Influence of decline of occlusal support on bilateral striatal dopamine release in rats

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Sensory inputs from the periodontal membrane play a role in brain activation and regulate various functions or reflexive feedback. We investigated the possibility of a correlation between striatal function and loss of occlusal support. Eighteen male Sprague-Dawley rats were divided into three groups: the EXT Group (n=6) had all three maxillary molars on both sides extracted; the EXT-R Group (n=6) had the right maxillary molars extracted; and the CON Group (n=6) served as a control. Dopamine levels in the bilateral striatum were measured using in vivo microdialysis. Once the extracellular basal concentrations of dopamine were stable, the rats were fed pellets and the food-induced changes in dopamine concentrations were monitored every 10 min for 2 h. In the CON group, feeding induced a significant increase in dopamine concentrations on both sides of the striatum. However in the EXT and EXT-R Groups, no significant increase was observed. This result indicated that even if rats lost occlusal support unilaterally, food-induced dopamine release was impaired bilaterally in the dorsal striatum, and this impairment was associated with occlusal support loss that affected the contralateral striatum.

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INTRODUCTION

Masticatory function is controlled through the coordination of the nervous systems of the teeth (periodontal ligament), temporomandibular joints, masticatory muscles and tongue, which together regulate sensorimotor integration. The control of mastication is adjusted by afferent impulses from periodontal mechanoreceptors and masseter muscle spindles to the cerebral cortex. Teeth play a major role in the perception of foods, and masticatory movements depend on the properties of the food. A reduction in afferent inputs from tooth pulp and periodontal ligaments is known to affect both the central and peripheral nervous systems. Gobel et al. removed the pulps of feline teeth to investigate degenerative changes in the neurons in the sensory nucleus of the trigeminal nerve by deafferentation. They found that degeneration occurred not only in the damaged primary afferent neurons of the pulp, but also in the secondary afferent neurons in the trigeminal nerve spinal tract nucleus caudal region subnucleus beyond the synapses. In addition, one study reported that degeneration of the hippocampal CA 1 region of rats resulted from feeding them a soft diet. Moreover, degenerative changes were found in the cerebral cortex and hippocampus after extraction of rat molars, and these changes were associated with impairment of learning and memory in many studies. Loss of molars resulted in loss of occlusal contacts and supports. Loss of occlusal support caused degenerative changes in the periodontal mechanoreceptors of opposing teeth, as well as a reduction in afferent information. Thus, it was revealed that loss of molars caused not only a large deterioration in masticatory function resulting from a lack of occlusal support, but also caused degenerative changes in the peripheral and central...
nervous systems, resulting in various impairments in brain function.

Water and food intake play an important role as natural rewards that are necessary for survival in rodents. In addition, water and food can be rewards that affect decision making by the expectation that they will be received after completion of a learning a task. The dopamine nervous system is related to the control of feeding behavior. Although the striatum receives major afferent inputs from the brain cortex and thalamus," it also receives important inputs from dopamine nerves in the midbrain. The striatum is composed of the dorsal region, including the caudate putamen, and the ventral region, including the core and shell of the nucleus accumbens. Although both regions are related to decision making, it has been reported that the role of each region is different.12

The caudate putamen is served by the substantia nigra pars compacta in the midbrain, and the nucleus accumbens receives innervation from the ventral tegmental area. Many previous studies that focused on the nucleus accumbens, reported that activity in this region was related to feeding behavior.15-17 However, a recent study revealed that the caudate putamen is also involved with feeding behavior. In that study, dopamine-deficient mice gradually became hypoactive and showed stunted growth due to inadequate feeding, perishing by 4 weeks of age.18 Another study found that when the function of the caudate putamen in dopamine-deficient mice was recovered by gene therapy, normal feeding behavior resumed.19 These results indicate that the dorsal striatum plays an indispensable role in maintaining the behavior necessary to obtain calories for survival, thus having a strong impact on feeding behavior. The results of previous studies indicated that the ventral striatum controls the reward value reflected by palatability. It has been reported that the dopamine level in the dorsal striatum increases according to feeding rewards,20 suggesting that loss of occlusal support may have a certain effect on the dopamine levels in the caudate putamen. On the other hand, no study has reported an effect on the bilateral striata of a decline in occlusal support.

In this study, we changed the degree of occlusal support by extracting the maxillary molars of rats either unilaterally or bilaterally, and measured their dopamine levels in the dorsal striatum by in vivo microdialysis. The effects of different levels of occlusal support loss on the bilateral striata were investigated by recording the changes in the dopamine levels in the dorsal striatum simultaneously on both sides during feeding.

MATERIALS AND METHODS

Experimental animals
A total of eighteen 5-week-old male rats (SLC Japan, Hamamatsu, Japan) with a mean body weight of 139 g (128–152 g) were used in this study. The animals were provided free access to food pellets (MF; Oriental Yeast, Tokyo, Japan) and tap water throughout the study and their body weight changes were recorded every week. All experiments were performed in accordance with the Guidelines for Animal Experiments of Osaka Dental University (approval number: 11-03039).

Experimental groups
The rats were assigned to three groups that at 5 weeks of age underwent bilateral maxillary molar extraction (EXT Group, n = 6), right side extraction (EXT-R Group, n = 6) or no extraction (control group, CON Group, n = 6), respectively. The molars in the two experimental groups were extracted using forceps (Delicate C; GC, Tokyo, Japan) after cutting the periodontal ligament with a dental explorer under intraperitoneal anesthesia with 50 mg/kg pentobarbital (Nembutal; Dainippon Sumitomo Pharma, Osaka, Japan). The CON Group underwent anesthesia using the same method, but no tooth extraction was performed.

Measurement of dopamine levels in the striatum
Figure 1 illustrates the experimental procedures. Dopamine levels in the striatum were measured using in vivo microdialysis. The probes used for microdialysis were 0.22 mm in external diameter, with a total length of 8 mm, and a membrane length of 3
mm (CX-I-8-3; Eicom, Kyoto, Japan). Prior to the experiment, the rats were implanted with guide cannulae (CXG-8; Eicom) under 50 mg/kg pentobarbital-induced intraperitoneal anesthesia at nine weeks of age. Referring to the rat brain atlas (Paxinos and Watson, 1986), the tip was placed 0.2 mm anterior, ±3.0 mm external and 3.5 mm ventral from the bregma (Fig 2), to allow placement directly above the bilateral striata. The inserted guide cannulae were fixed with two anchor screws placed on the surrounding cranial bone and covered with dental acrylic resin (Unifast III; GC). Dummy cannulae (CXD-8; Eicom) had been inserted into the guide cannulae until the day of measurement. After a one week recuperation period and confirmation of no abnormalities, the rats were used in the study. The 10-week-old rats were fasted beginning two days before measurements, after which dummy cannulae were replaced with microdialysis probes (CX-I-8-3; Eicom) under inhalation anesthesia using sevoflurane (Maruiishi Pharm Accutical, Osaka, Japan). The rats were left in a transparent acrylic cage (45 cm × 45 cm × 45 cm) without any other restrictions.

Immediately after probe insertion, the probes on both sides were simultaneously perfused with an artificial cerebrospinal fluid (Harvard Apparatus, Holliston, MA, USA) from a syringe stabilized at a microsyringe pump (ESP-64; Eicom) at a flow rate of 2.0 μL/min. After a 2-hour recuperation period and confirmation that no abnormalities were present, measurements were initiated. The perfusate was collected using a microfraction collector (EFC-82; Eicom); 15 samples from each side were collected every 10 minutes. After 20 minutes, the rats were fed 10 food pellets of 45 mg each, (Dustless Precision Pellets; Bio Serv, Frenchtown, NJ, Canada), and changes in dopamine levels of the bilateral striata were recorded.

After all samples had been collected, they were sequentially injected into a high-performance liquid chromatography system (HTEC-500; Eicom), using an auto-sampling injector (Model 234; Gilson, Middleton, WI, USA), to measure dopamine levels in the samples. A PP-ODS separation column of 30 mm × 4.6 mmø (Eicom) was used. The mobile phase comprised 0.1 M of sodium phosphate buffer (pH 6.0) with 1% added methanol, 500 mg/L sodium-1-decanesulfonate, and 50 mg/L ethylene dinitrilotetraacetate. The mobile phase flow rate was 500 μL/min. The rats were euthanized by a pentobarbital overdose administered by intraperitoneal injection after completion of the procedures.
The brain sections were then extracted to grossly confirm the secure placement of the probes in the striata.

**Statistical analysis**
Changes in the body weight of the rats and dopamine levels in their samples were analyzed using statistical software (SPSS Statistics 19; IBM, Armonk, NY, USA) and Bonferroni/Dunn tests were conducted for multiple comparison after analysis of repeated measures ANOVA, with time and experimental groups as the main variation factors.

**RESULTS**

**Changes in body weight**
Changes in body weight are shown in Figure 3. The results of repeated measures ANOVA with time and experimental groups as main variation factors showed a significant increase in body weight in all rats (p < 0.001). No significant difference was observed among the experimental groups (p = 0.952) or interactions (p = 0.207). All three groups showed increases in body weight in the same manner.

**Changes in dopamine levels of the striatum**
Figures 4 and 5 present changes in dopamine levels in the bilateral striata. The changes in dopamine levels were calculated by considering the mean

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![Fig. 3](image_url) Changes in rat weight throughout the experiment.

![Fig. 4](image_url) Extracellular levels of DA during microdialysis sessions in the right CPu.

*DA release after intake of pellets significantly higher in the CON Group than in the EXT Group (Bonferroni’s multiple comparison tests, p < 0.05). **DA release after intake of pellets significantly higher in the CON Group than in the EXT and EXT-R Group (Bonferroni’s multiple comparison tests, p < 0.05).
value of two samples as 100% immediately after starting measurements. The results of repeated measures ANOVA on the right striata, with time and experimental groups as main variation factors, showed significant differences between experimental groups (p = 0.009), time (p < 0.001) and interactions (p < 0.001). After conducting the Bonferroni/Dunn multiple comparison test, there was a significant difference between the CON and EXT Groups (p = 0.007). However, no significant difference was observed between the CON and EXT-R (p = 0.112), or between the EXT and EXT-R (p = 0.337) Groups. The results of comparison between groups by time showed significant differences between the CON and EXT Groups and between the CON and EXT-R Groups 40 minutes after feeding (sample: No.7). Also there was a significant difference between the CON and EXT Groups from 30 to 120 minutes after feeding (sample: No.6–15). No significant difference was observed between the EXT and EXT-R Groups throughout the study.

Similarly on the left striatum, significant differences were observed in the experimental groups (p = 0.001), time (p < 0.001) and interactions (p < 0.001). The multiple comparison showed significant differences between the CON and EXT Groups (p = 0.019), and between the CON and EXT-R Groups (p = 0.01). However, no significant difference was observed between the EXT and EXT-R Groups (p = 0.269). The results of comparison between groups by time showed significant differences between the CON and EXT Groups and between the CON and EXT-R Groups 40 minutes after feeding (sample: No.6–7). Also there was a significant difference between the CON and EXT-R Groups from 30 to 120 minutes after feeding (sample: No.6–15). No significant difference was observed between the EXT and EXT-R Groups throughout the study.

**DISCUSSION**

In the present study, rats were given free access to pellets and water. Since all three groups showed the same level of body weight changes, surgical stress and changes in occlusal conditions after molar extraction did not affect body weight. Previous studies reported that extraction of maxillary molars in rats was not an impairment in feeding. The results of the present study support these findings. Since rats crush pellets using their anterior teeth, groups with molar extraction were able to feed in the same manner as those in the control group. Therefore, it is suggested that the extractions did not affect their feeding behavior or nutritional status.

Afferent inputs from teeth are conveyed to the central nervous system using trigeminal sensory branches as primary afferent fibers. Secondary neurons that are present in the principal sensory nucleus of the trigeminal nerve and trigeminal spinal nucleus act as the sensory nucleus of the trigeminal nerve. Pain sensations from the dental pulp are mainly sent to the trigeminal spinal nucleus. The trigeminal spinal nucleus is divided by the subnucleus oralis, subnucleus interpolaris and subnucleus caudalis. Pain sensation is conveyed to the primary somatosensory area in the cerebral cortex via the ventral posteromedial thalamic nucleus after passing the subnucleus caudalis. Afferent inputs from the periodontal ligament are mainly sent to the principal sensory nucleus of the trigeminal nerve and the mesencephalic nucleus of the trigeminal nerve. The mesencephalic nucleus of the trigeminal nerve is thought to regulate masticatory movements by sending collateral branches to the motor nucleus of the trigeminal nerve. On the other hand, tactile and pressure sensations are sent to the principal sensory nucleus of the trigeminal nerve. There are crossing and non-crossing type fibers from the principal sensory nucleus; the sensation is projected to the primary somatosensory area in the brain cortex via the same and opposite sides of the posterior medial nucleus of the thalamus.

Tooth eruption in rats starts at around two weeks of age, at the time their neural connection to the somatosensory area in the brain cortex begins to develop. The effects of extraction change depending on the timing of tooth loss—i.e., loosing teeth before or after completion of the neural connection.
Yoshimura et al. conducted a study on the effects of multiple tooth loss on the oral somatosensory cortex during the developmental period. They reported that the layering structure of the somatosensory area in the brain cortex was completed at five weeks of age, and inhibitory changes in the oral somatosensory cortex were induced between the ages of two and three weeks. Based on these results, we conducted molar extractions at five weeks of age. In addition, evaluation of the effects on the striatum was conducted after ten weeks, considering the healing and habitation period for the new mastication pattern after extraction.

The striatum receives inputs from the thalamus and the cerebral cortex controlled by dopamine neurons in the midbrain. Dopamine neurons are activated by new and unexpected stimulation; without stimulation enabling reward prediction, dopamine neurons are activated during learning as a primary reward. An increase in dopamine levels in the bilateral striata was observed especially in the control group. The dopamine levels were slightly increased in both sides of the striata in the EXT-R Group and on the left side of the striatum in the EXT Group. The increase of the impairment and range in dopamine release with the expansion of occlusal support loss indicated that afferent inputs from the periodontal ligament play a significant role in gaining a primary reward. Moreover, the considerable decrease of dopamine release in the bilateral striata in EXT-R as well as EXT suggested the necessity of maintaining sufficient bilateral occlusal support in order to gain a primary reward.

A significant difference between the CON and EXT Groups was observed in dopamine level changes in the right striatum during feeding. In the left striatum, there were significant differences between CON and EXT, and between CON and EXT-R. This result indicates that right occlusal support loss has a strong impact on the left striatum because when crossing and bilateral projections of different inputs from the periodontal ligament via the secondary neurons occur, the crossing projection is predominant. Additionally, a decrease in bilaterally projected inputs may have caused a decreased dopamine release in the right striatum.

Loss of unilateral or bilateral occlusal support by molar extraction apparently caused a decreased dopamine release from the bilateral striata during feeding. Previous studies have reported that loss of occlusal support resulting in a decrease of afferent information inputs from opposing teeth caused degeneration of the trigeminal ganglion and cerebral cortex, as well as denaturation of the periodontal ligament of opposing teeth. These results suggest insufficient transmission of afferent inputs to the bilateral striata.

Sensory inputs from periodontal ligaments are important for feeding. Soft-diet feeding of rats decreased the sensory inputs from periodontal ligaments and synapse density on the cerebral cortex and hippocampus, resulting in deterioration in the ability of spatial memory. Furthermore, a number of studies on how molar crown resection and extraction affect the cerebral cortex and hippocampus have reported decreases of hippocampal pyramidal cells and the Ach synapse, and impairment of learning and memory skills. In the present study, we suggest that synapse density was decreased in the cerebral cortex after molar extraction, causing a reduction in afferent inputs to the striata. In addition, the pellets used in the present study were much smaller than those normally used, enabling the rats to feed without using their incisors. It is suggested therefore that only very little sensory information was input during pellet feeding of the rats without molars. In order for the animals to recognize pellet feeding as a primary reward, information should be conducted through afferent inputs from somatognathic organs and hematogenously from internal organs. The loss of molars may have strongly reduced afferent inputs from somatognathic organs.

During mastication, afferent information related to coordination with maxillofacial muscles and mandibular movements is important. Using electro-
myography of the rat masseter during mastication, one study reported that rhythmicity of mastication had deteriorated after molar extraction.\textsuperscript{29} It is thought that afferent information from maxillofacial muscles during mastication is considerably affected by loss of occlusal support, possibly causing a reduction in dopamine levels in the striatum.

Another study reported that mastication was related to changes in cerebral blood flow.\textsuperscript{29} An imbalance in the bilateral masticatory muscles in the group where teeth were extracted on the right side may have caused a difference in cerebral blood flows of the left and right cerebral hemispheres. Dopamine levels in the left striatum of the EXT-R Group temporarily showed a considerable decrease. The reason for this phenomenon is unclear. Since such a rapid decrease was not observed in the EXT group, it is possible that differences in bilateral neural inputs during feeding or an imbalance in occlusion by unilateral mastication activated a neural system and caused the dopamine levels to decrease. From these results, it may be concluded that occlusal imbalance caused by unilateral loss of occlusal support could possibly induce different effects than those associated with complete (bilateral) loss of occlusal support. To correct this imbalance, restoration of neural inputs from opposing teeth by reconstructing occlusal support through prosthodontic treatment is needed. Although the effects of loss of occlusal support are thought to be alleviated by this treatment, further investigation is needed.

A recent study revealed that dopamine is also involved in regulating obesity. A study using dopamine-deficient mice revealed that dopamine itself may be involved in regulating feeding and obesity via leptin.\textsuperscript{30} In addition, a study using functional MRI has also indicated that dopamine levels in the neural system of the dorsal striatum of obese individuals who dieted were lower than those of non-obese individuals.\textsuperscript{31} This indicates that obese individuals overeat to obtain satisfaction resulting from the deterioration of functions that induce a feeling of happiness while eating. In the present study, we did not investigate reactions of the dopamine receptors. However, there are similarities with the aforementioned study in terms of the reduction in satisfaction during feeding through dopamine at the dorsal striatum. In obese patients, the sensitivity of dopamine receptors is changed, resulting in a reduction in satisfaction after feeding. Similarly, loss of occlusal support in our study may have caused a reduction in satisfaction with the primary reward. It is not appropriate to simply conclude that a loss of occlusal support is directly related to obesity, but we suggest that the loss of occlusal support may trigger obesity by changing the sensitivity of dopamine receptors.

Our results suggest that it is important to maintain appropriate occlusal support and transmit afferent inputs from the periodontal ligaments and maxillofacial organs to the striatum for proper function of the dopamine neural systems. In addition, we found that even unilateral occlusal support loss affects both sides of the striata. This suggests that an imbalance in stomatognathic systems caused by unilateral mastication affects dopamine levels, although the route is unclear. Although loss of occlusal support has been discussed mostly in relation to learning and memory, there seems to be a close relationship between occlusal support, reward and feeding behaviors. Maintaining occlusal support and restoring it if it has been lost appears to be crucial for appropriate and satisfactory feeding.

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