BRAIN RESEARCH 1446 (2012) 34–39

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BRAIN RESEARCH

Research Report

Chewing ameliorates stress-induced suppression of spatial memory by increasing glucocorticoid receptor expression in the hippocampus

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ARTICLE INFO

Article history:
Accepted 7 January 2012
Available online 16 January 2012

Keywords:
Chewing
Spatial memory
Water maze
Glucocorticoid receptor
HPA axis
Immobilization stress

ABSTRACT

Chewing alters hypothalamic–pituitary–adrenal axis function and improves the ability to cope with stress in rodents. Given that stress negatively influences hippocampus-dependent learning and memory, we aimed to elucidate whether masticatory movements, namely chewing, improve the stress-induced impairment of spatial memory in conjunction with increased hippocampal glucocorticoid receptor expression. Male Sprague–Dawley rats were subjected to restraint stress by immobilization for 2 h: the stress with chewing (SC) group were allowed to chew on a wooden stick during the latter half of the immobilization period, whereas the stress without chewing (ST) group were not allowed to do so. Performance in the Morris water maze test was significantly impaired in the ST group compared with the SC group. Further, the numbers of glucocorticoid receptor immunopositive neurons in the hippocampal cornu ammonis 1 region were significantly lower in the ST group than in the control and SC groups. The control and SC rats showed no significant differences in both the water maze performance and the numbers of glucocorticoid receptor-immunopositive neurons. The immunohistochemical finding correlated with the performance in the water maze test. These results suggest that chewing is a behavioral mechanism to cope with stress by increasing hippocampal glucocorticoid receptor expression.

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Abbreviations: HPA, hypothalamic-pituitary-adrenal; ACTH, adrenocorticotropic hormone; GR, glucocorticoid receptor; MWM, Morris water maze; ANOVA, analysis of variance; CT, control; ST, stress without chewing; SC, stress with chewing; SC, CA1, cornu ammonis 1; PBS, phosphate buffer containing saline; PBS-BSAT, PBS containing bovine serum albumin and Triton X-100; DAB, 3,3′-diaminobenzidine tetrahydrochloride

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doi:10.1016/j.brainres.2012.01.011

1. Introduction

It has been reported that decreased masticatory function caused by the loss of molars, tooth attrition, or long-term intake of soft foods leads to impaired learning and memory processes and inhibits the negative-feedback response by the downregulation of GR protein and mRNA expression in the hippocampus (Ichihashi et al., 2007; Kubo et al., 2005; Onozuka et al., 1999, 2000, 2002; Tsutsui et al., 2007; Watanabe et al., 2001, 2002). The hippocampus is sensitive to stress and aging, and is one of the first regions to be structurally and functionally affected by severe and inescapable stress (McEwen, 2000). Stress stimulates the hypothalamic–pituitary–adrenal (HPA) axis and thereby causes the pituitary gland to secrete adrenocorticotropic hormone (ACTH). Elevated concentrations of this hormone, in turn, cause the adrenal cortex to secrete corticosterone. The hippocampus is a target region of corticosterone, and an elevated concentration of corticosterone suppresses hippocampus-dependent learning and memory (Bodnoff et al., 1995; de Kloet et al., 1998; Diamond et al., 2004; Kim and Diamond, 2002; Kim et al., 2006; Woodson et al., 2003).

The hippocampus has two types of adrenal steroid receptors: Type I (mineralocorticoid receptor) and Type II (glucocorticoid receptor, GR). They play important roles in the HPA axis through their effects on glucocorticoid negative feedback (Herman et al., 1989). The direct and principal mechanism by which glucocorticoids inhibit the HPA axis by negative feedback is through the inhibition of both the hypothalamus and the hypophysis. The indirect negative feedback mechanism of the HPA axis reduces the secretion of glucocorticoids by their binding to GRs in the hippocampus (Sapolsky et al., 1984). Chronic secretion of glucocorticoids stimulated by chronic stress downregulates GR protein and mRNA levels in the hippocampus (Freeman et al., 2004; Herman et al., 1995; Sapolsky et al., 1984, 1986). Previous reports suggest that masticatory movements reduce the negative influence of stress on hippocampal-dependent memory. For example, chewing ameliorates the stress-induced impairment of *N*-methyl-D-aspartate receptor-mediated long-term potentiation (Ono et al., 2008). Further, activation of the histamine H1 receptor by chewing, mediates the recovery of stress-suppressed hippocampal synaptic plasticity (Ono et al., 2009).

The aim of this study was to elucidate whether chewing improves stress-induced suppression of spatial memory in conjunction with increased hippocampal GR expression. We hypothesized that chewing improves stress-induced spatial memory performance in Morris water maze tasks and increases the GR protein level in hippocampus.

2. Results

2.1. *Spatial learning*

In the hippocampus-dependent hidden platform version of the MWM task, all groups exhibited significantly short latencies to find the hidden platform during the 32 training trials over four consecutive days (4trials/block, 2 blocks/day). The rate of acquisition was comparable among the groups (repeated measures two-way analysis of variance [ANOVA]; group × day interaction: F (2, 21) = 0.73, $P > 0.05$; main effect of group: F (2, 21) = 3.31, $P > 0.05$; main effect of day: F (2, 21) = 53.0, $P < 0.05$) (Fig. 1).

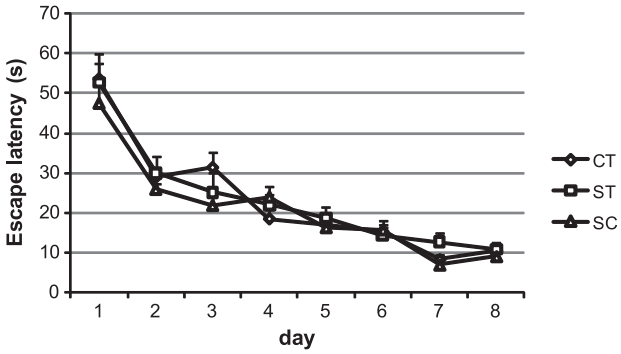

Fig. 1 – Spatial learning in the MWM test. The results represent the mean score ± SEM (n = 9, 7, and 8 rats in the CT, ST, and SC groups, respectively) of four trials per block.

2.2. *Spatial memory*

In the spatial memory test 24 h after the immobilization and chewing condition, the stress without chewing (ST) group (30.0 ± 4.3 s, n = 7) exhibited significantly longer latencies to swim to the original location of the platform than the control (CT) group (11.5 ± 2.1 s, n = 9) and the stress with chewing (SC) group (16.3 ± 4.3 s, n = 8) (one-way ANOVA: F (2, 21) = 6.78, $P < 0.01$; post hoc Tukey–Kramer test: $P < 0.01$ for ST group vs. CT group, $P < 0.05$ for ST group vs. SC group). The CT and SC groups showed no significant difference (Fig. 2).

2.3. *GR immunopositivity*

Photomicrographs showed GR induction in the cornu ammonis 1 (CA1) regions of all the groups after the spatial memory test. Further, GR-specific immunostaining was observed in the hippocampus of all the animals examined (Fig. 3).

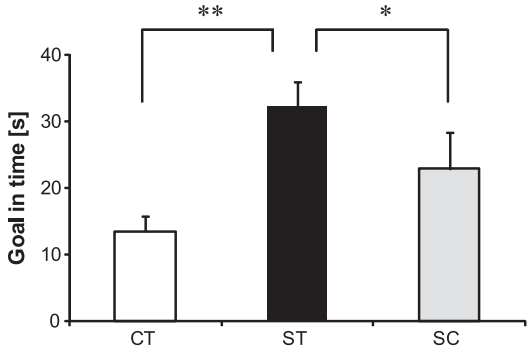

Fig. 2 – Effect of chewing on impaired memory caused by restraint stress. The results represent the mean score SEM (n = 9, 7, and 8 rats in the CT, ST, and SC groups, respectively). *P < 0.05, **P < 0.01.