matter density on radiotracer uptake. VBM was done with SPM12 (<https://www.fil.ion.ucl.ac.uk/spm/software/spm12/>) which enables automated spatial normalization, tissue classification, and radio-frequency bias correction to be combined with the segmentation step. Cut-off of spatial normalization was 25 mm and medium affine regularization 0.01 was used. Following normalization and segmentation into GM and WM, a modulation step was incorporated to take into account volume changes caused by spatial normalization and to correct for the differences in total brain size across subjects. Finally, the segmented, normalized, and modulated GM images were smoothed using 8-mm FWHM Gaussian kernel. Regional GM densities were extracted and used to predict [¹¹C]carfentanil BP_{ND} alongside with the BDI-II and STAI-X scores.

Data analysis

The population-level full-volume statistical analysis was done using SPM12. The normalized and smoothed BP_{ND} images were entered into general linear model, where BP_{ND} was predicted with depression and anxiety scores, respectively, while sex, age, and PET scanner were entered into the models as nuisance covariates. Statistical threshold was set at $p < 0.05$, FDR corrected at cluster level. In a complementary methodological approach, the data were analyzed by averaging voxelwise BP_{ND} within regions of interest (ROIs). Atlas-based ROIs were generated in the MOR-rich regions in the brain (amygdala, hippocampus, ventral striatum, dorsal caudate, thalamus, insula, orbitofrontal cortex (OFC), ACC, middle cingulate cortex, and posterior cingulate cortex using AAL [46] and Anatomy [47] toolboxes). The ROI data were analyzed with R statistical software (<https://cran.r-project.org>). Mean regional [¹¹C]carfentanil BP_{ND} and GM densities from VBM were extracted for each region.

Regional effects were estimated using Bayesian hierarchical modelling using the R package BRMS [48] that uses the efficient Markov chain Monte Carlo sampling tools of RStan (<https://mc-stan.org/users/interfaces/rstan>). We first fitted the models separately for BDI-II scores (135 subjects) and STAI-X (105 subjects) scores. Finally, we also fitted a model with both BDI-II and STAI-X scores (105 subjects) to test the unique contribution of depression and anxiety on MOR availability. We used weakly informative priors: For intercepts, we used the default of BRMS, i.e. Student's *t*-distribution with scale 3 and 10 degrees of freedom. For predictors, a Gaussian distribution with standard deviation of 1 was used to provide weak regularization. The BRMS default prior half Student's *t*-distribution with 3 degrees of freedom was used for standard deviations of group-level effects; BRMS automatically selects the scale parameter to improve convergence and sampling efficiency. The BRMS default prior LKJ [1] was used for correlations of group-level random effects. The ROI-level models were estimated using ten chains, each of which had 1000 warmup samples and 2000 post-warmup samples, thus totalling 20,000 post-warmup samples. The sampling parameters were slightly modified to facilitate convergence (*adapt_delta* = 0.999;

Table 1. Subject characteristics.

	Males (<i>n</i> = 113)	Females (<i>n</i> = 22)
Age	25.23 (6.23)	45.50 (10.70)
BDI-II score	3.01 (2.78)	3.27 (3.13)
STAI-X score	33.57 (7.36)	32.52 (5.16)
Siemens High Resolution Research Tool scans	8	9
GE Healthcare Discovery 690 scans	10	13
GE Discovery VCT PET/CT scanner	43	0
Philips Ingenuity PET/MR scans	52	0

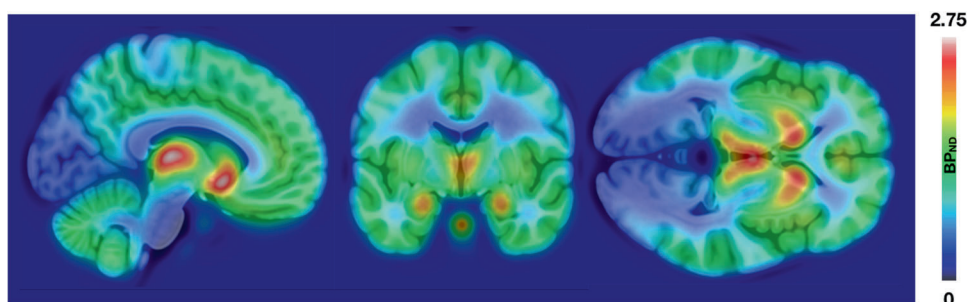


Fig. 1 Mean [^{11}C]-carfentanil BP_{ND} distribution in the sample ($n = 135$). .

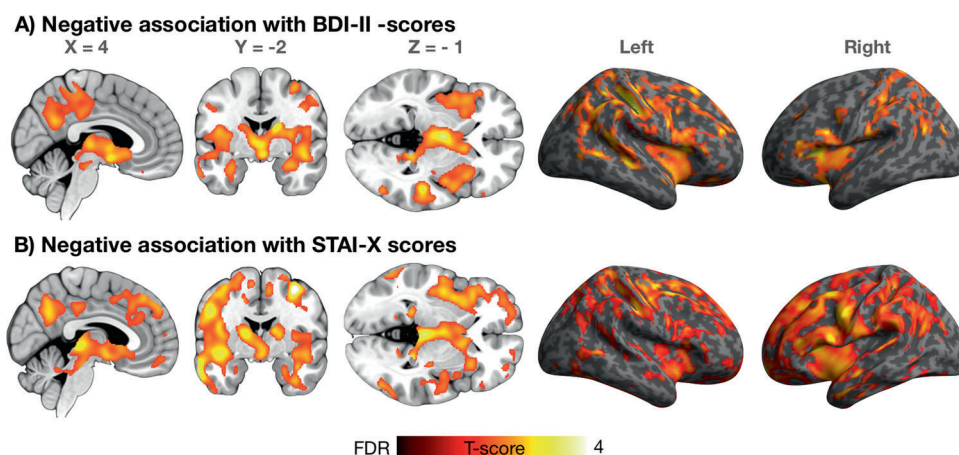


Fig. 2 Regions where BDI-II (top row; $n = 135$) and STAI-X scores (bottom row, $n = 105$) were associated with MOR availability. The data are thresholded at $p < 0.05$, FDR corrected.

$\text{max_treedepth} = 20$). The sampling produced no divergent iterations and the Rhats were all 1.0, suggesting that the chains converged successfully. Before model estimation, continuous predictors were standardized to have zero mean and unit variance, thus making the regression coefficients comparable across the predictors. Binding potentials were log-transformed because posterior predictive checking [49, 50] indicated that log-transformation significantly improves model fit. The log-transformation essentially switches the model from additive to multiplicative; it also helps in model fitting because the assumption of linear additivity works poorly when the dependent variable is restricted to positive values [51].

RESULTS

Figure 1 shows the spatial distribution of MOR availability in the sample. BDI-II and STAI-X scores were positively correlated ($r = 0.65$, $p < 0.001$, two-sided) but independent of age and sex (p 's > 0.05). Males were older than the females ($p < 0.05$). Both depression and anxiety were associated with reductions in MOR availability when controlling for age and sex (Fig. 2). For depression, these effects were widespread and bilateral. Most profound subcortical effects were found in thalamus, amygdala, hippocampus, and ventral striatum. Cortically the strongest effects were found in the insula, precuneus, and in temporoparietal regions. For anxiety, the pattern was anatomically comparable, yet the magnitude of the effect was stronger with additional clusters observed in the midcingulate and lateral prefrontal cortices. The effects for anxiety remained similar when controlling for the PET scanner. For depression, the effects remained significant in the right hemisphere. Including the four subjects with BDI-II scores > 12 did not alter the results.

Because STAI-X scores were not available for all the subjects, we also re-ran the BDI-II analyses for the sample for which both scores were available. These data essentially replicated those for the whole sample. No regions showed positive association between MOR availability and the BDI-II and STAI scores. Due to imbalanced sex distribution, we also reanalysed the data separately in the male and female samples. The effects for the male sample were similar to those as in the whole dataset (Fig. S-1), whereas for females the associations were not significant, most likely due to compromised statistical power ($n = 22$ for BDI-II and 21 for STAI-X).

The results of the full-volume analyses were corroborated in the ROI analysis (Figs. 3, 4 and Table S-2). In general, associations between BP_{ND} and BDI-II and STAI scores were negative in all the tested regions even when accounting for subjects' age, sex, regional GMD density, and PET camera. We also estimated a model that included both questionnaire scores simultaneously. In that model, the posterior distributions for the regression coefficients overlapped clearly with zero, suggesting that the contributions of the questionnaire scores are likely not independent. We also estimated a model where grey matter density was modelled with age, sex, and the questionnaire scores, and found no contributions for the BDI-II and STAI-X scales.

DISCUSSION

Our main finding was that subclinical symptoms of birth anxiety and depression were associated with lower MOR availability in a predominantly male sample. These effects were observed in widespread cortical and subcortical areas, most notably in thalamus, amygdala, hippocampus, ventral striatum, and insular cortices. These effects were also independent of sex and age, and could not be explained with mesoscopic changes in cortical

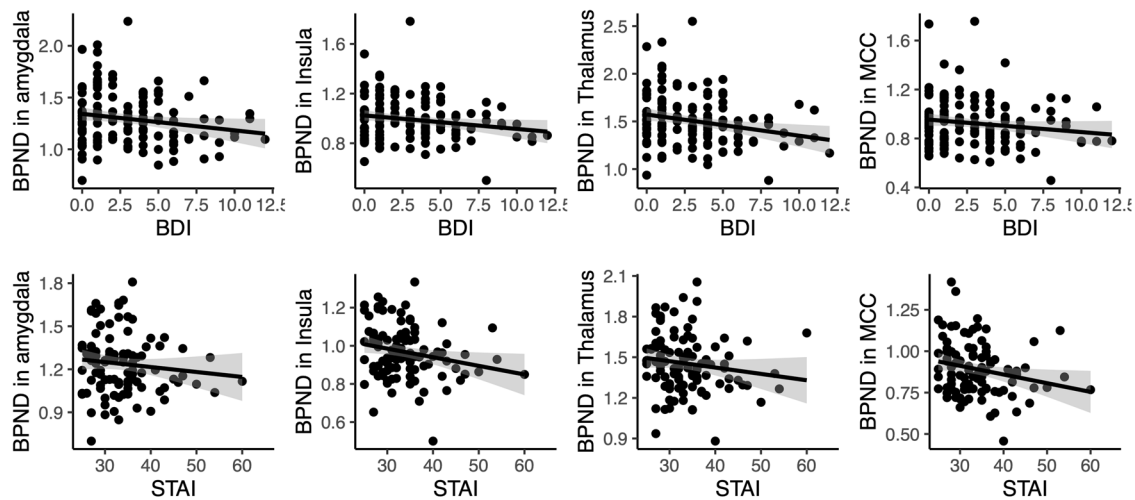


Fig. 3 Association between BDI-II and STAI-X scores on selected regions of interest. The plots show least-squares regression lines with 95% confidence intervals. Statistically significant correlations are flagged with an asterisk.

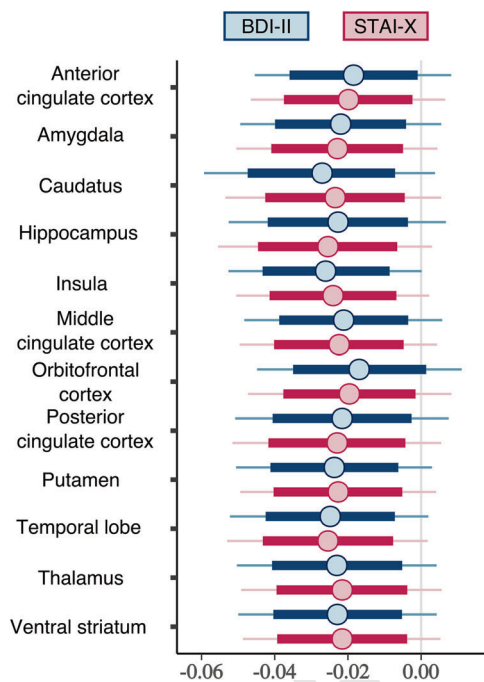


Fig. 4 Posterior distributions of the regression coefficients for the BDI-II and STAI-X scales. Thick lines show 80% posterior intervals, and the lines extend until 99% posterior intervals.

integrity, as quantified with VBM. As expected, depression and anxiety scores were also highly correlated ($r = 0.65$), and we could not find unique associations with the observed alterations in MOR availability in the present sample. This effect is consistent with the shared pathophysiology and concomitant comorbidity of depression and anxiety in general [4]. We did not find associations between grey matter density and the questionnaire scores, suggesting that the observed association between the MOR system and depressive/anxious symptomology cannot be explained by general structural changes in the brain. Instead, it is more specific to the MOR system. MORs are expressed abundantly in the brain circuits governing mood and emotion regulation [1] and exogenous opioid agonists are potent

modulators of mood [3]. These findings thus suggest that individual differences MOR-mediated neurotransmission might underlie sustained changes in negative affectivity, as indexed with the depressive and anxious symptomology.

Depressive and anxious symptoms are associated with low MOR availability
MOR availability, as measured with [11 C]carfentanil, has high spatial autocorrelation [52] and it is thus not surprising that the depressive and anxious symptoms were associated with MOR availability in large clusters. Clear peaks were however found in thalamus, insulae, and across the cingulate cortex. Structural alterations in the dACC and insula are commonly observed across a wide range of mental illnesses, and their structural integrity is associated with cognitive function [41]. Meta-analysis of metabolic PET studies have also revealed lowered glucose metabolism in the ACC in MDD [53]. These results based on T1-weighted MR imaging or [18F]-FDG-PET are however unspecific with respect to the underlying neurobiology, whereas the present data point towards altered opioidergic neurotransmission as a potential molecular mechanism. Because both ACC and insulae are involved in both affective and executive functions impaired in MDD [54, 55], this also fits with the data showing that opioidergic system modulates both executive [2] and affective functions [1]. Age is strong predictor of MOR availability [35, 56] and this effect—negative associations in midbrain/amygdala and positive associations in frontal and temporal cortex—was also observed in this sample (data not shown). However, age is unlikely to explain the associations between MOR, depression, and anxiety, because we adjusted for age in the statistical models, and because the correlations between age and BDI-II and STAI-X scores were not significant.

Because MOR agonists (such as β -endorphin) suppress the activity of the HPA axis by inhibiting neurons releasing corticotropin-releasing hormone in the paraventricular nucleus of the hypothalamus [57, 58], it is possible that dysfunctional MOR system might make an individual vulnerable to psychological stressors subsequently leading to development of depressive and anxiety disorders. As such, the present findings are also important when considering that subclinical depressive symptoms increase the risk for both depression and anxiety, whereas subclinical anxiety symptoms constitute primarily a risk factor for anxious psychopathology [28]: it is possible that dysregulated MOR system could be the key shared neurobiological mechanism predisposing individuals to both anxious and depressive psychopathology.

Given the centrality of opioid system in hedonia and reward [59] the present results might reflect lowered mood and/or anhedonia in the subjects scoring high on the depression and anxiety scales [60]. Indeed, human PET studies have found that MOR system responds to a variety of rewards ranging from feeding to social interaction and physical exercise [61–65]. In animal models, opioid agonists, and partial agonist buprenorphine reduce symptoms of anxiety and depression [9, 10] as well as alleviate the effects of psychological stressors such as separation distress [66]. Similarly in humans, fusion imaging studies with [¹¹C] carfentanil-PET and functional magnetic resonance imaging have found that high OR availability in the striatum and limbic system suppresses haemodynamic brain responses while viewing distressing videos [67, 68]. Moreover, opioid agonists make humans evaluate external stimuli more positively [69]. Altogether these data fit with the view that MOR neurotransmission acts as an important buffer against psychological stressors. This accords with the data showing that mental health disorders are consistently associated with increased prescription opioid use, and majority of patients with opioid prescription for pain in the US also have comorbid depression and anxiety [70]. It is thus possible the lowered mood and MOR system tone might—either deliberately or inadvertently—lead to self-medication of emotional pain and dysphoria with MOR agonists, potentially aggravating the misuse potential of opioids [71].

Because none of the subjects had depression scores in the clinical range, these results do not necessarily parallel those obtained in clinically depressed patients, and future imaging studies need to address the relationship between MDD, anxiety disorders and MOR system functioning. However, our data clearly point towards the role of MOR neurotransmission in lowered mood, here indexed by depressive and anxious symptomatology. These large-scale data from a subclinical sample accord with the currently limited imaging data on the role of opioidergic neurotransmission in depression in humans: First, experimentally induced negative moods deactivate the μ -system in healthy volunteers, suggesting opioidergic regulation of mood [18]. Some prior small-scale clinical PET studies have also found MOR dysregulation in MDD [20] and PTSD [23]. These present data show that integrating subclinical data for well-powered statistical analysis can complement the picture drawn from clinical populations.

Limitations

Our study was based on historical data that were acquired with different PET cameras that may, despite cross-calibration, produce different binding potential estimates (See Table S-3). All images were however reanalysed using the same analysis pipeline, and potential scanner-related biases were statistically controlled for in the analyses. Not all the subjects ($n = 135$) who had completed the BDI-II had completed also STAI-X questionnaire ($n = 105$) thus the sample sizes were not equal for these two analyses. However, we also validated the effects of BDI on the sample who had completed both questionnaires, and found essentially same results as in the whole sample. Even though well-powered for the primary outcome measure, the data however do not allow establishing reliable sex differences in the depression and anxiety linked MOR expression due to imbalanced sex distribution in the sample, and due to low number of females in the sample the present results can mostly be interpreted to represent MOR function in males. Finally, the data are cross-sectional thus we cannot conclude whether the links between MOR availability and depression/anxiety scores reflect (i) genetically determined individual differences in MOR availability [72] contributing to vulnerability endophenotypes for depressive/anxious mood or (ii) downregulation of MOR neurotransmission resulting from prodromal depression or anxiety.

CONCLUSIONS

We conclude that central opioidergic neurotransmission is involved in the maintenance of depressed and anxious mood, potentially predisposing individuals to affective psychopathology. This neurobiological correlate of risk factors for MDD/anxiety disorders is relevant for pre-emptive paradigms in affective disorders. MOR system might be the critical brain system accountable for dysregulated mood in depression, and possibly further predisposing individuals for exogenous opioid abuse.

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AUTHOR CONTRIBUTIONS

Acquired the data: TK, VK, JJ, PN, KK, JH, and JR. Analyzed the data: LN, ToK, JI, TK, and JT. Designed the study: LN, ToK, and JR. Wrote the paper: all authors.

ADDITIONAL INFORMATION

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