

Immediate Communication:

It still hurts: altered opioid activity in the brain during social rejection and acceptance in major depressive disorder

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ABSTRACT

The μ -opioid receptor (MOR) system, well known for dampening physical pain, is also hypothesized to dampen “social pain.” We used positron emission tomography scanning with the selective MOR radioligand [^{11}C]carfentanil to test the hypothesis that MOR system activation in response to social rejection and acceptance is altered in medication-free patients diagnosed with current major depressive disorder (MDD, $n = 17$) compared to healthy controls (HCs, $n = 18$). During rejection, MDD patients showed reduced MOR activation (e.g., reduced endogenous opioid release) in brain regions regulating stress, mood, and motivation, and slower emotional recovery compared to HCs. During acceptance, only HCs showed increased social motivation, which was positively correlated with MOR activation in the nucleus accumbens, a reward structure. Abnormal MOR function in MDD may hinder emotional recovery from negative social interactions and decrease pleasure derived from positive interactions. Both effects may reinforce depression, trigger relapse, and contribute to poor treatment outcomes.

Key words: depression, mu, opioid, PET, social, rejection, acceptance, stress

INTRODUCTION

Major depressive disorder (MDD) often develops in the context of negative social environments including childhood abuse and neglect^{1,2}, adolescent peer victimization^{3,4}, and romantic break-ups^{5,6}. In particular, rejection-related stressors have been shown to be among the best predictors of MDD compared to other types of stressors^{5–10}. One reason why rejection may be particularly depressogenic is that devaluation of the self by others, real or perceived, leads directly to low self-esteem^{11,12}, a causal factor for MDD^{13,14}. Once MDD develops, poor emotional regulation during rejection can continue to reinforce symptoms, contributing to the maintenance of MDD^{15,16}. Furthermore, in MDD reduced pleasure from social interactions can contribute to withdrawal, reduced social support, and the persistence of a depressive episode^{15,17,18}.

Endogenous opioid peptides acting at μ -opioid receptors (MORs) have been shown in animal models to both alleviate distress behaviors following social separation^{19–23}, and promote social play behaviors in the presence of conspecifics^{24–28}. Our recent study in healthy humans demonstrated that social rejection activated the MOR system in structures involved in mood and motivation including the amygdala, thalamus, and ventral striatum²⁹. This pattern of MOR activation was similar to that during physical pain^{29,30}, supporting the theory that emotional “hurt” during rejection is regulated by opioid pathways for physical pain^{31,32}. In addition, during social acceptance MOR activation in the ventral striatum was correlated with increased social motivation²⁹, supporting the theory that social rewards are regulated by opioid pathways^{26,28,33}.

The present study examined the function of the MOR system in response to social rejection and acceptance in patients with MDD, compared to a matched sample of healthy controls (HCs). Given the adaptive role of the opioid system in reducing social distress and promoting social motivation, we hypothesized that MDD patients would show deficient MOR activation during rejection and acceptance, with associated alterations in behavior and levels of the stress hormone cortisol. We tested this hypothesis using a salient, ecologically-relevant task for social

rejection and acceptance during positron emission tomography (PET) scanning with the selective MOR radioligand [^{11}C]carfentanil.

MATERIALS AND METHODS

Subjects

Participants were 18 HCs from a previous study²⁹ and 17 patients with current MDD, recruited through local advertisements. HCs and MDD patients were group-matched for gender, age, education, ethnicity, sexual orientation, and relationship status (**Supplementary Table 1**, P s > 0.05). HCs were free of psychiatric disorders as assessed using the Structured Clinical Interview for DSM-IV (SCID-I) for non-patients, version 2.0. Patients were diagnosed with current MDD using the SCID-I for patients, version 2.0, were free of psychotropic medication for at least six months at the time of the study, and had moderate to severe depression (mean score \pm SD for 17-item Hamilton Depression Rating Scale, 18.5 ± 5.6). No subjects were taking hormones or hormonal contraception in the three months prior to study. Phase of menstrual cycle was not controlled – hormonal fluctuations may impact sensitivity to social rejection, but MOR binding potential *in vivo* is not influenced by phase in the menstrual cycle³⁴. All protocols were approved by the Institutional Review Board of the University of Michigan Medical School, and written informed consent was obtained.

Social Feedback Task

The social feedback task with PET has been previously described²⁹ (**Supplementary Methods**). After each feedback trial participants rated how “sad,” “rejected,” “happy,” and “accepted” they felt. The scores for “sad” and “rejected,” and “happy” and “accepted” during each trial were averaged for analysis. Word order was randomized in each trial. After each block, subjects completed the Rosenberg Self-Esteem Scale³⁵, the Desire for Social Interaction Scale²⁹, and again rated how “sad,” “rejected,” “happy,” and “accepted” they felt. All items were

presented on a personal computer, and responses were obtained using a five-button response box. Scores for Ego Resiliency³⁶, a trait for successful psychological adjustment³⁷, were obtained prior to scanning. Planned two-tailed *t*-tests were performed to compare changes in ratings within subjects (paired analysis) and between groups.

PET and Magnetic Resonance Imaging

Acquisition and reconstruction of PET images, quantification of binding potential, and co-registration with structural MRs have been previously described²⁹ (**Supplementary Methods**).

Image Data Analysis

A priori volumes of interest (VOIs) included structures that are rich in MORs, respond to social rejection and/or physical pain^{29–31,38,39} and were identical to those in a previous study²⁹ (**Supplementary Methods**). “MOR activation” was defined as the reduction in MOR binding potential from baseline to rejection or acceptance block (i.e., baseline-rejection, baseline-acceptance). This metric represents competition between radiotracer and endogenous opioids, changes in the conformational state of the receptor after activation, and/or changes in receptor concentration (e.g., via internalization, trafficking), all of which are related to endogenous opioid neurotransmission^{40,41}.

Blood Collection and Plasma Cortisol Analysis

All scans were conducted in the afternoon (1:30pm - 5:00pm), when cortisol levels are more stable and approaching their nadir. Blood samples were collected from an indwelling venous catheter every 10 min for a total of 10 samples per scan (0-90 min). Samples were collected on ice and centrifuged at 3,000 rpm for 10 min. Plasma was collected and stored at -80°C until assay. Samples were not collected in four HCs and three MDD patients due to failed venous access, leaving a total of fourteen subjects in each group for cortisol analysis.

Plasma cortisol assays were performed using IMMULITE 1000 (Siemens Medical Solutions Diagnostic Division), a solid-phase competitive chemiluminescent enzyme immunoassay system. Intra- and inter-assay variabilities were < 8%. Areas under the curve (AUCs) were calculated for the last 4 time points (of 5 total) in each block in order to minimize potential carry-over effects from the previous block. Planned two-tailed *t*-tests were performed to compare changes in AUCs within subjects (paired analysis) and between groups.

RESULTS

HCs and MDD patients reported feeling more “sad and rejected” during rejection relative to baseline (HC, $t_{16} = 5.11$, $P = 0.0001$; MDD, $t_{16} = 5.47$, $P = 0.00005$); these increases were not statistically different between groups (**Fig. 1a,b, Supplementary Table 2**). During acceptance, both groups reported feeling more “happy and accepted” (HC, $t_{16} = 3.71$, $P = 0.002$; MDD, $t_{15} = 8.89$, $P < 0.0001$); these increases were greater in MDD patients compared to HCs ($t_{32} = 2.79$, $P = 0.009$) (**Fig. 1c,d, Supplementary Table 2**).

After rejection, MDDs but not HCs reported a significant decrease in self-esteem (MDD, $t_{16} = 2.51$, $P = 0.02$). In addition, after rejection both groups reported a significant decrease in desire for social interaction (HC, $t_{16} = 2.14$, $P = 0.048$; MDD, $t_{16} = 5.38$, $P = 0.00006$); these decreases were not statistically different between groups (**Supplementary Table 2**). After acceptance, HCs but not MDD patients reported an increase in self-esteem (HCs, $t_{15} = 2.16$, $P = 0.048$) and desire for social interaction (HCs, $t_{15} = 2.91$, $P = 0.01$) (**Supplementary Table 2**).

Ratings for “sad and rejected” were measured again five minutes after the last rejection trial, indicating how quickly their ratings returned toward baseline. At that time point, HCs returned toward baseline levels whereas MDD patients remained elevated ($t_{31} = 3.02$, $P = 0.005$) (**Fig. 1e**). Ratings for “happy and accepted” were also measured five minutes after the last acceptance trial. At this time point, both HCs and MDD patients returned toward baseline levels (**Fig. 1f**).

During rejection, MOR activation was significant in the right nucleus accumbens (NAcc), left and right amygdala, midline thalamus, and periaqueductal gray (PAG) in HCs. Significant activation was not found in MDD patients (**Fig. 2a,b, Table 1**). MOR *deactivation* was not found in HCs, but was significant in the left and right amygdala in MDD patients (**Fig. 2c,d, Table 1**). Expected patterns of MOR activation were obtained from group comparisons (**Supplementary Table 3**).

During acceptance, MOR activation was significant in the right anterior insula and left amygdala in HCs (**Fig. 2e, Table 1**), and in the midline thalamus in MDD patients (**Fig. 2f, Table 1**). MOR deactivation was significant in the midline thalamus and subgenual anterior cingulate cortex (sgACC) in HCs (**Fig. 2g, Table 1**), and in the left NAcc in MDD patients (**Fig. 2h, Table 1**). Expected patterns of MOR activation were obtained from group comparisons (**Supplementary Table 3**).

Ego Resiliency ratings were higher in HCs compared to MDD patients ($t_{33} = 5.52$, $P = 4 \times 10^{-6}$), and were positively correlated with MOR activation during rejection in the amygdala (left, $r = 0.48$, $P = 0.04$; right, $r = 0.54$, $P = 0.02$), PAG ($r = 0.66$, $P = 0.003$), and sgACC ($r = 0.65$, $P = 0.003$) in HCs but not MDD patients (**Fig. 3a-c**). In HCs, those with higher Ego Resiliency had smaller reductions in self-esteem following rejection ($r = 0.67$, $P = 0.003$). This relationship was not found in MDD patients (**Fig. 3d**). During acceptance, no significant correlations were found between Ego Resiliency and MOR activation or changes in self-esteem in HCs or MDD patients ($P_s > 0.24$).

Ratings for “sad and rejected” during rejection relative to baseline were negatively correlated with MOR activation in the pgACC in HCs ($r = -0.73$, $P < 0.001$), but not MDD patients ($P = 0.69$) (**Fig. 3e**). Increased desire for social interaction was positively correlated with MOR activation in the left NAcc following acceptance in HCs ($r = 0.60$, $P = 0.01$) but not MDD patients (**Fig. 3f**).

Plasma cortisol levels were not statistically different between rejection or acceptance relative to baseline in either HCs or MDD patients, and no group differences were found (**Supplementary Table 2**). In HCs but not MDD patients, MOR activation was negatively correlated with cortisol changes during rejection. This relationship was found in the right amygdala ($r = -0.69$, $P = 0.006$), and NAcc (left, $r = -0.60$, $P = 0.02$; right, $r = -0.59$, $P = 0.03$) (**Fig. 4**).

DISCUSSION

Altered endogenous opioid activity may be a mechanism for impaired emotion regulation during social rejection and acceptance in MDD. Despite strong, sustained negative affect during rejection in both groups, MOR activation in multiple brain regions was found only in HCs, whereas MDD patients showed MOR deactivation in the amygdala and slower emotional recovery from rejection. During acceptance, both groups reported increased positive affect, with MDD patients showing greater increases from baseline compared to HCs. However, this increase returned rapidly toward baseline after acceptance trials had ended. In MDD patients, MOR deactivation during acceptance was found in the NAcc, a reward structure. MOR activation in the NAcc in HCs but not MDD patients was positively correlated with increases in the desire for social interaction, suggesting opioid involvement in the motivation to seek out positive social interaction during acceptance in HCs, but not MDD patients.

During social rejection, MDD patients did not show significant activation in VOIs, whereas in HCs, MOR activation was found in the right NAcc, left and right amygdala, midline thalamus, and PAG (**Fig. 2a,b, Table 1**), as previously described²⁹. These structures are high in MOR concentrations and part of a pathway by which stressors can influence mood and motivation⁴²; thus, MOR activation in these structures may reduce the negative impact of stressors. In contrast, MDD patients showed MOR deactivation in the amygdala (**Fig. 2c**), which may contribute to blood-oxygen-level dependent (BOLD) hyperactivity in the amygdala in MDD

patients in response to negative social cues such as peer rejection⁴³. The present study also found a strong negative correlation between MOR activation in the pgACC, an area involved in emotion regulation⁴⁴, and increased ratings of negative affect during rejection in HCs but not in MDD patients (**Fig. 3e**). Similarly, previous studies found a strong negative correlation between MOR activation in the pgACC and increased ratings of negative affect during self-induced sadness in HCs⁴⁰ but not MDD patients⁴⁵. Thus, in MDD an absence of MOR activation plus greater MOR deactivation in the amygdala, and the lack of relationship between MOR activation in the pgACC and negative affect may contribute to sustained negative affect after rejection.

Ego Resiliency is a trait conceptualized by Block³⁶ as the ability to psychologically adapt across situations, and has been shown to correlate with faster emotional and physiological recovery from threat³⁷. Consistent with this concept, levels of Ego Resiliency were positively correlated with MOR activation in the amygdala, PAG, and sgACC in HCs during rejection, as previously described²⁹. This relationship was not found in any VOI in MDD patients (**Fig. 3a-c**), possibly due to significantly lower Ego Resiliency ratings in MDD patients. The positive relationship between Ego Resiliency and MOR activation in HCs suggests that MOR activation during rejection is protective or adaptive. This hypothesis is consistent with the finding that Ego Resiliency was positively correlated with changes in self-esteem in HCs but not MDD patients during rejection (**Fig. 3d**). Path analyses in a larger sample size may test the hypothesis that MOR activation mediates the relationship between Ego Resiliency and changes in self-esteem during rejection.

As with social rejection, there were marked differences between groups during social acceptance, including MOR activation/deactivation, changes in affect, and relationships between those measures. HCs showed activation in the left anterior insula and right amygdala, and deactivation in the midline thalamus and sgACC, whereas MDD patients showed activation in the midline thalamus and deactivation in the left NAcc (**Fig. 2e-h**). In HCs, this pattern of MOR activation is consistent with increased MOR activation in the anterior insula following

amphetamine administration⁴⁶ and in the amygdala during an amusing video clip⁴⁷, suggesting that MOR activation in these areas is related to positive affect. Also in HCs, MOR deactivation during acceptance in the midline thalamus and sgACC, both of which project heavily to the NAcc^{42,48}, is a possible mechanism for facilitating positive affect. In rats, a MOR agonist injected into the medial thalamus raised the threshold for both pain and reward⁴⁹. Similarly, MOR deactivation in the sgACC may facilitate increased NAcc activity when one is liked⁵⁰. In contrast, MDD patients showed MOR activation in the midline thalamus, which may impede sustained positive affect. MDD patients also did not show MOR deactivation in the sgACC, a region shown to be functionally associated with anhedonia^{51,52}. Unexpectedly, MDD patients reported a greater increase in positive affect relative to baseline during acceptance compared to HCs, however this increase was short-lived (**Fig. 1f**), consistent with a recent study showing that MDD patients can indeed experience positive affect, but with a shorter duration compared to HCs⁵³. Moreover, only HCs showed significant increases in self-esteem and the desire for social interaction after acceptance (**Supplementary Table 2**). Thus, in response to social acceptance MDD patients showed short-lived increases in positive affect that did not significantly increase self-esteem or social motivation.

As previously reported in HCs, increased MOR activation in the NAcc was positively correlated with an increased desire for social interaction²⁹, a finding consistent with a report in rats showing that MORs in the NAcc mediate social play behavior²⁸. The present study showed that after acceptance, HCs but not MDD patients reported a greater desire for social interaction, and that MOR activation in the left NAcc was positively correlated with increased desire for social interaction (**Fig. 3f**). In contrast, MDD patients showed MOR *deactivation* in the left NAcc, which may contribute to abnormal NAcc activity related to anhedonia in MDD patients⁵⁴. Thus, in addition to having short-lived positive affect, MDD patients did not show increased social motivation, which in HCs was related to MOR activation in the NAcc.

There were no significant differences in plasma cortisol levels between rejection or acceptance relative to baseline within groups, and no differences were found between groups. In HCs a significant negative correlation was found between MOR activation in the amygdala and NAcc and changes in cortisol levels during rejection, suggesting top-down MOR regulation of the hypothalamic-pituitary-adrenal (HPA) axis. Previous studies suggest that the MOR system plays a role in dampening stress-induced HPA axis activity by inhibiting corticotropin-releasing hormone in the hypothalamus^{55,56}. Consistent with the hypothesis, MOR activation in the right amygdala was negatively correlated with cortisol levels during rejection (**Fig. 4a**). Thus, MOR regulation of amygdala activity during rejection may dampen HPA axis activity, most likely through projections to the bed nucleus of the stria terminalis, which in turn projects to the hypothalamus⁵⁷. MOR activation in the NAcc was also negatively correlated with cortisol (**Fig. 4b,c**), although the pathway from the NAcc to the paraventricular nucleus of the hypothalamus is less clear and likely involves multisynaptic pathways. The inhibitory influence of MOR activation on cortisol levels has also been reported in HCs during placebo administration for pain⁵⁸. Thus, MOR activation may dampen HPA activity during rejection, a mechanism impaired in MDD by the lack of MOR activation and/or the uncoupling of the MOR system and HPA axis.

In HCs, the pattern of MOR activation during rejection was similar to that found during physical pain^{30,59}, supporting the theory that the regulation of social rejection and physical pain share overlapping neural pathways^{29,31,32,38,39,60–62}. In contrast to the present findings, previous studies found opposite patterns of MOR activity in HCs and MDD patients during recall of a sad autobiographical event (e.g., death of a friend or family member, romantic breakups or divorce). These studies found MOR *deactivation* in HCs (pgACC, ventral pallidum, amygdala, and inferior temporal cortex)⁴⁰, and MOR *activation* in MDD patients (anterior insula, thalamus, ventral basal ganglia, and periamygdalar cortex)⁴⁵. It is likely that different patterns of MOR activation are involved in responding to exteroceptive cues (i.e., pain, rejection) versus permissive, interoceptive cues (i.e., self-induced sadness). For example, in fMRI studies where subjects

viewed a photo of a romantic ex-partner (exteroceptive cue), increased BOLD signal was found in the ventral striatum, thalamus, anterior insula, and ACC^{38,63}. In contrast, recalling sad thoughts about a recent romantic breakup (interoceptive cue) resulted in deactivation in similar areas⁶⁴.

Conclusions

MDD patients showed a lack of regional activation as well as a greater deactivation of the MOR system during social rejection and acceptance. This may be a mechanism for slower/incomplete recovery from rejection and poorly sustained engagement in positive social interactions. At the present time, it is unclear whether these alterations precede and therefore represent a risk factor for the development of MDD, or develop as a consequence of MDD. These data support further investigation of the interaction between the endogenous opioid system, social environment, and pathophysiology and maintenance of MDD.

CONFLICTS OF INTEREST

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Supplementary information is available at Molecular Psychiatry's website.

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Figure 1. Changes in affect during PET scans. Ratings for “sad and rejected” during (a) baseline and (b) rejection. Ratings for “happy and accepted” during (c) baseline and (d) acceptance. (e) Ratings for “sad and rejected” during rejection relative to baseline (trial ratings averaged), and measured again after each block. f) Ratings for “happy and accepted” during acceptance relative to baseline (trial ratings averaged), and measured again after each block.

**** $P < 0.01$, two-tailed t -test (HC vs. MDD)**

Figure 2. MOR activation/deactivation. MOR activation during rejection in (a) HCs and (b) MDD patients, and deactivation during rejection in (c) HCs and (d) MDD patients. MOR activation during acceptance in (e) HCs and (f) MDD patients, and deactivation in (g) HCs and (h) MDD patients. For all images, contrast t maps are rendered onto a template brain in MNI space. Display threshold: $P < 0.01$, uncorrected. NAcc, nucleus accumbens; sgACC, subgenual anterior cingulate cortex; R, right

VOI	MOR Activation (Baseline – Rejection)				MOR Deactivation (Rejection – Baseline)			
	HC		MDD		HC		MDD	
	Peak	<i>t</i>	Peak	<i>t</i>	Peak	<i>t</i>	Peak	<i>t</i>
NAcc (R)	16, 12, -6	3.90*	--	--	--	--	--	--
Amygdala (L)	-26, -4, -23	4.53**	--	--	--	--	-20, -3, -27	3.61*
Amygdala (R)	23, 2, -17	3.62*	--	--	--	--	16, 3, -18	5.45**
Midline Thalamus	3, -18, 6	3.68**	--	--	--	--	--	--
PAG	0, -33, -12	2.30*	--	--	--	--	--	--
	MOR Activation (Baseline – Acceptance)				MOR Deactivation (Acceptance – Baseline)			
	HC		MDD		HC		MDD	
	Peak	<i>t</i>	Peak	<i>t</i>	Peak	<i>t</i>	Peak	<i>t</i>
NAcc (L)	--	--	--	--	--	--	-10, 15, -12	4.26**
Amygdala (L)	-22, -3, -17	3.91*	--	--	--	--	--	--
Midline Thalamus	--	--	2, -16, 9	4.18**	0, -12, 4	3.83**	--	--
Anterior Insula (R)	44, 8, -6	3.91*	--	--	--	--	--	--
sgACC	--	--	--	--	0, 9, -6	6.09***	--	--

Table 1. MOR activation/deactivation during rejection and acceptance: within-group

analyses. Locations of peaks shown in *x, y, z* coordinates (mm) in MNI space. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, small volume correction. Dashes indicate no clusters detected at $P < 0.05$.

Significant MOR activation/deactivation were not found in the left anterior insula, dACC, or pgACC. VOI, volume of interest; MOR, μ -opioid receptor; HC, healthy control; MDD, major depressive disorder; NAcc, nucleus accumbens; PAG, periaqueductal gray; sgACC, subgenual anterior cingulate cortex; L, left; R, right

Figure 3. Trait Ego Resiliency and state changes. Ego Resiliency ratings vs. MOR activation during rejection in VOIs (red outlines) in the **a)** amygdala, **b)** PAG, and **c)** sgACC. **d)** Ego Resiliency vs. changes in self-esteem during rejection. **e)** Ratings for “sad and rejected” vs. MOR activation in the pgACC during rejection. **f)** Ratings for the desire for social interaction vs. MOR activation in the left NAcc during acceptance. PAG, periaqueductal gray; sgACC, subgenual anterior cingulate cortex; pgACC, pregenual anterior cingulate cortex; NAcc, nucleus accumbens

Figure 4. MOR activation vs. plasma cortisol. MOR activation during rejection vs. plasma cortisol levels in the **a)** right amygdala, **b)** left NAcc, and **c)** right NAcc. NAcc, nucleus accumbens; AUC, area under the curve

Figure 1

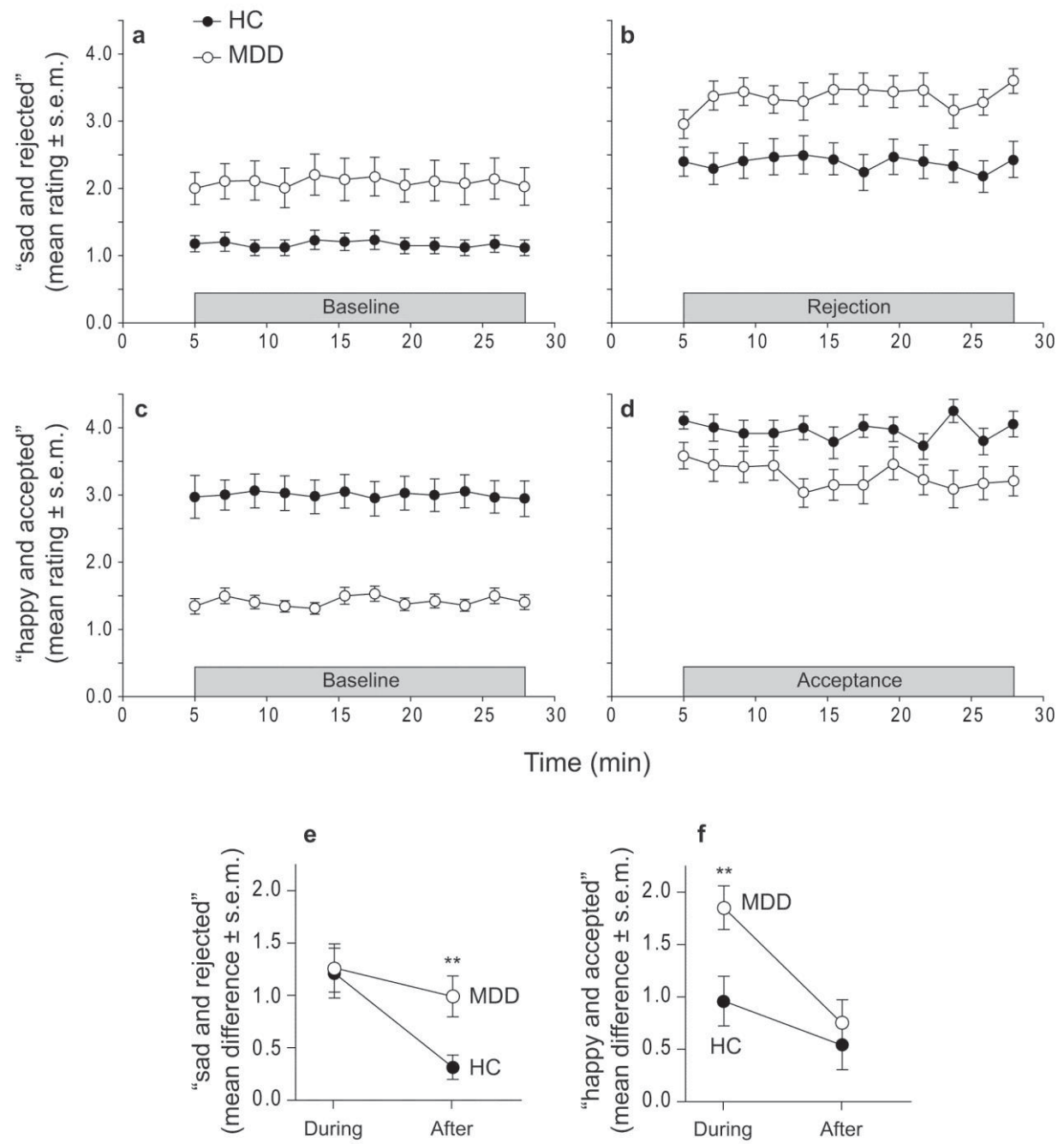


Figure 2 displays brain activation and deactivation maps for MOR and MDD groups. The figure is organized into four panels (a, b, c, d) and two rows (MOR Activation and MOR Deactivation).

MOR Activation (Baseline-Rejection):

- a Healthy Controls:** Shows significant activation in the NAcc, amygdala, and midline thalamus. The color scale ranges from $t = 0$ to 6.
- b MDD Patients:** Shows significantly reduced activation in the NAcc, amygdala, and midline thalamus compared to healthy controls. The color scale ranges from $t = 0$ to 5.

MOR Deactivation (Rejection-Baseline):

- c Healthy Controls:** Shows significant deactivation in the NAcc, amygdala, and midline thalamus. The color scale ranges from $t = 0$ to 5.
- d MDD Patients:** Shows significantly reduced deactivation in the NAcc, amygdala, and midline thalamus compared to healthy controls. The color scale ranges from $t = 0$ to 5.

MOR Activation (Baseline-Acceptance):

- e Healthy Controls:** Shows significant activation in the anterior insula and amygdala. The color scale ranges from $t = 0$ to 4.
- f MDD Patients:** Shows significantly reduced activation in the anterior insula and amygdala compared to healthy controls. The color scale ranges from $t = 0$ to 5.

MOR Deactivation (Acceptance-Baseline):

- g Healthy Controls:** Shows significant deactivation in the NAcc, midline thalamus, and sgACC. The color scale ranges from $t = 0$ to 6.
- h MDD Patients:** Shows significantly reduced deactivation in the NAcc, midline thalamus, and sgACC compared to healthy controls. The color scale ranges from $t = 0$ to 5.

Color scales indicate t -values, with red representing higher values and blue representing lower values. The maps are presented in axial and sagittal views.

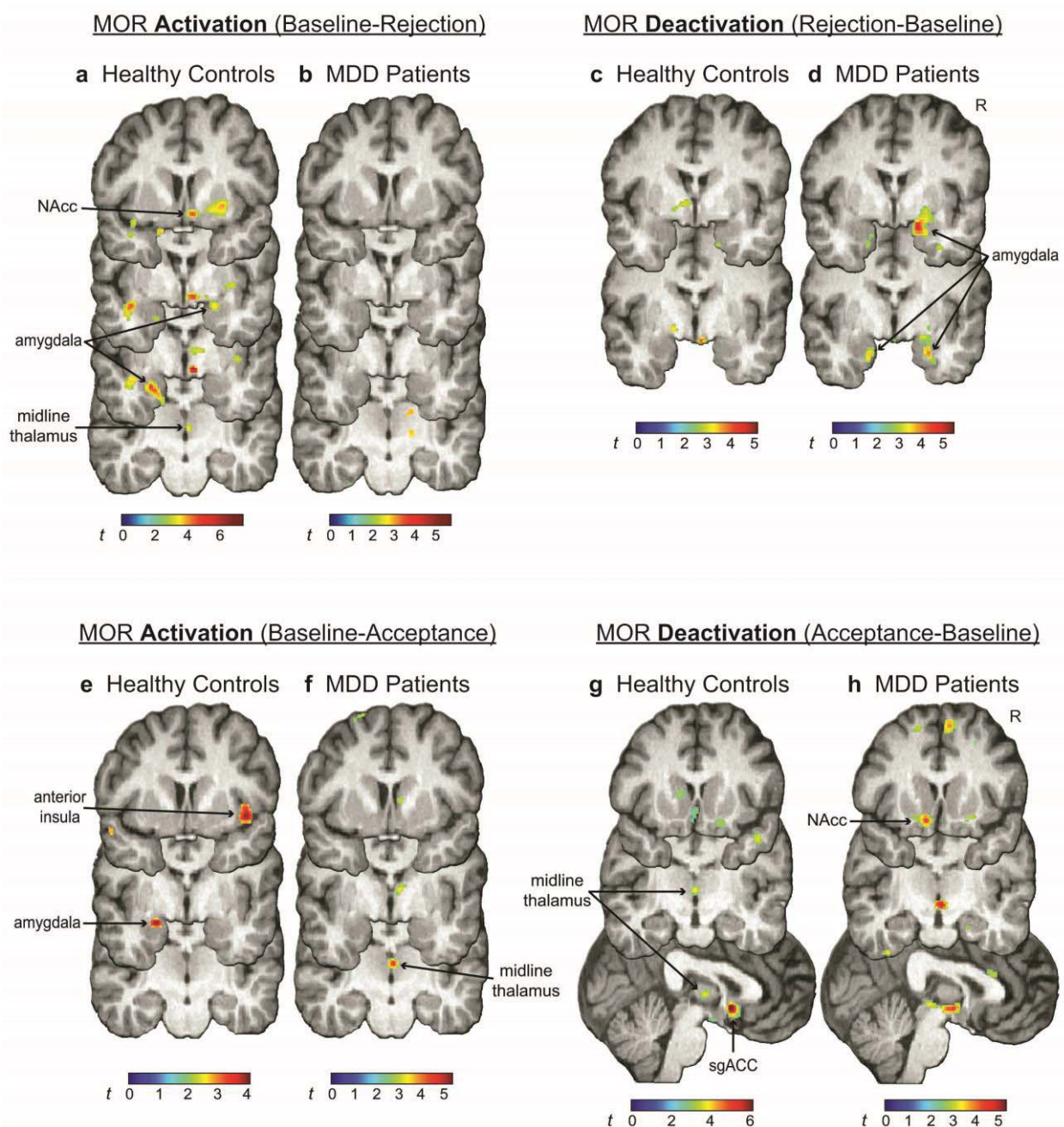


Figure 3

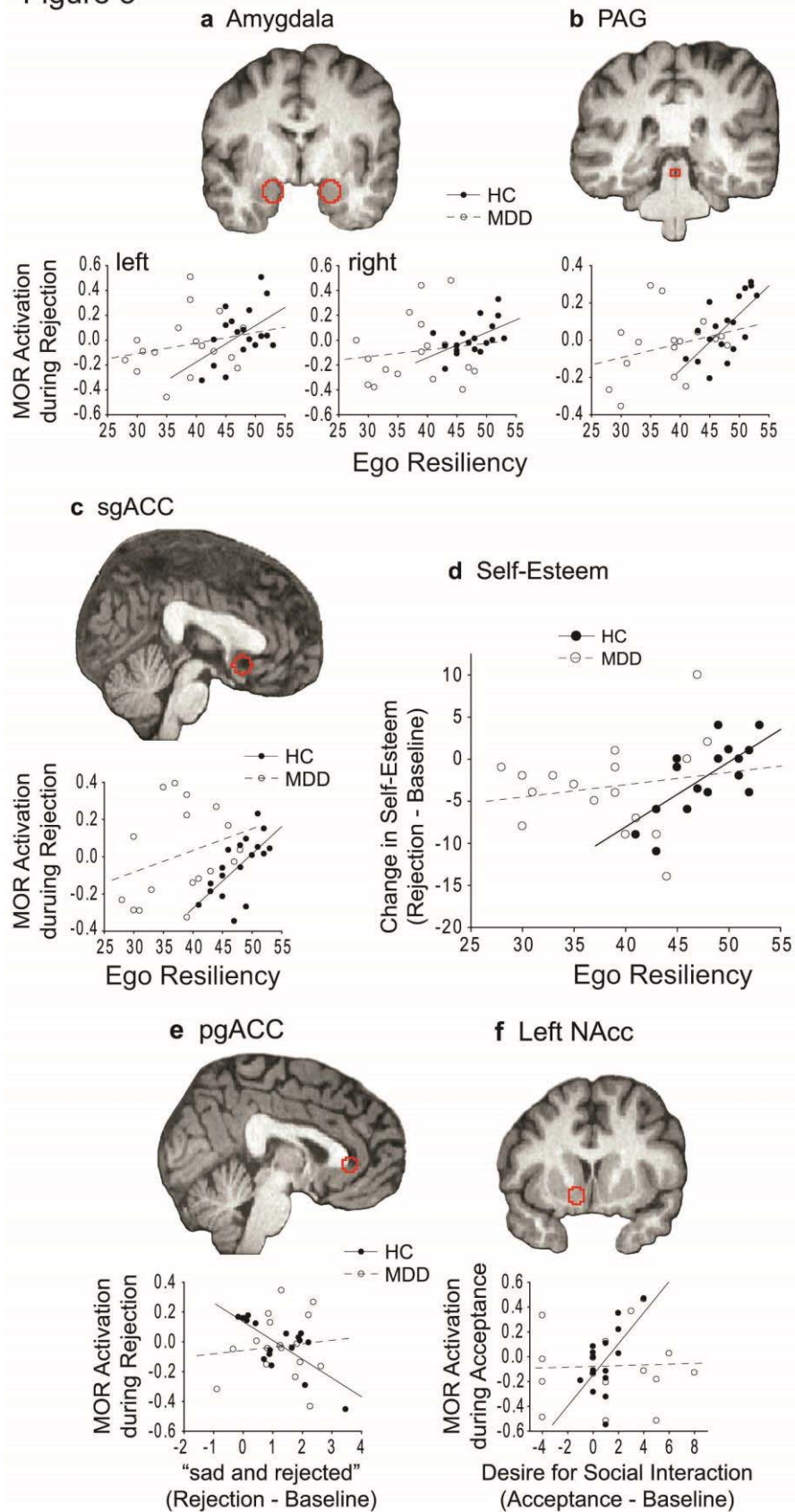


Figure 4

