Tohoku J. Exp. Med., 2013, **230**, 49-57

Occlusal Disharmony Transiently Impairs Learning and Memory in the Mouse by Increasing Dynorphin A Levels in the Amygdala

Kentaro Yamada,[1,2] Yumie Ono,[1,2,*] Kin-ya Kubo,[3] Toshiharu Yamamoto[2,4] and Minoru Onozuka[1,2]

[1]Department of Physiology and Neuroscience, Kanagawa Dental College, Yokosuka, Kanagawa, Japan
[2]Research Center of Brain and Oral Science, Kanagawa Dental College, Yokosuka, Kanagawa, Japan
[3]Seijoh University Graduate School of Health Care Studies, Tokai, Aichi, Japan
[4]Department of Human Biology, Kanagawa Dental College, Yokosuka, Kanagawa, Japan

Occlusal disharmony sometimes causes not only stiffness of neck but also psychiatric depression, suggesting that the condition of oral cavity may affect the central nervous system. Dynorphin A is an endogenous opioid peptide that specifically binds the κ-opioid receptor and has a protective role against stress. Dynorphinergic nervous system is intensely distributed in the amygdala and hippocampus that are coping areas with stress. As a model of malocclusion, we placed dental resin on the molars to increase the occlusal vertical dimension (bite-raise). After various survival times, we analyzed the amygdala and hippocampus by immunohistochemistry and immunosorbent assay (ELISA). Furthermore, the effects on learning and memory were assessed by Morris water maze test. In the amygdala, the levels of dynorphin A were increased on the 1st day after increasing the vertical dimension as indicated by immunohistochemical and ELISA assessments. The levels of dynorphin A returned to control levels on the 5th day. In the hippocampus, there were no noticeable changes in dynorphin A levels. The water maze test indicated that increasing the vertical dimension caused longer escape latency times on the 3rd day compared to those of sham-operated group. However, the bite-raised mice treated with a dynorphin antagonist, nor-binaltorphimine, showed similar escape latency times to the times of sham-operated group, even on the 3rd day. These results suggest that occlusal disharmony causes stress resulting in a transient increase of dynorphin A levels at least in the amygdala and that the increased dynorphin A levels transiently impair learning and memory.

Keywords: amygdala; dynorphin; hippocampus; malocclusion; stress
Tohoku J. Exp. Med., 2013 May, **230** (1), 49-57.

Introduction

Dynorphin is an opioid peptide. Its precursor, prodynorphin, produces α- and β-neoendorphin, dynorphin A and B (rimorphin), Leu-enkephalin, and a C-terminal-extended dynorphin B (leumorphin) (Day and Akil 1989; Day et al. 1998). Dynorphin is widely distributed in the brain, especially in the limbic system, hypothalamus, striatum, locus coeruleus and nucleus accumbens in the rat (Fallon and Leslie 1986; Ma et al. 2003) and mouse (Jamensky and Gianoulakis 1997; Ploj et al. 2000). Dynorphin specifically binds the κ-opioid receptor and has a protective role for stress through the κ-receptor. Gene expression of prodynorphin is increased in the limbic system by stress (Shirayama et al. 2004). In addition, injection of an antagonist of the κ-receptor causes stress-inducing behavior similar to those caused by immobility and forced swim stress (Mague et al. 2003; McLaughlin et al. 2003; Shirayama et al. 2004).

The amygdala, a part of the limbic system, forms the floor of the telencephalon in most mammals and locates in deep to the uncus in humans (Voogd et al. 1998). Information associated with emotion are sent to the thalamus and cerebral cortex, from which nerve fibers project to the hypothalamus, hippocampus and cingulate cortex, and affect emotional behavior, learning and memory (Davis 1992; LeDoux 1996; Adolphs and Tranel 2000). Anatomically, the amygdala is subdivided into central, medial, cortical, basomedial and basolateral nuclei. Among these nuclei, the central nucleus has abundant proenkepha-

Received February 12, 2013; revised and accepted May 15, 2013. Published online June 5, 2013; doi: 10.1620/tjem.230.49.
Correspondence: Toshiharu Yamamoto, Department of Human Biology, Kanagawa Dental College, 82 Inaoka-cho, Yokosuka, Kanagawa 238-8580, Japan.
e-mail: t.yamamoto@kdu.ac.jp
*Present address: Department of Electronics and Bioinformatics, Meiji University, 1-1-1 Higashi-Mita, Tama-Ku, Kawasaki, Kanagawa 214-8571, Japan.

lin, proopiomelanocortin (POMC) and prodynorphin nerve fibers (Finley et al. 1981; Khachaturian et al. 1982; Fallon and Leslie 1986; Cassell and Gray 1989; Cassell et al. 1999; Poulin et al. 2006). The opioid nervous system in the central amygdala associates with pain, fear, anxiety, stress and feeding behavior (Davis et al. 1994; Bernard et al. 1996; Pitkanen et al. 1997; LeDoux 2000; Swanson 2000; Petrovich and Gallagher 2003).

The hippocampal formation can be divided in the dentate gyrus, CA1, CA2, CA3 and hilus. Three major fiber systems, the angular bundle, fimbria-fornix pathway and hippocampal commissure, are involved in hippocampal functions such as information processing and certain forms of learning and memory (Amaral and Lavenex 2007). For example, hippocampal lesions in rats induce an impairment in various learning and memory tasks (Morris et al. 1982), and lesions of the CA3 region in the hippocampus disrupt spatial memory (Handleman and Olton 1981). The different regions in the hippocampus are innervated by both dynorphinergic and enkephalinergic neurons. Dynorphinergic neurons are present in the mossy fiber projection from the dentate gyrus to the CA3 region (McGinty et al. 1982; Chavkin et al. 1983). Enkephalinergic neurons are mainly present in the perforant path, which supplies the hippocampus and dentate gyrus with fibers from the entorhinal cortex (McGinty et al. 1982).

Occlusal disharmony occasionally causes stiffness of neck or shoulder, fatigue, or psychiatric depression (De Boever and Adriaens 1983). Recent studies suggest that deterioration of the oral environment causes various effects on the central nervous system, such as a decrease of spine numbers in the hippocampal CA1 area (Kubo et al. 2008), an increase of plasma corticosterone (Yoshihara et al. 2001), and transient activation of microglia (Kojo et al. 2010). Furthermore, masticatory disorders reduce input activities in the hippocampus, thereby leading to deficits in learning and memory in aged senescence-accelerated prone 8 mice (Onozuka et al. 1999, 2000, 2002; Kubo et al. 2005). In this report, we investigated the effects of increasing the occlusal vertical dimension on the mouse amygdaloid and hippocampal dynorphinergic nervous system using immunohistochemical, biochemical and behavioral techniques.

Materials and Methods

Increase of molar vertical dimension

Animal treatment protocols were approved by the Ethics Committee of Kanagawa Dental College, employing guidelines established by the committee. DDy male mice were housed in a condition under a 12-h light/12-h dark cycle and temperature at 22 ± 3°C with free access to pelleted chow and water until experiment. As a model of dysfunctional mastication, we increased the vertical dimension of mouse molar dentition, the condition of which was referred as "bite-raise" in the subsequent text. Procedures were performed under sodium pentobarbital anesthesia (35 mg/kg i.p.; Wako Pure Chemical Industries, Ltd., Osaka, Japan). The vertical dimension of the bite was increased approximately 0.1 mm by placing ultraviolet-ray polymerization resin (UniFil®LoFlo, GC corporation, Tokyo, Japan) on the all upper molars (6 maxillary molars) after treatment with a Single Bond Dental Adhesive System (3M Dental Product, Irvine, CA, USA). We confirmed remaining of the resin when these mice were sacrificed. Control animals (sham-operation) underwent the same anesthetic procedure, but no resin was applied. After the procedure, the mice were housed under the above conditions.

Immunohistochemistry

Total 20 mice were used for immunohistochemistry (n = 5 each). At 1, 3, and 5 days after the bite-raise, we examined the distribution of dynorphin A-like immunoreactivity in the amygdala and hippocampus of experimental animals in comparison with sham-operated mice (n = 5; 1 day survival time). The mice were deeply anesthetized by pentobarbital sodium (35 mg/kg, i.p. Wako Pure Chemical Industries, Ltd.), followed by perfusion with 0.9% NaCl and, subsequently, with 4% formaldehyde and 0.2% picric acid in 0.1 M sodium phosphate buffer (PB, pH 6.9). The brain was dissected out from the skull, blocked into three parts by cutting transversely at the levels of anterior commissure and caudal to the mammillary body, and further fixed for 1 or 2 days at 4°C in the same fixative. After washing in PB and immersing in 20% sucrose, the middle blocks were cut into 20-μm-thick transverse sections using a sliding microtome equipped with a frozen stage. Every third section was serially collected and these sections (approximately 55 sections per animal) were immunostained using the free-floating method. Immunohistochemistry was performed according to our routine method (Yamada et al. 2008). Briefly, the sections were washed overnight in 0.1 M PB (pH 7.4) containing 0.9% saline (PBS), and incubated for 24 h at 4°C with rabbit anti-porcine dynorphin A (1-17) serum (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) diluted 1:10,000 in PBS containing 1% bovine serum albumin (BSA; Sigma-Aldrich Co., St. Lous, MO, USA) and 0.3% Triton X-100 (PBS-BSAT). This primary antiserum cross-reacts less than 1.0% with porcine dynorphin A (1-13), and shows no cross-reaction with porcine dynorphin A (1-8), porcine dynorphin B (1-13), human β-endorphin, porcine neo-endorphin and Leu-enkephalin, according to the manufacturer's description. To test antiserum specificity, preabsorbed antiserum with human dynorphin A (20 μg/ml; Peptide Institute, Osaka, Japan) was used as a primary antibody. After washing in PBS, the sections were incubated for 1 h at room temperature with a secondary antibody (biotinylated goat anti-rabbit IgG, Vector Laboratories, Burlingame, CA, USA) diluted 1:100 in PBS-BSAT. The sections were then washed in PBS and incubated for 30 min at room temperature with avidin-biotin-horseradish peroxidase complex (ABC; Vector Laboratories) diluted 1:200 in PBS-BSAT. After a final wash in PBS, the sections were reacted with 0.02% 3,3′-diaminobenzidine tetrahydrochloride (DAB) and 0.005% hydrogen peroxide in 0.05 M Tris-HCl buffer solution (pH 7.4). The sections were counterstained with thionin and coverslipped with Malinol (Muto Pure Chemicals, Tokyo, Japan).

ELISA

Total 32 mice were used for enzyme-linked immunosorbent assay (ELISA) analysis. To collect amygdala samples, mouse brains were cut into approximately 1.5 mm-thick slices using a mouse brain slicer (Muromachi Corporation, Tokyo, Japan). Slices containing amygdala tissue were collected and the amygdala region was isolated by removing tissue lateral to the external capsule, medial to the optic

tract, and dorsal to the ventral border of the caudate putamen. For hippocampus collection, brains were removed from the skull, and the hippocampal formation was isolated from the cerebral cortex after cutting the corpus callosum along with the longitudinal cerebral fissure and reversing the cerebral hemispheres. Samples were weighed, proportional volumes of 1% trifluoroacetic acid to the weight were added, and the tissue was homogenized. Homogenates were centrifuged at 10,000 × g for 20 min at 4°C. Supernatants were corrected and applied to a C18 column (Waters Corporation, Massachusetts, Ireland). Samples were eluted from the C18 column with 1 ml of 1% trifluoroacetic acid. Eluted samples were collected and freeze dried. At the time of quantification, the samples were diluted in volumes of Tris-HCl buffer (pH 7.4) proportional to the weight. The following procedures were performed according to the manufacturer's instruction for dynorphin A enzyme immunoassay kit (Peninsula Laboratories, LLC, CA, USA). Briefly, each well of an immunoplate was incubated with anti-dynorphin A serum for 1h at room temperature and further added duplicated standard dynorphin A (ten different concentrations) or duplicated samples. After 2 h, each well was added with biotinylated dynorphin A and left overnight at 4°C. After washing, each well was incubated with streptavidin-horseradish peroxide (HRP) for 1 h at room temperature. After further washing, each well was added with tetramethyl benzidine dihydrochloride solution and the reaction was terminated by adding 2 N HCl solution. Absorbance at 450 nm as HRP activity was measured with an iMark Microplate Absorbance Reader (Bio-Rad Laboratories Inc., CA, USA).

Morris water maze leaning test

Total 32 mice were used for water maze analysis. The Morris water maze test is a sensitive behavioral assay for forebrain abnormalities (Morris 1984). Morris water maze training was performed as described previously (Onozuka et al. 1999; Watanabe et al. 2002; Kubo et al. 2005). A stainless steel tank with 90 cm in diameter and 30 cm in depth was filled with water (approximately 26°C) to a height of 16 cm, and the water surface was covered with floating polystyrene foam granules (approximately 2 mm in diameter). A column-shaped platform with 10 cm in diameter and 15 cm in height 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. In the training period, the mice were placed into the water at 1 of 4 points at the perimeter of the tank for 20 trials over 5 consecutive days (4 trials per day). The sequence of the starting positions was randomized daily. After 5 days of training, half of the mice were deeply anesthetized by pentobarbital sodium (35 mg/kg, i.p. Wako Pure Chemical Industries, Ltd.) and the molar vertical dimension was increased as described above. The remaining mice were anesthetized with pentobarbital sodium, but their molar vertical dimension was not increased (controls). The vertical dimension-increased group was further subdivided randomly into two groups: those injected with nor-binaltorphimine (nor-BNI), a highly selective κ-opioid antagonist (McLaughlin et al. 2003) and those injected with saline alone. Similarly, the control group was also randomly subdivided into nor-BNI and saline injection groups. Two hours before the first test of each assessment day of the Morris water maze test, the mice by group were peritoneally injected with either saline (10 ml/kg, Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan) or with nor-BNI-dissolved saline (10 ml/kg). Doses of nor-BNI (10 mg/kg; Sigma-Aldrich Corporation, Gillingham, UK) were referred to the paper by McLaughlin et al. (2003). On each assessment day, mice were placed on the one of four submerged perimeter starting points. Four trials were performed on the each assessment day, and the starting positions were changed randomly. The time to reach the platform (escape latency time) was recorded. The mean of four escape latency times was considered to be the mouse score. Means of 8 mouse scores were plotted on the X-axis. These assessments were performed on the days 1, 2, 3, 4 and 5 after the vertical dimension was increased.

Statistical analyses were performed using a one-way analysis of variance (ANOVA) to compare the dynorphin A levels and the escape latency times. The value of $p < 0.05$ considered to be statistically significant.

Results

Amygdala

In the control mice, dense dynorphin A immunoreactive fibers were observed in the central amygdala (Fig. 1A, B). However, relatively fewer immunoreactive fibers were seen in the basolateral, basomedial, medial and cortical amygdala. In comparison with the control, the density of immunoreactive fibers in the bite-raised mice seemed to be increased at 1 day after the bite-raise (Fig. 1C, D). Immunoreactive soma-like structures became remarkable at 3 days after increasing the vertical dimension (Fig. 1E, F), while somal staining was very faint in the control animals (Fig. 1A). At 5 days after the bite-raise, dynorphin A immunoreactive profiles were similar to those of control animals (Fig. 1G, H). Preabsorption of the antiserum with dynorphin A abolished these staining profiles (not shown). Consistent with the immunohistochemical data, the levels of dynorphin A in the amygdala were increased at 1 day after the vertical dimension was increased (5.09 ± 0.37 pg/mg ; $p < 0.01$) over that of control mice (2.27 ± 0.30 pg/mg) (Fig. 2). Thereafter, the levels of dynorphin A were decreased at 3 (3.30 ± 0.43 pg/mg) and 5 (1.69 ± 0.40 pg/mg) days after the bite-raise (Fig. 2).

Hippocampus

In the hippocampus, dynorphin A immunoreactive fibers were present in the hilus and the zone of mossy fibers (Fig. 3). The latter is a fiber bundle of projections from the dentate gyrus to the CA3 region of the hippocampus. In comparison with control mice (Fig. 3A), the width of the zone and density of immunoreactive fibers were similar to those at 1, 3, and 5 days after the vertical dimension was increased (Fig. 3B, C, D). Preabsorption of the antiserum with dynorphin A abolished these staining profiles (not shown). ELISA analysis indicated that the levels of dynorphin A in the hippocampus were slightly increased at 1 day (1.22 ± 0.04 pg/mg) after increasing the vertical dimension (Fig. 4). However, the difference in dynorphin A levels was not statistically significant compared with that of control animals (1.06 ± 0.10 pg/mg). At 3 (1.12 ± 0.04 pg/mg) and 5 days (1.07 ± 0.05 pg/mg) after the bite-raise, the levels of dynorphin A were similar to control levels (Fig. 4).

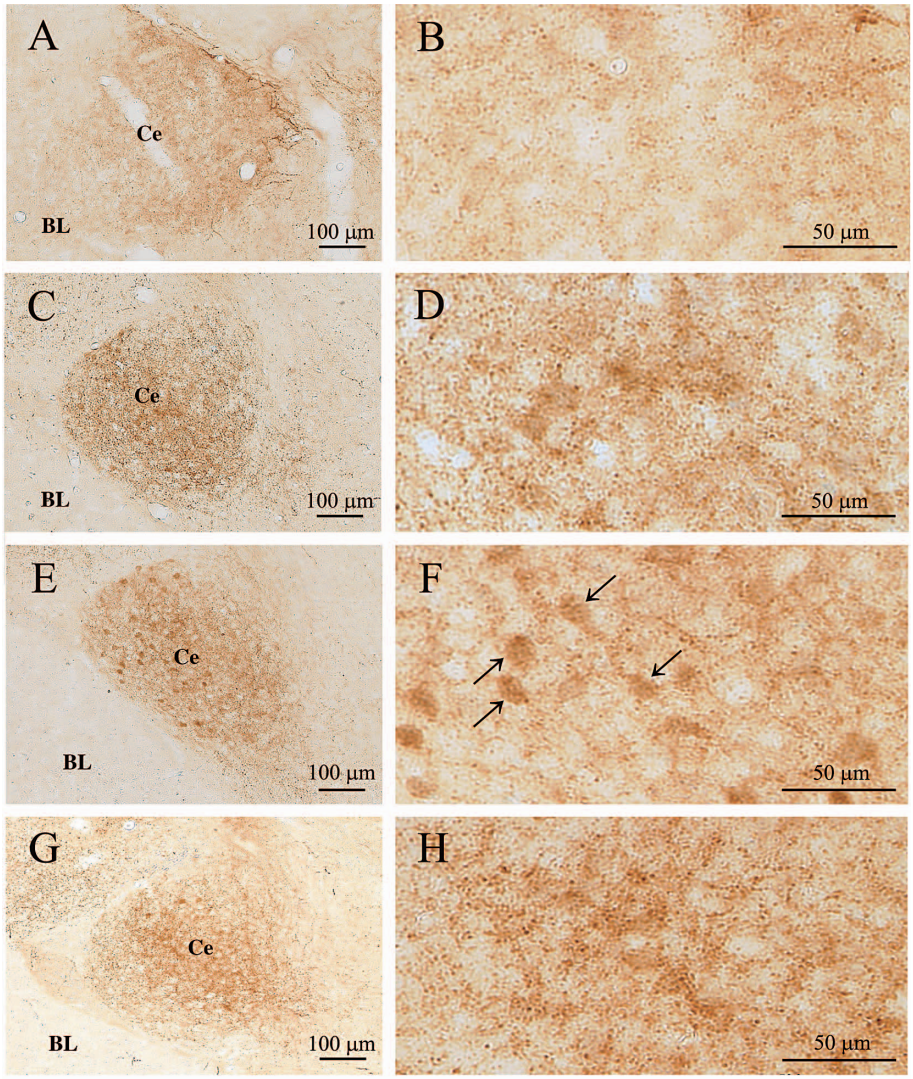

Fig. 1. Changes of dynorphin A immunoreactivity in the amygdala.
Low (A, C, E, G) and high (B, D, F, H) magnification photographs showing dynorphin A immunoreactive somata and fibers in the central amygdala of control animals (A, B), and at 1 (C, D), 3 (E, F), and 5 (G, H) days after increasing the vertical dimension. Note the denser distribution of dynorphin A immunoreactive fibers at 1 day (C, D) after the bite-raise and that dynorphin A immunoreactive somata (arrows in F) are conspicuous at 3 days (E, F) after the bite-raise. Bars: 100 μm (A, C, E, G), 50 μm (B, D, F, H). Abbreviations: BL, basolateral amygdaloid nucleus; Ce, central amygdaloid nucleus.

Morris water maze test

During the training period, all of the animals tended to improve their scores day by day (Fig. 5). On the first assessment day, the group of normal vertical dimension with nor-BNI injection showed the shortest escape latency times among the four groups (Fig. 6). The bite-raised animals with nor-BNI injection showed the longest escape latency times among the four groups. However, these differences were not statistically significant. From the 2nd to 5th assessment day, the bite-raised animals with saline injection tended to show longer escape latency times compared to the other three groups. This was particularly apparent on the 3rd day, where values were statistically significant ($p < 0.05$) in comparison with the other three groups (Fig. 6). On the 5th assessment day, all four groups showed similar escape latency times (Fig. 6).

Discussion

In this study, we found that increasing the occlusal vertical dimension caused a transient increase of dynorphin A levels in the amygdala but not in the hippocampus based on the morphological and ELISA analyses, both of which have technical limitations (see later discussion). Furthermore, the transient increase of dynorphin A caused by the increase of vertical dimension seemed to impair memory and learning ability as assessed by the Morris water maze test.

The influence of the bite-raise on the brain has been demonstrated in catecholamine levels (Budts-Jφrgensen et al. 1981; Areso et al. 1999; Yoshihara et al. 2001), c-Fos

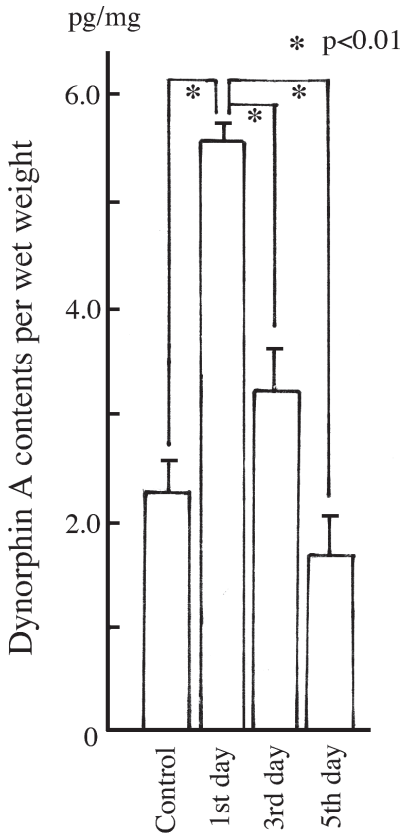

Fig. 2. ELISA assessment in the amygdala.
Changes in dynorphin A contents in the amygdala (mean ± S.E.) of control animals and at various survival times (1, 3, and 5 days) after increasing the vertical dimension. Note that the increased contents of dynorphin A in 1 day survival animals are statistically significant compared with control, 3 and 5 day survival animals.

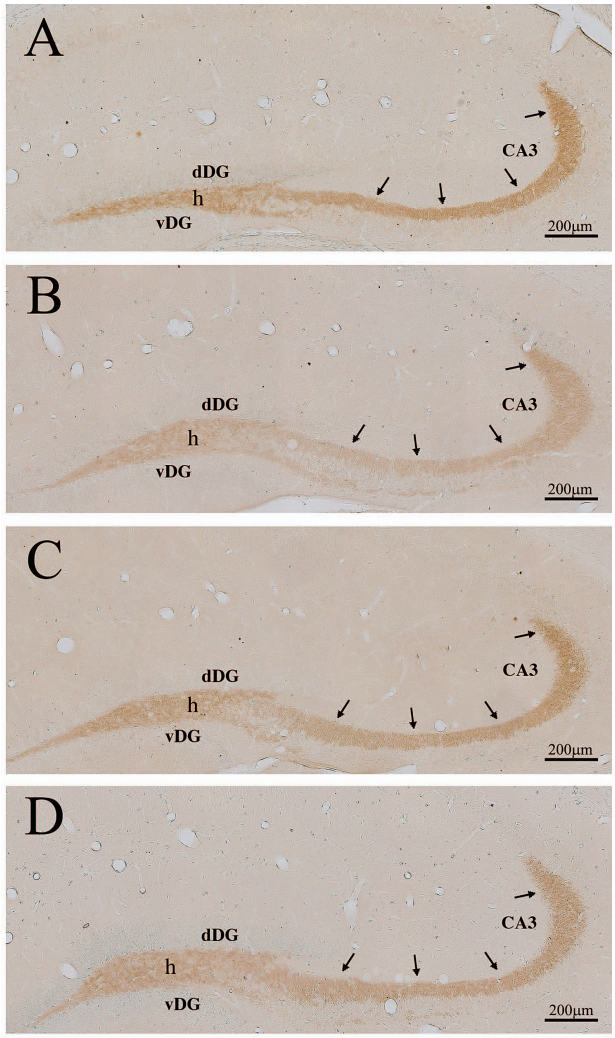

Fig. 3. Dynorphin A immunoreactivity in the hippocampus.
Low magnification photographs showing dynorphin A immunoreactive fiber bundles in the hippocampal hilus (h) and mossy fiber bundles (arrows) of control animals (A), and animals at 1 (B), 3 (C) and 5 (D) days after increasing the vertical dimension. Note that it appears there are no differences in staining density among control and various survival times. Bars: 200 μm. Abbreviations: CA3, CA3 field of Ammon's horn; dDG, dorsal blade of the dentate gyrus; vDG, ventral blade of the dentate gyrus.

and its mRNA (Kobayashi et al. 2004), and glial cells (Kubo et al. 2007; Kojo et al. 2010). All of these effects tend to be transient, similar to the present results. It is well known that various stresses increase brain dynorphin levels. For example, immunobilization and forced swim stress increase dynorphin A levels in the hippocampus and nucleus accumbens (Shirayama et al. 2004), and infusion of an antagonist of dynorphin receptor, nor-BNI produces an antidepressant effect (Pliakas et al. 2001; Mague et al. 2003). In general, stress is classified as either acute and clonical. A recent paper classified the κ-opioid receptor-mediated reactions for stresses into three categories: acute and delayed responses, and multiple rounds of adaptation (Knoll et al. 2010). The bite-raise increases plasma corticosterone levels, which is a marker indicating stress (Kubo et al. 2007), suggesting that this condition is a type of stress. The increase of dynorphin A levels in the amygdala may be a neural adaptation. In contrast to results obtained from forced swim, immobilization and learned helplessness (Shirayama et al. 2004), dynorphin changes in the hippocampus in response to the bite-raise were not conspicuous, suggesting the increase of vertical dimension is a less robust stress than other stress tasks.

The amygdala plays an important role in emotional perception and expression, and in fear response (Davis 1992; LeDoux 1996; Adolphs and Tranel 2000). Peak times of elevated dynorphin A levels by morphological inspection differed from those by ELISA analysis. In general, morphological assessment is more inaccurate than biochemical quantity. However, we isolated the whole amygdala although morphological inspection suggested that the increased dynorpin A levels limited only to the central amygdala. Furthermore, there is a technical limitation to equalize sampling for ELISA. In any case, dynorphin A levels are transiently increased by the bite-raise within sev-

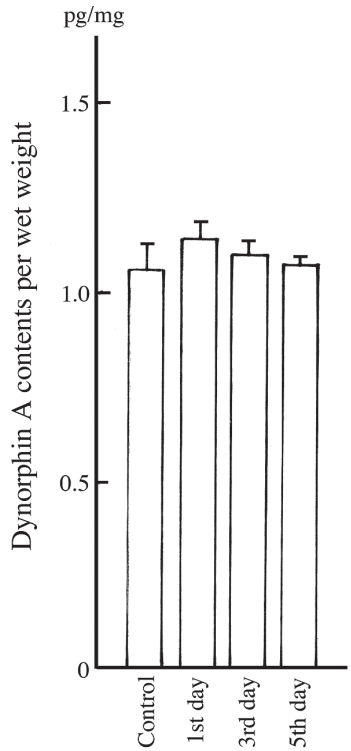

Fig. 4. ELISA assessment in the hippocampus.
Changes in dynorphin A contents in the hippocampus (mean ± S.E.) of control animals and in animals at various survival times (1, 3 and 5 days) after increasing the vertical dimension. Note that the dynorphin contents at 1 day survival tend to increase although the increase is not statistically significant.

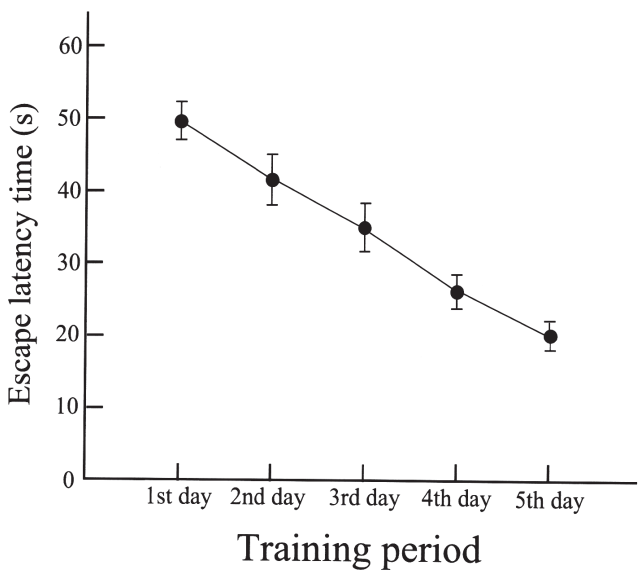

Fig. 5. Scores of water maze test in training period.
Changes of escape latency times during 5 days training periods in the water maze test. The results are expressed as the mean score (mean ± S.E., $n = 32$) of four trials per day. Note that mice improved their scores day by day.

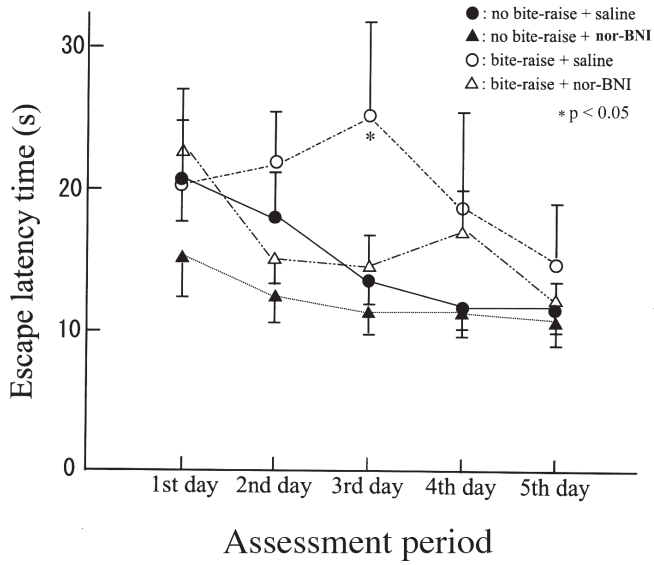

Fig. 6. Scores of water maze test in assessment period.
The results are expressed as the mean score (mean ± S.E., $n = 8$ for each group) of four trials per day. After 5 days training period, mice were divided into four groups: no bite-raise with saline injection (open circles), no bite-raise with nor-BNI injection (open triangles), bite-raise with saline injection (filled circles), and bite-raise with nor-BNI injection (filled triangles). Note that the bite-raise with saline injection group required a significantly longer time to reach the platform than other groups on the 3rd day.

eral days. The presence of the dynorphin A-containing nervous system in the amygdala is well documented in the rat (Fallon and Leslie 1986; Ma et al. 2003) and mouse (Jamensky and Gianoulakis 1997; Ploj et al. 2000). Interestingly, dynorphin-containing neurons in the amygdala coexist with corticotroph releasing hormone (CRH) (Marchant et al. 2007), which serves as a neurotransmitter regulating diverse extra-hypophysial systems under stress (Vale et al. 1981). Therefore, dynorphin with CRH may play a fundamental role in the stress response. The lateral amygdaloid nucleus receives projections from sensory cortices (Pitkanen et al. 1997; McDonald 1998), which receive oral cavity sensory information and projects to the central amygdaloid nucleus. This pathway is one route by which the bite-raise increases dynorphin levels in the central amygdala in addition to other direct and indirect routes from the oral cavity to the central amygdala. Furthermore, the amygdaloid complex projects to the hippocampus (Pikkarainen et al. 1999; Pitkanen et al. 2000). Therefore, the amygdala may affect hippocampal functions through the increased dynorphin A in the amygdala. This pathway may be responsible for the phenomena that emotional states affect learning and memory.

The hippocampus is often implicated in the neurobiology of stress (Knoll and Carlezon 2010) and is essential for certain forms of learning and memory, such as visuospatial and content-dependent learning and memory (Squire 1992;

Ergorul and Eichenbaum 2004). The present data did not show a statistical significance in the differences in dynorphin levels in the hippocampus by immunohistochemistry and ELISA between control and bite-raised animals. As mentioned above, immobilization and forced swimming increases hippocampal dynorphin A level (Shirayama et al. 2004). However, we could not detect the statistically significant increase of dynorphin A in the hippocampus, suggesting the increase of vertical dimension targets the amygdala rather than the hippocampus. Alternatively, small changes are difficult to detect by morphological assessment. Dynorphin immunoreactive fibers are restricted in mossy fibers and perforant pathway terminals. For ELISA, we used the whole hippocampal formation owing to difficulty of selective dissection of these fiber bundles, which may underestimate changes of dynorphin levels. Therefore, the tendency of dynorphin to increase on the 1st day after the bite-raise may be substantial. Dynorphin is co-localized with glutamate, the primary neurotransmitter in granule cells in the dentate gyrus, and the synaptic release of dynorphin is reported to cause pre-synaptic inhibition of glutamate release from the mossy fibers and perforant pathway terminals (Drake et al. 2007). Opioid receptors are expressed at moderate to high levels in the hippocampus (Clarke et al. 2001; Drake et al. 2007). These lines suggest that the hippocampus is a targeting area for the increased dynorphin in the amygdala and/or hippocampus associated with glutamate-mediated hippocampal functions.

The water maze task has long been regarded as a hippocampal dependent task based on studies of special impairment after selective lesioning of the hippocampus (Jarrard 1978; Morris et al. 1982). The present data from the water maze task indicated that the increase of vertical dimension transiently impaired learning and memory, suggesting that increased dynorphin impairs learning and memory. Supporting this hypothesis, injection of a specific antagonist for κ-opioid receptor improved the impaired learning and memory. At the present time, the detailed mechanism that dynorphin affects learning and memory is not known. However, the increased levels of dynorphin resulting from stress may block glutamate-mediated functional plasticity of the hippocampal formation, and dynorphin antagonism with nor-BNI may restore this response. Spatial memory tasks activate hippocampal subfields, and plasticity of the mossy fiber projections is involved in and required for the storage of spatial representations (McClelland and Goddard 1996; Vann et al. 2000; Ramirez-Amaya et al. 2001). The presence of time lag between the highest dynorphin A levels in the amygdala and the impairment of spatial learning and memory assessed by water maze test suggests that behavioral expression of the amygdala and/or hippocampus is delayed from the neurochemical expression probably owing to establishment of effects of dynorphin A on the glutamate-mediated hippocampal functions. Taken together, results suggest that the induction of dynorphin A by stress may block the plasticity of hippocampal pathways that is required for spatial learning, and this activity may underlie the transient behavioral deficit. Although there is no clinical evidence that occlusal disharmony directly affects the learning and memory, a part of patient's complaints, such as shoulder stiffness, fatigue and psychiatric depress (De Boever and Adriaens 1983), may associate with the transient increase of dynorphin A levels.

Acknowledgements

A part of this work was supported by JSPS KAKENHI Grant Number 24792119 and MEXT-supported Program for the Strategic Research Foundation at Private Universities, S1203004, 2012-2014.

Conflict of Interest

The authors declare no conflict of interest.

References

Adolphs, R. & Tranel, D. (2000) Emotion recognition and the human amygdala. In *The Amygdala: A Functional Analysis*, 2nd ed., edited by J.A. Aggleton. Oxford University Press, Oxford, pp. 587-630.

Amaral, D. & Lavenex, P. (2007) Hippocampal neuroanatomy. In *The hippocampus book*, edited by P. Andersen, R. Morris, D. Amaral, T. Bliss & J. O'Keefe. Oxford University Press, Oxford, pp. 37-114.

Areso, M.P., Giralt, M.T., Sainz, B., Prieto, M., Garcia-Vallejo, P. & Gomez, F.M. (1999) Occlusal disharmonies modulate central catecholaminergic activity in the rat. *J. Dent. Res*., **78**, 1204-1213.

Bernard, J.F., Bester, H. & Besson, J.M. (1996) Involvement of the spinoparabrachio-amygdaloid and -hypothalamic pathways in the autonomic and affective emotional aspects of pain. *Prog. Brain Res.*, **107**, 243-255.

Budts-Jϕrgensen, E. (1981) Occlusal dysfunction and stress. An experimental study in macaque monkeys. *J. Oral Rehabil.*, **8**, 1-9.

Cassell, M.D., Freedman, L.J. & Shi, C. (1999) The intrinsic organization of the central extended amygdala. *Ann. NY Acad. Sci.*, **877**, 217-241.

Cassell, M.D. & Gray, T.S. (1989) Morphology of peptide-immunoreactive neurons in the rat central nucleus of the amygdala. *J. Comp. Neurol.*, **281**, 320-333.

Chavkin, C., Bakhit, C., Weber, E. & Bloom, F.E. (1983) Relative contents and concomitant release of prodynorphin/neodynorphin-derived peptides in rat hippocampus. *Proc. Natl. Acad. Sci. USA*, **80**, 7669-7673.

Clarke, S., Chen, Z., Hsu, M.S., Pintar, J., Hill, R. & Kitchen, I. (2001) Quantitative autoradiographic mapping of the ORL1, mu-, delta- and kappa-receptors in the brains of knockout mice lacking the ORL1 receptor gene. *Brain Res.*, **906**, 13-24.

Davis, M. (1992) The role of the amygdala in fear and anxiety. *Annu. Rev. Neurosci.*, **15**, 353-375.

Davis, M., Rainnie, D. & Cassell, M. (1994) Neurotransmission in the rat amygdala related to fear and anxiety. *Trends Neurosci.*, **17**, 208-214.

Day, R. & Akil, H. (1989) The posttranslational processing of prodynorphin in the rat anterior pituitary. *Endocrinology*, **124**, 2392-2405.

Day, R., Lazure, C., Basak, A., Boudreault, A., Limperis, P., Dong, W. & Lindberg, I. (1998) Prodynorphin processing by proprotein convertase 2. *J. Biol. Chem.*, **273**, 829-836.

De Boever, J.A. & Adriaens, P.A. (1983) Occlusal relationship in patients with pain-dysfunction symptoms in the temporomandibular joints. *J. Oral Rehabil.*, **10**, 1-7.

Drake, C.T., Chavkin, C. & Milner, T.A. (2007) Opioid systems in the dentate gyrus. *Prog. Brain Res.*, **163**, 245-263.

Ergorul, C. & Eichenbaum, H. (2004) The hippocampus and memory for "what", "where" and "when". *Learn. Mem.*, **11**, 397-405.

Fallon, J.H. & Leslie, F.M. (1986) Distribution of dynorphin and enkephalin peptides in the rat brain. *J. Comp. Neurol.*, **249**, 293-336.

Finley, J.C., Maderdrut, J.L. & Petrusz, P. (1981) The immunocytochemical localization of enkephalin in the central nervous system of the rat. *J. Comp. Neurol.*, **198**, 541-565.

Handleman, G.E. & Olton, D.A. (1981) Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training. *Brain Res*., **217**, 41-58.

Jamensky, N. & Gianoulakis, C. (1997) Content of dynorphins and κ-opioid receptors in distinct brain regions of C67BL/6 and DBA/2 mice. *Alcohol Clin. Exp. Res.*, **21**, 1455-1464.

Jarrard, L.E. (1978) Selective hippocampal lesions: differential effects on performance by rats of a spatial task with preoperative versus postoperative training. *J. Comp. Physiol. Psychol.*, **92**, 1119-1127.

Khachaturian, H., Watson, S.J., Lewis, M.E., Coy, D., Goldstein, A. & Akil, H. (1982) Dynorphin immunocytochemistry in the rat central nervous system. *Peptides*, **3**, 941-954.

Knoll, A.T. & Carlezon, W.A. Jr. (2010) Dynorphin, stress, and depression. *Brain Res.*, **1314**, 56-73.

Kobayashi, T. & Mushimoto, E. (2004) Daily change of stress induced expression of c-fos mRNA in rat brain by experimental occlusal interference. *J. Jpn. Prosthodont. Soc.*, **48**, 203-211.

Kojo, A., Yamada, K., Kubo, K., Yamashita, A. & Yamamoto, T. (2010) Occlusal disharmony in mice transiently activates microglia in hippocampal CA1 region but not in dentate gyrus. *Tohoku J. Exp. Med.*, **221**, 237-243.

Kubo, K., Ichihashi, Y., Iinuma, M., Iwaku, F., Tamura, Y., Karasawa, N., Nagatsu, I. & Onozuka, M. (2007) Involvement of glucocorticoid response in hippocampal activities in aged SAMP8 mice with occlusal disharmony. *Biogenic Amines*, **21**, 273-282.

Kubo, K., Iwaku, F., Watanabe, K., Fujita, M. & Onozuka, M. (2005) Molarless induced changes of spines in hippocampal region of SAMP8 mice. *Brain Res.*, **1057**, 191-195.

Kubo, K., Kojo, A., Yamamoto, T. & Onozuka, M. (2008) The bite-raised condition in aged SAMP8 mice induces dendritic spine changes in the hippocampal region. *Neurosci. Lett.*, **441**, 141-144.

LeDoux, J.E. (1996) Emotional networks and motor control: a fearful view. *Prog. Brain Res.*, **107**, 437-460.

LeDoux, J.E. (2000) Emotion circuits in the brain. *Annu. Rev. Neurosci*., **23**, 155-184.

Ma, J., Ye, N., Lange, N. & Cohen, B.M. (2003) Dynorphinergic GABA neurons are a target of both typical and atypical antipsychotic drugs in the nucleus accumbens shell, central amygdaloid nucleus and thalamic central medial nucleus. *Neuroscience*, **121**, 991-998.

Mague, D.D., Pliakas, A.M., Todtenkopf, M.S., Tomasiewicz, H.C., Zhang, Y., Stevens, W.C. Jr., Jones, R.M., Portoghese, P.S. & Cariezon, W.A. Jr. (2003) Antidepressant-like effects of kappa-opioid receptor antagonists in the forced swim test in rats. *J. Pharmacol. Exp. Ther.*, **305**, 323-330.

Marchant, J.N., Densmore, V.S. & Osborne, P.B. (2007) Coexpression of prodynorphin and corticotrophin-releasing hormone in the rat central amygdala: Evidence of two distinct endogenous opioid systems in the lateral division. *J. Comp. Neurol.*, **504**, 702-715.

McClelland, J.L. & Goddard, N.H. (1996) Considerations arising from a complementary learning systems perspective on hippocampus and neocortex. *Hippocampus*, **6**, 654-665.

McDonald, A.J. (1998) Cortical pathways to the mammalian amygdala. *Prog. Neurobiol.*, **55**, 257-332.

McGinty, J.F., Henriksen, S.J., Goldstein, A., Terenius, L. & Bloom, F.E. (1982) Dynorphin is contained within hippocampal mossy fibers: immunochemical alterations after kainic acid administration and colchicine-induced neurotoxicity. *Proc. Natl. Acad. Sci. USA*, **31**, 1797-1800.

McLaughlin, J.P., Marton-Popovici, M. & Chavkin, C. (2003) Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. *J. Neurosci.*, **23**, 5674-5683.

Morris, R.G.M. (1984) Development of a water-maze procedure for studying spatial learning in the rat. *J. Neurosci. Methods*, **11**, 47-60.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P. & O'Keefe, J. (1982) Place navigation impaired in rats with hippocampal lesions. *Nature*, **297**, 681-683.

Onozuka, M., Watanabe, K., Fujita, M., Tomida, M. & Ozono, S. (2002) Changes in the septohippocampal cholinergic system following removal of molar teeth in the aged SAMP8 mouse. *Behav. Brain Res*., **133**, 197-204.

Onozuka, M., Watanabe, K., Mirbord, S.M., Ozono, S., Nishiyama, K., Karasawa, N. & Nagatsu, I. (1999) Reduced mastication stimulates impairment of spatial memory and degeneration of hippocampal neurons in aged SAMP8. *Brain Res.*, **826**, 148-153.

Onozuka, M., Watanabe, K., Nagasaki, S., Jiang, Y., Ozono, S., Nishiyama, K., Kawase, T., Karasawa, N. & Nagatsu, I. (2000) Impairment of spatial memory and changes in astroglial responsiveness following loss of molar teeth in aged SAMP8 mice. *Behav. Brain Res.*, **108**, 145-155.

Petrovich, G.D. & Gallagher, M. (2003) Amygdala subsystems and control of feeding behavior by learned cues. *Ann. NY Acad. Sci.*, **985**, 251-262.

Pikkarainen, M., Ronkko, S., Savander, V., Insausti, R. & Pitkanen, A. (1999) Projections from the lateral, basal, and accessory basal nuclei of the amygdala to the hippocampal formation in rat. *J. Comp. Neurol*., **403**, 229-260.

Pitkanen, A., Pikkarainen, M., Nurminen, N. & Ylinen, A. (2000) Reciprocal connections between the amygdala and the hippocampal formation, perirhinal cortex and postrhinal cortex in the rat. *Ann. NY Acad. Sci.*, **911**, 369-391.

Pitkanen, A., Savander, V. & LeDoux, J.E. (1997) Organization of intra-amygdaloid circuitries in the rat: an emerging framework for understanding function of the amygdala. *Trends Neurosci.*, **20**, 517-523.

Pliakas, A.M., Carlson, R.R., Neve, R.L., Konradi, C., Nestler, E.J. & Carlezon, W.A. Jr. (2001) Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J. Neurosci.*, **21**, 7397-7403.

Ploj, K., Roman, E., Gustavsson, L. & Nylander, I. (2000) Basal levels and alcohol-induced changes in nociceptin/orphanin FQ, dynorphin, and enkephalin levels in C57BL/6J mice. *Brain Res. Bull.*, **53**, 219-226.

Poulin, J.F., Chevalier, B., Laforest, S. & Drolet, G. (2006) Enkephalinergic afferents of the centromedial amygdala in the rat. *J. Comp. Neurol.*, **496**, 859-876.

Ramirez-Amaya, V., Balderas, I., Sandoval, J., Escobar, M.L. & Bermudez-Rattoni, F. (2001) Spatial long-term memory is related to mossy fiber synaptogenesis. *J. Neurosci.*, **21**, 7340-7348.

Shirayama, Y., Ishida, H., Iwata, M., Hazama, G.I., Kawahara, R. & Duman, R.S. (2004) Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant-like effects. *J. Neurochem.*, **90**, 1258-1268.

Squire, L.R. (1992) Memory and the hippocampus: a synthesis

from findings with rats, monkeys, and humans. *Psychol. Rev.*, **99**, 195-231.

Swanson, L.W. (2000) Cerebral hemisphere regulation of motivated behavior. *Brain Res.*, **886**, 113-164.

Vale, W., Spiess, J., Rivier, C. & Rivier, J. (1981) Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotrophin and beta-endorphin. *Science*, **213**, 1394-1397.

Vann, S., Brown, M.W., Erichsen, J.T. & Aggleton, J.P. (2000) Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to differential spatial memory tests. *J. Neurosci*., **20**, 2711-2718.

Voogd, J., Nieuwenhuys, R., van Dongen, P.A.M. & ten Donkelaar, H.J. (1998) Mammals. In *The central nervous system of vertebrates*, vol. 3, edited by Nieuwenhuys, R., ten Donkelaar, H.J. & Nicholson, C. Springer-Verlag, Berlin/Heidelberg/New York/Tokyo, pp. 1637-2097.

Watanabe, K., Ozono, S., Nishiyama, K., Saito, S., Tonosaki, K., Fujita, M. & Onozuka, M. (2002) The molarless condition in aged SAMP8 mice attenuates hippocampal Fos induction linked to water maze performance. *Behav. Brain Res.*, **128**, 19-25.

Yamada, K., Park, H., Sato, S., Onozuka, M., Kubo, K. & Yamamoto, T. (2008) Dynorphin-A immunoreactive terminals on the neuronal somata of rat mesencephalic trigeminal nucleus. *Neurosci. Lett.*, **438**, 150-154.

Yoshihara, T., Matsumoto, Y. & Ogura, T. (2001) Occlusal disharmony affects plasma corticosterone and hypothalamic noradrenaline release in rats. *J. Dent. Res.*, **80**, 2089-2092.