Occlusal disharmony attenuates glucocorticoid negative feedback in aged SAMP8 mice

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Abstract
To evaluate the mechanism underlying impaired cognitive function due to occlusal disharmony, we examined the effect of the bite-raised condition on spatial performance and hippocampal expression of glucocorticoid receptors (GR) and glucocorticoid receptor messenger ribonucleic acid (GRmRNA) using behavioral, immunohistochemical, and in situ hybridization techniques. Learning ability in the water maze test was significantly impaired in aged bite-raised mice compared with age-matched control mice. There was no difference between control and bite-raised young and middle-aged mice. Also, immunohistochemical and in situ hybridization analysis showed that the bite-raised condition enhanced the age-related decrease in GR and GRmRNA expression in the hippocampus. In particular, GR and GRmRNA expressions were significantly decreased in aged bite-raised mice compared to age-matched control mice. These findings suggest that the bite-raised condition in aged SAMP8 mice decreases GR and GRmRNA, which impairs the hypothalamic-pituitary-adrenal feedback inhibition, thereby leading to memory deficits.

Keywords: Occlusal disharmony; Hippocampus; Stress; Glucocorticoid receptor; Glucocorticoid receptor mRNA; SAMP8 mice

Occlusal disharmony, such as loss of teeth and/or increases in the vertical dimension of crowns, bridges, or dentures, causes bruxism or pain in the masticatory muscles and temporomandibular joint [2], and general malaise [22]. The application of occlusal splints elevates urinary cortisol excretion rates in monkeys [1], and the placement of acrylic caps on the lower incisors increases plasma corticosterone levels in rats [25]. In recent studies using accelerated senescence-prone mice (SAMP8), the bite-raised condition in aged mice increased plasma corticosterone levels and neuronal death in the hippocampal CA3 region, and impaired spatial learning in the Morris water maze test [9]. These findings suggest that stress is involved in the occlusal disharmony-induced hippocampal-dependent changes.

Glucocorticoid (GC) secretion is negatively regulated by GCs at the level of the anterior pituitary gland [14], suprathyroidic limbic structures (e.g., hypothalamus, hippocampus, and amygdale), and the mesoprefrontal system [3,11]. In particular, hippocampal corticosteroid receptors appear to be sensitive to elevated GC levels [17] and these receptors or their mRNA are downregulated by chronic stress [5,18]. These findings suggest that the bite-raised condition-induced impairment of spatial memory in the aged mice might be due to reduced negative feedback response to chronic stress.

In this study, using immunohistochemical and in situ hybridization techniques, we examined the effect of the bite-raised condition on the expression of GR and GRmRNA, together with changes in learning ability in the Morris water maze.

Male SAMP8 mice (3-, 5-, and 9-month-old; n = 12 for each age) were used in the study. The animals were bred and maintained under conventional conditions; housed in groups of five in plastic cages under temperature (23 ± 1 °C), humidity (55 ± 2%), and light (12 h; light period, 6:00–18:00; dark period, 18:00–6:00) controlled conditions; and allowed free access to food and

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water. The mice were treated in accordance with the principles approved by the Council of the Japanese Neuroscience Society.

The bite-raising procedure was performed under sodium pentobarbital anesthesia (35 mg/kg). The vertical dimension in the bite was raised as previously described [9]. The vertical dimension in the bite was raised approximately 0.1 mm by placing ultraviolet-ray polymerization resin (UniFil®LoFlo, GC Corporation, Tokyo, Japan) on the upper molars after treatment with a Single Bond Dental Adhesive System (3M Dental Product, USA). Control animals underwent the same anesthetic procedure except that no resin was applied. After the procedure, the mice were allowed free access to pelleted chow and water.

The Morris water maze test is a sensitive behavioral assay for brain abnormalities in the hippocampus [20]. Eight days after the bite-raising procedure, Morris water maze training was performed as described previously [8,9,24]. Briefly, a stainless steel tank (90 cm in diameter, 30 cm deep) was filled with water (approximately 28 °C) to a height of 22 cm and the water surface was covered with floating polystyrene foam granules (approximately 2 mm in diameter). A platform (12-cm in diameter) was submerged 1 cm under the water surface and located at a constant position near the center of one of the four quadrants of the pool. Seven days after the bite-raising procedure, the mice were placed into the water from one of four points at the perimeter of the tank with an intertrial interval of approximately 5 min and given 28 trials over 7 consecutive days (4 trials per day). The sequence of the starting positions was randomized daily. A CCD video camera linked to a computer system (Move-tr/2D, Library Co., Ltd., Tokyo, Japan) was used to measure the latency of each mouse to reach the platform. In our previous study [7], there was no significant difference in the probe trial performance between the control and bite-raised condition in 9-mo-old SAMP8 mice.

For immunohistochemical analysis of GR induction in the hippocampal formation, on the final training day (day 7), 5 mice of each group were anesthetized with pentobarbital sodium (40 mg/kg) and perfused transcardially with 30 ml of saline at 37 °C, followed by 100 ml of 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. The brain was then removed and immersed briefly in 5 × SSC, then washed in 50% formamide/2 × SSC for 30 min at 55 °C. After rinsing in TNE (10 nmol/l Tris–HCl, pH 7.6; 1 mmol/l EDTA, 0.5 M NaCl) for 10 min at 37 °C, the sections were treated with 10 μg/ml RNase A (Roche Diagnostics) for 30 min at 37 °C. After rinsing in TNE for 10 min at 37 °C, the sections were washed sequentially in 2 × SSC, 0.2 × SSC, and 0.1 × SSC for 20 min each at 55 °C. After rinsing in TBS (0.01 mol/l Tris–HCl, pH 7.5, 150 mmol/l NaCl) for 20 min, the sections were reacted with a 1:400 diluted horseradish peroxidase-conjugated rabbit anti-DIG F(ab') fragment antibody (Dako), 0.07 μmol/l biotinylated tyramide solution, and avidin (Dako Cytomation) diluted 1:500 with TBS for 1 h at room temperature. Bound complex was visualized using 3,3′-diaminobenzidine (0.5 mg/ml) and hydrogen peroxide (0.01%) in TBS. Controls sections were treated with non-immune rabbit immunoglobulin instead of primary antibody.

Fig. 1. Spatial learning in the water maze test. The results are expressed as the mean score (mean ± S.E., n = 6 for each group) of four trials per day. Note that 9-mo-old bite-raised mice required a significantly longer time to reach the platform than age-matched controls.
Fig. 2. Photomicrographs showing GR immunoreactivity in the hippocampal formation (A) and CA1, CA3, and DG subfields (B). Note that there were no GR-immunoreactive cells (arrow in A) and there were fewer GR-immunoreactive cells within the CA1 and DG regions of 9-mo-old bite-raised mice, but there were no differences in CA1 and DG regions of 3- and 5-mo-old bite-raised mice (B). Bars: 200 μm (A) and 50 μm (B).

Table 1

<table>
<thead>
<tr>
<th>Time at 7 days</th>
<th>GRCA1</th>
<th>GRDG</th>
<th>GRmRNACA1</th>
<th>GRmRNACA3</th>
<th>GRmRNADG</th>
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<td>GRCA1</td>
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<td></td>
<td></td>
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<td>GRDG</td>
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<td>0.89**</td>
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<td>0.712**</td>
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<tr>
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<td>0.778**</td>
<td>0.77**</td>
<td>0.862**</td>
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<tr>
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<td>0.718**</td>
<td>0.735**</td>
<td>0.822**</td>
<td>0.892**</td>
</tr>
</tbody>
</table>

Time at 7 days, the time to reach the platform at 7 days; GRCA1 and GRDG, the number of GR positive cells in hippocampal CA1 and DG subfields, respectively; GRmRNACA1, GRmRNACA3, and GRmRNADG, the number of GRmRNA signals in hippocampal CA1, CA3, and DG subfields, respectively. **P<0.01, note there were strong correlations between all factors.
Because this finding was identical to that of other reports [14], the GR immunoreactivity in hippocampal CA1 and DG subfields of the bite-raised group (mean number of GR-immunoreactive cells of the control was higher than that in the bite-raised groups in 3 and 5 month existed (P < 0.0005) and no interaction on the number of DG subfields (P > 0.1), the number of GR-immunoreactive cells of the control was higher than that of the bite-raised group (P < 0.05 by multiple comparison).

There was a strong correlation between the time to reach the platform at 7 days, the number of GR-positive cells in the hippocampal CA1 and DG subfields, and the number of GRmRNA signals in the hippocampal CA1, CA3, and DG subfields was investigated using the correlation coefficient test.

First, we examined the effect of raising the bite on spatial cognitive performance in the Morris water maze. In agreement with our previous observation in molarless mice [9], an age-dependent increase in escape latency (F(2,30) = 17.8, P < 0.001) was seen. There was a statistically significant increase only in the 9-mo-old mice (P < 0.05 by multiple comparison; Fig. 1). There was an interaction between the day and group on the spatial cognitive performance (F(12, 180) = 2.6, P < 0.02).

We next examined the effect of the bite-raised condition on GR-immunohistochemistry in the hippocampus. Light microscopic analysis revealed that GR-immunoreactive cells in the CA1 and DG subfields, but not in CA3 (Fig. 2 Panel A). Because this finding was identical to that of other reports [14], the GR immunoreactivity in hippocampal CA1 and DG subfields was assessed. The results are shown in Fig. 3. There was an interaction between the age and group on the number of GR-immunoreactive cells in the CA1 (F(2, 24) = 10.5, P < 0.0005) and no interaction on the number of DG subfields (P > 0.1). While no difference between the control and bite-raised groups in 3 and 5 month existed (P > 0.1), the number of GR-immunoreactive cells of the control was higher than that of the bite-raised group (P < 0.05 by multiple comparison).

There was an age-dependent decrease in the number of GR-immunoreactive cells (F(2, 24) = 27.0, P < 0.001) and difference between the two groups (F(1, 24) = 29.5, P < 0.001) in the DG subfields.

Finally, we examined the effect of bite-raising on GRmRNA expression in the hippocampus. GRmRNA was detected in hippocampal CA1, CA3, and DG subfields by in situ hybridization (Fig. 4). There was a difference of the expression in CA3 between the control and bite-raised (F(1, 24) = 10.5, P < 0.008). The 9-mo-old mice had a significant lower number of positive cells than the age-matched control mice (CA1 and DG: P = 0.05 by multiple comparison).

Table 1 shows the correlation coefficient test results. There was a strong correlation between the time to reach the platform at 7 days, the number of GR-positive cells in the hippocampal CA1 and DG subfields, and the number of GRmRNA signals in the hippocampal CA1, CA3, and DG subfields.

In this study, GR immunoreactivity was not observed, although GRmRNA was detected in the hippocampal CA3 region, which is consistent with other reports [15]. This difference is thought to depend on differences in the synthesis or metabolic rates of GR and GRmRNA in the hippocampal CA3 region [15].

The present study, using behavioral and morphologic techniques, also demonstrated that raising the bite induces an age-related deficit in spatial learning with a concomitant decrease in GR immunoreactivity in CA1 and DG, and decreased expression of GRmRNA-positive cells in the hippocampal CA1, CA3 and DG subfields. These findings indicate that in aged SAMP8 mice, the bite-raised condition downregulates GR and GRmRNA in the hippocampus, which is very similar to previous reports on chronic stress [6,18].

GCs are believed to be key mediators of the perception and management of stressful stimuli. It is generally accepted that...
neuroendocrine activation of the hypothalamic-pituitary-adrenal (HPA) axis is counteracted by GR-mediated negative feedback, which results in the termination of the stress response. In addition, GR are distributed throughout a wide variety of brain regions (in particular, the hippocampus) [15], where GR appear to be sensitive to elevated GC levels [17]. The hippocampus is a region of negative feedback of GC on the HPA axis [18]. Taken together, these findings suggest that the downregulation of GR and GRmRNA expression induced by the bite-raised condition reduces inhibition of the negative feedback response, ultimately resulting in increased plasma corticosterone levels.

Our previous study showed that the bite-raised condition in aged SAMP8 mice not only increases plasma corticosterone levels, but also impairs spatial memory and a decreased number of neurons in the hippocampal CA3 region [9]. Also, chronic stress or glucocorticoid (GC) exposure impairs maze learning performance [10,23] and hippocampal neuronal degeneration [10]. Furthermore, in rats, hippocampal neuronal degeneration is blocked when a GC synthesis inhibitor is administrated, even in conditions of chronic stress [13]. It is very possible that, under chronic stress, the hippocampal degeneration and impaired spatial performance are caused by increased GC
through impairment of the HPA axis due to the downregulation of GR and GRmRNA.

Corticosteroids regulate the activity of the HPA axis and modulate learning and memory processes through two corticosteroid receptor types in brain, i.e., GR and mineralocorticoid receptors (MR). MRs are close to being saturated at basal concentrations of corticosterone, while high corticosterone concentrations during stress occupy mainly GR. Oitzl et al. [16] reported that unlike the effect of overexposure to corticosteroids, the continuous blockade of brain GR facilitates learning and memory processes, suggesting that improvement versus impairment of cognitive function is critically dependent on the context and duration of GR activation.

There are reports, however that chronic stress facilitates the ACTH response to novel acute stressors, which is uncovered after corticosterone removal in rats [12], and that the amount of negative feed back from the hippocampus through GR to the HPA axis is relatively small.

In conclusion, in aged SAMP8 mice, raising the bite induced a reduction of hippocampal GR and GRmRNA expression, leading to HPA axis impairment.

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References