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Effects of chronic social defeat stress on MAP kinase cascade

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ABSTRACT

Chronic psychological and social stress can cause psychiatric disorders in humans. In this study, we analyzed the mitogen-activated protein kinase (MAPK) cascade in the hippocampus of chronically socially defeated rats. The rats that were subjected to social defeat every day for 5 weeks showed physiological and behavioral changes, including a reduced rate of weight gain, enlarged adrenal glands, and increased immobility in the forced swim test without concomitant changes in locomotor activity in the open field test. Altered body weight and enlarged adrenal glands are typical symptoms of human depression. Prolonged immobility in the forced swim test indicates behavioral despair, a well-established index of depression. Because the MAPK cascade plays a pivotal role in depression, we quantified the expression of these molecules in the hippocampus of chronically defeated rats using western blot analysis. We found that phospho-MAPK kinases 1/2 (MEK1/2) and phospho-extracellular signal-regulated kinases 1/2 (ERK1/2) were decreased, whereas MAPK phosphatase-1 (MKP-1) was increased in the hippocampus of chronically defeated rats compared to the control group. These results were consistent with findings in depressed patients and other animal models of depression. In conclusion, our findings suggest that chronic psychosocial stress in Wistar rats induced depression-like behavior and downregulated the MAPK cascade in the hippocampus.

Chronic stress can precipitate psychopathologies such as depression [1]. Various rodent models of depression have been developed based on the exposure to stressful conditions that induce depression-like symptoms. The most common stressors in humans are thought to have a psychological or social nature [1]; therefore, the use of social conflicts among members of the same species and strains to generate stress has an obvious advantage over animal models that require aversive physical or chemical stimuli, such as restraint. Social defeat in rodents can be generated using the resident-intruder paradigm in which the subject, or "intruder", is placed in the cage of a dominant "resident" [1,10]. Social defeat induces a variety of behavioral changes, including decreased locomotor activity [10], reduced aggression [14], and increased submissive behavior [23]. Physiologically, social defeat activates the hypothalamic-pituitary-adrenal axis [2] and induces molecular changes in the brain, including altered expression of brain-derived neurotrophic factor (BDNF) [11,29], extracellular signal-regulated kinase (ERK) [11], and cyclic AMP-responsive-element-binding protein (CREB) [30].

BDNF is involved in the pathophysiology of stress-related mood disorders, and BDNF expression decreases after exposure to stress, including social-defeat stress [17,27,29]. Conversely, infusion of BDNF into the hippocampus has shown antidepressant effects [25]. BDNF influences cellular function via activation of the tyrosine kinase receptor B (TrkB) and mitogen-activated protein kinase (MAPK) cascade, which includes ERK signaling [4,28]. A postmortem study reported that the levels of ERK activity and expression are decreased in the hippocampus and cerebral cortex of depressed suicide victims, providing additional support for the downregulation of neurotrophic factor function in depression [8]. Recently, Duric et al. reported that expression of MAPK phosphatase-1 (MKP-1), which dephosphorylates MAPK, is increased in the hippocampus of depressed humans and chronically mildly stressed mice [7].

In this study, we analyzed the MAPK cascade, including the expression of MKP-1, in the hippocampus of chronic social defeated rats.

We used male Wistar rats ($n = 8$, 9 weeks, Charles River) as the intruder. The rats were housed individually at room temperature ($22 \pm 1\,^{\circ}C$), with lights on from 06:00 to 18:00 and *ad libitum* access to food and water. Before the beginning of the stress exposure, they were handled daily for 1 week to habituate them to the environment. We used male Wistar rats ($n = 8$, 12 weeks, our colonies of Ibaraki University) as residents, and they were housed in a large

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cage (L 60 cm × W 45 cm × H 45 cm) with a 12-week-old sterilized female rat. Before the beginning of the stress exposure, the residents were paired with sterilized females for 3 weeks to establish territorial dominance. All experimental procedures followed the Guidelines of the Animal Care and Use Committee of Ibaraki University.

Social defeat using the resident-intruder paradigm was induced as previously described, with some modifications [3,24]. The female rats were removed from their home cage, and an intruder rat was introduced into each resident's home cage for up to 1 h. Within the first 10 min, the intruder was usually attacked and defeated by the resident and would show subordinate behaviors, including vocalization, jumping, freezing and a submissive posture [15]. As soon as those behaviors appeared, the intruder was removed and kept for the remainder of the hour in a wire-mesh cage (L 17 cm × W 22 cm × H 23 cm) within the resident's home cage. Rats from the stress group were subjected to this social defeat procedure on a daily basis for 5 weeks, whereas the rats from the control group were handled at the same time every day.

Locomotor activity was measured using an open field test (OF). Each rat was placed in the same corner of the open field apparatus (L 60 cm × W 60 cm × H 60 cm; O'Hara & Co. Ltd., Tokyo, Japan). The total distance traveled (in cm) during the 10-min session was recorded, and the results were analyzed on a Windows computer using Image J XX (O'Hara & Co. Ltd.), a modified software program based on the public domain Image J program. The OF was performed at the end of the control phase (baseline) and again after 1 and 5 weeks of stress exposure.

The forced swim test (FS) was performed as previously described, with some modifications [18]. Each rat was placed into an acrylic cylinder (50 cm high, 22 cm diameter) filled with water (24 ± 1 °C) to a height of 18 cm. After 15 min, the animal was transferred to a 35 °C environment for another 15 min (pre-test). Twenty-four hours later, the rat was placed into the cylinder again for 5 min (test). The FS was performed after 5 weeks of stress exposure.

Body weight was measured at the end of the control phase (baseline) and at weekly intervals during the stress phase, and weight gain was calculated by subtracting the baseline body weight from the weight at the end of each week. At the end of the experiment, the animals were anesthetized and decapitated. The brains and adrenal glands were dissected quickly, the adrenal glands were weighed, and their weight as a percentage of body weight was calculated.

The rat brains were rapidly removed and chilled on ice, and the hippocampus was dissected out. The tissue was homogenized in ice-cold RIPA buffer (50 mM Tris–HCl pH 7.4, 150 mM NaCl, 1% NP-40, 0.75% sodium deoxycholate, 1 mM EDTA, 100 mM NaF, 2 mM Na_3VO_4 and a protease inhibitor mix (GE Healthcare)) with a Polytron homogenizer. The homogenate was centrifuged at 800 × *g* for 15 min at 4 °C, and the supernatant was collected.

SDS-PAGE, western blotting, and protein determination were performed as described elsewhere [9]. The primary antibodies used were anti-actin (1:1000), anti-MEK1/2 (1:1000), anti-phospho-MEK1/2 (1:1000), anti-ERK1/2 (1:2500), anti-phospho-ERK1/2 (1:2000) and anti-MKP-1 (1:500). Detection was performed using ECL plus western blotting detection reagents (GE Healthcare) and LAS-3000mini (FUJIFILM).

Data were analyzed using Excel Toukei 2006 for Windows (Social Survey Research Information Co. Ltd., Tokyo, Japan). The weight of the adrenal glands, immobility time in FS and western blotting data were analyzed using Student's *t*-test. The body weight gain and locomotor activity in the OF were analyzed using two-way repeated measures ANOVAs followed by Bonferroni post-hoc tests.

Socially defeated rats gained less body weight than control rats (Fig. 1A). Before exposure to stress, the body weights of the stress and control groups were similar (365.5 ± 13.7 g versus 373.6 ± 19.0 g, $p > 0.1$). A two-way repeated measures ANOVA revealed that stress ($F(1, 70) = 26.12$, $p < 0.001$) and the stress × time interaction ($F(5, 70) = 12.35$, $p < 0.001$) had significant effects on body weight gain. Subsequently, the Bonferroni post-hoc tests revealed a significant difference between the stressed and control rats for each week ($p < 0.001$). The inhibition of body weight gain is a major symptom of depression that is observed in various depression models in rodents [21,22,24]. Accordingly, we observed that the body weight gain of rats in our social-defeat model of depression rats was suppressed. We also observed that socially defeated rats had a significantly higher ratio of adrenal gland weight to body weight than the control rats (0.0160 ± 0.0008% versus 0.0130 ± 0.0011%, $p < 0.05$; Fig. 1B). Hypertrophy of the adrenal glands is also a major symptom of depression in depression-model animals [24].

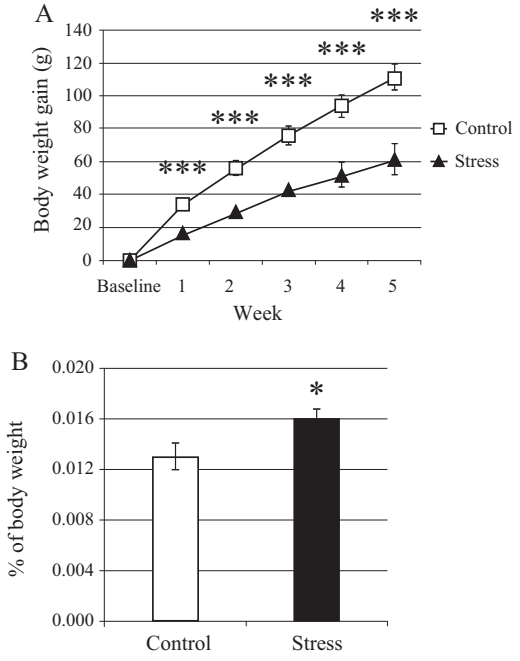

Fig. 1. Effects of chronic social stress on body weight gain and adrenal weight. (A) Body weight gain was calculated relative to the initial (baseline) body weight. A two-way repeated measures ANOVA showed that stress ($p < 0.001$) and the stress × time interaction ($p < 0.001$) had significant effects on body weight gain. ***$p < 0.001$ (Bonferroni post-hoc test). (B) Adrenal gland weight was calculated as a ratio of the total body weight at the end of the experiment. ***$p < 0.001$ (Student's *t*-test). Data represent the mean ± S.E.M. ($n = 8$/group).

Further, we tested the effects of chronic social stress on locomotor activity in the OF and immobility in FS. The locomotor activities in the OF were similar in the stress and control groups (Fig. 2A). A two-way repeated measures ANOVA showed that stress ($F(1, 28) = 1.93$, $p > 0.1$) and the stress × time interaction ($F(2, 28) = 0.14$, $p > 0.1$) did not significantly affect locomotor activity in the OF (Fig. 2A), and the time spent in the center of the OF or the entries into the center of the OF was similar in the stress and control groups (data not shown). We did not observe decreased locomotor activity in the stressed rats, whereas other studies using OF showed decreased [24] or unchanged [29] locomotor activity in socially defeated rats. In another depression model, the locomotor activity of mice receiving chronic corticosterone treatment was unchanged in the OF [6]. These discrepancies may be due to variations in animals, stresses and environments [5]. In contrast, the immobility time in the FS was significantly longer in the stress than in the control group (245.3 ± 6.1 s versus 206.0 ± 8.9 s, $p < 0.01$; Fig. 2B). Prolonged immobility time in the FS was used as a proxy for the

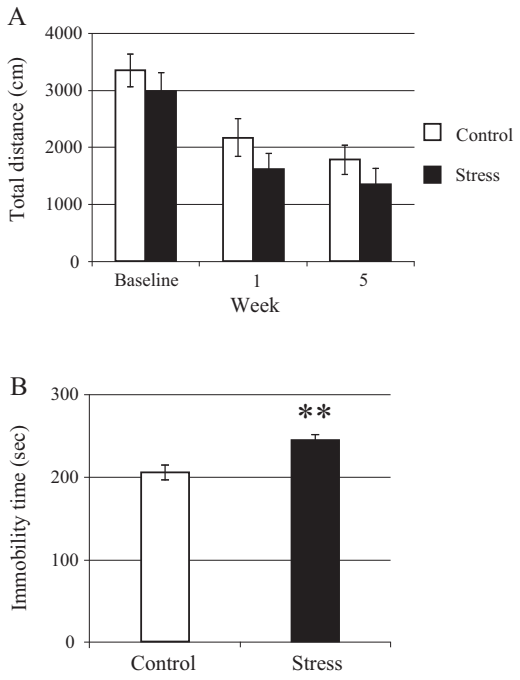

Fig. 2. Effects of chronic social stress on behavioral tests. (A) The effects of 1 and 5 weeks of chronic social stress on locomotor activity in the open field test. The total distance traveled was measured during a 10-min open field test. (B) The effects of 5 weeks of chronic social stress on behavioral despair in the forced swim test. The immobility time was measured during a 5-min forced swim test. $^{**}p < 0.01$ (Student's *t*-test). Data represent the mean ± S.E.M. ($n = 8$/group).

symptom of decreased motivation and behavioral despair [18], and prolonged immobility in the FS has been observed in chronically defeated rats [24], which was consistent with our results.

The expression of actin, MEK1/2 and ERK1/2 in the hippocampus was similar in the stress and control groups (data not shown), whereas the hippocampal ratio of phospho-MEK1/2/MEK1/2 (0.676 ± 0.063 versus 1.000 ± 0.098, $p < 0.05$), phospho-ERK1/ERK1 (0.563 ± 0.059 versus 1.000 ± 0.093, $p < 0.01$) and phospho-ERK2/ERK2 (0.643 ± 0.053 versus 1.000 ± 0.058, $p < 0.01$) were significantly lower in the stressed rats compared to the control rats (Fig. 3). Conversely, the expression of MKP-1 in the hippocampus was significantly higher in the stressed rats compared to the control rats (1.402 ± 0.041 versus 1.000 ± 0.109, $p < 0.05$) (Fig. 3).

Various molecular changes in the brain were reported in animal models of depression [29]. ERK1/2 are downstream targets of serotonin and BDNF, which are pivotal molecules in depression [13]. ERK1/2 are phosphorylated by MEK1/2, leading to CREB phosphorylation [19]. BDNF, phospho-ERK1/2 and phospho-CREB were all found to be decreased in some brain regions in animal models of depression [16,20], and previous studies have shown that rats with depressive-like characteristics have decreased phospho-ERK2 levels in the hippocampus and prefrontal cortex [21]. Moreover, infusion of the MEK inhibitor U0124 into the hippocampus and prefrontal cortex of rats resulted in depression-like molecular, physiological and behavioral changes [22]. Decreased levels of phospho-ERK1/2 and decreased expression of ERK1/2 mRNA have been observed in the hippocampus and the frontal cortex of depressed suicide victims [8], whereas infusion of BDNF into the hippocampus has antidepressant effects [25]. Conversely, activated MAPK is dephosphorylated and inactivated by MAPK phosphatases (MKPs). MKP-1 is one of the dual specific MKPs that dephosphorylates ERK2, JNK and p38 [26]. MKP-1 is phosphorylated by ERK, and phospho-MKP-1 is ubiquitinated and degraded [12]. Recent reports showed that depressed patients and mice exposed to chronic mild stress have increased expression of MKP-1 in the hippocampus [7]. We found that phospho-MEK1/2 and phospho-ERK1/2 were decreased, and the expression of MKP-1 was increased in the hippocampus of rats exposed to chronic social defeat.

In conclusion, our data show that chronic social defeat using the resident-intruder paradigm induced depressive-like physiological, behavioral and molecular changes. Decreased levels of phosphorylated MEK1/2 and ERK1/2 in the hippocampus, increased expression of hippocampal MKP-1, suppressed body weight gain, enlarged adrenal glands, and increased the immobility time in the FS were all observed using this paradigm. Because these changes

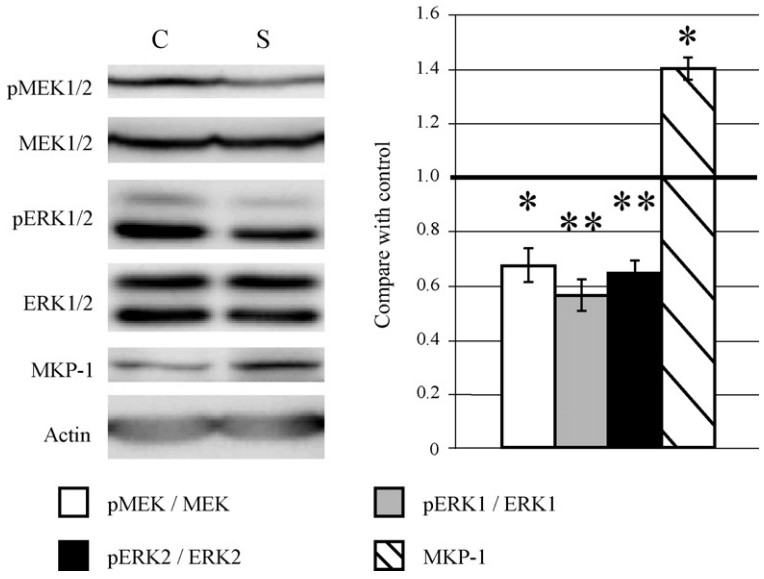

Fig. 3. Effects of chronic social stress on the MAPK cascade in the hippocampus. Western blotting of phospho-MEK1/2, MEK1/2, phospho-ERK1/2, ERK1/2, MKP-1, and actin are shown in the left panel. The ratio of each molecule in the hippocampus of stressed rats is shown in the right panel. Bands were normalized relative to actin and compared quantitatively using Image J software. $^{*}p < 0.05$, $^{**}p < 0.01$ (Student's *t*-test). Data represent the mean ± S.E.M.; C, control; S, stress.

represent some of the core symptoms of depression, our findings suggest that chronic social defeat stress induced depressive-like behavior and disrupted MAPK signaling in the hippocampus.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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