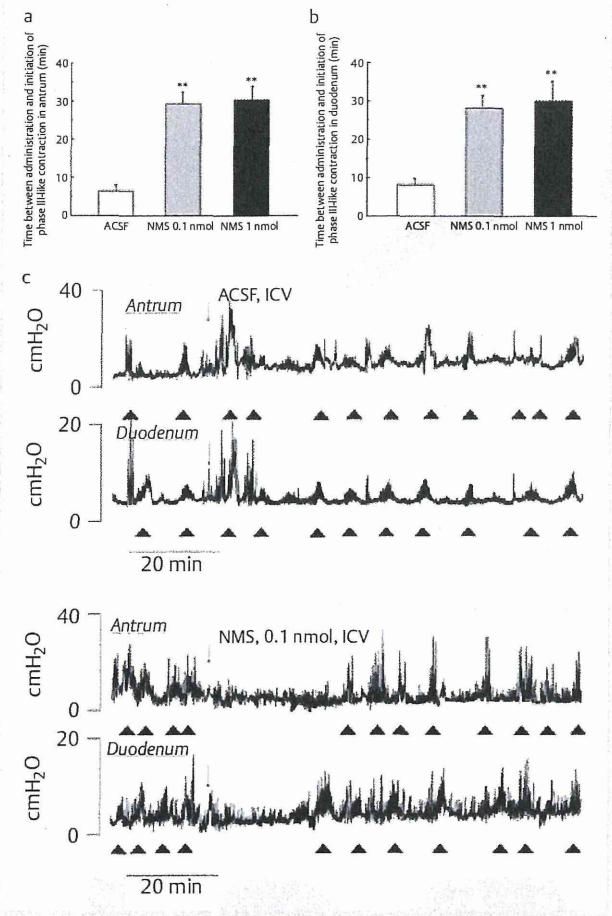

Fig. 2 Effects of intracerebroventricular (ICV) administration of neuromedin S (NMS) (0.1 and 1 nmol/mouse) on the fasted motor activity in the antrum (**a**) and duodenum (**b**). Time between the administration of increasing doses of NMS and the initiation of phase-like contractions in the antrum and duodenum. **c**: Representative tracing of antral and duodenal motility in fasted mice treated with ICV administration of NMS (0.1 nmol/mouse) or artificial cerebrospinal fluid (ACSF). The fasted motor pattern in the antrum and duodenum were disrupted by ICV administration of NMS. The phase-like contractions in fasted motility are indicated by arrowheads. Results are expressed as mean ± SEM (n=4–9); n indicates the number of mice used. ** $p < 0.01$ compared with ACSF treated control by Bonferroni's *t*-test.

Effect of ICV administration of NMS on gastroduodenal motility

When ICV administration of NMS (0.1 and 1.0 nmol/mouse) was given in the fasted state, the fasted motor pattern observed in the antrum and duodenum was replaced by the fed motor pattern, which consisted of irregular contractions that continued for 29.3 ± 2.9 min (0.1 nmol/mouse, n=4) and 30.4 ± 3.5 min (1 nmol/mouse, n=9) in the antrum and for 28.1 ± 3.0 min (0.1 nmol/mouse, n=4) and 29.7 ± 5.2 min (1 nmol/mouse, n=9) in the duodenum. These values were significantly longer ($p < 0.01$) compared with ICV administration of ACSF in the antrum (6.5 ± 1.6 min, n=8) and duodenum (8.0 ± 1.7 min, n=8) (◉ **Fig. 2a, b**). Representative tracing of antral and duodenal motility in fasted mice treated with ICV administration of NMS (0.1 nmol/mouse) is shown in ◉ **Fig. 2c**.

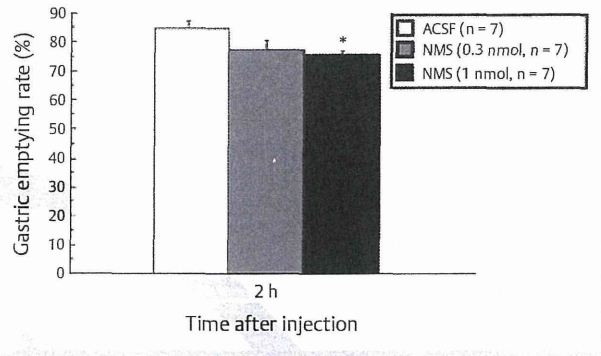

Fig. 3 Effects of intracerebroventricular (ICV) administration of neuromedin S (NMS) (0.3 and 1.0 nmol/mouse) on gastric emptying rate 2 h after administration. Results are expressed as mean ± SEM; n indicates the number of mice used. * $p < 0.05$ compared with artificial cerebrospinal fluid (ACSF) treated control by Bonferroni's *t*-test.

Effect of ICV administration of NMS on gastric emptying

Neuromedin S (1.0 nmol/mouse) produced a significant decrease in gastric emptying rate 2 h after ICV administration compared with ACSF treated control [75.8 ± 5.2% vs. 84.7 ± 3.0% (control); $p < 0.05$] (◉ **Fig. 3**). Administration of 0.3 nmol/mouse had a tendency to decrease gastric emptying 2 h after ICV administration, although this failed to reach statistical significance (77.5 ± 1.3%; $p < 0.07$).

Discussion

Feeding behavior is regulated by various hormones, including neuropeptide Y (NPY), leptin, and ghrelin. In particular, central neuropeptides in the hypothalamus play an important role in feeding behavior. In our study, centrally administered NMS decreased the food intake in both food-deprived mice and non-food-deprived mice. These results indicate that NMS has an anorexigenic activity, similar to those of other anorexigenic peptides such as corticotropin-releasing factor (CRF) peptides.

Very recently, Yayou et al. showed that centrally administered NMS increases plasma cortisol concentrations, thereby indicating that NMS participates in the control of the hypothalamo-pituitary-adrenal axis [24]. Additionally, it has been reported that central administration of NMS decreases food intake in food-deprived mice. The effects induced by NMS on feeding behavior were absent in NMUR-2-deficient mice [11]. Moreover, repeated administration of NMS was found to decrease body weight gain [25].

It has been shown that feeding behavior and gastrointestinal motility are closely related; feeding stimulatory peptides such as NPY and ghrelin induce motor activity in the gastrointestional tract in food-deprived mice [26,27], whereas feeding inhibitory peptides such as CRF, urocortin [28–30], bombesin [31], and cholecystokinin [32] disrupt the motor activity in the gastrointestional tract of food-deprived mice.

Very recently, we have reported manometric methods to measure the physiologically fed and fasted motor activities in the gastrointestinal tract of conscious mice [33]. When NMS was administered ICV in the food-deprived state, the patterns of antral and duodenal motility were disrupted and motor patterns like those observed in fed state ware observed. These results indicate that

NMS influences gastroduodenal motility, similar to the effect of other feeding inhibitory peptides. In addition, it has been shown that the gastric emptying rate is decreased by the ICV administration of anorexigenic peptides such as those belonging to the CRF peptide family. In our study, the ICV administration of NMS decreased gastric emptying rate. Neuromedin S at the lower dose (0.1 nmol/mouse) affected fasted motor pattern in the antrum and duodenum, whereas NMS only at the higher dose (1.0 nmol/mouse) delayed gastric emptying in conscious mice. These findings may represent different sensitivity between manometric method and solid gastric emptying in detecting the inhibitory effects of anorexigenic peptides on the upper gut motility.

In conclusion, we have described here for the first time the effects of NMS on gastroduodenal motility and gastric emptying in mice. Our findings indicate that NMS influences feeding behavior and gut motility. A better understanding of the role of NMS may provide an entirely new therapeutic approach for the treatment of various diseases, including obesity, diabetes mellitus, eating disorders, and functional gastrointestional disorders, all of which have become increasingly prevalent globally.

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Journal of Neuroscience Research

Journal of Neuroscience Research 88:1890–1897 (2010)

Chronic Foot-Shock Stress Potentiates the Influx of Bone Marrow-Derived Microglia Into Hippocampus

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For several years, a new population of microglia derived from bone marrow has been described in multiple settings such as infection, trauma, and neurodegenerative disease. The aim of this study was to investigate the migration of bone marrow-derived cells to the brain parenchyma after stress exposure. Stress exposure was performed in mice that had received bone marrow transplantation from GFP mice, allowing identification of blood-derived elements within the brain. Electric foot-shock exposure was chosen because of its ability to serve as fundamental and physical stress in mice. Bone marrow-derived GFP^+ cells migrated to the ventral part of the hippocampus and acquired a ramified microglia-like morphology. Microglia marker Iba1 was expressed by 100% of the ramified cells, whereas ramified cells were negative for the astrocyte marker GFAP. Compared with the case in the control group, ramified cells significantly increased after chronic exposure to stress (5 days). One month after 5 days of stress exposure, ramified cells significantly decreased in ventral hippocampus compared with the group examined immediately after the last stress exposure. We report for the first time the migration of bone marrow-derived cells to the ventral hippocampus after stress exposure. These cells have the characteristics of microglia. Mechanisms responsible for this migration and their roles in the brain remain to be determined.

Key words: bone marrow-derived microglia; stress; hippocampus; mice

Stress exposure modulates a behavioral and neuroendocrine response pattern, producing many responses that are elicited by infectious or inflammatory stimuli, including fever, aphagia, hypothalamic–pituitary–adrenal axis activation, reduced social interaction, and changes in acute-phase proteins (O'Connor et al., 2003). Microglia are described as the resident macrophages of the brain parenchyma, and there is increasing interest regarding their implications in different pathological conditions such as infection, ischemia, neurodegenerative disorder, brain injury, feeding behavior, and stress. Indeed, stress conditions induce proliferation of microglia (Nair and Bonneau, 2006), morphological changes (Sugama et al., 2007), expression of phagocytic markers, or activation of major histocompatibility complex II (MHCII; Frank et al., 2007), and production of proinflammatory cytokines (O'Connor et al., 2003).

Microglia response is commonly associated with the proliferation of resident microglia, migration from other areas of the brain, and blood monocyte migration (Bechmann et al., 2005). Bone marrow-derived microglia have already been studied during infection conditions (Djukic et al., 2006), after axonal degeneration (Bechmann et al., 2005), and in some models of neurode

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Contract grant sponsor: University Hospital and the Faculty of Medicine of Amiens (to M.B.); Contract grant sponsor: Le Conseil regional de Picardie (to M.B.); Contract grant sponsor: Ministry of Education, Culture, Sports, Science and Technology, Japan; Contract grant number: 18390100 (to H.Ko.); Contract grant sponsor: President's Discretionary Fund from Shiga University of Medical Science; Contract grant number: 1515508B (to H.Ko.).

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Received 24 August 2009; Revised 16 November 2009; Accepted 21 November 2009

Published online 12 February 2010 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jnr.22362

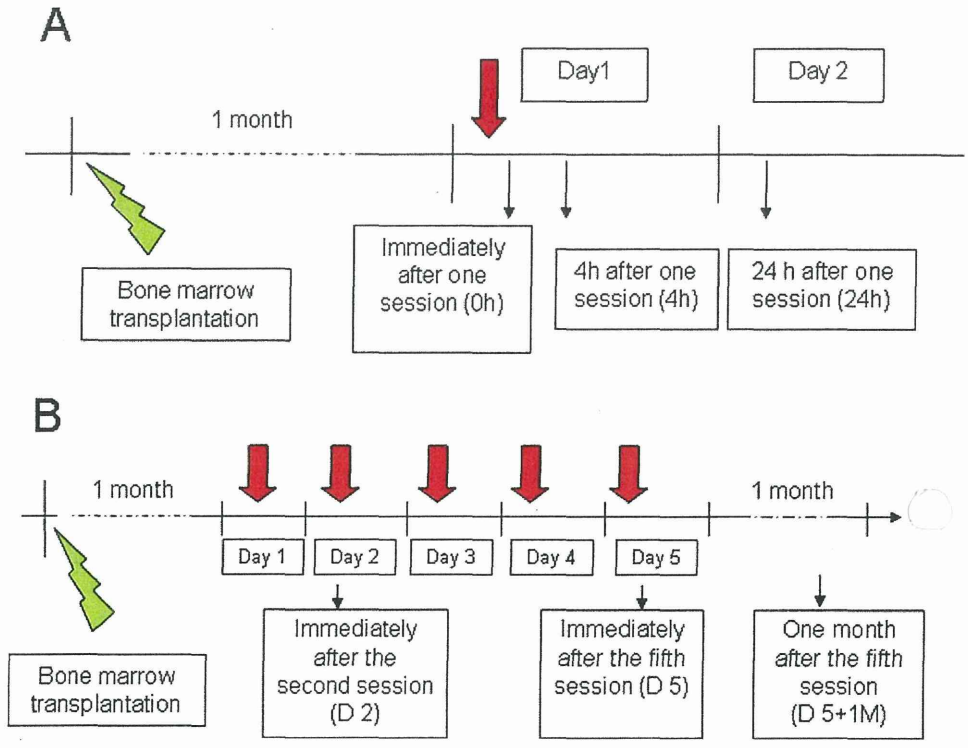


Fig. 1. **A:** Acute stress exposure. A 1-hr stress session is given one time, and mice are killed immediately, 4 hr, or 24 hr after the session. **B:** Chronic stress exposure. One-hour stress session is given two or five times, and mice are killed immediately after the second session, immediately after the fifth session, or 1 month after the fifth session.

generative disease (Wu et al., 2000; Simard et al., 2006; Priller et al., 2006; Rodriguez et al., 2007); however, the contribution of bone marrow-derived microglia in stress conditions has never been examined.

Among various stress models, including restraint or immobilization, water avoidance, and force swimming stress, we chose electrical foot-shock stress in the present study. Such a foot-shock model is based on the pain paradigm, which has been used for decades as a fundamental approach for stress induction (Blackburm-Munro and Blackburm-Munro, 2001). Therefore foot-shock stress seems to be a physical stimulus compared with fear- and anxiety-based paradigms, such as water avoidance or force swimming stress model. The aim of this study was to examine the migration of bone marrow-derived microglia into the brain after stress exposure. Because we sought to examine a totally new hypothesis, we chose a foot-shock stress model because of its ability to serve as a fundamental and physical stimulus in mice.

MATERIALS AND METHODS

Preparation of Animals and Stress Exposure

Animals. Adult female C57BL/6 mice were purchased from Clea Japan Inc. and adapted to standard laboratory conditions, then subjected to bone marrow transplantation at 10–12 weeks of age. GFP mice (C57BL/6-Tg (UBC-GFP, 30 Scha; The Jackson Laboratory, Bar Harbor, ME) were used as donors at 2 months of age. All procedures were approved by the Ethics Committee of Shiga University of Medical Science.

Bone marrow transplantation. The bone marrow cells of GFP mice were aseptically harvested by flushing the femur, tibia, and humerus with PBS using a syringe with a 26-ga needle. The samples were filtered through a 70-μm mesh and then centrifuged. Recovered cells were resuspended in PBS at a concentration of 4×10^6 cells/100 μl. Recipient mice were exposed to 9 Gray total-body irradiation administered in one fraction. Between 30 min and 2 hr later, the animals were injected via a tail vein with 4×10^6 bone marrow cells freshly collected from GFP mice.

Foot-shock stress exposure. One month after the bone marrow transplantation, mice were randomly separated. Those that were exposed to stress were individually placed into a foot-shock stress box (Trentani et al., 2003). In this device, they received, during a 1-hr session, 120 electrical foot-shocks (0.15 mA, shocks of 5 sec every 30 sec) through an electrified grid floor. For the acute stress exposure, mice received one session of stress and were sacrificed immediately or at 4 hr or 24 hr after the end of the stress session (Fig. 1A). For the chronic stress exposure, mice underwent a 1-hr session of stress (same parameters) each day at the same time and were sacrificed immediately after the second stress session (day 2), immediately after the fifth stress session (day 5), or 1 month after the fifth stress session (day 5 + 1 month; Fig. 1B). The number of mice was five in each group.

For control mice, the stress session was replaced by 1 hr in the same foot-shock stress box without electrical foot shock. Another control was an unmanipulated group that was left undisturbed in the home cage. For all experiments, stress exposure was given between 9:00 AM and 3:00 PM.

Morphological Analysis

Tissue preparation. For morphological analysis, animals were anesthetized at the indicated times after the stress session or control session, and transcardially perfused with 0.01 M PBS for 5 min, followed by ice-cold 4% paraformaldehyde, 0.5% glutaraldehyde, and 0.2% picric acid in 0.1 M PB for 10 min. The brain was removed, postfixed with 4% paraformaldehyde and 0.2% picric acid in 0.1 M PB for 24 hr at 4°C, and then placed in 15% sucrose solution. Coronal sections through the entire brain were cut into 20-μm slices in a cryostat. All sections were collected in 0.1 M PBS containing 0.3% Triton X-100 (PBST) and stored a 4°C until histological analysis. Every tenth section was mounted on a gelatin-coated glass slide, and nuclear staining was achieved with propidium iodide. The sections were then observed under a confocal laser scanning microscope (LSM 510; Carl Zeiss).

Quantitative analysis for GFP$^+$ cells. To exclude blood cells, perivascular cells, and brain macrophages, only ramified GFP$^+$ cells within brain parenchyma were analyzed. In our preliminary study, GFP$^+$ cells in every tenth sections of whole brain were counted in three specific areas including the amygdala (basolateral nuclei), the hypothalamus, and the hippocampus (at the position of −0.94 to −3.54 mm from bregma). Total number of GFP$^+$ cells in all sections from each brain region was compared between controls and stress groups in 0 hr, 4 hr, 24 hr, day 2, and day 5 conditions. In the second step of the experiments, only the ventral hippocampus was selected for further analysis. Total number of GFP$^+$ cells in every tenth sections between −2.2 to −3.54 mm from bregma was compared between control and stress groups in 0 hr, 4 hr, 24 hr, day 2, day 5 and day 5 + 1 month conditions. Both sides of the hippocampus were examined.

Immunofluorescence staining. Free-floating sections from day 5 mice, from both control and stress groups, were incubated with primary antibodies (ionized calcium binding adaptor protein 1 (anti-Iba1), rabbit polyclonal antibody (sc-98468, Santa Cruz Biotechnology, 1/1,000), anti-glial fibrillary acidic protein (GFAP), goat polyclonal antibody (sc-6171, Santa Cruz Biotechnology, 1/1,000), or anti-MHCII NIMR-4, rat monoclonal antibody (sc-52548, Santa Cruz Biotechnology, 1/1,000) for 2 days at 4°C; rinsed three times in Triton X-100 containing PBS; then incubated with fluorescence-labelled secondary antibodies for 2 hr at room temperature (dilution 1/1,000). Photomicrographs were obtained from 20-μm-thick slices of the specimen observed under a confocal laser scanning microscope (LSM 510; Carl Zeiss)

Morphometric Analysis

For morphometric analysis, free-floating sections from control day 5 and stress day 5 groups were stained with anti-Iba1 antibody. Using the Image-Pro Plus software, we measured the area, the perimeter, the major axis, and the minor axis of Iba1$^+$/GFP$^+$ cells and Iba1$^+$/GFP$^-$ cells in the control and stress groups (n = 12 cells in each group).

Statistical Analysis

For quantitative statistical analysis, the total number of GFP$^+$ ramified cells in every tenth section of the ventral hippocampus of each mouse (from −2.2 to −3.54 mm from bregma) was considered. The quantitative histogram was created using the mean number of GFP$^+$ cells per ventral hippocampus. ANOVA tests from Origin 7 software were used to compare these groups (control vs. stress 0 hr groups; control vs. stress 4 hr groups; control vs. stress 24 hr groups; control vs. stress day 2 groups; control vs. stress day 5 groups; control vs. stress day 5 + 1 month groups; control 0 hr vs. control day 5 groups; stress 0 hr vs. stress day 5 groups and stress day 5 vs. stress day 5 + 1 month groups). ANOVA tests were also used to compare the different groups in the morphometric analysis (for each parameter: control vs. stress groups and comparison of GFP$^+$/Iba-1$^+$ vs. GFP$^+$/Iba-1$^-$ cells). Fisher tests (two-tailed, χ^2) were used in GraphPad software. A correlation was considered significant at $P < 0.05$.

RESULTS

Enhancement of Blood-Derived Microglia in Hippocampus After Stress Exposure

Different shapes of GFP$^+$ cells were observed in the brain 4 weeks after bone marrow transplantation. Elongated cells or round cells attached to the vessels were observed both in control and in stress groups. Brain macrophages are spindel-shaped or rounded (Davoust 2008) and localized next to the basal lamina or the small vessels or next to the meninges. Microglia, on the other hand, are localized within the brain parenchyma and present a ramified morphology. In the present study, we analyzed ramified cells as follows. More characteristically, we observed cells with ramified shape in different areas of the brain (amygdala, hypothalamus, hippocampus). Our preliminary study showed an increase of these ramified cells in the hippocampus compared with other areas, specifically between the control day 5 and the stress day 5 groups (data not shown). Inside the hippocampus, cells migrate preferentially to the ventral part, in the CA1 and CA3 areas (data not shown). Therefore, we concentrated our work on the ventral hippocampus as described in Materials and Methods.

Ramified cells were observed more frequently along the full length of the longitudinal axis of the ventral hippocampus, in proximity to the blood vessels (Fig. 2). They infiltrate the parenchyma and migrate into the molecular layer, the stratum radiatum, and the pyramidal cell layer of CA1 and CA3. Only a few GFP$^+$ cells were observed in the dorsal hippocampus in the control, as in the stress group.

After five sessions of stress, ramified GFP$^+$ cells were significantly increased in the hippocampus (Fig. 3). At day 5, ramified cells were significantly increased in the stress group compared with the control group ($P = 0.004$, n = 5). Furthermore, ramified cells were increased significantly in the stress day 5 group vs. the stress 0 h group ($P = 0.0005$, n = 5). There was no difference between stress and control groups in 0, 4, 24 hr or day 2 conditions (Fig. 3).

One month after five stress sessions (day 5 + 1 M), ramified cells were significantly decreased in the ventral hippocampus compared with the stress day 5 group ($P = 0.003$, n = 5; Fig. 3). There was a statistical difference between control 0 hr group and control day 5

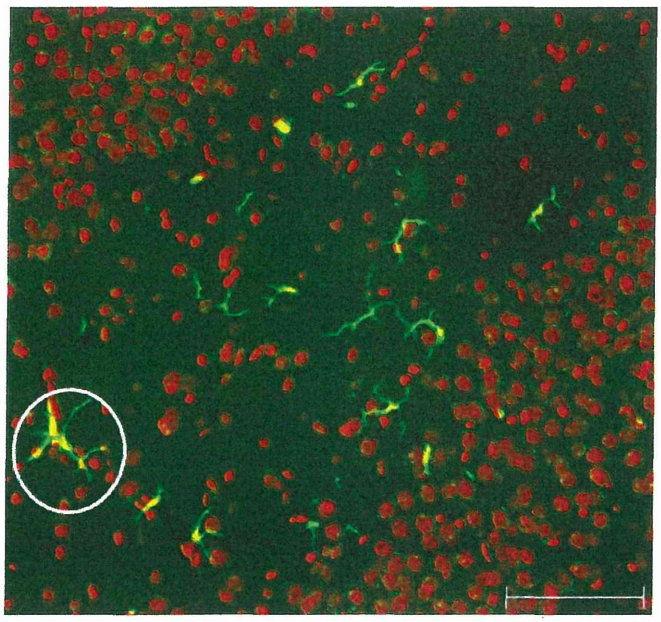


Fig. 2. Migration by extravasations of GFP^+ cells in the ventral part of the hippocampus. GFP^+ cells arrive from the vessel (indicated by a circle) and migrate into the brain parenchyma. Nuclei are colored in red by propidium iodide. Scale bar = 100 μm.

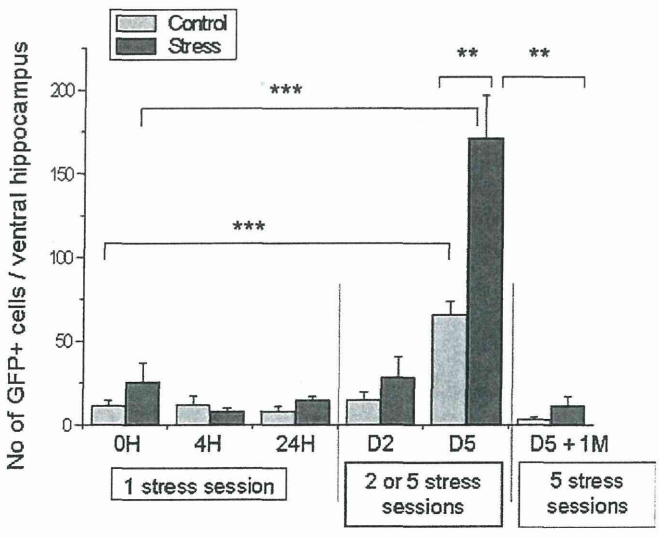
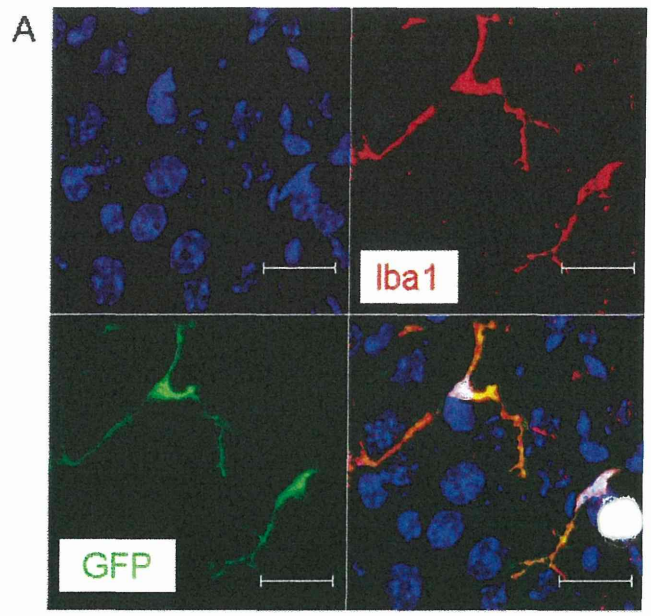


Fig. 3. Quantitative analysis for density of GFP^+ cells after stress exposure. There is a significant increase in GFP^+ cells in the ventral hippocampus of mice killed immediately after five sessions of stress compared with the control day 5 group ($P = 0.004$). Significant differences are found between stress 0 hr and stress day 5 ($P = 0.0005$) and between control 0 h and control day 5 ($P = 0.0002$). When the mice are killed after 1 month without any stress, GFP^+ cells are decreased compared with mice killed immediately after the last, fifth session ($P = 0.003$). Values are means ± SD from five mice. $^{**}P < 0.005$, $^{***}P < 0.0005$

groups ($P = 0.0002$, n = 5; Fig. 3). Furthermore, significant difference was detected between unmanipulated control group (12.4 ± 3.5, n = 5) and control day 5 group (66.0 ± 8.2, $P = 0.0003$, n = 5).



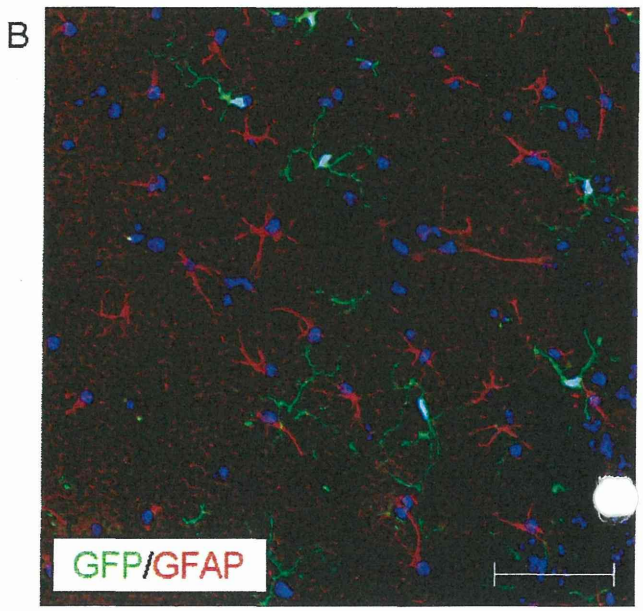


Fig. 4. Characterization of the GFP^+ cells. **A:** GFP^+ cells express Iba1, a marker for microglia (red). Nuclei are stained in blue by DAPI. **B:** GFP^+ cells do not express GFAP (red), a marker for astrocytes. Nuclei are stained in blue by DAPI. Scale bars = 20 μm in A; 100 μm in B.

Immunoexpression of Blood-Derived Microglia

First, we identified GFP^+ ramified cells observed in the brain by immunohistochemistry. Results showed that 100% of these cells were strongly positive for Iba-1, a marker of microglia as well as monocytes–macrophages (Fig. 4A). GFAP is usually expressed by astrocytes. None of the ramified GFP^+ cells was positive for GFAP (Fig. 4B, GFAP stained red).

With Iba-1 antibody reaction, we observed a significant increase in the number of microglia ($Iba1^+$ cells) in the stress day 5 group (55.4 cells ± 3.2/field × 10) compared with the control day 5 group (43.5 cells ± 2.1/field × 10; $P = 0.01$). Among $Iba1^+$ cells, $Iba1^+/GFP^+$ cells accounted for 4.5% in the control day 5 group and 9.6% in the stress day 5 group.

We next examined the activation of microglia after five sessions of stress exposure by MHCII staining. Although we failed to observe $MHCII^+/Iba1^+/GFP^+$ cells in the control day 5 group (0/14 of $Iba1^+/GFP^+$ cells), we observed that 9.9% of $Iba1^+/GFP^+$ cells were $MHCII^+$ in the stress day 5 group (10/101 of $Iba1^+/GFP^+$ cells). On the other hand, resident microglia ($Iba1^+/GFP^-$ cells) were negative for MHCII in both control and stress day 5 groups (0/50). There was a statistically significant difference in $MHCII^+$ staining between GFP^+ microglial cells and GFP^- microglial cells in the stress day 5 group (10/101 vs. 0/50, respectively, $P = 0.03$). This difference was not observed in the control day 5 group because of a low number of cells available for analysis.

Difference of Morphology Between Blood-Derived Microglia and Resident Microglia

To examine the activation of bone marrow-derived microglia, we measured the area, the perimeter, the major axis, and the minor axis of this population and compared these data with those from resident microglia. A statistically significant difference was observed between the morphology of resident microglia ($Iba1^+/GFP^-$ cells) and that of bone marrow-derived microglia ($Iba1^+/GFP^+$ cells), which were smaller than resident microglia ($P < 0.005$ for all parameters). However, there was no difference observed between control and stress groups (Fig. 5A–E).

DISCUSSION

In the present study, we report for the first time that chronic foot-shock stress exposure potentiates the influx of bone marrow-derived cells into the brain parenchyma compared with unstressed animals. Bone marrow-derived cells migrate by extravasation into the ventral hippocampus and differentiate into microglia.

Recruitment of bone marrow-derived microglia in the brain has been studied in various conditions, such as infection (Djukic et al., 2006), axonal degeneration (Bechmann et al., 2005), and neurodegenerative disease (Wu et al., 2000; Simard et al., 2006; Priller et al., 2006; Rodriguez et al., 2007). For these conditions, however, although the exact role of the bone marrow-derived microglia in these brain diseases remains unclear, it can be suggested that, because of the brain lesions, immunological signals might induce the migration of blood-derived microglia to help the resident microglia in repair of the brain. Indeed, bone marrow-derived microglia could play a role in phagocytosis after neuron degeneration (Djukic et al., 2006) or may be able to prevent the formation of amyloid deposits in models of mice with Alzheimer's disease (Simard et al., 2006).

In this study, after five sessions of electrical foot-shock exposure, we observed the migration of bone marrow-derived microglia to the ventral hippocampus. The hippocampus plays a prominent role in some forms of trace conditioning, and the ventral hippocampus, in particular, plays an important role in trace fear conditioning (Yoon and Otto, 2007). Anatomically, the ventral, but not the dorsal, hippocampus makes a direct connection to the amygdala, which is implicated in anxiety and fear behavior (Yoon and Otto, 2007). Hippocampal atrophy, mediated by glucocorticoid secretion, has been suggested after stress exposure (Sapolsky, 2001; Conrad, 2006). However a recent study demonstrated that the decrease in hippocampal volume occurs only after 14 days of corticosteroid secretion (Murray et al., 2008). It is unlikely that the increased number of GFP^+ cells observed in this study was due to hippocampal atrophy.

The migration of the bone marrow-derived microglia increased significantly after five sessions of foot-shock stress exposure. No difference was observed in other groups after only one session. In the literature, 48 hr seems to be the minimum of delay to observe a migration of blood-derived microglia to the brain (Bechmann et al., 2005; Djukic et al., 2006). That could explain the absence of migration at 0, 4, or 24 hr after one stress session.

It is interesting that there is a significant increase in the migration of bone marrow-derived microglia to the ventral hippocampus in the control day 5 group compared with the control 0 hr group. To deny the possibility that the influx of GFP^+ cells into the hippocampus simply constitutes a basal repopulation of brain microglia resulting from routine turnover of resident microglia, we compared the control day 5 group and the unmanipulated control group. Results showed that the control day 5 group showed a significant increase in migration of bone marrow-derived cells compared with unmanipulated controls. Therefore, the routine manipulation of mice such as from the cage to the foot-shock stress box for 5 days possibly constitutes a chronic "psychological" stress and may be sufficient to provoke a migration of bone marrow-derived cells into the brain.

Because it was suggested that microglia could present an activated morphology after stress exposure (Simard and Rivest, 2004; Djukic et al., 2006; Frank et al., 2007), a morphometric analysis of microglial cells was done, and we also looked for an expression of MHCII. The morphometric analysis showed that GFP^+ microglia were smaller than resident microglia, which possessed long and thin arms. This morphology implies an activation of GFP^+ cells, and in fact there was a statistically significant increase in the expression of MHCII in GFP^+ cells compared with GFP^- cells in the stress day 5 group. Nevertheless, morphometric parameters such as area, perimeter, and major and minor axes in GFP^+ cells between control day 5 and stress day 5

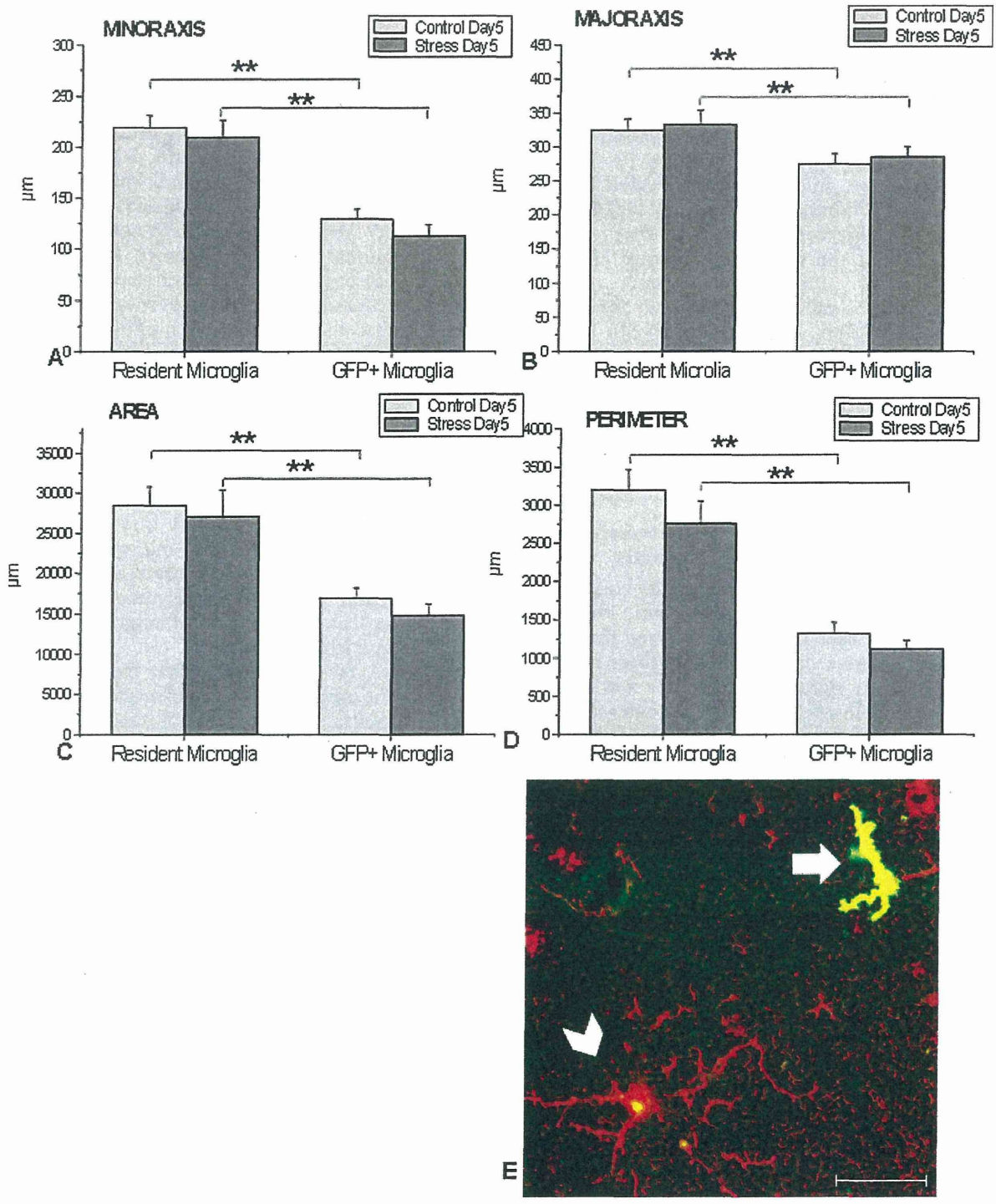


Fig. 5. Morphometric analysis study for GFP^+ and resident (GFP^-) microglia. Minor axis (**A**), major axis (**B**), area (**C**), and perimeter (**D**) are measured in control day 5 and stress day 5 groups (n = 12 cells per group). A significant difference ($P < 0.005$) is found between GFP^+ and resident microglia in both stress and control groups for all parameters. However, no difference is found between control and stress groups in both GFP^+ and resident microglia for all parameters. **E:** Difference in morphology between resident microglia (arrowhead) and GFP^+ microglia (arrow). Microglia is stained by Iba-1. GFP^+ cells are smaller than resident microglial cells, which have long, thin arms. Scale bar = 20 µm.

groups were identical, suggesting that morphological change of microglia may not occur in different types of stress, including foot-shock stress or psychological stress. In this study, we failed to observe any resident microglia positive for MHCII, suggesting that there was a difference in MHCII expression between resident microglia and bone marrow-derived microglia.

Frank et al. (2007) reported that MHCII expression was not detectable in quiescent microglia but was induced by inescapable shocks in the hippocampus parenchyma (Frank et al., 2007). On the other hand, Sugama et al. (2007) failed to observe any expression of specific markers for activation after stress exposure, suggesting that stress exposure induced only morphological change but not functional activation of chemical mediator in microglia (Sugama et al., 2007). It seems clear that "activated morphology" in GFP^+ cells is not always associated with the activation state (Davoust et al., 2008). With models of chimeric mice, Djukic et al. (2006) observed an expression of MHCII in GFP^+ microglial cells after meningitis, whereas Simard and Rivest (2004) observed the expression of MHCII in GFP^+ microglial cells without any pathological conditions. In our study, results showed that MCHII expression was not found in resident microglia but was found only in GFP^+ cells after foot-shock stress exposure. These phenomena might be important in considering functional significance in the microglial population newly recruited from the bone marrow.

In the control as in the stress groups, migration of bone marrow-derived microglia occurs without any brain lesions, suggesting that only physiological mechanisms are sufficient to promote an infiltration of blood monocytes. Furthermore, this new population represents only 4.5–13.5% of the total microglial population in the brain [4.5–9.6% in our study, 5–13.5% in the study of Djukic et al. (2006)]. In this condition, what roles do these newly recruited microglia play? Chronic stress induces CA3 dendrite atrophy, which is caused by secretion of glucocorticoid and reduced levels of brain-derived neurotrophic factor (Conrad, 2006). However, because the dendritic retraction occurs in 6 days following the stress exposure (Conrad, 2006), it seems unlikely that the bone marrow-derived cells could play a role in the dendritic retraction. A recent study suggests that the immune system is involved in the maintenance of spatial memory (Ron-Harel et al., 2008). Another study also demonstrates that the mitochondrial DNA damage in microglia could be involved in the impairment of age-dependent memory in mice, by impairment of hippocampal long-term potentiation (Hayashi et al., 2008). These findings support the idea that the microglial population plays a role in hippocampus-dependent memory. It seems too early to know whether the bone marrow-derived microglia plays a particular role in this memory acquisition.

In models of neuron degeneration, the bone marrow-derived microglia remains in the brain and is involved in the resolution of tissue damage (Djukic et al., 2006). In our model, the GFP^+ cells disappear from the hippocampus after removal of the stress stimulus. Is this due to a reverse migration from the brain to the blood or to normal apoptosis of the cells without continuous migration? Further studies will be necessary to answer this question, but the decrease of GFP^+ cells 1 month after removal of the stress stimulus supports the fact that a brain lesion does not occur after foot-shock stress exposure. Compared with other studies with chimeric mice, this point is most important, meaning that cell recruitment could occur without brain damage or inflammatory changes. The mechanisms involved in the migration of blood monocytes into the brain are unknown; however, neurotransmitters and chemoattractive molecules such as monocyte chemoattractive protein-1 (MCP-1 or CCL2) and its receptor CCR2 (Zhang et al., 2007) might be involved.

In conclusion, we report for the first time the migration of bone marrow-derived cells into the brain parenchyma after chronic electrical foot-shock stress exposure and show a decrease of these bone marrow-derived microglia after removal of the stress exposure. These cells migrate into the hippocampus and immediately acquire the characteristics of microglia. The function of these newly recruited cells and the molecules responsible for migration are unknown. Further investigation will be necessary to elucidate these mechanisms.

ACKNOWLEDGMENTS

The authors thank Dr. Hiroshi Urabe for advice and Dr. Lawrence Chan for reviewing the manuscript. The authors report no conflicts of interest.

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