Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Short-term mastication after weaning upregulates GABAergic signalling and reduces dendritic spine in thalamus

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ARTICLE INFO

Article history:
Received 20 February 2018
Accepted 6 March 2018
Available online xxx

Keywords:
Mastication
GABA
Dendritic spine
Thalamus
Transcriptome

ABSTRACT

Mastication enhances brain function and mental health, but little is known about the molecular mechanisms underlying the effects of mastication on neural development in early childhood. Therefore, we analysed the gene expression in juvenile neural circuits in rats fed with a soft or chow diet immediately after weaning. We observed that the gene expression patterns in the thalamus varied depending on the diet. Furthermore, gene ontology analysis revealed that two terms were significantly enhanced: chemical synaptic transmission and positive regulation of dendritic spine morphogenesis. With respect to chemical synaptic transmission, glutamate decarboxylase and GABA receptors were upregulated in the chow diet group. The related genes, including vesicular GABA transporter, were also upregulated, suggesting that mastication activates GABAergic signalling. With respect to dendritic spine morphogenesis, Ingenuity Pathway Analysis predicted fewer extension of neurites and neurons and fewer number of branches in the chow diet group. The numbers of spines in the ventral posterolateral and posteromedial regions were significantly decreased. These results suggest that mastication in the early developing period upregulates GABAergic signalling genes, with a decrease of spines in the thalamus.

1. Introduction

Mastication, the first step of digestion and absorption, evokes many effects in the body [1–3]. The relationships between mastication and development of brain function for memory, attention, and stress mitigation have been phenomenologically studied in rodents, primates, and humans [1,2,4]. In studies on rodents continuously fed a soft or solid diet, behavioural tests showed that a solid diet improved memory-related functions [5,6], whereas a soft diet increased the risk of mental disorders [7]. However, the effects of mastication on the central nervous system at the molecular [8–10] and morphological [11–13] levels have been restricted to the hippocampus. Conversely, a human study revealed changes in the cerebral blood flow rate in many areas, including the sensorimotor cortex, supplementary motor area, cerebellum, insula, and thalamus [14,15]. This indicates that mastication has some influence on regions other than the hippocampus.

The thalamus plays an important role as a relay point in the transmission of various perceptual sensory stimuli from auditory, somatic, visceral, gustatory, and visual systems to the basal ganglia, cerebral cortex, and hippocampus [16–18]. Neural circuit plasticity during early life, a critical period, is well-studied with regard to thalamocortical connections with the visual system [19–21]. In addition, the thalamus was reported to control nerve activity. For example, by causing alerts and controlling the position memory, the prefrontal–thalamo–hippocampal circuit navigates to the next action, interprets, and modifies the input signal and sends it to higher-order regions, such as the cerebral cortex, basal ganglia, and hippocampus [22].

With respect to mastication, studies have reported a bidirectional circuit between the thalamus and cerebral cortex [23] to

Abbreviations: DAVID, Database for Annotation, Visualization and Integrated Discovery; DEGs, Differentially Expressed Genes; GO, Gene Ontology; VPL, Ventral Posterolateral; VPM, Ventral Posteromedial.

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https://doi.org/10.1016/j.bbrc.2018.03.032

control rhythmic jaw movements in various mammalian species, including rodents and primates [24,25]. The development of this coordinated system may require appropriate mastication in the growing stage because mice fed a liquid diet showed less ability to chew compared with those fed a hard diet [26]. The former mice were less able to discriminate the hardness of the ingested food and could not perform appropriate masticatory exercise because of an unstable mastication path and rhythm. In this developmental period, the neural network is highly established, and we predict that the stimulation of mastication in this period largely affects the cranial nerve circuit morphology. In the present study, we analysed the gene expression in juvenile neural circuits immediately after weaning. We investigated the effects of mastication on the brain, particularly the thalamus, during the developmental period.

2. Materials and methods

2.1. Feed, animals, and breeding method

10 three-week-old male Wistar rats, about 35 g weight, (CLEA Japan, Tokyo, Japan) were bred in a room conditioned at 22 ± 1 °C and 50% humidity, a 12-h light:dark cycle. To avoid chewing any materials other than the administered diet, the two plastic cages were stacked with the one cage upside down. As a preparatory phase of the breeding process, the rats were administered a powdered feed and water for 2 days *ad libitum*. Then, the rats were fed with a powdered feed (powder, P) or a solid feed (chow, C) for another 8 days (Supplementary Table 1). Animals' individual IDs were assigned to P1–P5 (n = 5) and C1–C5 (n = 5), respectively. The rats were allowed to consume the diet and water at any time of the day. This experiment was conducted under strict adherence to the regulations on animal experiments, as defined by the University of Tokyo, and the study protocol was approved by an ethics board of this university.

2.2. Measurement of biochemical parameters

Body weight, feed intake, and water ingestion were measured daily at 10 a.m. After breeding for 8 days, the rats were anesthetised with isoflurane (Wako Pure Chemical Industries, Osaka, Japan) during the dissection process; thereafter, the thalamus was extirpated, and their blood was collected. Serum was separated using a Venoject blood collection tube (Terumo Corporation, Tokyo, Japan) and preserved at −80 °C. The serum corticosterone concentration was measured with a corticosterone EIA kit (Arbor Assays, Ann Arbor, MI, USA).

2.3. Extraction of total RNA from thalamus

RNA extraction was performed as described previously [27]. The total RNA was extracted with the TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and purified with RNeasy Mini Kit (Qiagen, Hilden, Germany). While 10 rats were included in the study, the following analyses were conducted only on nine individuals because RNA extracted from one rat in the chow diet group had insufficient purity.

2.4. DNA microarray assay and data analysis

Sample preparation and analysis were performed as described previously [27]. Briefly, Affymetryx GeneChip Rat Genome 230 2.0 Array (Thermo Fisher Scientific) was used and the obtained CEL file was normalised using the statistical software R Version 2.10.1 with the method of factor analysis for robust microarray summarisation. Hierarchical clustering was performed using the signal values of the normalised probe set. Two groups were compared using the rank products method in R Version 3.1.2, and a probe set with false discovery rate (FDR) < 0.05 was extracted as differentially expressed genes (DEGs).

Gene ontology (GO) analysis was performed using Database for Annotation, Visualization and Integrated Discovery (DAVID) 6.8 (https://david.ncifcrf.gov/). GO terms, where the EASE score was corrected using a multiple comparison by the Benjamini and Hochberg FDR of < 0.05, were extracted as significantly concentrated. Ingenuity Pathway Analysis (Qiagen) was performed on the probe set extracted by the comparison between the two diet groups. This was followed by an extraction of a diseases and functions of the nervous system that satisfied the following conditions: $p < 0.05$ and $|\text{Z-score}| \geq 1.5$.

2.5. Golgi–Cox staining

Ten animals, five with chow diet and five with powder diet, were bred according to the method of feed, animals, and breeding method section in this paper. A Golgi–Cox staining was performed using a slice Golgi Kit (Bioenno Lifesciences, Santa Ana, CA, USA). During the dissection process, the rats were perfused and fixated with 0.9% saline solution and a fixative solution contained in the fixative kit under anaesthesia. Their brains were removed and a block with a dimension of 1 cm × 1.5 cm × 0.6 cm was prepared for each brain. The blocks were immersed in the fixative solution at 4 °C overnight.

From the block immersed in the fixative kit, continuous coronal slices with a 100-μm thickness were prepared using a vibratome, VT1000S (Leica, Wetzlar, Germany); the slices were subsequently post-fixed for 1 h. Thereafter, the fixed slices were immersed in an impregnation solution (1% mercury II chloride; Bioenno Lifesciences) for 7 days and stained with Solution C (Bioenno Lifesciences). The stained slices were mounted on slides coated with a gelatin solution (0.5% gelatin; Nacalai Tesque, Kyoto, Japan and 0.05% potassium sulphate chromium III-12-water; Wako Pure Chemical Industries), dehydrated with 100% ethanol (Kanto Chemical, Tokyo, Japan), dissolved in xylene (Kanto Chemical), and enclosed with Entellan New (Wako Pure Chemical Industries).

The section closest to the center of the ventral posteromedial region per slice, between the bregma (−3.36 to −4.08 mm), was analysed. For each rat, seven cell bodies were randomly selected from the aforementioned section. Images were captured using

Table 1
Significantly enriched GO terms in differentially expressed genes in the thalamus.

Go-ID	Go Term	Benjamini's *p*-value
GO:0021987	Cerebral cortex development	6.73E-03
GO:0007268	Chemical synaptic transmission	1.44E-02
GO:0007626	Locomotory behavior	9.73E-04
GO:0000226	Microtubule cytoskeleton organization	3.11E-02
GO:0050885	Neuromuscular process controlling balance	2.94E-02
GO:0061003	Positive regulation of dendritic spine morphogenesis	1.93E-02

Please cite this article in press as: M. Ogawa, et al., Short-term mastication after weaning upregulates GABAergic signalling and reduces dendritic spine in thalamus, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.032