

Effect of masticatory function on central nervous system regeneration in the acute phase of cerebral infarction

Eikichi Matsumoto¹ and *Yoshihiro Momota²

¹Graduate School of Dentistry (Department of Anesthesiology), and ²Department of Anesthesiology, Osaka Dental University, 8-1 Kuzuhahanazono-cho, Hirakata-shi, Osaka 573-1121, Japan

*E-mail: momota@cc.osaka-dent.ac.jp

We examined the influence of masticatory function on central nervous system regeneration after the onset of cerebral infarction. For this experiment, we used a CB17 mouse cerebral ischemia model, which has high reproducibility. The mice were divided into two groups: the hard diet group (the left middle cerebral artery was occluded, and hard, solid food was given after the ischemic operation), and the soft diet group (the left middle cerebral artery was occluded, the upper and lower incisors were extracted, and powdery food was given after the ischemic operation). Brain tissue sections were prepared in both groups at 3, 7 and 14 days after the ischemic operation, and the pattern of nerve, glial and neural stem cell expression was investigated using immunohistochemical staining.

In the hard diet group, although the expression of astrocytes and nestin-positive neural stem cells was confirmed in infarcted foci up to 7 days after the ischemic operation, there was no expression of astrocytes or neural stem cells in infarcted foci at 14 days after the operation. On the other hand, in the soft diet group, although the expression of astrocytes was confirmed in infarcted foci up to 14 days after the ischemic operation, nestin-positive neural stem cells were confirmed in infarcted foci 3 days after ischemia. In the penumbra area, neural stem cell expression was noted in the two groups, suggesting that the nerve regeneration capacity was maintained.

In the hippocampal dentate gyrus, nestin-positive and GFAP-positive cells were observed up to 14 days after ischemia in both groups. In the hard diet group, the expressions of nestin-positive and GFAP-positive cells reached a peak at 14 days after the ischemic operation, confirming the enhancement of reactive astrocyte expression. On the other hand, in the soft diet group, the expressions of nestin-positive and GFAP-positive cells reached a peak at 7 days after the ischemic operation.

The results of this experiment confirmed that intrinsic neural stem cells were induced in the infarcted area and hippocampal dentate gyrus in the acute phase of cerebral infarction, suggesting that the nerve regeneration capacity in the hippocampal dentate gyrus is enhanced during the early phase in the presence of masticatory hypofunction, in comparison with a state in which the masticatory function is maintained. In addition, we found that reactive astrocytes were closely involved in the mechanism of nerve regeneration in the hippocampal area. (J Osaka Dent Univ 2022; 56: 79-86)

Key words: Neural stem cell; Ischemic stroke; Mastication; Hippocampal dentate gyrus

INTRODUCTION

It has been reported that cerebrovascular disorders

and their sequelae account for more than one-third of the bedridden in Japan. Dysphagia or masticatory hypofunction may worsen the conditions asso-

ciated with cerebrovascular disorders, increase the risk of dementia, and influence nerve activity/synapse formation and neurotrophic factor expression, markedly influencing nerve function recovery. Rehabilitation should be started within 48 hours after onset of the acute phase of cerebral infarction. In the field of dentistry, although rehabilitation for preventing oral function disuse is often performed, standard diet ingestion is difficult during the acute phase of cerebral infarction, even when oral ingestion is possible. This is because masticatory function may be restricted.

It has recently been reported that the frequency of mastication has decreased with the widespread use of soft, highly nutritious processed foods, inducing various types of higher brain dysfunction, including memory/learning disorders, in addition to a reduction in masticatory muscle strength.^{1,2} It has also been noted that the maintenance of masticatory movement increases proliferation of neural stem cells in the hippocampal area, enhancing the capacity to produce new neurons. This suggests a relationship with NO-producing cells in the hippocampal dentate gyrus.³⁻⁵ Many studies on neurogenesis have investigated intracellular signal transmission by intrinsic neural stem cells in the hippocampal area, motor hypofunction-related hypoxic stress, oxidative stress, and glutamic acid stress. It is unclear now masticatory locomotor stimulation is related to cellular character changes in the infarcted or hippocampal areas, or to stem cell formation.

In this study, we focused on the pattern of nestin-positive neural stem/glial cell expression in infarcted foci of the cerebral cortex and hippocampal area at 3, 7 and 14 days after cerebral infarction. We divided mice into two groups: a hard diet group in which solid food was given, and a soft diet group in which powdery food was given after extraction of the upper and lower incisors following cerebrovascular occlusion using a mouse acute-phase cerebral infarction model. We then examined the influence of the masticatory function on central nervous regeneration.

MATERIALS AND METHODS

Animal studies

Six-to-10-week-old male CB 17 mice (CB-17/Icr-+/+Jcl; CLEA Japan, Tokyo, Japan) were used in all experiments. All procedures were performed in accordance with a protocol that was approved by the Animal Research Committee of Osaka Dental University (Approval no. 21-06001) and complied with the Animal Research Guidelines of the University. The experiments were designed to minimize pain and the number of animals used. Mice had free access to water and food during the study period, and were not under any restrictions.

Experiments

The induction of infarction areas in the cerebral cortices of mice has been shown to be highly reproducible and limited to the ipsilateral cerebral cortex.^{6,7} Permanent middle cerebral artery (MCA) occlusion was induced according to our modification of the Tamura method.^{8,9} MCA occlusion was induced and maintained by inhalation of 1-5% sevoflurane. Skin incision was made between the left eyeball and left auditory meatus. The left salivary gland and veins were carefully removed to visualize the zygoma. The left zygoma was dissected under an operating microscope (KOM300S; Konan Medical, Nishinomiya, Japan), and the jaw joint was detached to allow visualization of the MCA through the cranial bone. A hole 1.5 mm in diameter was made in the bone using a dental drill.

Subsequently, the dura matter was carefully removed so as not to damage the surface of the brain. The MCA was then isolated, electrocauterized, and disconnected distal to where it crossed the olfactory tract. It is notable that very mild and gradual coagulation is required in order for electrocoagulation to avoid bleeding from the MCA. We used an electrocoagulator designed for ophthalmologic surgery (Aaron 940; Bovie Medical, Clearwater, FL, USA) and bipolar forceps at an output level of 1.0 Watt. Sequential, very brief coagulation with the top of the bipolar forceps was carried out, starting from the distal portion of the MCA to the proxi-

mal. Two to four repetitions of this procedure usually stopped blood flow in the vessel. The MCA was then coagulated from the distal to the proximal. This method resulted in a very low risk of bleeding from the MCA. Body temperature was maintained at 37-37.5°C using a heat lamp during the operation.

This experiment was conducted by dividing mice into two groups. In the hard diet group cefmetazole sodium was applied after MCA occlusion to the brain surface, and the skin was sutured before recovery from anesthesia. In the soft diet group cefmetazole sodium was applied to the brain surface after MCA occlusion, and the skin was sutured. Subsequently, the upper and lower incisors were extracted using forceps. After confirming hemostasis, the mice were allowed to recover from anesthesia.

Immunohistochemistry

For the histochemical analysis of post-permanent cortical ischemic tissues, the mice were anesthetized with sodium pentobarbital (50 mg/kg) and transcardially perfused with 100 mL 4% paraformaldehyde at 3, 7 and 14 days after MCA occlusion. After overnight fixation, brain samples were cryoprotected in 30 % sucrose and frozen at -80°C. Coronal sections of 16 µm were then prepared using a cryostat and subjected to immunohistochemistry with antibodies against microtubule-associated protein 2 (MAP2) at 1:500 (Sigma-Aldrich, St. Louis, MO, USA), glial fibrillary acidic protein (GFAP) at 1:200 (Bioss Antibodies, Woburn, MA, USA), and nestin at 1:200 (Merck Millipore, Darmstadt, Germany). Primary antibodies were visualized using Alexa Fluor 488- or 555-conjugated secondary antibodies at 1:500 (Invitrogen, Carlsbad, CA, USA). Nuclei were stained with 4', 6-diamidino-2-phenylidole (DAPI) at 1:1000 (Sera Care, Gaithersburg, MD, USA). The stained slides were examined using a DP74 Fluorescence Microscope (Olympus, Tokyo, Japan) and images were acquired using the Olympus software cellSens.

RESULTS

Cell expression at the site of cerebral cortex infarction (Figs. 1-4)

In both the hard and soft diet groups, MAP2-positive mature nerve cell loss in infarcted foci was observed at 3, 7 and 14 days after the ischemic operation. The expression of GFAP-positive astrocytes in the penumbra was noted from 3 until 14 days after the operation in both groups. However, in the hard diet group, decidual cells were observed in infarcted foci at 14 days after the operation. In this group, the expression of nestin-positive cells in the infarcted foci and penumbra was noted at 3 and 7 days after the ischemic operation, but not in the infarcted foci at 14 days. In the soft diet group, although the expression of nestin-positive cells in the penumbra and infarcted foci was observed at 3 days after the operation, there were no nestin-positive cells in any infarcted focus at 7 or 14 days. In addition, nestin + GFAP-positive astrocytes were detected in the penumbra and infarcted foci until 7 days after the ischemic operation in the hard diet group. In the soft diet group, although they were confirmed in the penumbra and infarcted foci until 3 days after the operation, their expression was noted in the penumbra only at 7 and 14 days after ischemia.

Cell expression in the hippocampal dentate gyrus (Figs. 5 and 6)

In both the hard and soft diet groups, nestin- and nestin + GFAP-positive astrocytes were detected from 3 until 14 days after the ischemic operation. In the former, the expression of nestin-positive cells reached a peak at 14 days after the operation. In the latter, it reached a peak at 7 days after the operation. There was no difference in the pattern of GFAP-positive cell expression between the two groups.

DISCUSSION

For rehabilitation during the acute phase of a cerebral infarction, in the field of dentistry an attempt is made to regain masticatory function as early as

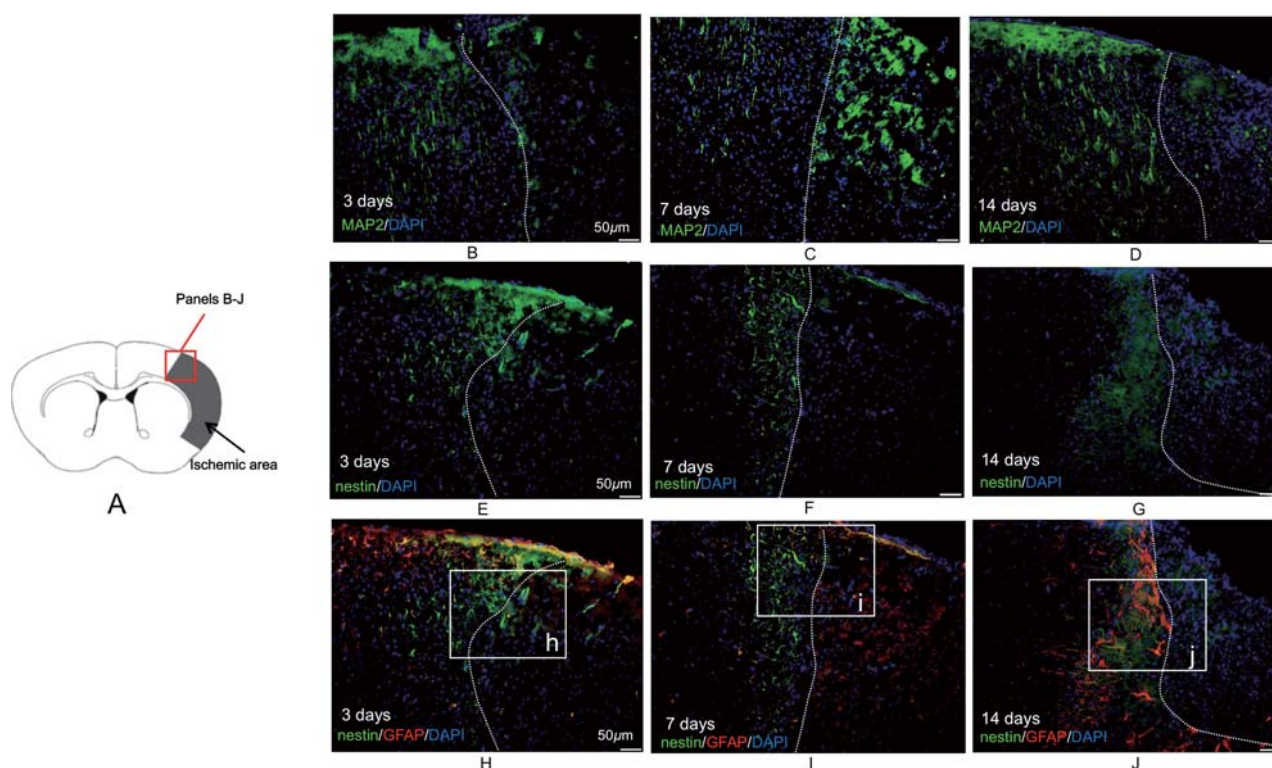


Fig. 1 Ischemic area of the cerebral cortex in the hard diet group (A).

The loss of MAP2-positive neurons was observed in infarcted foci at 3, 7 and 14 days after ischemia (B, C and D). Although nestin-positive neural stem cells were noted in infarcted foci and penumbra areas at 3 days (E) and 7 days (F) after ischemia, they were seen only in the penumbra at 14 days after ischemia (G). Although GFAP-positive astrocytes were observed in infarcted foci and penumbra areas at 3 days (H) and 7 days (I) after ischemia, they were only in the penumbra at 14 days (J) (Scale bar = 50 μm).

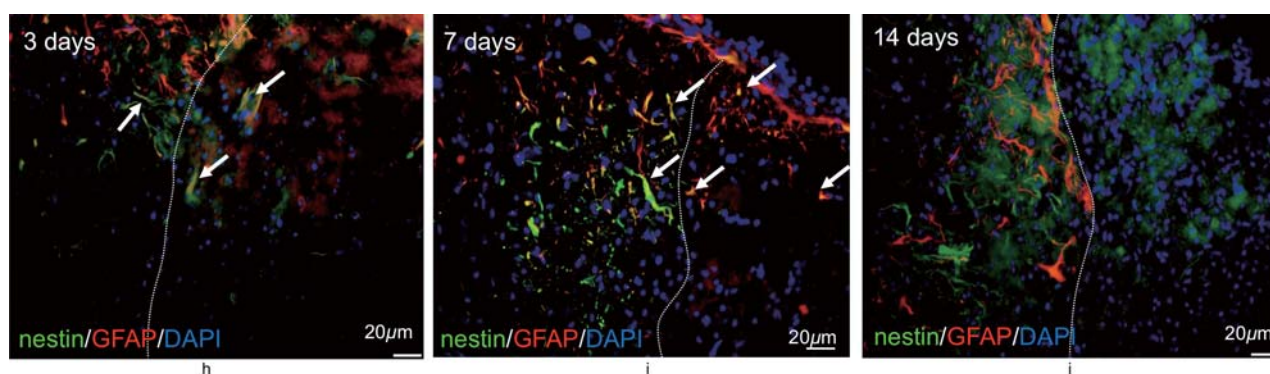


Fig. 2 Ischemic area of the cerebral cortex in the hard diet group.

Although nestin-positive cells merging with GFAP-positive cells (white arrows) were confirmed in the penumbra and infarcted foci at 3 days (h) and 7 days (i) after ischemia, they were not seen at 14 days (j) (Scale bar = 20 μm).

possible by promoting solid food ingestion (not a liquid diet) to prevent oral function disuse. Masticatory movement is important for maintaining cognitive/learning functions. It has been reported that nerve regeneration capacity is primarily maintained

in the hippocampal area of the adult brain even in daily living.¹⁰ We previously found that neural stem cells were induced around the microvessels of an ischemic area under lethal/non-lethal cerebral ischemia loading, and reported the expression of

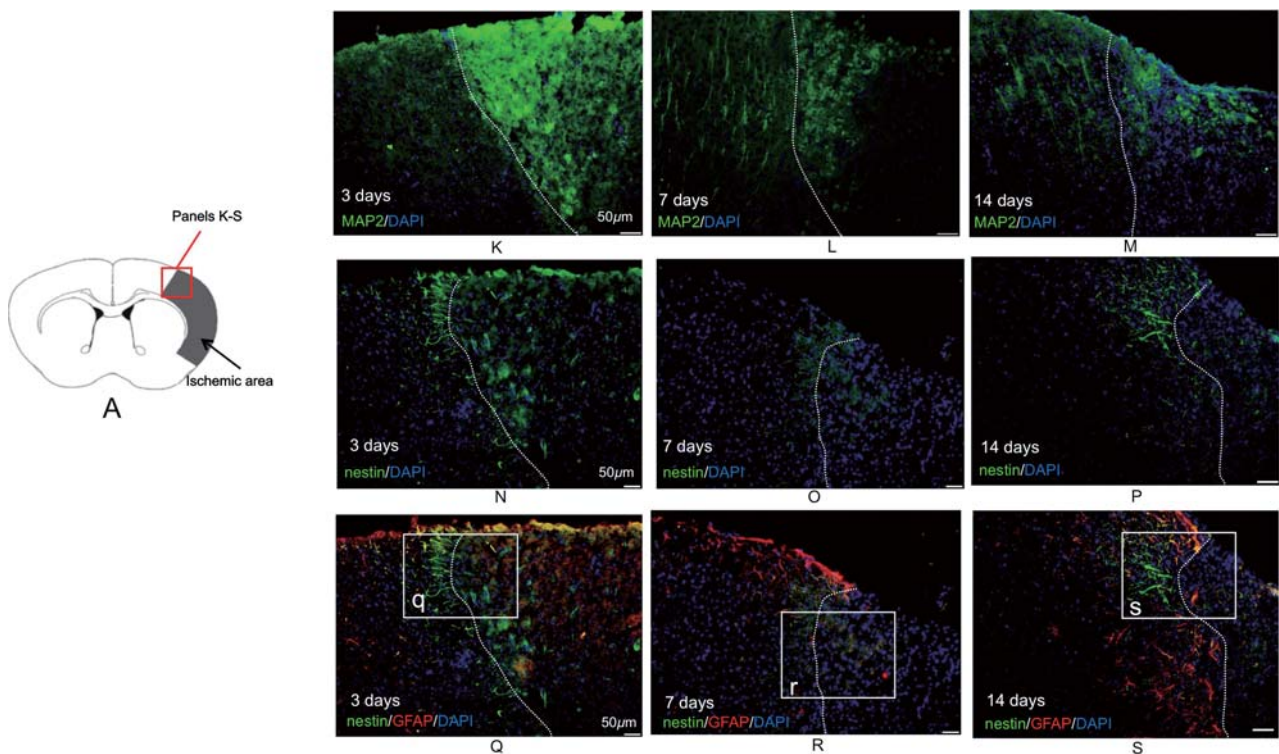


Fig. 3 Ischemic area of the cerebral cortex in the soft diet group (A).

The loss of MAP2-positive neurons was observed in infarcted foci at 3, 7 and 14 days after ischemia (K, L and M). Nestin-positive neural stem cells were noted in infarcted foci and penumbra areas 3 days after ischemia (N), but only in the penumbra at 7 days (O) and 14 days (P). GFAP-positive astrocytes were observed in infarcted foci and penumbra areas at 3 days (Q), 7 days (R), and 14 days (S) after ischemia (Scale bar=50 μ m).

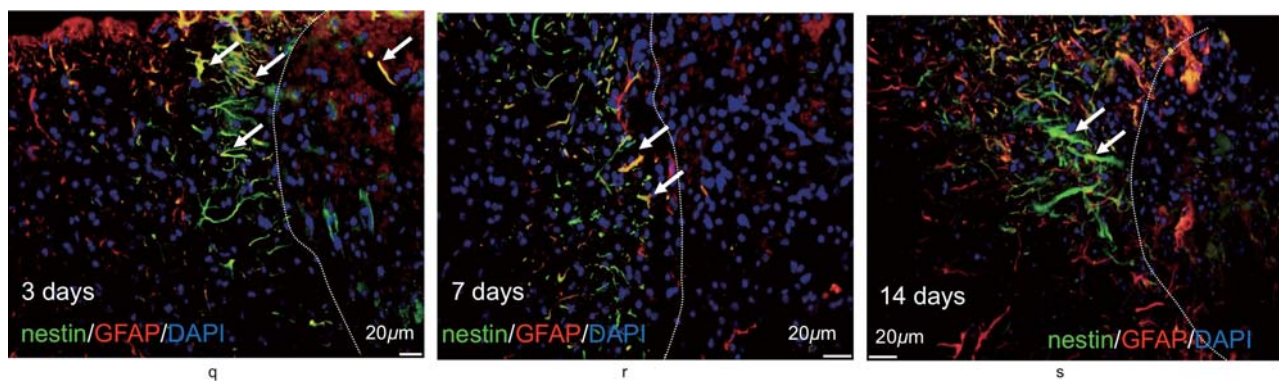


Fig. 4 Ischemic area of the cerebral cortex in the soft diet group.

Nestin-positive cells merging with GFAP-positive cells (white arrows) were confirmed in the penumbra and infarcted foci at 3 days after ischemia (q), but only in the penumbra at 7 days (r) and 14 days (s) (Scale bar=20 μ m).

vascular pericyte-derived neural stem cells in infarcted cerebral foci. We found that the nerve regeneration capacity by intrinsic neural stem cells was enhanced in the presence of ischemic stress.^{11, 12}

On the other hand, concerning neurogenesis in the hippocampal dentate gyrus, it has been reported that exercise induces acetylcholine stimulation,¹³ enhancing neuron production. In addition, biochemical stimuli associated with masticatory-

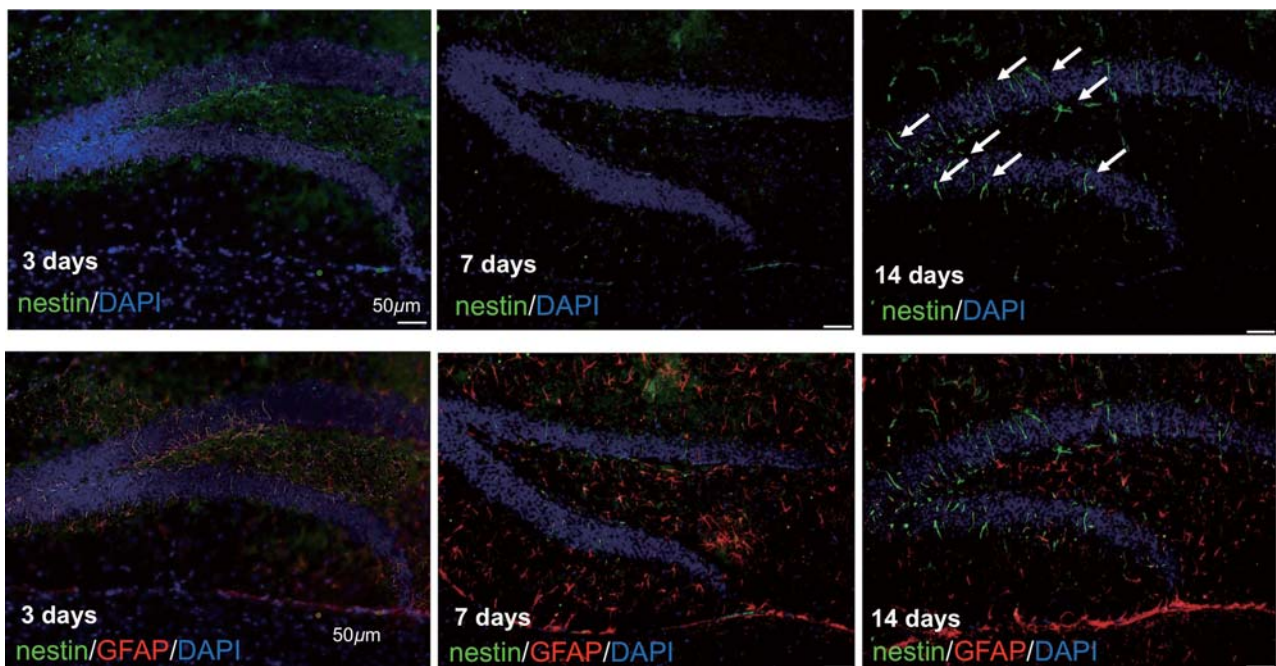


Fig. 5 Hippocampal dentate gyrus in the hard diet group.

Nestin-positive cells and nestin+GFAP-positive cells appeared at 3, 7 and 14 days after ischemia. The expression of nestin-positive cells was the most marked at 14 days (white arrows) (Scale bar=50 μm).

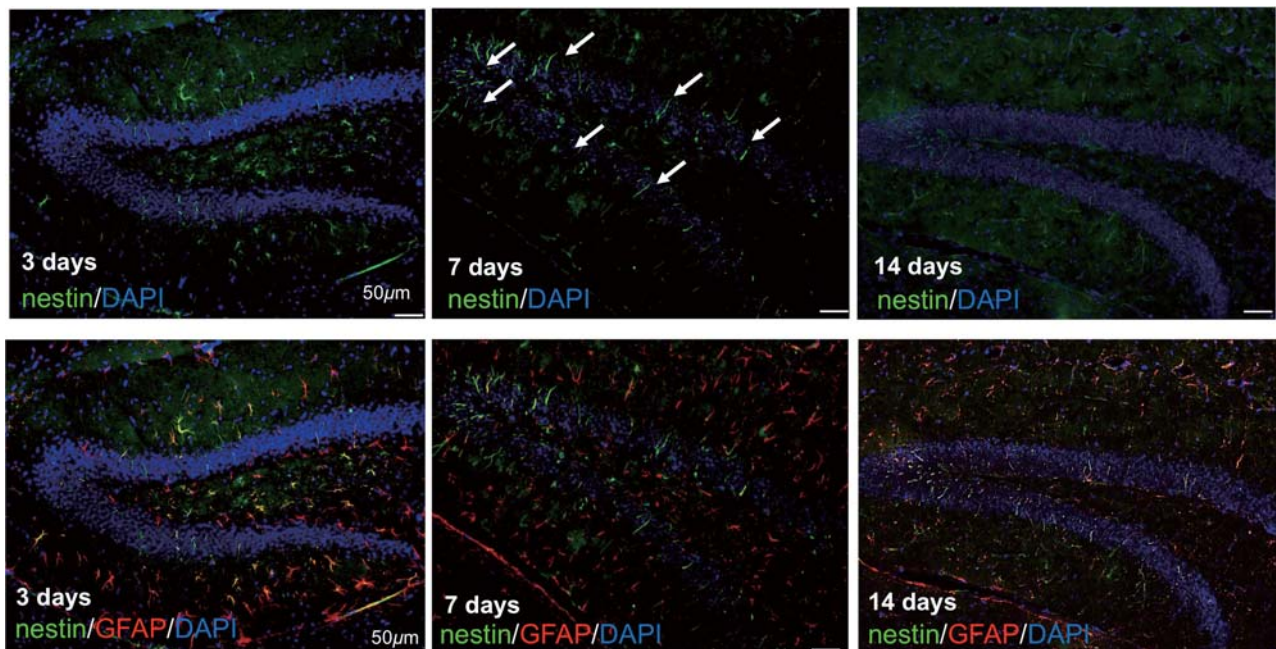


Fig. 6 Hippocampal dentate gyrus in the soft diet group.

Nestin-positive cells and nestin+GFAP-positive cells appeared at 3, 7 and 14 days after ischemia. The expression of nestin-positive cells was the most marked at 7 days (white arrows) (Scale bar=50 μm).

movement-related increases in the heart rate and cerebral blood flow may be involved in hippocampal neurogenesis.⁴ Glial and neural stem cells are activated by ischemic loading. In addition, maintenance of sufficient masticatory function during the acute phase of a cerebral infarction may be advantageous for central nervous regeneration. However, in clinical practice, it is often difficult to ingest solid food by mastication during the acute phase of a cerebral infarction. Furthermore, the use of dentures is difficult; sufficient masticatory function may not be possible.

In this study, we confirmed the expression of glial/neural stem cells in ischemia-loaded areas even in the soft diet group, similar to that in the hard diet group. In particular, reactive astrocytes having the cellular character of neural stem cells in the penumbra area may be closely involved in the maintenance of nerve regeneration capacity. This suggests that the maintenance of masticatory function in some form contributes to central nervous regeneration capacity regardless of the amount of masticatory movement. In adults, neural stem cells are present in the subventricular zone and hippocampal dentate gyrus, and ischemia-related neuron damage enhances the mechanism of nerve regeneration through chemotaxis. In particular, neurogenesis in the hippocampal dentate gyrus is important for the development of higher brain function responsible for memory and learning.

A reduction in masticatory stimulation during a growth period influences neurons, reducing memory/cognitive functions.¹⁴ In our study, in the hard diet group, which was established to create sufficient masticatory function, the peak of nestin-positive cell/reactive astrocyte expression in the hippocampal dentate gyrus was delayed when compared with the soft diet group. This suggests that, if masticatory function is maintained, neurogenesis in the hippocampal dentate gyrus is enhanced even after the mechanism of nerve regeneration at the site of ischemic damage had decreased at 7 days, enhancing the subsequent mechanism of nerve regeneration. Previous studies have indicated that the survival rate of neural stem cells in the hippocam-

pal dentate gyrus decreases with a reduction in masticatory function.¹⁵ However, in the acute phase of cerebral infarction, nerve regeneration capacity may be enhanced from the early phase, (up to 7 days after ischemia). This occurred even in the hippocampal dentate gyrus in the presence of masticatory hypofunction in the soft diet group, with the activation of the nerve regeneration capacity at the site of ischemic damage starting 3 days after ischemic loading. This indicates that functional factors have no influence, such as chewing force or frequency of mastication, during the acute phase of cerebral infarction. During this phase, it is often difficult to ingest solid food as sufficient masticatory function may not be achieved. However, it seems that neurogenesis by glial/neural stem cells in the hippocampal dentate gyrus is promoted by enhancement of the mechanism of nerve regeneration at the site of ischemic damage.

CONCLUSION

Our results suggest that, when food ingestion can be started during the acute phase of a cerebral infarction, nerve regeneration capacity at the site of ischemic damage and the hippocampus can be maintained through masticatory movement, such as mastication of solid food, even under circumstances in which sufficient masticatory function is not exhibited, such as the ingestion of a fluid-diet.

Acknowledgments

The author would like to thank the members of the Institute for Advanced Medical Sciences, Department of Therapeutic Progress in Brain Diseases, Hyogo College of Medicine, for their support and guidance.

REFERENCES

1. Konaka K, Kondo J, Hirota N, Tamine K, Hori K, Ono T, Maeda Y, Sakoda S, Naritomi H. Relationship between tongue pressure production and dysphagia in stroke patients, *Euro Neurol* 2010; **64**: 101-107.
2. Smith N, Miquel-Kergoat S, Thuret S. The impact of mastication on cognition: Evidence for intervention and the role of adult hippocampal neurogenesis. *Nutrition and Aging* 2015; **3**: 115-123.
3. Mitome M, Hasegawa T, Shirakawa T. Mastication influences the survival of newly generated cells in mouse dentate gyrus. *Neuroreport* 2005; **16**: 249-252.
4. Weijenberg RAF, Scherder EJA, Lobbezoo F. Mastication for the mind- the relationship between mastication and cog-

- nition in ageing and dementia. *Neuroscience & Biobehavioral Reviews* 2011; **35**: 483-497.
5. Yamamoto T, Hirayama A, Hosoe N, Furube M, Hirano S. Soft-diet feeding inhibits adult neurogenesis in hippocampus of mice. *Bull Tokyo Coll* 2009; **50**: 117-124.
 6. Taguchi A, Kasahara Y, Nakagomi T, Stern DM, Fukunaga M, Ishikawa M, Matsuyama T. A reproducible and simple model of permanent cerebral ischemia in CB-17 and SCID mice. *J Exp Stroke Transl Med* 2010; **3**: 28-33.
 7. Nakagomi T, Taguchi A, Fujimori Y, Saino O, Nakano-Doi A, Kubo S, Gotoh A, Soma T, Yoshikawa H, Nishizaki T, Nakagomi N, Stern DM, Matsuyama T. Isolation and characterization of neural stem/progenitor cells from post-stroke cerebral cortex in mice. *Eur J of Neurosci* 2009; **29**: 1842-1852.
 8. Tamura A, Gotoh O, Sano K. Focal cerebral infarction in the rat: I. Operative technique and physiological monitorings for chronic model. *No To Shinkei* 1986; **38**: 747-751.
 9. Kasahara Y, Ihara M, Nakagomi T, Momota Y, Stern DM, Matsuyama T, Taguchi A. A highly reproducible model of cerebral ischemia/reperfusion with extended survival in CB-17 mice. *Neurosci Res* 2013; **76**: 163-168.
 10. Fukushima-Nakayama Y, Ono T, Hayashi M, Inoue M, Wake H, Ono T, Nakashima T. Reduced mastication impairs memory function. *J Dent Res* 2017; **96**: 1058-1066.
 11. Nakata M, Nakagomi T, Maeda M, Nakano-Doi A, Momota Y, Matsuyama T. Induction of perivascular neural stem cell and possible contribution to neurogenesis following transient brain ischemia/reperfusion injury. *Transl Stroke Res* 2017; **8**: 131-143.
 12. Momota Y. Induction of pericyte-derived neural stem cells following transient brain ischemia/reperfusion injury. *Cerebral Blood Flow and Metabolism* 2017; **28**: 347-351.
 13. Itoh Y, Nochi R, Kuribayashi H, Saito Y, Hisatsune T. Cholinergic activation of hippocampal neural stem cells in aged dentate gyrus. *Hippocampus* 2011; **21**: 446-459.
 14. Koehl M, Abrous DM. A new chapter in the field of memory: Adult hippocampal neurogenesis. *Eur J Neurosci* 2011; **33**: 1101-1114.
 15. Akazawa Y, Kitamura T, Fujihara Y, Yoshimura Y, Mitome M, Hasegawa T. Forced mastication increases survival of adult neural stem cells in the hippocampal dentate gyrus. *Int J Mol Med* 2013; **31**: 307-314.