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Decreased response to social defeat stress in μ-opioid-receptor knockout mice

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ABSTRACT

Substantial evidence exists that opioid systems are involved in stress response and that changes in opioid systems in response to stressors affect both reward and analgesia. Reportedly, mice suffering chronic social defeat stress subsequently show aversion to social contact with unfamiliar mice. To further examine the role of opioid systems in stress response, the behavioral and neurochemical effects of chronic social defeat stress (psychosocial stress) were evaluated in μ-opioid-receptor knockout (MOR-KO) mice. Aversion to social contact was induced by chronic social defeat stress in wild-type mice but was reduced in MOR-KO mice. Moreover, basal expression of brain-derived neurotrophic factor (BDNF) mRNA in MOR-KO mice hippocampi was significantly lower than in wild-type mice. Psychosocial stress significantly decreased BDNF mRNA expression in wild-type mice but did not affect BDNF expression in MOR-KO mice; no difference in basal levels of plasma corticosterone was observed. These results suggest that the μ-opioid receptor is involved in the behavioral sequelae of psychosocial stress and consequent regulation of BDNF expression in the hippocampus, and may play an important role in psychiatric disorders for which stress is an important predisposing or precipitating factor, such as depression, posttraumatic stress disorder, and social anxiety disorder.

1. Introduction

Physiological and psychological adaptations to stressful life events are essential for maintenance of homeostasis, although excessive stress may contribute to the development of stress-related psychiatric disorders such as depression, posttraumatic stress disorder (PTSD), general anxiety disorder, and social anxiety disorder. Acute stress activates the hypothalamic-pituitary-adrenal (HPA) axis, as well as monoaminergic neurotransmission in the brain, and affects the expression of numerous genes, including brain-derived neurotrophic factor (*BDNF*) (Pacák and Palkovits, 2001). Dysfunctions of the HPA axis (Phillips et al., 2006; Ströhle and Holsboer, 2003), monoaminergic neurotransmission (Delgado and Moreno, 2000; Graef, 2003; Southwick et al., 1999), GABAergic neurotransmission (Cameron and Nesse, 1988), and abnormal expression of *BDNF* transcripts (Duman and Monteggia, 2006) have been observed in some stress-related psychiatric disorders. However, it remains to be seen which of these changes are critical causal events mediating the pathophysiological mechanisms underlying stress-related psychiatric disorders.

Opioid systems have been well studied with regard to mechanisms of reward and analgesia, both of which are affected by stressful experiences. In addition, some researchers have reported that differences in opioid function modify acute stress responses, and stress-related neurochemical and behavioral changes have been documented. For example, in healthy human subjects, allelic variation in the single nucleotide polymorphism (SNP) A118G in exon 1 of the μ-opioid-receptor (*MOR*) gene is a predictor of HPA axis responsiveness (Chong et al., 2006; Hernandez-Avila et al., 2003; Wand et al., 2002). The polymorphism A118G is a functional SNP encoding an exon 1 variant resulting in an Asn40Asp substitution in the extracellular N-terminal domain of MOR that influences the binding affinity of β-endorphin.

Consistent with a link between genetic variation in opioid systems and stress responsiveness, MOR-knockout (MOR-KO) mice are less anxious, exhibit reduced depressive-like trait features, and show region-specific changes in 5-HT1A receptor function (Filliol et al., 2000; Yoo et al., 2004) than wild-type mice. These features may represent chronic adaptations resulting from differences in HPA axis function, as MOR-KO mice also have decreased activity of the HPA axis in response to chronic restraint stress (Wang et al., 2002). However,

Abbreviations: BDNF, brain-derived neurotrophic factor; CRH, corticotropin-releasing hormone; GR, glucocorticoid receptor; HPA, hypothalamic-pituitary-adrenal; MOR, μ-opiate receptor; PTSD, posttraumatic stress disorder; SNP, single nucleotide polymorphism; VTA, ventral tegmental area.

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the generality of the changes in HPA axis function in MOR-KO mice for various types of stressors and the subsequent consequences of different types of stressful experiences remain to be determined.

Previous research has suggested that corticotropin-releasing hormone (CRH) receptor subtypes 1 and 2 as well as the glucocorticoid receptor (GR) regulate stress-related anxiety and depression-like behavior (Bale et al., 2000; Froger et al., 2004; Timpl et al., 1998; Todorovic et al., 2005). However, the expression levels of these genes in MOR-KO mice are unknown. Mice that experience chronic social defeat exhibit subsequent behavioral and neurochemical changes, including aversion to social contact with unfamiliar mice (Berton et al., 2006; Krishnan et al., 2007), decreased hippocampal expression of BDNF mRNA (Tsankova et al., 2006), and reduced body weight (Bartolomucci et al., 2004). Rats that have suffered from chronic social defeat stress showed anxiety-like behavior (Becker et al., 2008; Frank et al., 2006), despair behavior, anhedonia (Rygula et al., 2005) and reduced body weight (Meerlo et al., 1996). Foster et al. (2006) reported that chronic social defeat increases food intake, body mass, and adiposity in Syrian hamsters. Kinsey et al. (2006) demonstrated that repeated social disruption stress increased anxiety-like behavior in mice. Moreover, social defeat stress in mice induced long-lasted analgesia (Miczek et al., 1982), and opioid antagonists involving action via MORs influenced the display of agonistic interactions such as defensive and submissive behaviors in rodents (Rodgers and Randall, 1985). These earlier results indicate a profound impact by opioid peptides on regulation of opioid receptors activated by the stress of social confrontation.

Therefore, to further elucidate the role of the opioid system in psychosocial stress responses, behavioral and neurochemical changes consequent to chronic social defeat stress were examined in MOR-KO mice.

2. Materials and methods

2.1. Animal subjects

All procedures and experiments involving animals were approved by the Institutional Animal and Care committee of Tohoku University.

2.1.1. Intruder mice

We used congenic homozygote MOR-KO mice and wild-type mice that were generated by heterozygote–heterozygote crosses of MOR-KO mice on C57BL/6J backgrounds (Hall et al., 2003; Sora et al., 1997). Congenic homozygote MOR-KO mice and wild-type mice with a C57BL/6J genetic background were used as "intruder" mice that were attacked by resident mice in a chronic social defeat stress model.

All intruder mice were housed at the Institute for Animal Experimentation, Tohoku University Graduate School of Medicine, in a colony maintained at an ambient temperature of 22±2 °C, on a 12-h light:12-h dark cycle (lights on, 8:00–20:00) with food and water available ad libitum. Intruder mice were housed 2–4 per cage and used at 8–10 weeks of age.

2.1.2. Resident mice

ICR-1 male mice were used as resident mice (i.e., intruder mice were inserted into the cages of residents to induce social defeat in the intruders). ICR-1 strain male and female mice at 12–15 weeks of age (male body weight, 35–45 g; Japan CLEA Inc., Shizuoka, Japan) were housed together for at least 14 days to increase aggressive and territorial behavior in the male resident mice before they were used for experimentation.

2.1.3. Unfamiliar target mice

C57BL/6J male mice at 8–12 weeks of age were used as unfamiliar target mice to assess psychosocial stress-induced aversion of the intruder mice to social contact.

2.1.4. Control mice

Congenic homozygote MOR-KO mice (n=7–10) and wild-type C57BL/6J mice (n=7–12) were used as controls for the intruder mice. Control mice were handled at the same time and placed in a new empty cage when intruder mice were placed in the home cage of an aggressive resident mouse. Control mice were used for corticosterone and hippocampal gene expression studies only and were not involved in the behavioral studies. They were age- and weight-matched to the experimental animals of the corresponding genotypes.

2.2. Chronic social defeat stress procedure

A chronic social defeat stress paradigm similar to a procedure described previously (Berton et al., 2006) was employed with some slight modifications as noted below. Mice designated as intruder mice (MOR-KO mice or wild-type mice) were placed in the home cage of an aggressive resident mouse for 10 min (physical stress). During this time, the resident exhibited aggressive behavior, including physical attacks such as biting, towards the intruder. Immediately after the period of physical contact, the intruder mice were separated for 24 h from the aggressive resident mouse by a perforated steel partition that allowed visual, auditory, and olfactory contact between the resident and intruder mice but prevented physical contact (psychological stress). After this period of psychological stress, the intruder mice were placed in the cage of a different resident mouse each day, and the same procedure was followed: 10 min of physical stress between 10:30 and 11:30 AM, followed by 24 h of psychological stress. This procedure was continued for 11 consecutive days. On the tenth day of chronic social defeat stress, after 10 min of physical stress exposure and 6 h of psychological stress, social interaction with an unfamiliar target mouse was measured for 10 min to assess the aversion of the intruder mice to social contact. This procedure is described in detail below. On the eleventh day, after 10 min of physical stress between 10:30 and 11:30 AM and 3 h of psychological stress, the intruder mice were decapitated.

Trunk blood was collected for measurement of plasma corticosterone, and whole hippocampi were dissected on an ice-cold plate to measure expression of stress-related gene mRNA. Blood collection and hippocampal dissection were performed between 1:30 and 2:30 PM to reduce variation produced by the circadian rhythms of plasma corticosterone and *BDNF* mRNA expression. Blood samples were immediately centrifuged at 4 °C for 15 min at 2500 rpm, and 50 µl of plasma was stored at −20 °C. Hippocampal samples were immediately frozen on dry ice and stored at −80 °C until subsequent analysis.

2.3. Social interaction test

To evaluate social aversion in the intruder mice (MOR-KO mice or wild-type mice) after chronic social defeat stress, social interaction was measured for 10 min between 10 and 11 PM the day before beginning the chronic social defeat stress regimen and immediately after exposure to physical stress on the tenth day of chronic social defeat stress. Social interaction was measured in an open field (42 cm×42 cm×48 cm) divided into 5 zones: 1 social interaction zone and 4 corner zones (Fig. 1). A perforated plastic box (8 cm×8 cm×8 cm) was placed on 1 side of the open field, defining the social interaction zone. Testing was conducted under bright-light conditions. Time spent in each zone and the total distance traveled were measured by analysis of a digital image recording for each subject. An initial open-field session was conducted in which there was no target mouse in the social interaction zone, followed by a social interaction session with target mice in the zone. All mice were exposed to an open field with and without a target mouse before and after chronic social defeat stress. The same target mice were used before and after chronic social defeat stress. To begin each social interaction test, an intruder mouse was placed at the center of the open

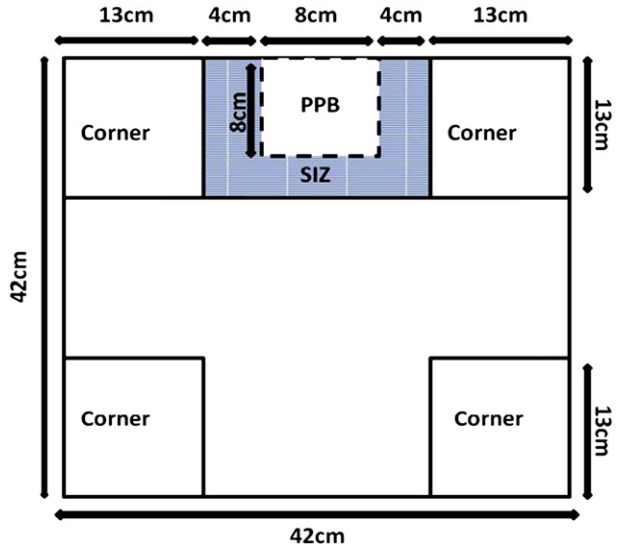

Fig. 1. Experimental design. Social interaction was measured in an open field (42 cm × 42 cm × 48 cm) for 10 min to evaluate the effects of chronic social defeat stress on social interaction and anxiety-like behavior of intruder mice. The open field was divided into 5 zones (a social interaction zone and 4 corner zones). A perforated clear plastic box (PPB) was placed in the social interaction zone (SIZ) in which the target mouse was placed. With no target mouse in the social interaction zone, the behavior of intruder mice was measured for 10 min, followed by measurement for 10 min with a target mouse in the box. The social interaction test was conducted before and after chronic social defeat stress.

field, and movements were recorded with a video recorder (NV-GS100; Matsushita Electric Industrial Co. Ltd., Osaka, Japan) for 10 min. Recordings were transformed into black and white images with appropriate contrast and brightness adjustments so that a mouse on the floor was visualized as a black spot moving against a white background. These data were then transformed into a sequence of still images (9.99 fps; 5994 still images per 10 min). The center coordinate of the black spot in each still image was calculated; 5994 sequential coordinates were used to generate locus chart images that summarized the total track record of each mouse for the 10-min observation period. Time spent in each zone and the total movement distance were calculated from these data.

2.4. Measurement of plasma corticosterone levels

Levels of plasma corticosterone were measured using a commercial EIA kit (Assay Designs, Inc., Ann Arbor, MI, USA) after extraction of corticosterone with ethyl acetate from 50 μl of plasma stored at −20 °C.

2.5. Quantification of stress-related gene mRNA by real-time reverse transcription-polymerase chain reaction

Total RNA was extracted from hippocampi using an RNeasy Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions. To remove traces of genomic contamination, RNase-free DNase I (RNase-Free DNase Set; Qiagen, Hilden, Germany) was added directly to a spin column containing the total RNA. Total RNA was eluted from the spin column to a total volume of 30 μl of RNase-free water: 27 μl of total RNA solution was frozen at −80 °C for synthesis of cDNA, and 3 μl was used for measurement of total RNA by optical density measurement using a UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). Total RNA (1 μg) was reverse transcribed into cDNA by oligo-dT priming using a ThermoScript real-time reverse transcription-polymerase chain reaction RT-PCR system (Invitrogen, Carlsbad, CA, USA). Total RNA and oligo-dT primer were first heated to 65 °C for 5 min, followed by addition of the RT reaction components. The reaction mixture was incubated at 50 °C for 1 h and terminated at 37 °C for 20 min with 2 U of RNase H. The *BDNF* gene contains 9 exons composed of 8 5′-untranslated exons and 1 3′-protein-coding exon (Aid et al., 2007). Each of the 8 5′-untranslated exons is spliced to the common 3′-protein-coding exon IX. In addition, transcription can be initiated in the intron before the protein-coding exon, which results in transcripts containing only the 5′-extended protein-coding exon. Thus, since all *BDNF* transcript variants contain the protein-coding exon, *BDNF* primer pairs were designed to match the sequence of the protein-coding exon of the *BDNF* gene to measure total *BDNF* transcripts. Primers were designed with the aid of DNASTAR software (DNASTAR Inc., Madison, WI, USA). The primer sequences for *BDNF*; the genes for GR, CRH1R, CRH2R; and the housekeeping protein β-actin were as follows: *BDNF* forward primer 5′-CCCGGTATCCAAGGCCAACTGA-3′ and reverse primer 5′-CGAGTTC-CAGTGCCTTTTGTC-3′ (product length, 110 bp); CRH1R forward primer 5′-CCTCCTGGTGGCCTTTGTCCTC-3′ and reverse primer 5′-ATGGG-GCCCTGGTAGATGTAGTAC-3′ (product length, 429 bp); CRH2R forward primer 5′-CTGGGCCTCAAGGGTCAACTACTC-3′ and reverse primer 5′-CCAACTGCCCAGGCGATGATGATA-3′ (product length, 468 bp); GR forward primer 5′-CAGCCAGATTTATCCAAAGCCGTTTC-3′ and reverse primer 5′-CCAAGGACTCTCGTTTGTCTCTTT-3′ (product length, 417 bp); and β-actin forward primer 5′-AACCCTAAGGCCAACCGTGAAAAGAT-3′ and reverse primer 5′-GATTCCATACCCAAGAAGGAAGGCTG-3′ (product length, 482 bp). The PCR reaction was carried out using an ABI Prism 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with 10 μl of 2× SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan), 0.5 μl of 50× ROX Reference Dye, 1 μl of each of the primer pairs (10 μM), and 5 μl of the synthesized cDNA. Distilled water was added to produce a final reaction volume of 50 μl. A dilution curve was calculated by performing PCR with various concentrations of cDNA. Signals were determined using ABI Prism 7700 SDS software version 1.6.3 (Applied Biosystems). The threshold cycle value Ct was calculated from the exponential phase of each PCR, and a standard curve for each gene of interest and for β-actin was plotted (Ct vs. log DNA concentration). There were no differences in β-actin expression between the groups. The amounts of *BDNF*, GR, CRH1R, CRH2R, and β-actin mRNA were calculated using standard curves. Relative levels of stress-related gene mRNA were reported after normalization against levels of β-actin mRNA.

2.6. Statistical analysis

All data are reported as means ± S.E.M. Mice that died during the course of the experiment were excluded from subsequent analyses. The number of MOR-KO and wild-type mice excluded from the experiments was 2 and 3, respectively, in the social interaction test and 3 and 3, respectively, in the neurochemical experiments. There were no differences in mortality between genotypes. Data from the social interaction test were subjected to a repeated-measures analysis of variance (ANOVA), followed by least-squares difference (LSD) post-hoc testing, with genotype and social defeat stress as between-subjects factors. The basal body weights of 8-week-old unstressed mice were analyzed using an unpaired Student's *t*-test. Body weight gain, plasma corticosterone, and hippocampal gene expression data were analyzed using a two-way ANOVA, followed by LSD post-hoc testing, with genotype and chronic social defeat stress as between-subjects factors. Statistical significance was set at $p<0.05$.

3. Results

3.1. Effects of chronic social defeat stress on aversion response and anxiety behavior

3.1.1. Time spent in the social interaction zone

Unstressed mice of both genotypes spent more time in the social interaction zone with a target mouse present than when no target

mouse was in the zone (target, $F(1,11)=14.325$, $p=0.003$; genotype, $F(1,11)=0.89$, $p=0.366$; target × genotype interaction, $F(1,11)=0.266$, $p=0.616$; Fig. 2A). When no target mouse was present, neither MOR-KO nor wild-type mice exhibited differences in the amount of time spent in the social interaction zone after social defeat stress (defeat stress, $F(1,11)=0.2$, $p=0.6$; genotype, $F(1,11)=0.007$, $p=0.94$; genotype × defeat stress interaction, $F(1,11)=0.8$, $p=0.39$). In contrast, when a target mouse was present, wild-type mice spent significantly less time in the social interaction zone after social defeat stress than MOR-KO mice (defeat stress, $F(1,11)=3.3$, $p=0.09$; genotype, $F(1,11)=0.1$, $p=0.67$; genotype × defeat stress interaction, $F(1,11)=12.9$, $p=0.004$; Fig. 2B).

3.1.2. Time spent in corner zones

When no target mouse was present in the open field, both MOR-KO and wild-type mice spent significantly more time in the corner zones after social defeat stress than before (defeat stress, $F(1,11)=19.3$, $p=0.001$; genotype, $F(1,11)=0.47$, $p=0.51$; genotype × defeat stress, $F(1,11)=0.91$, $p=0.36$). Similarly, when a target mouse was present in the open field, mice of both genotypes spent significantly more time in corner zones after social defeat stress (defeat stress, $F(1,11)=31.9$, $p<0.001$; genotype, $F(1,11)=4.2$, $p=0.07$; genotype × defeat stress interaction, $F(1,11)=6.1$, $p=0.03$; Fig. 2C). Thus, regardless of whether an unfamiliar mouse was present in the target zone, both groups of mice spent more time in the corners of the open field after stress.

3.1.3. Distance traveled

When no target mouse was present, both MOR-KO and wild-type mice displayed significantly less locomotion in terms of distance traveled after social defeat stress (defeat stress, $F(1,11)=30.9$, $p<0.001$; genotype, $F(1,11)=1.13$, $p=0.31$; defeat stress × genotype, $F(1,11)=0.77$, $p=0.40$). Similarly, when a target mouse was present, distance traveled was reduced in both genotypes after social defeat

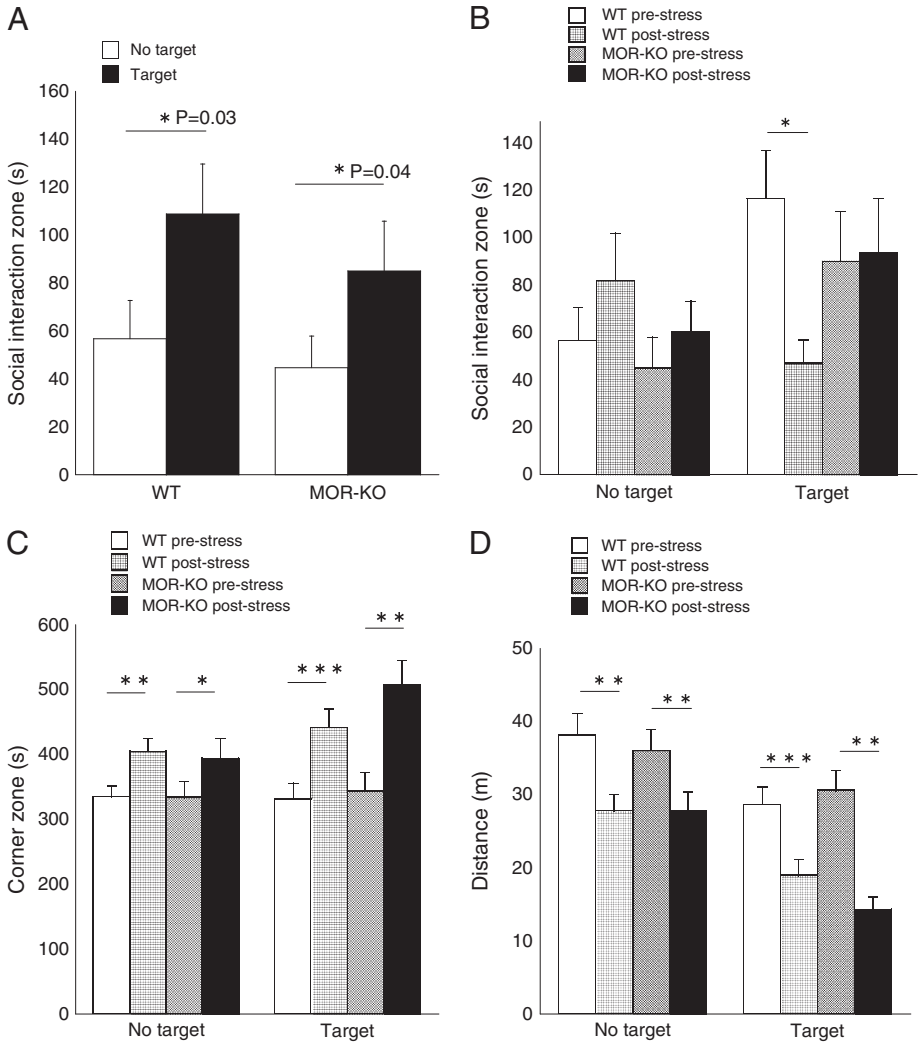

Fig. 2. Results of social interaction experiments. (A) Unstressed wild-type (WT) mice ($n=11$) and μ-opioid-receptor knockout (MOR-KO) mice ($n=15$) spent more time in the social interaction zone with a target mouse present than without a target mouse present. Values are expressed as means ± S.E.M. Least-squares difference (LSD) post-hoc test $^{*}p<0.05$. (B) Time spent in the social interaction zone by WT and MOR-KO mice measured before and after chronic social defeat stress (pre-stress and post-stress). With 45 no target mouse present, both MOR-KO ($n=13$) and WT ($n=12$) mice showed no change in time spent in the social interaction zone after social defeat stress, whereas when a target mouse was present in the social interaction zone, the time in the social interaction zone was reduced by social defeat stress in WT mice, but not in MOR-KO mice. Values are expressed as means ± S.E.M. LSD post-hoc test $^{*}p<0.05$. (C) Time spent in the corner zones in the social interaction test (10 min) in WT and MOR-KO mice before and after chronic social defeat stress (pre-stress and post-stress). With no target mouse in the social interaction zone, both MOR-KO ($n=13$) and WT ($n=12$) mice spent more time in the corner zones after social defeat stress. When a target mouse was present in the social interaction zone, a similar effect was observed after social defeat stress in both WT and MOR-KO mice. Values are expressed as means ± S.E.M. LSD post-hoc test $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. (D) Locomotion in terms of distance traveled during the social interaction test (10 min) by WT and MOR-KO mice before and after chronic social defeat stress (pre-stress and post-stress). With no target mouse in the social interaction zone, both MOR-KO ($n=13$) and WT ($n=12$) mice moved a shorter distance after social defeat stress. A similar effect was observed when a target mouse was present in the social interaction zone in both WT and MOR-KO mice. Values are expressed as means ± S.E.M. LSD post-hoc test: $^{**}p<0.01$, $^{***}p<0.001$.

stress (defeat stress, $F(1,11)=53.8$, $p<0.001$; genotype, $F(1,11)=0.26$, $p=0.62$; genotype × defeat stress, $F(1,11)=4.17$, $p=0.07$; Fig. 2d). Genotype did not affect locomotion under any conditions.

3.2. Effects of chronic social defeat stress on hormonal response and stress-related gene transcript levels in the hippocampus

3.2.1. Plasma corticosterone levels

Compared to control mice, mice that suffered chronic social defeat had increased levels of plasma corticosterone (defeat stress, $F(1,36)=0.35$, $p=0.022$; genotype, $F(1,36)<0.001$, $p=0.989$; genotype × defeat stress interaction, $F(1,36)=0.35$, $p=0.554$; Fig. 3). This effect appeared to be slightly greater in the MOR-KO mice (post-hoc analysis revealed a significant difference in MOR-KO, but not wild-type mice); however, there was no significant main effect of genotype or any significant genotype × defeat stress interaction according to the ANOVA.

3.2.2. Hippocampal expression of stress-related gene transcripts

Neither wild-type nor MOR-KO mice had a significant change in CRH 1R, CRH 2R, or GR gene transcript levels in the hippocampus after chronic social defeat stress. However, wild-type mice had significantly lower expression of *BDNF* mRNA in the hippocampus after chronic social defeat stress in comparison with control mice; this effect was not observed in MOR-KO mice. Moreover, compared with control (i.e., unstressed) mice, the *BDNF* gene transcript level was significantly lower in MOR-KO mice than in wild-type mice, although there was no significant effect of chronic social defeat stress (defeat stress, $F(1,42)=2.46$, $p=0.12$; genotype, $F(1,42)=0.89$, $p=0.35$; genotype × defeat stress, $F(1,42)=5.57$, $p=0.02$; Fig. 4).

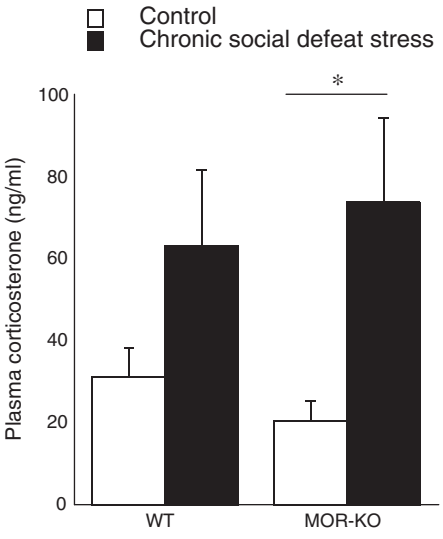

Fig. 3. Effects of chronic social defeat stress on plasma corticosterone levels of wild-type (WT) and μ-opioid-receptor knockout (MOR-KO) mice ($n=7$–13 per group). Chronic social defeat stress increased plasma corticosterone levels in both WT and MOR-KO mice, with no difference in plasma corticosterone levels between the groups. Values are expressed as means ± S.E.M. Least-squares difference post-hoc test: $^{*}p<0.05$.

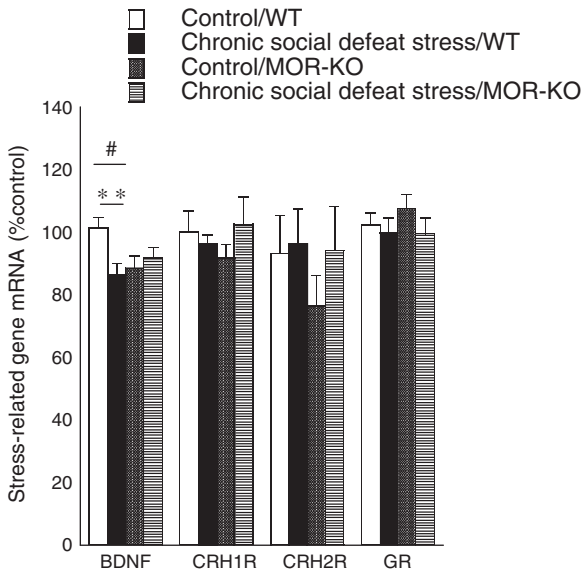

Fig. 4. Effects of chronic social defeat stress on hippocampal expression of stress-related gene mRNA. Results are shown for brain-derived neurotrophic factor (BDNF), corticotropin-releasing hormone (CRH) 1R, CRH 2R, and glucocorticoid receptor (GR) mRNA in wild-type (WT) and μ-opioid-receptor knockout (MOR-KO) mice ($n=10$–12). The levels of CRH 1R, CRH 2R, and GR mRNA in the hippocampus showed no significant change after chronic social defeat stress in either genotype. However, in WT mice, the level of BDNF mRNA was significantly lower after chronic social defeat stress compared with control mice, but this effect was eliminated in MOR-KO mice. Additionally, in unstressed control mice, the level of BDNF mRNA was significantly lower in MOR-KO mice than in WT mice. Values are expressed as 47 means ± S.E.M. Least-squares difference post-hoc test: $\#p<0.05$, $^{**}p<0.01$.

4. Discussion

The presence of a conspecific animal in a familiar environment elicits social interaction. In the present study, unstressed mice of both genotypes spent more time in the social interaction zone with a target mouse present than when no target mouse was present. Berton et al. (2006) reported that mice that experience chronic social defeat develop a long-lasting aversion to social contact and spend significantly decreased time in a social interaction zone with a target mouse in that zone. This effect was confirmed in the present study, which found that wild-type mice spent substantially less time in the social interaction zone of the open field in contact with an unfamiliar target mouse after chronic social defeat stress (social aversion). Although MOR-KO eliminated the effects of chronic social defeat stress on social aversion, other behavioral consequences of social defeat did not appear to be affected by MOR-KO. That is, both wild-type and MOR-KO mice showed a reduction in overall locomotion as well as in the amount of time spent in the center of the open field after chronic social defeat, independent of whether a target mouse was present. Time spent in the social interaction zone appeared to be a good indicator for social investigation since the time spent in this zone was unchanged in either genotype if there was no target mouse present. This observation therefore supports the conclusion that the decrease in time spent in the social interaction zone after chronic social defeat in wild-type mice represents acquisition of aversion to social contact, not a more general change in anxiety as reflected by the other measures.

While reduction in time spent in the social interaction zone appeared to reflect social aversion resulting from defeat stress, a general change in anxiety level also appeared to result from social defeat, as indicated by increased time spent in the corner zones. This effect was observed regardless of whether an unfamiliar target mouse was present in the social interaction zone. As this effect was observed in both genotypes—wild-type and MOR-KO mice—it would appear that the μ-opioid receptor does not have a general effect on anxiety behavior (as indicated by change in time spent in the corner zones without a target mouse in a social interaction zone), but instead may be specifically involved in social anxiety. Unlike the pronounced social aversion observed in wild-type mice, MOR-KO mice did not exhibit decreases in the amount of time spent in the social interaction zone after chronic social defeat stress. Thus, MOR-KO mice do not acquire aversion to social contact as a consequence of social defeat even though more general aspects of anxiety, as assessed in the open field, are affected by social defeat in MOR-KO mice. Similarly, it would

appear that not all responses to stressors are affected by MOR-KO. We evaluated the time spent in corner zones as a general anxiety parameter. However, it has previously been reported that MOR-KO mice exhibit decreased anxiety-like behavior in an elevated plus maze and a light-dark box. These discrepancies may be due to differences in the general anxiety parameter. It has been reported previously that MOR-KO mice have impaired acquisition of Pavlovian contextual fear conditioning (Sanders et al., 2005), which may indicate either impairment of conditioning or reduced response to aversive stimuli. In any case, these differences in the effects of social defeat on social aversion suggest that manipulation of the μ-opioid receptor gene affects not only positive emotions such as reward, but also negative emotions such as social aversion.

Most stressors increase the release of glucocorticoids consequent to activation of the HPA axis (Keeney et al., 2001; Pacák and Palkovits, 2001). A gene polymorphism of the human μ-opioid receptor affects responsiveness of the HPA axis to stress (Chong et al., 2006; Hernandez-Avila et al., 2003; Wand et al., 2002). Furthermore, decreased response of the HPA axis after chronic restraint stress has been reported in MOR-KO mice compared with wild-type mice (Wang et al., 2002). However, in the present study, the increase in plasma corticosterone levels after chronic psychosocial stress was similar between wild-type and MOR-KO mice. A previous study found that MOR-KO mice showed no change in plasma corticosterone levels after a forced swim stress, while plasma corticosterone levels were substantially affected in wild-type mice (Contet et al., 2006). These results, including the results of the present experiment, suggest that the functional role of MOR in responses of the HPA axis to stress may depend on the particular stress paradigm utilized.

GR and the CRH receptor subtypes CRH 1R and CRH 2R have been reported to regulate anxiety and depression-like behavior (Bale et al., 2000; Froger et al., 2004; Todorovic et al., 2005). In the present study, neither wild-type nor MOR-KO mice showed changes in the levels of GR, CRH 1R, or CRH 2R mRNA in the hippocampus after chronic social defeat stress. However, stress has also been shown to decrease expression of *BDNF* mRNA in brain tissue (Duman and Monteggia, 2006), and specifically, social defeat stress decreases expression of *BDNF* mRNA in the hippocampus of wild-type mice (Pizarro et al., 2004; Tsankova et al., 2006). These findings were confirmed in the present study, which found that chronic social defeat stress decreased expression of *BDNF* mRNA in the hippocampus of wild-type mice, while MOR-KO eliminated this marker of chronic social defeat. In addition, the expression of *BDNF* mRNA in the hippocampi of naive MOR-KO mice was lower than that of naive wild-type mice. Therefore, there may be a floor effect such that chronic stress cannot decrease *BDNF* expression any lower in MOR-KO mice. This difference in the basal expression level of *BDNF* mRNA in the hippocampi of MOR-KO mice may reflect a differential response to normal social experience in these mice. Consistent with the idea that opioid peptides released in response to social experiences may be a necessary mediator of changes in *BDNF* expression consequent to those experiences, intracerebroventricular administration of endogenous opioid ligands, such as endomorphin-1 and -2 or β-endorphin, has been shown to increase *BDNF* mRNA expression in the hippocampus (Zhang et al., 2006). The hippocampus plays an important role in emotional learning and has been shown to mediate the long-term consequences of various types of social experiences (Buwalda et al., 2005; LaBar and Cabeza, 2006; Maren and Quirk, 2004). Therefore, dysfunction of the hippocampus caused by decreased expression of the *BDNF* gene in MOR-KO mice may underlie decreased social aversion to unfamiliar mice related to the role of opioid signaling in long-term modification of hippocampal gene expression.

Transcription of the *BDNF* gene involves the production of 11 splice variants that vary in expression patterns across tissues (Aid et al., 2007; Qing et al., 2006). It was recently reported that chromatin remodeling, histone modification, and CpG methylation of the *BDNF* promoter are involved in regulation of the expression of *BDNF* transcript variants (Martinowich et al., 2003; Tsankova et al., 2004, 2006). Future studies should look for such changes in *BDNF* promoter function consequent to opioid receptor signaling that mediate changes in *BDNF* expression.

In the present study, we evaluated neurochemical status only in the hippocampus. Previous research has shown that a single social defeat stress episode in rats induced rapid up-regulation of MOR mRNA expression in the ventral tegmental area (VTA), which lasted at least 6 h (Nikulina et al., 1999). Moreover, it has been reported that repeated social defeat stress induces a higher expression of MOR mRNA selectively in the VTA in defeated rats relative to handled control animals, which is detectable several weeks after stress termination (Nikulina et al., 2008). Therefore, it may be important to assess neurochemical change in other brain areas such as the VTA to identify the mechanism of behavioral change after chronic social defeat stress in MOR-KO mice.

In conclusion, the present study documented that deletion of the μ-opioid receptor diminished chronic social defeat stress-induced aversion response to social contact and decreased the basal level of hippocampal *BDNF* transcripts; there was no change in the level of hippocampal *BDNF* transcripts after psychosocial stress. These data suggest that μ-opioid systems play an important role in the pathology of stress-related psychiatric disorders. Further research to reveal the functional role of the μ-opioid receptor in emotional responsivity may help to elucidate the mechanisms underlying pathological changes in neural function that contribute to the development of several psychiatric disorders involving impairment of emotional regulation in response to social stimuli, such as depression, PTSD, and social anxiety disorder.

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