Review article

Occlusal-masticatory function and learning and memory: Immunohistochemical, biochemical, behavioral and electrophysiological studies in rats

Toshihiro Hirai a,*, Youngnam Kang c, Hisashi Koshino a, Katsuya Kawanishi a, Yoshifumi Toyoshita a, Yasuhiro Ikeda b, Mitsuru Saito c

a Department of Removable Prosthodontics, School of Dentistry, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-0293, Japan
b Department of Dental Science, Institute of Personalized Medical Science, Health Sciences University of Hokkaido, 2-5 Ainosato, Kita-Ku, Sapporo, Hokkaido 002-8072, Japan
c Department of Neuroscience and Oral Physiology, Osaka University Graduate School of Dentistry, 1-8, Yamadaoka, Suita, Osaka 565-0871, Japan

Received 29 July 2009; received in revised form 14 December 2009; accepted 25 December 2009

KEYWORDS
Mastication; Cholinergic neurons; Acetylcholine; Nucleus of the diagonal band; Pedunculopontine tegmental nucleus; Learning and memory function

Summary This review paper discusses the relationship between the mastication and learning and memory in rats. Immunohistochemistry of choline acetyltransferase (ChAT) showed that the numbers of ChAT-positive neurons in the nucleus of the diagonal band/medial septal nucleus (NDB/MS) of a powder diet group (PDG) and a molar crown-less powder diet group (MCPDG) were smaller than those of solid diet group (SDG). Subsequently, the concentrations of acetylcholine (ACh) in the hippocampus and in the cerebral cortex in MCPDG were significantly lower than that of SDG. The response latency of the MCPDG group was significantly shorter in passive avoidance tests. The impact of mastication on recovery of learning and memory function rats receiving permanent middle cerebral artery occlusion (MCAO) was also evaluated. In a Morris water maze task, rats fed a solid diet after MCAO surgery had greater functional recovery than rats fed a liquid. Finally, a whole-cell patch-clamp study revealed crucial roles of nitric oxide (NO) in the physiological control of cholinergic neurons in NDB/MS and in the pathogenic mechanism for cell death. Collectively, these results suggest key roles for NO both in the learning and memory function, and potentially in dementia. Mastication may have a significant impact on the maintenance and recovery of learning and memory, presumably through pathogenic and physiological mechanisms of NO action.

© 2010 Japanese Association for Dental Science. Published by Elsevier Ireland. All rights reserved.

* Corresponding author. Tel.: +81 133 23 1425; fax: +81 133 23 1425.
E-mail address: hirai@hoku-iryo-u.ac.jp (T. Hirai).

1882-7616/$ — see front matter © 2010 Japanese Association for Dental Science. Published by Elsevier Ireland. All rights reserved.
Contents

1. Introduction.................................................................................................................. 144
2. Mastication and cholinergic neurons in rats ............................................................... 144
3. Mastication and learning/memory function, and acetylcholine (ACh) concentration of the cerebral cortex and hippocampus in rats ........................................................................................................... 144
4. Recovery effects of mastication on learning and memory after middle cerebral artery occlusion (MCAO) in rats ................................................................. 145
5. Nitric oxide (NO) in basal forebrain cholinergic (BFC) neurons and learning/memory .......................................................................................................................... 147
6. Conclusion ..................................................................................................................... 148
Acknowledgements .......................................................................................................... 148
References .......................................................................................................................... 148

1. Introduction

A sharp increase in the population of elderly persons in recent years, along with the report of tooth loss being a risk factor for Alzheimer-type dementia [1] led relationship between mastication and higher brain function. The dysfunction of occlusion and/or mastication has been implicated in impairment of learning and memory in a number of studies. Tooth extraction in aged (135 weeks old) rats decreased the acetylcholine (ACh) level in the cerebral cortex, subsequently causing an impairment of spatial memory [2]. A similar impairment of spatial memory in aged rats has been reported to be due to extracting or cutting the molar teeth at young ages [3], and also by feeding soft-diet after the weaning period in adult rats [4]. In these studies, the neuronal density in the CA1 hippocampus [3] and the synaptic formation in the hippocampus and the parietal cortex [4] were found to be decreased.

Our previous studies suggested that the molar crown-less rat fed powder diet developed Alzheimer-type dementia, consistent with a significantly lower activity of ChAT in the cerebral cortex and hippocampus of patients with Alzheimer-type dementia, as described by Iizuka [5–7]. Furthermore, it is reported that the central cholinergic system is disturbed in patients with vascular dementia [8] as well as in Alzheimer’s patients [9,10]. Therefore, we have examined whether mastication can impact recovery of learning and memory function in rats that permanent middle cerebral artery occlusion (MCAO) by intraluminal suture [11]. Finally, we addressed the possible cellular mechanisms underlying functional impairment of cholinergic neurons in NDB/MS. It is well known that basal forebrain cholinergic (BFC) neurons are highly susceptible to nitric oxide (NO) toxicity [12,13], while NO also plays crucial roles in learning and memory [14,15]. Therefore, we examined whether NO plays any physiological roles in spite of its toxicity in neurons in the basal forebrain.

This review paper discusses the relationship between the mastication and learning and memory, along with the possible cellular mechanisms.

2. Mastication and cholinergic neurons in rats

In our recent study using ChAT immunohistochemistry, the tooth loss was found to have a significant impact on the viability of cholinergic neurons in NDB/MS. Tooth loss was achieved by removing all molar crowns of rats. These rats with crown-less molars (molar crown-less group) were fed a powder diet and compared with two groups of rats with normal molar teeth; one fed a solid diet and the other fed a powder diet, categorized as the control and soft-diet groups, respectively. As shown in Table 1, when the three groups of rats were fed their respective diets, beginning on the 25th postnatal week, the numbers of ChAT-positive neurons in NDB/MS of the soft diet and the molar crown-less groups were significantly fewer than those of the control group, at 15 and 35 weeks post-treatment. The pedunculopontine tegmental nucleus (PPTN) is another source of major cholinergic projection neurons in the central nervous system. At 15 and 35 weeks post-treatment, there were no significant differences in the number of ChAT-positive neurons in the trigeminal motor nucleus (Vmo) and in the PPTN between any two groups. However, there appeared to be a slight shrinkage in dendrites of the molar crown-less group of the Vmo, but not in PPTN, in comparison with the control group.

The possible loss of cholinergic neurons in NDB/MS may have resulted in decreases in the concentrations of ACh and choline (Ch) in the dissected hippocampus [16], which receives projections from cholinergic neurons in NDB/MS. As shown in Table 2, there were no significant differences in Ch concentration among all the groups, and a significant decrease in ACh concentration in the hippocampus was seen only in the molar crown-less group. Therefore, it is likely that the ACh concentration was decreased following removal of molar crowns. Taken together, a decrease of oral sensory information may have caused a reduction in the number of ChAT-positive neurons selectively in NDB/MS, which in turn caused a decline of ACh concentrations in the hippocampus.

3. Mastication and learning/memory function, and acetylcholine (ACh) concentration of the cerebral cortex and hippocampus in rats

Learning and memory ability in rats with all molars removed at 25 weeks post natal and then fed a powder diet (molarless group) was significantly lower compared to rats with intact molars fed with a solid diet (control group) in our study. Learning and memory was assessed with a one-way step through type of passive avoidance apparatus divided into light and dark chambers [17]. As shown in Fig. 1, the response latency of the molarless group was significantly shorter than that in the control group at the 4th and 7th day in the passive avoidance test. Moreover, ACh levels in the cerebral cortex
and hippocampal were significantly lower in the molarless group compared to than the control group after the passive avoidance test (Fig. 2A and B).

A disturbance in the cholinergic system is reported in the patients with senile dementia of an Alzheimer-type [8,18—20]. It is also reported that the response latency during passive avoidance tasks of stroke-prone spontaneously hypertensive rats (SHRSP) were shorter than that of the normotensive control Wistar Kyoto rats (WKY). Furthermore, cerebral ACh levels correlated positively with the total response latency [21]. SHRSP is one of the animal models of vascular dementia and exhibits histopathological, behavioral and biochemical abnormalities similar to those found in the patients with vascular dementia [22]. It is well known that cerebral blood flow carries the oxygen and the glucose to brain neurons and excretes waste matter and carbon dioxide. The hippocampal neurons are reported to be weak when a deficiency of oxygen exists. It can be speculated that cerebral blood flow impediment contributes to a decrease in learning and memory function. A increase in cerebral blood flow was observed in the lower part of the bilateral pre-and post central gyri, extending to the operculum and the insula, both in gum and candy chewing compared to an "at rest" condition in all subjects by Senda et al. [23].

From these results, the shorter response latency in the molarless group compared to the control group in this study may be brought about by the decrease of brain blood flow caused by a decrease of masticatory function. It was suggested that the decrease of masticatory function caused by tooth loss leads to a decrease of ACh synthesis resulting in a learning memory disorder.

4. Recovery effects of mastication on learning and memory after middle cerebral artery occlusion (MCAO) in rats

Neuroplastic adaptations in the mammalian brain have been reported [24—26], and exposure to different tasks has been

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The number of choline acetyltransferase immunoreactive neurons.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-operative week (post-natal week)</td>
<td>0 (25th)</td>
</tr>
<tr>
<td><strong>NDB/MS</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1496 ± 176.1</td>
</tr>
<tr>
<td>Soft diet</td>
<td>1207 ± 97.1</td>
</tr>
<tr>
<td>Molar crown-less</td>
<td>1040 ± 91.1</td>
</tr>
<tr>
<td><strong>PPTN</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>564 ± 33.8</td>
</tr>
<tr>
<td>Soft diet</td>
<td>475 ± 18.9</td>
</tr>
<tr>
<td>Molar crown-less</td>
<td>503 ± 77.5</td>
</tr>
<tr>
<td><strong>Vmo</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1215 ± 263.4</td>
</tr>
<tr>
<td>Soft diet</td>
<td>1095 ± 83.4</td>
</tr>
<tr>
<td>Molar crown-less</td>
<td>1209 ± 68.1</td>
</tr>
</tbody>
</table>

Mean ± S.D. values of the number of ChAT-positive neurons in the nucleus of the diagonal band/medial septal nucleus (NDB/MS). There were significant differences between the control group and the molar crown-less group both at 15 and 35 weeks post-treatment (\(p < 0.01\)). Six rats were examined for each group. Values are mean ± S.D.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The concentration of acetylcholine (Ach) and (Ch) in the hippocampus.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-operative week (post-natal week)</td>
<td>0 (25th)</td>
</tr>
<tr>
<td><strong>Ach (nmol/mg protein)</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.293 ± 0.038</td>
</tr>
<tr>
<td>Soft diet</td>
<td>0.222 ± 0.020</td>
</tr>
<tr>
<td>Molar crown-less</td>
<td>0.210 ± 0.024</td>
</tr>
<tr>
<td><strong>Ch (nmol/mg protein)</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.183 ± 0.035</td>
</tr>
<tr>
<td>Soft diet</td>
<td>0.226 ± 0.025</td>
</tr>
<tr>
<td>Molar crown-less</td>
<td>0.196 ± 0.024</td>
</tr>
</tbody>
</table>

Mean ± S.D. values of the concentration of ACh and Ch in the hippocampus. At 15 weeks post-treatment, the concentration of ACh in the molar crown-less rats was significantly decreased in comparison with the control group (\(p < 0.05\)). Six rats were examined for each group. Values are mean ± S.D.

\(a p < 0.05\) compared with the control group.
shown to increase cortical representation in humans [27, 28]. Moreover, it has been suggested that early implementation of intensive stroke rehabilitation is associated with enhanced improvement of the performance of activities after stroke [29]. From this point of view, mastication of solid feed in rats is speculated to be more effective for performance of activities after stroke, compared to that of soft feed. To test this hypothesis, we examined the effect of mastication on recovery of learning and memory in rats that received the permanent middle cerebral artery occlusion (MCAO) using the intraluminal suture technique (Longa et al. [30]).

In our study, sensorimotor deficits evaluated by the limb placement test (LPT) [31] after the MCAO surgery in rats were found not to differ between the solid feed group of MCAO (MCAO-S) and a liquid feed group (MCAO-L) (Fig. 3). In addition, a Morris water maze task (MWM) was used to evaluate spatial learning and memory [32, 33]. The thigmotaxis time spent in the periphery of the pool was expressed as a percentage of the escape latency in acquisition trials [34]. In the acquisition trials, statistically significant differences in escape latency were observed between MCAO-L and a solid feed sham group at all days, and between the MCAO-S and sham groups at days 3 and 4 of the trials (Fig. 4A). In the probe trial, statistically significant differences in time spent were observed between the MCAO-L and sham groups (Fig. 4B). The mean proportions of the escape latencies spent in the periphery of the pool for the three trials on days 1 and 5 was calculated. The rats in all groups spent more time in the periphery of the pool during the trials on day 1 than day 5. On day 5, there was significant difference among three groups (Fig. 4C).

Thus, solid feeding after MCAO surgery in rats was more effective for functional recoveries than liquid feeding. It can be said that solid diet mastication could be effective for the rehabilitation of sensorimotor learning/memory induced by cerebral infarction.
5. Nitric oxide (NO) in basal forebrain cholinergic (BFC) neurons and learning/memory

In the basal forebrain, neurons containing nitric oxide synthase (NOS) and/or nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-D, a histochemical marker for NOS) have been found among cholinergic as well as noncholinergic neurons [35,36]. Nitric oxide (NO) is known to play crucial roles in learning and memory [14,15], and activates postsynaptic and/or presynaptic cGMP-coupled targets [37,38]. Nevertheless, little is known about the roles of NO in the basal forebrain. On the other hand, cholinergic neurons in the basal forebrain are highly susceptible to NO. Superoxide dismutase (SOD) mRNA levels in BFC neurons were relatively lower than those in cholinergic neurons in the laterodorsal tegmental nucleus (LDTN) and PPTN [13]. In agreement with this low SOD level, BFC neurons are 300 times more susceptible to NO toxicity than those in LDTN/PPTN [12]. Thus, it is very important to address if NO affects the excitability of BFC neurons in spite of its toxicity.

Our recent study using a whole-cell patch-clamp method in in vitro slice preparations disclosed the dual effects of NO on BFC neurons [39]. Under a current-clamp condition in a presumed cholinergic neuron in the basal forebrain, the application of 1 mM S-nitroso-N-acetyl- DL-penicillamine (SNAP, an NO donor) induced hyperpolarization by opening Cs+-sensitive K+ channels through the action of NO (Fig. 5a), while it also caused a membrane depolarization when K+ channels were blocked by the presence of Cs+ (Fig. 5b). The SNAP-induced membrane hyperpolarization was found to be caused by generation of K+ current through activation of NO-cGMP-PKG pathway. In contrast, the SNAP-induced membrane depolarization was found to be mediated by Na+-K+ pump inhibition through the depletion of ATP.

It is well established that NO and peroxynitrite (ONOO-) causes reversible and irreversible impairments of mitochondrial respiration involved in ATP production, respectively [40–42]. In our study, NO-induced depolarization became irreversible after repetitive application of SNAP. This irreversible depolarization might be responsible for and/or induction of subsequent neuronal death, presumably because the irreversible membrane depolarization would increase [Ca2+], which in turn causes various effects such as activation of calpain [43,44] and facilitation of amyloid-β accumulation [45,46], leading to a cell death. In conclusion, BFC neurons may need NO to open leak K+ channels for the better performance of memory and learning, at the risk of their high susceptibility to NO toxicity [12,13]. Crucial roles of NO in the pathogenic mechanism of Alzheimer’s disease have been recognized [47]. Our present study would provide a cellular basis for the putative link between learning/memory and dementia. BFC neurons receive synaptic inputs arising from neurons in PPTN/LDTN [48] as a robust source of NO [49,50]. Since an adequate amount of NO plays an important physiological role while excessive NO exerts a toxic effect in BFC neurons, mastication or trigeminal sensory inputs may be
necessary for LDTN/PPTN neurons to be activated adequately. However, little is known about the neuronal connection between the trigeminal nervous system and LDTN/PPTN. PPTN neurons are involved in regulation of mastication [51] while receiving trigeminal sensory inputs [52].

6. Conclusion
The tooth loss and deficiency in mastication caused an impairment of learning and memory through the degeneration of cholinergic neurons in the basal forebrain, while facilitation of mastication exerted recovery effects on learning and memory in rats that received the permanent middle cerebral artery occlusion. Thus, mastication plays important roles in the maintenance and recovery of learning and memory, presumably through pathogenic and physiological mechanisms of NO action on cholinergic neurons in NDB/MS. In view of the rolls of mastication in higher brain function, it would not be an exaggeration to say that the ultimate aim of prosthetics and prosthetic treatments is the maintenance and improvement of quality of life (QOL).

Acknowledgements
Authors would like to express a great appreciation to Drs. Yoshie Dempo, Tetsuro Makiura, Hiderou Terasawa and the associates for their valuable contribution to the studies used for this review.

References


Lizasoain I, Moro MA, Knowles RG, Darley-Usmar V, Moncada S. Nitric oxide and peroxynitrite exert distinct effects on mitochondrial respiration which are differentially blocked by glutathione or glucose. Biochem J 1996;314:877—80.


