ORIGINAL ARTICLE

Regulation of Human Affective Responses by Anterior Cingulate and Limbic μ-Opioid Neurotransmission

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Background: Human affective responses appear to be regulated by limbic and paralimbic circuits. However, much less is known about the neurochemical systems engaged in this regulation. The μ-opioid neurotransmitter system is distributed in, and thought to regulate the function of, brain regions centrally implicated in affective processing.

Objective: To examine the involvement of μ-opioid neurotransmission in the regulation of affective states in healthy human volunteers.

Design: Measures of μ-opioid receptor availability in vivo were obtained with positron emission tomography and the μ-opioid receptor selective radiotracer [^{11}C]carfentanil during a neutral state and during a sustained sadness state. Subtraction analyses of the binding potential maps were then performed within subjects, between conditions, on a voxel-by-voxel basis.

Setting: Imaging center at a university medical center.

Participants: Fourteen healthy female volunteers.

Intervention: Sustained neutral and sadness states, randomized and counterbalanced in order, elicited by the cued recall of an autobiographical event associated with that emotion.

Main Outcome Measures: Changes in μ-opioid receptor availability and negative and positive affect ratings between conditions. Increases or reductions in the in vivo receptor measure reflect deactivation or activation of neurotransmitter release, respectively.

Results: The sustained sadness condition was associated with a statistically significant deactivation in μ-opioid neurotransmission in the rostral anterior cingulate, ventral pallidum, amygdala, and inferior temporal cortex. This deactivation was reflected by increases in μ-opioid receptor availability in vivo. The deactivation of μ-opioid neurotransmission in the rostral anterior cingulate, ventral pallidum, and amygdala was correlated with the increases in negative affect ratings and the reductions in positive affect ratings during the sustained sadness state.

Conclusions: These data demonstrate dynamic changes in μ-opioid neurotransmission in response to an experimentally induced negative affective state. The direction and localization of these responses confirms the role of the μ-opioid receptor system in the physiological regulation of affective experiences in humans.

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ADVANCES IN functional neuroimaging have made possible the identification of brain areas mediating the experience of emotions directly in human subjects, which complements data acquired in animal models and in neurological disorders. A distributed network of regions, both cortical (eg, prefrontal cortex, anterior cingulate cortex, insular cortex) and subcortical (eg, amygdala, thalamus, ventral striatum), increase their synaptic activity during the presentation of emotional stimuli or the experience of emotional states.[1-8] Functional and structural changes in some of these regions have also been implicated in the pathophysiology of mood disorders (eg, major depression).[9-12]

In comparison, relatively little information has been acquired about the neurotransmitter systems involved in the regulation of emotional and mood states in humans. The use of radiotracers labeling specific receptor sites and appropriate kinetic models allows the examination of neurotransmitter release in response to experimental challenges, which typically have been pharmacological in nature.[13] More recently, demonstration of neurotransmitter release was achieved with a physiological stimulus (a sustained pain challenge) and the μ-opioid receptor system. In that work, we showed that the ac-

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tivation of μ-opioid neurotransmission in the dorsal anterior cingulate, anterior thalamus, and ventral basal ganglia suppressed the affective quality of the pain stressor.[14] Subsequent data demonstrated sex differences in these responses and a deactivation of μ-opioid neurotransmission in the nucleus accumbens of women during the pain challenge, which was associated with hyperalgesia.[15]

Following up on those findings, the present article focuses on the endogenous opioid system and μ-opioid receptor–mediated neurotransmission as a possible common mechanism modulating affective responses to various stimuli. The μ-opioid receptors are present in high concentrations and thought to regulate the function of a number of brain regions and neurotransmitter systems involved in the processing of emotional information, stress responses, and reward.[16] These regions include, among others, the anterior cingulate,[17,18] prefrontal cortex,[19] insular cortex,[14] amygdala,[20] and striatopallidal circuitry (eg, nucleus accumbens, ventral pallidum[21-23]).

Studies in animal models have shown that the activation of μ-opioid receptor–mediated neurotransmission suppresses fear and stress responses to noxious threatening stimuli and mother-infant separation.[24-27] The μ-opioid receptors may also regulate the consolidation of emotional memory through interactions with noradrenergic terminals in the amygdala.[28] The involvement of μ-opioid and δ-opioid receptors in anxietylike responses has been examined in mice deficient for either of these receptor types. The data obtained supported a suppressive effect of μ-opioid receptors on emotional reactivity, with the opposite effects being found for the δ receptor.[29] These data are consistent with the findings in humans that the regional activation of μ-opioid neurotransmission is centrally implicated in the suppression of the affective qualities of a pain stressor, as well as the negative internal affective states induced by that challenge.[14,15,30]

The studies presented here further extend this line of research by examining whether the μ-opioid receptor system is also involved in the physiological dynamic regulation of internally generated emotional states in healthy human volunteers. The volunteer sample studied was restricted to one sex to reduce interindividual variability in the data. In this regard, sex differences have been encountered in responses to emotional stimuli,[31] amygdala-regulated emotional memory storage,[32] regional μ-opioid receptor concentrations,[33-35] and the magnitude and direction of μ-opioid system responses to a pain stressor.[15]

Measures of μ-opioid receptor availability in vivo were obtained with positron emission tomography (PET) and [^{11}C]carfentanil, a selective μ-opioid receptor radiotracer.[36] In the experimental conditions used, increases in endogenous opioid release and μ-opioid receptor activation are observed as reductions in μ-opioid receptor availability for the radiotracer in vivo.[14] Reductions in the baseline activity of this neurotransmitter system have the opposite effect (ie, increases in the receptor measure)[15] (**Figure 1**). Two receptor measures were acquired for each subject, during a neutral emotional state and while experiencing a state of sustained sadness induced by the recall of an autobiographical event associated with that emotion. The responses of μ-opioid neurotransmission during the self-induced sustained sadness state were then calculated as the difference in receptor availability in vivo between neutral and sadness conditions as measured with PET. If changes in regional μ-opioid system activation were to be detected, they were expected to be in the direction of activation suppressing and deactivation enhancing the negative affective state of the volunteers.

METHODS

SUBJECTS

Volunteers were 14 healthy, medication-free, right-handed women, mean ± SD 36 ± 9 years of age, with an educational level of mean ± SD 18 ± 2 years. Subjects had no personal history of medical illness, psychiatric illness, or substance abuse or dependence and no family history of inheritable illnesses or psychiatric illness in first-degree relatives, as ascertained by means of the *Structured Clinical Interview for DSM-IV* (nonpatient version[39]). None of the volunteers were taking psychotropic medications or hormonal treatments, including hormonal birth control, for at least 6 months, and all the women reported a history of regular menstrual cycles. Phase of the menstrual cycle was not controlled for in view of previous data demonstrating that μ-opioid receptor binding potential (BP) in vivo is not influenced by the phase of the menstrual cycle.[40] Protocols were approved by the University of Michigan Investigational Review Board and the Subcommittee for Human Use of Radioisotopes (Ann Arbor, Mich). Written informed consent was obtained in all subjects.

PET AND MAGNETIC RESONANCE IMAGING

We acquired PET scans with an ECAT Exact scanner (CTI-Siemens, Knoxville, Tenn) in 3-dimensional mode with septa retracted and with an intrinsic full width at half maximum resolution of approximately 6 mm in-plane and 5 mm in the z axis. Participants were positioned in the PET scanner gantry, and 2 antecubital intravenous catheters were placed. A light forehead restraint was used to eliminate intrascan movement. [^{11}C]carfentanil was synthesized at high specific activity (>2000 Ci/mmol [>74 TBq/mmol]) by the reaction of [^{11}C]methyl iodide and a nonmethyl precursor, as previously described[41,42]; 10 to 15 mCi (370-555 MBq) was administered in each subject. The total mass of carfentanil injected was mean ± SD 0.028 ± 0.004 μg per kilogram of body weight per scan to ensure that the compound was administered in tracer quantities (ie, subpharmacological doses). Receptor occupancy at peak carfentanil concentrations was calculated at 0.2% to 0.5% for brain regions with low (cerebellum), intermediate (prefrontal cortex), and high (thalamus) μ-opioid receptor concentrations. Fifty percent of the [^{11}C]carfentanil dose was administered as a bolus and the remainder as a continuous infusion by using a computer-controlled pump to more rapidly achieve steady-state tracer levels.

Twenty-two sets of scans were acquired during 100 minutes, with an increasing duration (30 seconds up to 10 minutes). Time points were decay-corrected by using a calculated method and reconstructed by using filtered back-projection with a Hanning 0.5 filter and included both measured attenuation and scatter corrections. The dynamic images were then coregistered by using automated computer routines.[43] Image data were transformed, on a voxel-by-voxel basis, into 3 sets of parametric maps: (1) a tracer transport measure (K_1 ratio) and (2) 2 receptor-related measures ("neutral" and "sad"). To avoid the need for arterial blood sampling, these measures were calculated with

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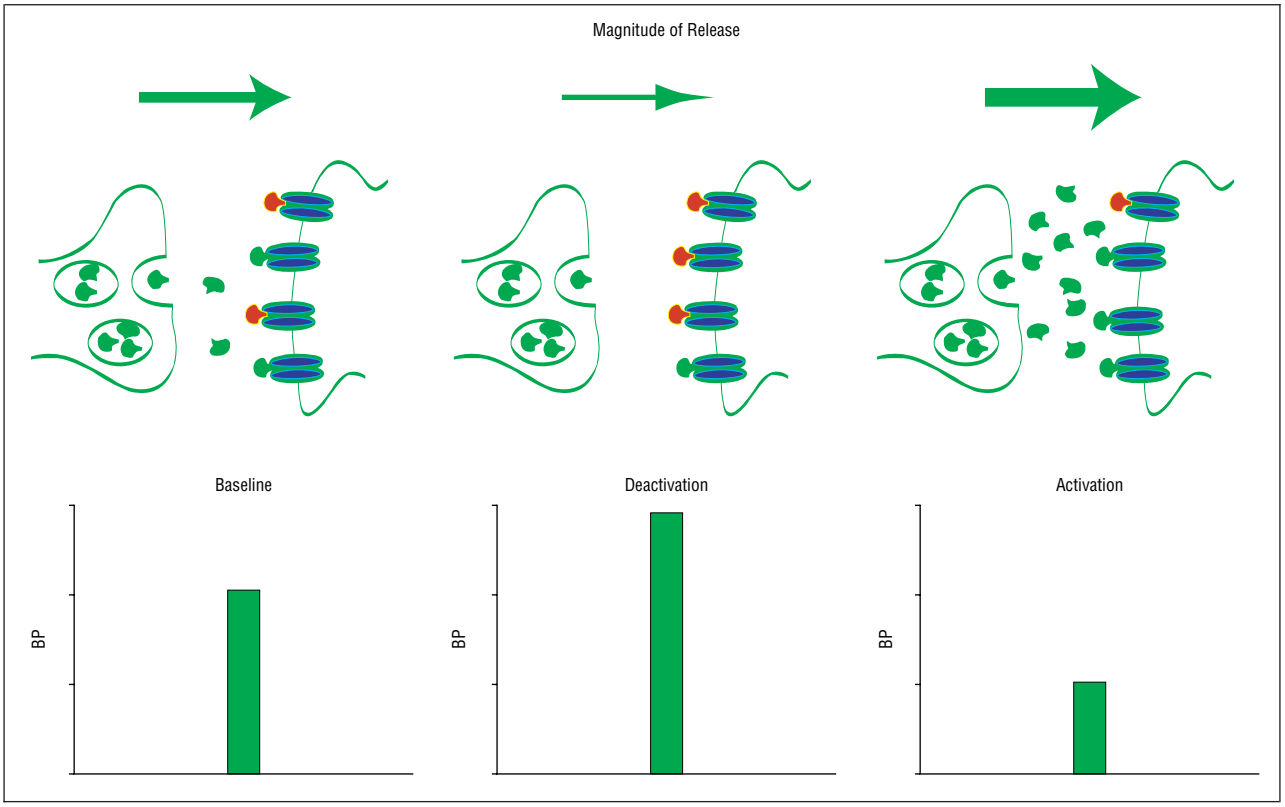

Figure 1. Relationship between activation and deactivation of endogenous neurotransmission and measures of μ-opioid receptor availability in vivo with positron emission tomography and [^{11}C]carfentanil. In the left graph, at baseline, the concentration of receptors available to bind the radiotracer (radioligand in red) depends on the tonic baseline activity of the neurotransmitter system (endogenous ligand in green). Intermediate binding potential (BP) values are obtained. In the center graph, the activation of neurotransmission in response to a challenge is associated with reductions in the concentration of radioligand bound to the receptors; lower BP measures are obtained. This reduction may reflect various processes, such as competition between endogenous neurotransmitter and radiotracer or fewer available cell surface receptors after they become activated by the endogenous neurotransmitter.[13] A receptor agonist introduced at tracer doses, [^{11}C]carfentanil is also likely to bind preferentially to receptors in high-affinity states (ie, located in the outer synaptic membrane and coupled with intracellular transduction mechanisms). Lower concentrations of these high-affinity receptors would be expected after their activation by the released endogenous opioid peptides.[37,38] In the right graph, the deactivation of μ-opioid neurotransmission is associated with higher concentrations of receptors available to bind the radioligand, which results in increases in the BP measure.

a modified Logan graphical analysis[44] and using the occipital cortex (an area devoid of μ-opioid receptors) as the reference region. With the partial bolus–continuous infusion radiotracer administration protocol used, the Logan plot became linear 5 to 7 minutes after the start of radiotracer administration, which allowed the calculation of receptor measures early after tracer administration.

The slope of the Logan plot is equal to the $(\text{Bmax}/K_d)+1$ for this receptor site, where Bmax is the receptor concentration and K_d is the receptor affinity for the radiotracer; this slope has also been referred to as the distribution volume ratio (DVR).[44] The Bmax/K_d (or DVR − 1) is the receptor-related measure (BP, or μ-opioid receptor availability in vivo). Because changes in Bmax/K_d will cause a change in the slope of the Logan plot, we measured DVR during both the neutral and sad phases of the study. The slope during the early phase was estimated from 10 to 45 minutes after injection, while the slope for the second phase was estimated from 50 to 100 minutes after injection.

Prior to PET, anatomical magnetic resonance images were acquired on a 1.5-T imager (Signa; GE Medical Systems, Milwaukee, Wis). The K_1 and DVR images for each experimental period and spoiled gradient-recalled-echo magnetic resonance images (echo time, 5.5 milliseconds; repetition time, 14 milliseconds; inversion time, 300 milliseconds; flip angle, 20°; number of signals acquired, 1; thickness, 1.5 mm) were coregistered to each other and to the International Consortium for Brain Mapping stereotactic atlas orientation.[45]

INDUCTION OF SUSTAINED SADNESS AND NEUTRAL STATES

Neutral and sadness states were initiated at either 5 or 45 minutes after radiotracer administration in a randomized counterbalanced fashion. Volunteers were blinded to the order of the experimental conditions until asked to self-induce neutral or sad emotional states. During the sadness condition, volunteers were instructed to focus on an autobiographical event associated with a profound feeling of sadness that was selected and rehearsed prior to the studies. Ten of them recalled the death of a friend or family member; 3, breakups with boyfriends; and 1, a recent argument and problems with a close friend. They were asked to recreate that sad emotion during imaging and to maintain it for the duration of the period. We did not constrain the selection of episodes to ensure that they were emotionally important for each subject.

For the neutral state, subjects were asked to relax and passively pay attention to current sensory experiences but not to actively involve in other processes, because we often find the intrusion of emotional events even during the recall of events seemingly neutral in emotional content. Subjects rated their experience every 10 minutes with the sadness subscale (sad, blue, downhearted, alone, lonely) of the Positive and Negative Affectivity Scale (PANAS[46]) to ascertain their ability to maintain that emotional state. The complete PANAS of 20 adjectives grouped into 2 main affective states, positive and negative, was

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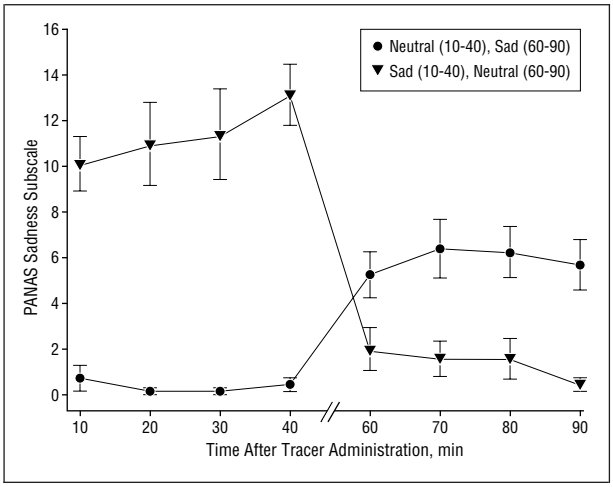

Figure 2. Time-course of sadness ratings. Data show the standard error of the mean for the Positive and Negative Affectivity Scale (PANAS) sadness subscale scores obtained every 10 minutes for the duration of the study. The order of conditions (sad, neutral) was randomized and counterbalanced between subjects. Sadness subscale scores tended to be higher for studies in which the sadness condition was performed first, which may reflect additional effects of experimental novelty or subject fatigue as the study progressed.

rated by the subjects at baseline, 45 minutes after tracer administration, and after completion of the study, the latter 2 being retrospective ratings of the preceding experimental period.

INDEPENDENCE OF RECEPTOR MEASURES FROM BLOOD FLOW EFFECTS: COMPUTER SIMULATIONS

To ensure that changes in the perceived receptor measure Bmax/K_d would not reflect alterations in regional cerebral blood flow (and therefore radiotracer transport across the blood-brain barrier) at the time of the switch from one affective phase to the other, a series of computer simulation studies were performed. The simulated (noise-added) rate of flow and hence blood-brain barrier transport rate of the radioligand was increased or decreased beginning at the time of the change in phases of the study. Simultaneous changes in Bmax/K_d of either the same or the opposite direction were also simulated. The Bmax/K_d measures resulting from those simulations were then tested for significant differences that could be ascribed to the change in radiotracer delivery. The results indicated that changes in flow have only minor effects on Bmax/K_d estimates. A simulated blood flow change of 5%, in the upper range achieved with cognitive-emotional challenges, caused at most a 0.6% change in the perceived Bmax/K_d measure. Regions of higher binding levels exhibited the largest errors. Most regions, including all cortical regions, showed less than a 0.5% bias in Bmax/K_d. The small error that was seen in Bmax/K_d was in the same direction as the change in blood flow, and there was no detectable interaction between this bias and a concurrent change in Bmax/K_d (R.A.K. unpublished data).

IMAGE DATA ANALYSIS

Parametric maps of differences between conditions (sad minus neutral) were generated by anatomically standardizing the spoiled gradient-recalled-echo magnetic resonance images of each subject to the International Consortium for Brain Mapping stereotactic atlas coordinates, with subsequent application of this transformation to the DVR maps.[45] The DVR images were then smoothed with a 3-dimensional Gaussian filter with a full width at half maximum resolution of 6 mm. Differences between conditions were mapped into stereotactic space by using z maps of statistical significance with Statistical Parametric Mapping version 99 (software available at http://www.fil.ion.ucl.ac.uk/spm/spm99.html) and Matlab (software available at http://www.math.utah.edu/lab/ms/matlab/matlab.html) software.[47] Only pixels with specific binding were included in the analyses (pixels with DVR values more than 1.2 times the mean global image value as calculated with Statistical Parametric Mapping version 99). For each subtraction analysis, 1-sample, 2-tailed t values were calculated for each pixel by using the pooled variance across pixels.[48] Significant differences and correlations were detected by using a statistical threshold that controls a type 1 error rate at $P = .05$ for multiple comparisons, estimated by using the Euler characteristic, the number of pixels in the gray matter, and image smoothness.[48] The z value typically varies from 4.4 to 4.6 in our studies for peak analyses at a final smoothed resolution of approximately 10 mm. The z scores were also deemed significant if they reached statistical thresholds after correction for the size of the cluster being considered.

Both absolute (Bmax/K_d) and normalized data were tested for statistical significance. The latter comparisons were performed to reduce global interexperimental variability within subjects that would obscure the presumably small changes in BP elicited by the emotional challenge. Normalized data were scaled to a global DVR of 1.6. The occipital cortex DVR values were held constant at 1 to avoid the introduction of errors in the calculation of Bmax/K_d in nonspecific binding regions, such as the occipital cortex, with following the formula: $\{[(1.6-1) / (\text{global DVR}-1)] \times (\text{pixel value}-1)\}+1$.

RESULTS

During task performance, PANAS sadness subscale ratings increased from a mean ± SD 0.60 ± 1.11 during the neutral condition to 7.9 ± 3.9 during the sad state (paired 2-tailed t test, $t_{13} = 7.31$; $P < .001$). The time-course and maintenance of the sadness state is illustrated in **Figure 2**. More prominent ratings of sadness were achieved during studies in which the emotional challenge was introduced first, which is perhaps a reflection of experimental novelty or more pronounced fatigue during sadness challenges performed later during imaging. The total PANAS negative affect scores rated at completion of each experimental period also increased during sadness (neutral 1.6 ± 2.4, sad 7.8 ± 4.5, $t_{13} = 5.49$; $P < .001$), while positive affect scores decreased somewhat (neutral 14.6 ± 7.3, sad 10.9 ± 6.4, $t_{13} = 2.07$; $P < .06$). Mean changes in PANAS negative and positive ratings and their coefficients of variation, within subjects, are presented in the inset of **Figure 3**.

Significant changes in μ-opioid receptor BP were detected between sad and neutral states. Increases in BP were uniformly observed during the sustained sadness condition, as compared with findings in the neutral state. Because the BP measure represents the availability of receptors for the radiotracer in vivo, this effect is consistent with a deactivation of μ-opioid neurotransmission (Figure 1). In the comparisons in which we used absolute data, statistically significant μ-opioid system deactivation was observed in the rostral anterior cingulate (Brodmann area 24/32) coordinates −3, 32, and 2 mm (x, y, and z, respectively), with $z = 5.47$ and $P < .001$ after correction for multiple comparisons.

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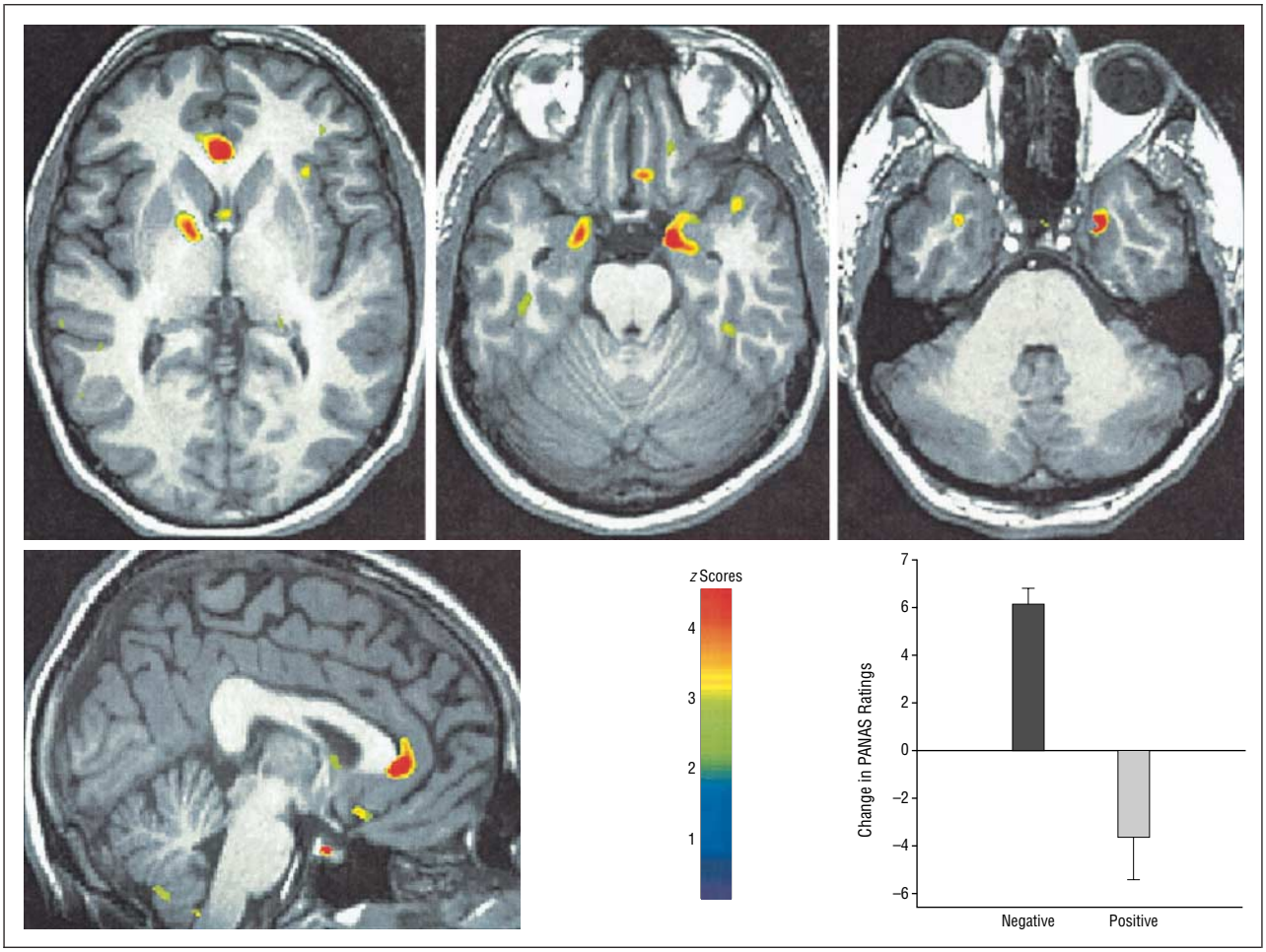

Figure 3. Reductions in μ-opioid receptor–mediated neurotransmission during a sustained sadness state. Brain areas where significant increases in regional μ-opioid receptor binding potential from neutral to sadness states were obtained, reflecting reductions in μ-opioid receptor–mediated neurotransmission. The *z* scores of statistical significance are represented by the pseudocolor scale on the lower row and are superimposed over an anatomically standardized magnetic resonance image in axial views (from left to right on the top row, anterior cingulate and right ventral pallidum; left amygdala; left inferior temporal cortex). A detail of the area of change in the anterior cingulate is shown in the lower left side in a sagittal 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 for the axial views. The graph inset shows the mean changes in the Positive and Negative Affectivity Scale (PANAS) negative and positive affect ratings between neutral and sadness conditions. The error bars show the coefficients of variation (SD/mean) of the changes in negative and positive affect ratings within subjects between conditions.

Below the statistically significant threshold after correction for multiple comparisons, high *z* scores were also obtained in the left inferior temporal cortex coordinates 25, 7, and −38 mm (x, y, and z, respectively), with $z=4.36$; in the left amygdala, 16, −4, and −21, with $z=4.19$; in the right amygdala, −20, 0, and −21, with $z=3.85$; in the left ventral pallidum, 14, 1, and −2, with $z=4.02$; and in the right ventral pallidum, −15, 2, and 3, with $z=3.93$. All these regions reached statistically significant differences between conditions once the data were normalized to whole brain values, which reduces interexperimental variability due to technical factors (**Table 1**, Figure 3). The only exceptions were the left ventral pallidum and the right amygdala, with *z* scores of 4.39 and 4.30, respectively, that did not reach multiple comparison–adjusted thresholds of significance by a small margin.

Possible relationships between μ-opioid system deactivation and PANAS affect ratings were then explored with linear regression analyses performed on a voxel-by-voxel basis (**Table 2**, **Figure 4**). The magnitude of μ-opioid system deactivation during sadness correlated with the increase in PANAS negative affect ratings in the rostral anterior cingulate and right ventral pallidum. The μ-opioid system deactivation also correlated with the reductions in PANAS positive affect scores in the ventral pallidum bilaterally and in the left amygdala. Two additional regions in which significant correlations were obtained but for which significant changes in receptor availability were not initially detected included the left insular cortex and left hypothalamus (Table 2, Figure 4). In all subjects, the effect encountered during the sustained sadness challenge, namely, regional reductions in the state of activation of the μ-opioid system, were associated with more prominent negative and lower positive affective ratings by the volunteers.

COMMENT

The present study is the first demonstration of dynamic changes in the function of a neurotransmitter system, in this case the endogenous opioid and μ-opioid receptors, during the experience of an internally generated negative affective state in human subjects. The self-induction of a

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Table 1. Normalized Data Comparisons Show Increases in in Vivo Regional μ-Opioid Receptor Availability in Healthy Human Volunteers From Neutral to Sad States

Region	Side of the Brain	State*		Cluster Size†	Coordinates, mm‡ (x,y,z)	*z* Score§	Percent Change‖
		Neutral	Sad				
Anterior cingulate	NA	0.94 ± 0.18	1.10 ± 0.33	2213	–3,31,2	6.21	16.4¶
Inferior temporal cortex	L	0.91 ± 0.25	1.04 ± 0.22	450	23,5,–37	5.97	19.9
Amygdala	L	1.11 ± 0.27	1.24 ± 0.33	1817	16,–3,–22	5.07	11.9
Ventral pallidum	R	1.42 ± 0.24	1.55 ± 0.40	1104	–14,1,4	4.44	9.0

Abbreviations: Bmax, receptor concentration; K_d, receptor affinity for radiotracer; L, left; NA, not applicable; R, right.
*Data represent the mean ± SD of binding potential values (Bmax/K_d) in regions showing significant increases in μ-opioid receptor availability in vivo (reflecting μ-opioid system deactivation) from neutral to sad states across subjects.
†Cluster size is expressed in 1-mm^3 voxels.
‡Location of the peak in stereotactic coordinates.
§The *z* values refer to the comparison between neutral and sadness Bmax/K_d values within subjects after global normalization. The *z* values corresponding to the absolute data comparisons are shown in the text.
‖Average percent change between Bmax/K_d values obtained in the neutral and sadness scans within subjects. All values were significant in the normalized data comparisons.
¶Significant in the absolute data comparisons.

Table 2. Correlations Between Increases in Regional μ-Opioid Receptor Availability in Vivo and Changes in Affect Ratings From Neutral to Sad States

Region	Side of the Brain	Change in Receptor Availability*	Cluster Size†	Coordinates, mm‡ (x,y,z)	*z* Score§	*P* Value‖
Change in negative affect¶						
Anterior cingulate	NA	0.14 ± 0.22	1053	–1,32,1	5.80	<.001#**
Insular cortex	L	0.01 ± 0.23	871	35,5,–8	4.43	<.02**
Ventral pallidum	R	0.08 ± 0.26	1477	–13,6,–3	5.20	<.005#**
Hypothalamus	L	0.07 ± 0.30	113	5,3,–7	5.68	<.001#
Change in positive affect¶						
Amygdala	L	0.04 ± 0.27	337	19,–2,–18	–4.96	<.02#
Ventral pallidum	R	0.08 ± 0.31	1113	–11,6,–1	–8.82	<.001#**
Ventral pallidum	L	0.01 ± 0.24	1212	6,4,–1	–6.68	<.001#**

Abbreviations: Bmax, receptor concentration; K_d, receptor affinity for radiotracer; L, left; NA, not applicable; R, right.
*Data represent the mean ± SD of binding potential values (Bmax/K_d) in regions identified as showing significant correlations between the increases in μ-opioid receptor availability in vivo from neutral to sad conditions (reflecting μ-opioid system deactivation) and the change in Positive and Negative Affectivity Scale ratings. Only absolute data (Bmax/K_d) were used for the calculation of correlational levels.
†Cluster size is expressed in 1-mm^3 voxels.
‡Location of the peak in stereotactic coordinates.
§Standardized *z* scores with the direction of the correlation (negative or positive).
‖*P* values after correction for multiple comparisons.
¶Change in negative affect ratings was a mean ± SD 6.2 ± 4.0 and in positive affect ratings was –3.7 ± 6.4.
#Significant according to peak analysis after correction for multiple comparisons.
**Significant according to cluster-level analysis after correction for multiple comparisons.

sustained sadness state was associated with significant reductions in μ-opioid receptor–mediated neurotransmission in the rostral anterior cingulate, ventral pallidum, amygdala, and inferior temporal cortex, as compared with findings in a neutral affect condition. Furthermore, these regional reductions in μ-opioid neurotransmission were significantly correlated with enhancements in negative affect and reductions in positive affect as rated by the volunteers.

The data obtained support the hypothesis that the μ-opioid receptor system is involved in the physiological regulation of affective states. The brain areas where these effects were observed form part of the neural circuits previously implicated in the representation and integration of emotional information. The experience of transient sadness has been shown to increase the synaptic activity of limbic and paralimbic regions, including the rostral anterior cingulate, insular cortex, and ventral basal ganglia, as measured by changes in regional cerebral blood flow.[1,7,49,50] The induction of a more sustained sad mood in a manner identical to that performed in this study has been associated with the opposite effect: reductions in the metabolic rate of glucose in the rostral anterior cingulate, basal ganglia, and insular cortex.[51,52] Brief presentations of stimuli with an affective valence, most frequently negative (eg, emotional faces, aversive pictures), also increase the synaptic activity of the anterior cingulate cortex, insular cortex, ventral pallidum, and amygdala.[3,4,53]

In this study, we found robust reductions in μ-opioid neurotransmission in the rostral anterior cingulate in response to the self-induced sadness state, which correlated with the increase in negative affect ratings by the volunteers. This brain region is thought to be a principal locus of representation and modulation of emotional and social behavior.[17] In its more dorsal subdivisions, the anterior cingulate cortex has also been implicated in the representation of pain affect,[54,55] a func-

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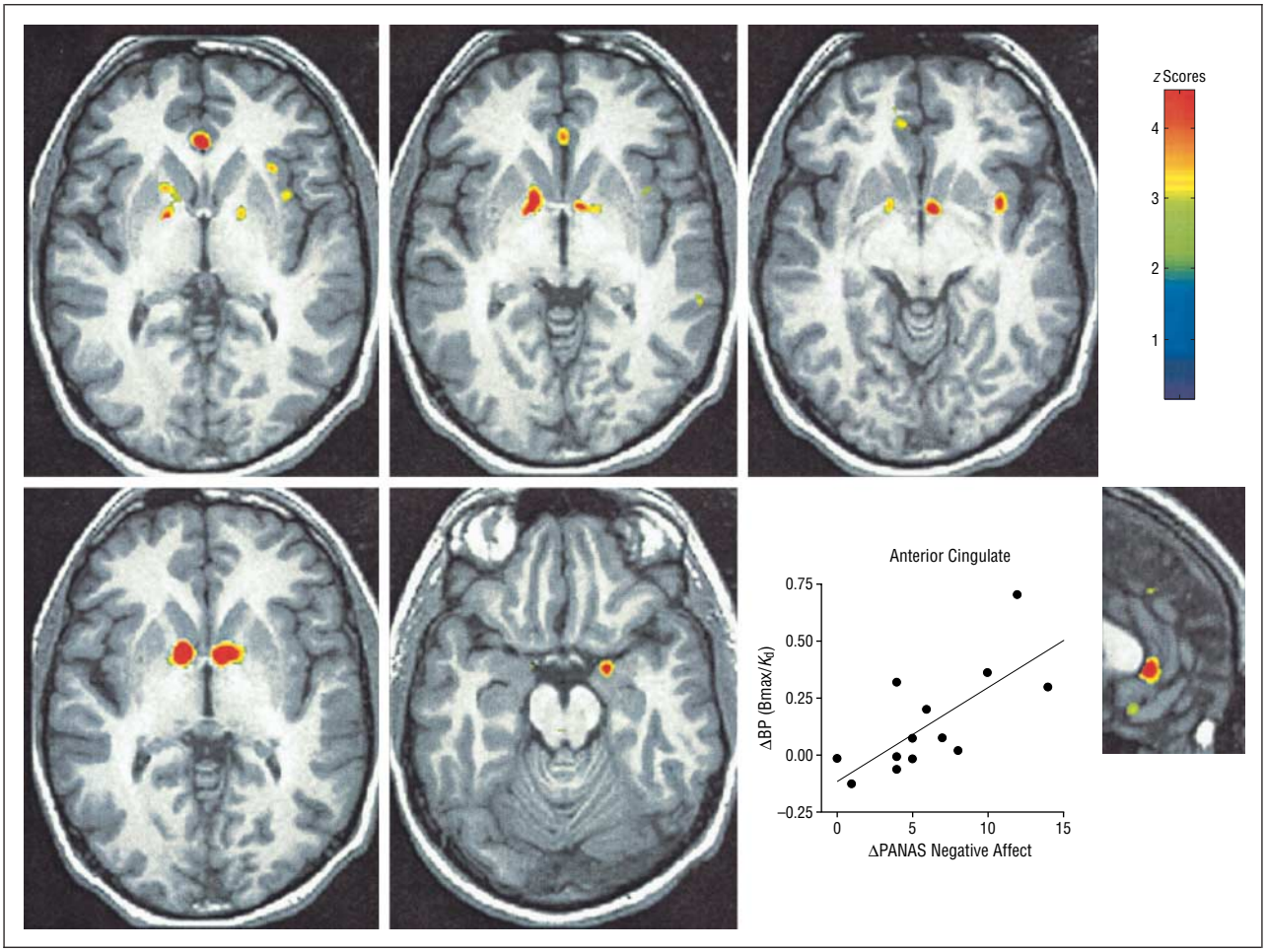

Figure 4. Correlations between deactivation of μ-opioid receptor–mediated neurotransmission and Positive and Negative Affectivity Scale (PANAS) negative and positive affect scores. Brain areas where significant correlations between the increases in μ-opioid receptor binding potential (BP; deactivation of neurotransmission) and the change in PANAS scores were obtained are shown in axial views superimposed over an anatomically standardized magnetic resonance image. Standardized *z* scores are represented by the pseudocolor scale on the upper right. 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. Upper row images depict significant correlations between the magnitude of μ-opioid system deactivation (increases in BP) and the enhancements in negative affect scores localized (left to right) in the rostral anterior cingulate and right ventral pallidum, left hypothalamus, and left insular cortex. Lower row images depict significant correlations between μ-opioid system deactivation and the reductions in positive affect scores (left to right) in the ventral pallidum bilaterally and in the left amygdala. The graph shows the individual values for the correlation in the anterior cingulate (lower row, right), with a detail of its regional localization in a sagittal view. Bmax indicates the receptor concentration and K_d the receptor affinity for the radiotracer.

tion that is also regulated by μ-opioid receptors.[14] Of relevance to the pathophysiology of mood disorders, reductions in the metabolic function of the rostral anterior cingulate have been associated with poorer responses to antidepressant medication in patients with a diagnosis of major depression, an illness associated with blunted hedonic and affective responsivity.[56,57]

Additional regions in which reductions in μ-opioid neurotransmission were observed during sustained sadness included the ventral pallidum, amygdala, and inferior temporal cortex. The ventral pallidum has been implicated in the assessment and responses to salient stimuli by integrating emotional, sensory, and cognitive information with motor responses.[4,53,58] This integrative role takes place through μ-opioid receptor-regulated afferent and efferent connections with the nucleus accumbens, amygdala, mediodorsal nucleus of the thalamus, prefrontal cortex, and ventral tegmental area.[21,23,59-61] In the present data, reductions in ventral pallidal μ-opioid neurotransmission were associated with higher negative and lower positive affect ratings by the volunteers. The direction of these relationships is consistent with that observed in the context of μ-opioid system activation during a sustained painful challenge, whereby enhancements in ventral pallidal μ-opioid neurotransmission correlated with the suppression of the negative affective state elicited by that stressor.[15]

The amygdala is centrally implicated in the evaluation and regulation of emotional information and responses. Both the central and basolateral nuclei of the amygdala present some of the highest concentrations of μ-opioid receptors in the mammalian brain.[16] At the level of the basolateral amygdala, μ-opioid receptor activation has been shown to reduce norepinephrine release, an effect thought to reduce emotional responses and the consolidation of emotional memory induced by arousing stimuli.[20,28] The opposite response (ie, a deactivation of μ-opioid neurotransmission) was observed in response to the self-induced sadness challenge, which suggests that the reduction in opioid input at this level may have a permissive role in the experience of negative affective states. The rela-

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tively prolonged duration of the challenge may have been of relevance to this finding, because synaptic activation in this area is most frequently observed during the passive presentation of brief emotional stimuli, as opposed to transient sadness induction.[62]

Regarding the reduction in baseline μ-opioid neurotransmitter activity observed in the left inferior temporal cortex, recent data have shown a direct inverse relationship between baseline μ-opioid receptor BP in this region and the cerebral blood flow responses and ratings of aversive visual stimuli.[63] These data suggested that μ-opioid receptor BP in regions involved in emotional regulation may be involved in determining their subsequent response in challenge conditions. Again, these findings appear to be in agreement with the contention that μ-opioid neurotransmission in certain limbic and paralimbic brain regions is centrally involved in the regulation of affective responses.

The μ-opioid neurotransmission in the insular cortex was not initially found to be affected by the sustained sadness induction; however, significant correlations with negative affective ratings were obtained in this region. This discrepancy may be because of small magnitudes of change in the binding measure and the small size of the region involved, not allowing for the detection of significant differences in the subtraction analyses, but which became apparent in the correlations. As noted earlier, this area is consistently found to be activated in response to affective stimuli and thought to reflect more visceral, or interoceptive, aspects of emotional experiences.[50,64] A similar situation was encountered in the hypothalamus. Both of these regions are part of the circuits involved in responses to emotionally salient stimuli and responses to stressors.[4,7,14,16,50,65]

The observations presented in this article are consistent with extensive data demonstrating that activation of the μ-opioid neurotransmitter system is associated with the dampening of emotional reactivity and responses to affectively salient and stressful stimuli.[14,15,20,24,26,66-70] Shown here is the opposite response, regional deactivations associated with a permissive effect on the experience of a negative emotional state. These results also imply the presence of significant baseline endogenous opioid peptide release during neutral experimental conditions. Authors of studies in animal models[71-73] and more recently in humans[15] demonstrated the presence of tonic endogenous opioid and μ-opioid receptor activity in various regions which, of relevance to the present article, included the ventral basal ganglia and the amygdala.

These findings are relevant for the understanding of the neurochemical mechanisms underlying the physiology of emotional processing and justify the examination of μ-opioid receptor–mediated neurotransmission in the pathophysiology of mood and anxiety disorders, both of which are precipitated by stressors.[74] In this regard, transgenic animal models devoid of μ-opioid receptors demonstrated exaggerated anxietylike behavior, as compared with findings in their wild-type counterparts.[29] More recently, a genetic polymorphism affecting the function of the catechol *O*-methyltransferase enzyme has been associated both with trait anxiety in women[75] and with dysregulation of μ-opioid neurotransmission and enhanced affective responses to a pain stressor.[30] Substantial increases in μ-opioid receptor binding without changes in receptor affinity have also been described in the prefrontal cortex, temporal cortex, and basal ganglia in postmortem studies in those who commit suicide.[33,34]

The results of this study demonstrate the feasibility of examining the function of neurochemical systems involved in the modulation of emotional states directly in human subjects and allowing translation of basic animal neurochemical findings into human experimental models. They also further illustrate the complexity of neurotransmitter systems and circuits involved in the regulation of mood and affective states. Examination of these processes will result in a greater understanding of the neurobiology of affect and mood regulation in a number of physiologic and pathologic conditions.

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REFERENCES

1. George MS, Ketter TA, Parekh PI, Horwitz B, Herscovitch P, Post RM. Brain activity during transient sadness and happiness in healthy women. *Am J Psychiatry.* 1995;152:341-351.
2. Adolphs R, Damasio H, Tranel D, Damasio A. Cortical systems for the recognition of emotion in facial expressions. *J Neurosci.* 1996;16:7678-7687.
3. Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, Strauss MM, Hyman SE, Rosen BR. Response and habituation of the human amygdala during visual processing of facial expression. *Neuron.* 1996;17:875-887.
4. Morris JS, Friston KJ, Buchel C, Frith CD, Young AW, Calder AJ, Dolan RJ. A neuromodulatory role for the human amygdala in processing emotional facial expressions. *Brain.* 1998;121:47-57.
5. Taylor S, Liberzon I, Fig L, Decker L, Minoshima S, Koeppe R. The effect of emotional content on visual recognition memory: a PET activation study. *Neuroimage.* 1998;8:188-197.
6. Davidson R, Irwin W. The functional neuroanatomy of emotion and affective style. *Trends Cogn Sci.* 1999;3:11-21.
7. Mayberg HS, Liotti M, Brannan SK, McGinnis S, Mahurin RK, Jerabek PA, Silva JA, Tekell JL, Martin CC, Lancaster JL, Fox PT. Reciprocal limbic-cortical function and negative mood: converging PET findings in depression and normal sadness. *Am J Psychiatry.* 1999;156:675-682.
8. Calder A, Lawrence A, Young A. Neuropsychology of fear and loathing. *Nat Rev Neurosci.* 2001;2:352-363.
9. Bench CJ, Frackowiak RS, Dolan RJ. Changes in regional cerebral blood flow on recovery from depression. *Psychol Med.* 1995;25:247-261.
10. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. *Proc Natl Acad Sci U S A.* 1996;93:3908-3913.
11. Drevets WC, Price JL, Simpson JR Jr, Todd RD, Reich T, Vannier M, Raichle ME. Subgenual prefrontal cortex abnormalities in mood disorders. *Nature.* 1997;386:824-827.
12. Mayberg HS. Limbic-cortical dysregulation: a proposed model of depression. *J Neuropsychiatry Clin Neurosci.* 1997;9:471-481.
13. Laruelle M. Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab.* 2000;20:423-451.

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14. Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. Regional mu opioid receptor regulation of sensory and affective dimensions of pain. *Science*. 2001;293:311-315.
15. Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. Mu-opioid receptor-mediated antinociception differs in men and women. *J Neurosci*. 2002;22:5100-5107.
16. Mansour A, Fox C, Akil H, Watson S. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. *Trends Neurosci*. 1995;18:22-29.
17. Devinsky O, Morrell MJ, Vogt BA. Contributions of anterior cingulate cortex to behaviour. *Brain*. 1995;118:279-306.
18. Vogt B, Wiley R, Jensen E. Localization of mu and delta opioid receptors to anterior cingulate afferents and projection neurons and input/output model of Mu regulation. *Exp Neurol*. 1995;135:83-92.
19. Marek G, Aghajanian G. 5-Hydroxytryptamine-induced excitatory postsynaptic currents in neocortical layer V pyramidal cells: suppression by mu-opiate receptor activation. *Neuroscience*. 1998;86:485-497.
20. McGaugh JL. Neuroscience-memory: a century of consolidation. *Science*. 2000; 287:248-251.
21. Mogenson G, Yang C. The contribution of the basal forebrain to limbic-motor integration and the mediation of motivation to action. In: Napier T, Kalivas P, Hanin I, eds. *The Basal Forebrain: Anatomy to Function*. New York, NY: Plenum Press; 1991:267-290.
22. Steiner H, Gerfen CR. Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. *Exp Brain Res*. 1998;123:60-76.
23. Napier T, Mitrovic I. Opioid modulation of ventral pallidal inputs. *Ann N Y Acad Sci*. 1999;877:176-201.
24. Akil H, Watson S, Young E, Lewis M, Khachaturian H, Walker J. Endogenous opioids: biology and function. *Annu Rev Neurosci*. 1984;7:223-255.
25. Kalin N, Shelton S. Defensive behaviors in infant rhesus monkeys: environmental cues and neurochemical regulation. *Science*. 1989;243:1718-1721.
26. Kalin N, Shelton S, Barksdale C. Opiate modulation of separation-induced distress in non-human primates. *Brain Res*. 1988;440:285-292.
27. Good A, Westbrook R. Effects of a microinjection of morphine into the amygdala on the acquisition and expression of conditioned fear and hypoalgesia in rats. *Behav Neurosci*. 1995;109:631-641.
28. Quirarte G, Galvez R, Roozendaal B, McGaugh J. Norepinephrine release in the amygdala in response to footshock and opioid peptidergic drugs. *Brain Res*. 1998; 808:134-140.
29. Filliol D, Ghozland S, Chluba J, Martin M, Matthes HW, Simonin F, Befort K, Gaveriaux-Ruff C, Dierich A, LeMeur M, Valverde O, Maldonado R, Kieffer BL. Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. *Nat Genet*. 2000;25:195-200.
30. Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D. COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. *Science*. 2003;299:1240-1243.
31. George MS, Ketter TA, Parekh PI, Herscovitch P, Post RM. Gender differences in regional cerebral blood flow during transient self-induced sadness or happiness. *Biol Psychiatry*. 1996;40:859-871.
32. Cahill L, Haier RJ, White NS, Fallon J, Kilpatrick L, Lawrence C, Potkin SG, Alkire MT. Sex-related difference in amygdala activity during emotionally influenced memory storage. *Neurobiol Learn Mem*. 2001;75:1-9.
33. Gross-Isseroff R, Dillon K, Israeli M, Biegon A. Regionally selective increases in mu opioid receptor density in the brains of suicide victims. *Brain Res*. 1990;530: 312-316.
34. Gabilondo A, Meana J, Garcia-Sevilla J. Increased density of mu-opioid receptors in the postmortem brain of suicide victims. *Brain Res*. 1995;682:245-250.
35. Zubieta J, Dannals R, Frost J. Gender and age influences on human brain mu opioid receptor binding measured by PET. *Am J Psychiatry*. 1999;156:842-848.
36. Titeler M, Lyon RA, Kuhar MJ, Frost JF, Dannals RF, Leonhardt S, Bullock A, Rydelek LT, Price DL, Struble RG. Mu opiate receptors are selectively labelled by [3H]carfentanil in human and rat brain. *Eur J Pharmacol*. 1989;167:221-228.
37. Sastre M, Garcia-Sevilla J. Density of alpha-2A adrenoceptors and Gi proteins in the human brain: ratio of high-affinity agonist sites to antagonist sites and effect of age. *J Pharmacol Exp Ther*. 1994;269:1062-1072.
38. Kenakin T. Differences between natural and recombinant G protein-coupled receptor systems with varying receptor/G protein stoichiometry. *Trends Pharmacol Sci*. 1997;18:456-464.
39. First MB, Spitzer RL, Gibbon M, Williams JBW. *Structured Clinical Interview for DSM-IV Axis I Disorders*. New York, NY: Biometric Research Department New York Psychiatric Institute; 1995.
40. Smith YR, Zubieta JK, del Carmen MG, Dannals RF, Ravert HT, Zacur HA, Frost JJ. Brain mu opioid receptor measurements by positron emission tomography in normal cycling women: relationship to LH pulsatility and gonadal steroid hormones. *J Clin Endocrinol Metab*. 1998;83:4498-4505.
41. Dannals R, Ravert H, Frost J, Wilson A, Burns H, Wagner HJ. Radiosynthesis of an opiate receptor binding radiotracer: [^{11}C]carfentanil. *Int J Appl Radiat Isot*. 1985;36:303-306.
42. Jewett D. A simple synthesis of [^{11}C]carfentanil. *Nucl Med Biol*. 2001;28:733-734.
43. Minoshima S, Koeppe RA, Mintun MA, Berger KL, Taylor SF, Frey KA, Kuhl DE. Automated detection of the intercommissural line for stereotactic localization of functional brain images. *J Nucl Med*. 1993;34:322-329.
44. Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL. Distribution volume ratios without blood sampling from graphical analysis of PET data. *J Cereb Blood Flow Metab*. 1996;16:834-840.
45. Meyer CR, Boes JL, Kim B, Bland PH, Zasadny KR, Kison PV, Koral K, Frey KA, Wahl RL. Demonstration of accuracy and clinical versatility of mutual information for automatic multimodality image fusion using affine and thin-plate spline warped geometric deformations. *Med Image Anal*. 1997;1:195-206.
46. Watson D, Clark L, Tellegen A. Development and validation of brief measures of positive and negative affect: the PANAS scales. *J Pers Soc Psychol*. 1988;54: 1063-1070.
47. Friston KJ, Holmes AP, Worsley KJ, Poline JP, Frith CD, Frackowiak RS. Statistical parametric maps in functional imaging: a general linear approach. *Hum Brain Mapp*. 1995;2:189-210.
48. Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional statistical analysis for CBF activation studies in human brain. *J Cereb Blood Flow Metab*. 1992; 12:900-918.
49. Lane R, Reiman E, Ahern G, Schwartz G, Davidson R. Neuroanatomical correlates of happiness, sadness, and disgust. *Am J Psychiatry*. 1997;154:926-933.
50. Damasio AR, Grabowski TJ, Bechara A, Damasio H, Ponto LL, Parvizi J, Hichwa RD. Subcortical and cortical brain activity during the feeling of self-generated emotions. *Nature Neurosci*. 2000;3:1049-1056.
51. Ketter T, Wang P, Lembke A, Sachs N. Physiological and pharmacological induction of affect. In: Davidson R, Scherer K, Goldsmith H, eds. *The Handbook of Affective Science*. New York, NY: Oxford University Press; 2002:930-962.
52. Ketter TA, Wang PW, Sachs N, Jimison JJ, Segall GM. *Commonalities and Dissociations in Anterior Paralimbic Metabolic Decreases With Sustained Sadness in Female Unipolar Depressives Versus Bipolar II Depressives and Healthy Volunteers*. San Juan, Puerto Rico: American College of Neuropsychopharmacology; 2002:147.
53. Taylor S, Liberzon I, Koeppe R. The effect of graded aversive stimuli on limbic and visual activation. *Neuropsychologia*. 2000;38:1415-1425.
54. Rainville P, Duncan G, Price D, Carrier B, Bushnell M. Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science*. 1997;277:968-971.
55. Tolle TR, Kaufmann T, Siessmeier T, Lautenbacher S, Berthele A, Munz F, Zieglgansberger W, Willoch F, Schwaiger M, Conrad B, Bartenstein P. Region-specific encoding of sensory and affective components of pain in the human brain: a positron emission tomography correlation analysis. *Ann Neurol*. 1999;45:40-47.
56. Mayberg HS, Brannan SK, Tekell JL, Silva JA, Mahurin RK, McGinnis S, Jerabek PA. Regional metabolic effects of fluoxetine in major depression: serial changes and relationship to clinical response. *Biol Psychiatry*. 2000;48:830-843.
57. Mayberg HS, Brannan SK, Mahurin RK, Jerabek PA, Brickman JS, Tekell JL, Silva JA, McGinnis S, Glass TG, Martin CC, Fox PT. Cingulate function in depression: a potential predictor of treatment response. *Neuroreport*. 1997;8:1057-1061.
58. Kalivas P, Churchill L, Romanides A. Involvement of the pallidal-thalamocortical circuit in adaptive behavior. *Ann N Y Acad Sci*. 1999;877:64-70.
59. Austin M, Kalivas P. Dopaminergic involvement in locomotion elicited from the ventral pallidum/substantia innominata. *Brain Res*. 1991;542:123-131.
60. Chrobak J, Napier T. Opioid and GABA modulation of accumbens-evoked ventral pallidal activity. *J Neural Transm Gen Sect*. 1993;93:123-143.
61. Johnson P, Napier T. Morphine modulation of GABA- and glutamate-induced changes of ventral pallidal neuronal activity. *Neuroscience*. 1997;77:187-197.
62. Phan KL, Wager T, Taylor SF, Liberzon I. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. *Neuroimage*. 2002; 16:331-348.
63. Liberzon I, Zubieta JK, Fig LM, Phan KL, Koeppe RA, Taylor SF. mu-Opioid receptors and limbic responses to aversive emotional stimuli. *Proc Natl Acad Sci U S A*. 2002;99:7084-7089.
64. Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, Yun LS, Chen K. Neuroanatomical correlates of externally and internally generated human emotion. *Am J Psychiatry*. 1997;154:918-925.
65. Davidson R, Putnam K, Larson C. Dysfunction in the neural circuitry of emotion regulation: a possible prelude to violence. *Science*. 2000;289:591-594.
66. Watkins L, Mayer D. Organization of endogenous opiate and nonopiate pain control systems. *Science*. 1982;216:1185-1192.
67. Rubinstein M, Mogil JS, Japon M, Chan EC, Allen RG, Low MJ. Absence of opioid stress-induced analgesia in mice lacking ß-endorphin by site directed mutagenesis. *Proc Natl Acad Sci U S A*. 1996;93:3995-4000.
68. La Buda C, Sora I, Uhl G, Fuchs P. Stress-induced analgesia in mu-opioid receptor knockout mice reveals normal function of the delta-opioid receptor system. *Brain Res*. 2000;869:1-5.
69. Curtis AL, Bello NT, Valentino RJ. Evidence for functional release of endogenous opioids in the locus ceruleus during stress termination. *J Neurosci*. 2001; 21:RC152.
70. Wand GS, McCaul M, Yang X, Reynolds J, Gotjen D, Lee S, Ali A. The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. *Neuropsychopharmacology*. 2002;26:106-114.
71. Gear R, Levine J. Antinociception produced by an ascending spino-supraspinal pathway. *J Neurosci*. 1995;15:3154-3161.
72. Kraus M, Piper J, Kornetsky C. Naloxone alters the local metabolic rate for glucose in discrete bran regions associated with opiate withdrawal. *Brain Res*. 1996; 724:33-40.
73. Gestreau C, Le GS, Besson J. Is there tonic activity in the endogenous opioid systems? A c-Fos study in the rat central nervous system after intravenous injection of naloxone or naloxone-methiodide. *J Comp Neurol*. 2000;427:285-301.
74. Kessler R. The effects of stressful life events on depression. *Annu Rev Psychol*. 1997;48:191-214.
75. Enoch MA, Xu K, Ferro E, Harris C, Goldman D. Genetic origins of anxiety in women: a role for a functional catechol-O-methyltransferase polymorphism. *Psychiatr Genet*. 2003;13:33-41.

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