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The Effect of Functional Mandibular Shift on the Muscle Spindle Systems in Head-Neck Muscles and the Related Neurotransmitter Histamine

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Abstract: The aim of this study is to explore the effects of abnormal occlusion and functional recovery caused by functional mandible deviation on the head and neck muscles and muscle spindle sensory-motor system by electrophysiological response and endogenous monoamine neurotransmitters' distribution in the nucleus of the spinal tract. Seven-week-old male Wistar rats were randomly divided into 7 groups: normal control group, 2W experimental control group, 2W functional mandible deviation group, 2W functional mandible deviation recovery group, 4W experimental control group, 4W functional mandible deviation group, 4W functional mandible deviation recovery group. Chewing muscles, digastric muscle, splenius, and trapezius muscle spindles electrophysiological response activities at the opening and closing state were recorded. And then the chewing muscles, digastric, splenius, trapezius, and neck trigeminal nucleus were taken for histidine decarboxylase (HDC) detection by high performance liquid chromatography (HPLC), immunofluorescence, and reverse-transcription polymerase chain reaction (RT-PCR). Histamine receptor proteins in the neck nucleus of the spinal tract were also examined by immunofluorescence and RT-PCR. Electromyography activity of chewing muscles, digastric, and splenius muscle was significantly asymmetric; the abnormal muscle electromyography activity was mainly detected at the ipsilateral side. After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle and splenius decreased, muscle excitement weakened, modulation depth decreased, and the muscle spindle afferent impulses of excitation transmission speed slowed down. Changes for digastric muscle electrical activity were contrary. The functions recovered at different extents after removing the deflector. However, trapezius in all the experimental groups and recovery groups exhibited bilateral symmetry electrophysiological responses, and no significant difference compared with the control group. After functional mandibular deviation, HDC protein and messenger ribonucleic acid (mRNA) levels on the ipsilateral sides of the chewing muscle and splenius increased significantly. HDC level changes for digastric muscle were contrary. After the removal of the mandibular position deflector, HDC protein and mRNA levels decreased on the ipsilateral sides of the chewing muscle and splenius while they increased in the digastric muscle. The difference of histamine decarboxylase content in the bilateral trapezius in each experimental group was small. After functional mandibular deviation, the temporomandibular joint mechanical receptors not only caused the fusimotor fiber hypoallergenic fatigue slow response on the ipsilateral sides of splenius, but also increased the injury neurotransmitter histamine release. The authors' results further support the opinion that the temporomandibular joint receptors may be involved in the mechanical theory of the head and neck muscles nervous system regulation.

Key Words: Functional mandible deviation, fusimotor fiber, histamine decarboxylase, neck trigeminal nucleus

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Mandibular asymmetry is a common finding in people with normal facial appearance.[1] However, severe asymmetry may create functional abnormality and esthetic concerns.[2] Recent observations in people with concomitant mandibular suggest a close functional relationship between the head-neck motor systems and the mandibular.[3] Previous researches on neck and stomatognathic system relationship focused on the following points: ''Functional jaw movements'' are the result of jaw and neck muscles activation, leading to simultaneous movements in the at lanto-occipital, temporomandibular, and the cervical spine joints.[3,4] Functional mandibular displacement caused by unilateral posterior cross bite could cause mandibular asymmetry.[5] A few studies have studied the morphological changes in the temporomandibular joint (TMJ), including smaller superior condylar space, smaller condylar head, and steeper eminence on the shifted side in asymmetric patients.[6] Other studies also suggested that there was a higher incidence of

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disk displacement and temporomandibular dysfunction symptom on the deviated side in mandibular asymmetric patients.[7] These findings imply that the asymmetric morphology of TMJ will interact with its dysfunction to some degree.[8]

Mechanical stimuli delivered by a functional shift produce a series of morphological and histological responses in the mandibular asymmetry in rats.[9] The upper quarter should be evaluated in patients with more complex or persistent symptoms in the head and neck region.[10] However, the muscle spindle sensory-motor system electrophysiological response and endogenous monoamine neurotransmitters' distribution in the central nucleus have not been clarified.

Proprioception and nociceptors distributed in the mouth jaw and neck muscle are the first step to transform the external stimuli into nerve impulses incoming. Muscle spindle is a kind of special receptor in skeletal muscle. The responses of the muscle spindle to stretch are produced by the sensory terminals' transduction and by impulse initiation in the sensory axon.[11] And the activity of the histidine decarboxylase (HDC) is a proposed marker of muscle fatigue in the masseter muscle.[12]

The aim of this study is to explore the effects of abnormal occlusion and functional recovery caused by functional mandible deviation on the head and neck muscles, muscle spindle sensory-motor system electrophysiological response and endogenous monoamine neurotransmitters distribution in the central nucleus. This study could also provide neurophysiological mechanisms for occlusion of head and neck disorders caused by stomatognathic system dysfunction. What is more, our study offers a theoretical basis for in-depth understanding of the adjustment of mandibular location and the associated neuromuscular relationship, and provides some experimental basis for the method of clinical orthodontic correction at the same time.

METHODS

Animal Preparation

All study protocols were approved by the Animal Testing Committee Guidelines at the Capital Medical University. Animal care and handling procedures were in accordance with Guiding Principles for the Care and Use of Animals in the Capital Medical University, China. Sixty seven-week-old male Wistar rats (180g ± 16 g) were randomly divided into 7 groups: Normal control group (NC, n = 10), 2W experimental control group (2W-EC, n = 5), 2W functional mandible deviation group (2W-FMD, n = 10), 2W functional mandible deviation recovery group (2W-FMD-R, n = 10), 4W experimental control group (4W-EC, n = 5), 4W functional mandible deviation group (4W-FMD, n = 10), 4W functional mandible deviation recovery group (4W-FMD-R, n = 10). Functional mandible deviation device consisted of 2 parts: upper induction device: 3-dimensional design of the plate stack wax with the size of 5 × 8 × 6 mm in a coronal to 45° chamfer. Mandibular incisors with ring holding steady shift to the left mandibular 2 mm while protecting from damage mandibular incisors. All casting device reinforcing glass ionomer cement bonded to the corresponding upper and lower incisors in rats.

Stimulation and Recording

In all experiments, the animals were anesthetized with thiamylal sodium (60 mg/kg i.p.). A supplemental injection of 5 mg/kg i.p. was given when necessary. We monitored the level of anesthesia by checking the animals' pupil size, flexion and corneal reflexes, and heart rate. The animals were placed in left lateral decubitus with their heads fixed to a stereotaxic frame (models RA-4 and SR-50, Narishige Scientific Instruments, Tokyo, Japan). To stimulate the masseter muscle, we fixed 1 end of a piece of cotton thread to the animals' lower incisors and the other end to an automatic pulling machine (modified from an artificial respirator, model SN-480-7, Shinano Manufactory, Tokyo, Japan) and applied cyclic sinusoidal stretches. The maximum jaw-opening distance was set at 5.0 mm, with a cycle duration of 4.0 seconds (jaw opening and -closing time of 2.0 seconds, followed by an interval of 2.0 seconds). We performed at least 5 trials for stimulation in each unit. Stretch responses of spindle endings were recorded from the fine filament of the masseteric nerve on the right side. We accessed the masseteric nerve after removing the temporalis muscle, and then tied the nerve with a piece of cotton thread, cut at the central end from the tying point. The nerve bundle was then divided into several filaments. We used a silver hook electrode (diameter, 0.7 mm) to record functional single-unit responses. The recording activity belonged to muscle spindle but not to any other receptor system. Chewing muscles, digastric muscle, splenius, and trapezius muscle spindles electrophysiological response activities at the opening and closing state under general anesthesia state were recorded by the Model 1800-type microelectrode amplifier (Dongle Nature Genetics Life Sciences).[13] All data were captured by means of a CED 1401 interface (Cambridge Electronic Design, Cambridge, UK) and were stored in a computer hard disc. The data were later analyzed offline with the Spike2 software for Windows, Version 4.02a (Cambridge Electronic Design, Cambridge, UK). After the electrophysiological testing, the rats were sacrificed and the chewing muscles, digastric, splenius, trapezius, and neck trigeminal nucleus were taken for HDC detection by high performance liquid chromatography (HPLC), immunofluorescence, and reverse-transcription polymerase chain reaction (RT-PCR). Histamine receptor proteins in the neck trigeminal nucleus were also examined by immunofluorescence and RT-PCR.

High-Performance Liquid Chromatography Analyses

A Waters 600E multisolvent delivery system with a Waters U6K injector was used for HPLC analysis.[14] The system was operated at room temperature. HPLC analyses were done on a Cosmosil 5SL column (4.6 mm I.D. × 150 mm, Nacalai, San Diego, CA) with a solvent system of a mixture of CHCl3/N, N-dimethylformamide/H2O (210:90:4) containing 0.4% acetic acid. The flow rate was at 0.8 mL/min, and monitored at 423 nm.

Immunofluorescence Assay

The tissue sections were prepared for the assay of immunofluorescence as described previously.[15] Serial cross sections of the muscle tissues were generated on a cryostatat 20 micron thickness (Microm, Heidelberg, Germany). Briefly, after the preparation of cell slides by blocking of endogenous peroxidase activity and nonspecific binding sites. The tissues were washed and incubated with normal goat serum for 2 hours at room temperature. Thereafter, anti-histidine decarboxylase antibody (ab37291) and Anti-HRH1 antibody (ab154158) were used as primary antibodies. The tissues were incubated at 4°C overnight. After washing again, the secondary antibodies (Anti-rabbit IgG (H+L), F (ab') 2 Fragment (Alexa Fluor 488 Conjugate) #4412, Cell Signaling Technology Inc, Danvers, MA) were applied according to the instruction and tissues were incubated at 4°C overnight. They were then rinsed with PBS 3 times for 10 minutes each. 250 μL DRAQ5 (DRAQ5 #4084, 1:5000, Cell Signaling Technology Inc, CST#4084) was applied on the slides to stain the nuclear. Finally, the sections were mounted with FlourSave (Calbiochem, La Jolla, CA) mounting reagent. Images of histological sections were collected using Northern Eclipse software (Empix Imaging Inc, Mississauga, ON, Canada) on a

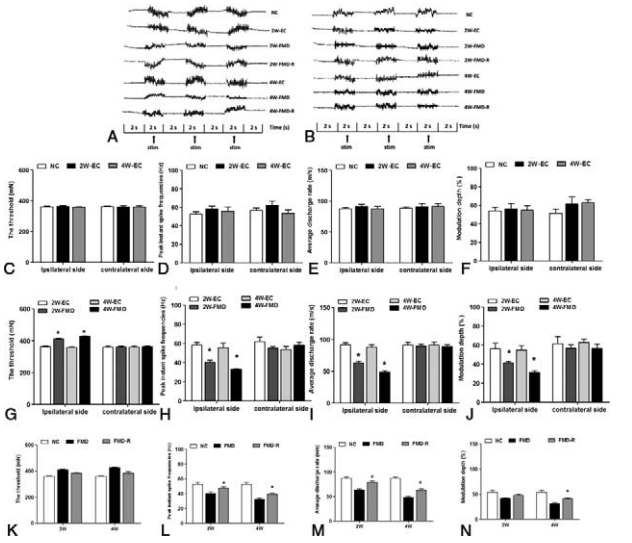

FIGURE 1. Electromyography (EMG) activity of chewing muscles induced by functional mandibular deviation. (A, B) Electromyography activity of chewing muscles was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side. (C–F) The weight of casting deflection device had no effect on the threshold, peak instant spike frequencies, and average discharge rate or modulation depth. (G) After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle decreased, (H) muscle excitement weakened (I) the muscle spindle afferent impulses of excitation transmission speed slowed down, and (J) modulation depth decreased. (K–N) The functions recovered at different extent after removing the deflector.

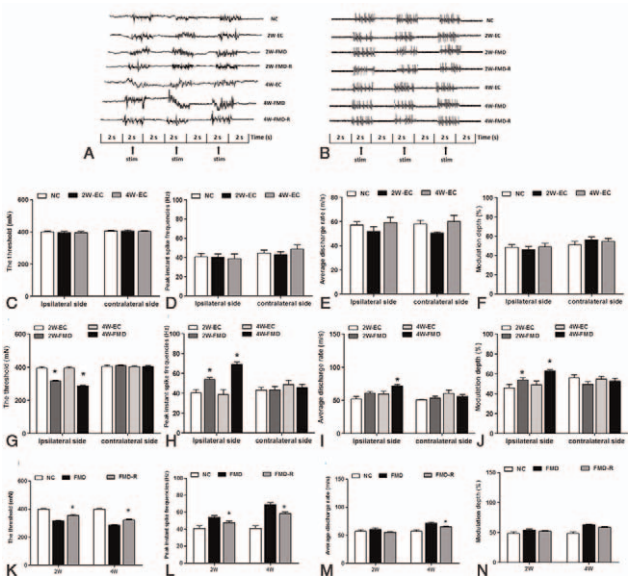

FIGURE 2. Electromyography (EMG) activity of digastric muscle induced by functional mandibular deviation. (A, B) Changes for digastric muscle electrical activity were contrary compared with the chewing muscle. Electromyography activity of digastric muscle was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side. (C–)The weight of casting deflection device had no effect on the threshold, peak instant spike frequencies, and average discharge rate or modulation depth. (G) After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle increased, (H) muscle excitement increased (I) the muscle spindle afferent impulses of excitation transmission speed accelerated, and (J) modulation depth increased. And the changes above increased with time. (K–N) The functions recovered at different extent after removing the deflector.

Zeiss Axioplan 2 imaging microscope (Carl Zeiss, Toronto, ON, Canada).

Real-Time PCR

After treatment, total RNA was prepared by using total RNA Kit (R6934, Omega Bio-tek Inc, Norcross, GA) and cDNA was synthesized from 5 μg RNA in cDNA Synthesis Kit (K1622, Fermentas International Inc, Waltham, MA) according to the manufacturer's instructions. Each PCR was performed in triplicate in a final volume of 20 μL solutions: 10 μL of SYBR Green dye, 1 μL of diluted cDNA products, 0.2 μM of each paired primer, 8.6 μL deionized water. Protocols were as follows: initial denaturation for 5 minutes at 94°C, followed by 40 cycles denaturation for 30 seconds at 94°C and extension for 30 seconds at 58°C. The last cycle for dissociation of SYBR Green probe was 15 seconds at 95°C, 30 seconds at 60°C, and 15 seconds at 95°C. The primer sequences for HDC were: sense, 5'- GTGAATACTACCGAGCTAGAGGG-3'; antisense, 5'-GACTCGTTCAATGTCCCCAAA -3'. The primer sequences for the histamine receptor H1 were: sense, 5'- CAGACCTGATTG-TAGGGGCAG-3'; antisense, 5'- CATAGAGAGCCAAAAGAGG-CAG-3'. The primer sequences for the control GAPDH were: sense, 5'-CCCCCAATGTATCCGTTGTG-3'; antisense, 5'-TAGCCCAG-GATGCCCTTTAGT-3'. Assays were performed in triplicate with the ABI7500 instrument. All data were normalized by GAPDH. Gene expression data was analyzed by the $2^{-\triangle\triangle}$CT method.[16]

Statistical Analysis

Statistical analysis was performed with SPSS17.0 statistical software. Comparison between the groups used Student *t* test or 1-way analysis of variance. The difference was considered significantly if $P < 0.05$. * means $P < 0.05$, ** means $P < 0.01$, and *** means $P < 0.001$.

RESULTS

Electromyography Activity of Chewing Muscles Induced by Functional Mandibular Deviation

The TMJ mechanoreceptors not only affect the neck muscles's motor unit activities, but also are concerned in the regulation of postural control of the head.[17] Electromyography activity of chewing muscles was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side (Fig. 1A and B). The weight of casting deflection device had no effect on the threshold (Fig. 1C), peak instant spike frequencies (Fig. 1D), average discharge rate (Fig. 1E), or modulation depth (Fig. 1F). After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle decreased (the threshold for 4W-EC group was 357.42 ± 7.55 mN, and the threshold for 4W-FMD was 426.30 ± 13.51 mN, $P < 0.05$) (Fig. 1G), muscle excitement weakened (the peak instant spike frequency for 2W-EC group was 58.42 ± 6.11 Hz, for 2W-FMD was 40.45 ± 6.77 Hz, $P < 0.05$; the peak instant spike frequency for 4W-EC group was 55.60 ± 10.49 Hz, for 4W-FMD was 32.66 ± 4.02 Hz, $P < 0.05$) (Fig. 1H), the muscle spindle afferent impulses of excitation transmission speed slowed down (the average discharge rate for 2W-EC group was 91.46 ± 7.65 m/s, for 2W-FMD was 63.32 ± 7.74 m/s, $P < 0.05$; the average discharge rate for 4W-EC group was 87.60 ± 8.75 m/s, for 4W-FMD was 48.62 ± 6.33 m/s, $P < 0.05$) (Fig. 1I) and modulation depth decreased (the modulation depth for 2W-EC group was 56.08 ± 12.96%, for 2W-FMD was 41.31 ± 4.79%, $P < 0.05$; the modulation depth for 4W-EC group was 54.86 ± 10.36%, for 4W-FMD was 31.28 ± 5.65%, $P < 0.05$) (Fig. 1J). And the changes above increased with time. The functions recovered at different extents after removing the deflector (Fig. 1K–N).

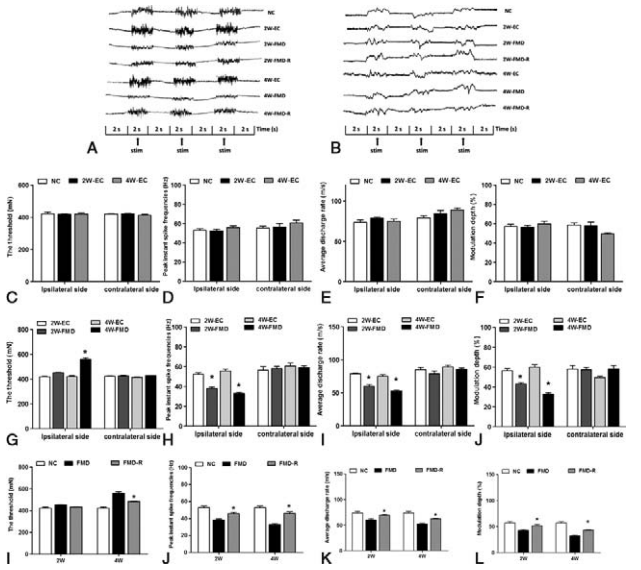

FIGURE 3. Electromyography activity of Splenius muscle induced by functional mandibular deviation. (A, B) Changes for splenius muscle electrical activity were similar with the chewing muscle. Electromyography activity of splenius muscle was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side. (C–F) The weight of casting deflection device had no effect on the threshold, peak instant spike frequencies, and average discharge rate or modulation depth. (G) After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle decreased, (H) muscle excitement weakened (I) the muscle spindle afferent impulses of excitation transmission speed slowed down, and (J) modulation depth decreased. (K–N) The functions recovered at different extents after removing the deflector.

Electromyography Activity of Digastric Muscle Induced by Functional Mandibular Deviation

Changes for digastric muscle electrical activity were contrary compared with the chewing muscle. Electromyography activity of digastric muscle was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side (Fig. 2A and B). The weight of casting deflection device had no effect on the threshold (Fig. 2C), peak instant spike frequencies (Fig. 2D), and average discharge rate (Fig. 2E) or modulation depth (Fig. 2F). After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle increased (the threshold for 2W-EC group was 394.08 ± 19.15 mN, the threshold for 2W-FMD was 315.23 ± 16.39 mN, $P < 0.05$; the threshold for 4W-EC group was 394.22 ± 18.76 mN, the threshold for 4W-FMD was 284.77 ± 18.82 mN, $P < 0.05$) (Fig. 2G), muscle excitement increased (the peak instant spike frequency for 2W-EC group was 40.24 ± 7.50 Hz, for 2W-FMD was 53.87 ± 6.62 Hz, $P < 0.05$; the peak instant spike frequency for 4W-EC group was 38.58 ± 10.93 Hz, for 4W-FMD was 69.11 ± 7.29 Hz, $P < 0.05$) (Fig. 2H), the muscle spindle afferent impulses of excitation transmission speed accelerated (the average discharge rate for 2W-EC group was 5.90 ± 8.65 m/s, for 2W-FMD was 60.68 ± 7.53 m/s, $P < 0.05$; the average discharge rate for 4W-EC group was 59.30 ± 9.26 m/s, for 4W-FMD was 71.38 ± 6.65 m/s, $P < 0.05$) (Fig. 2I) and modulation depth increased (the modulation depth for 2W-EC group was 45.62 ± 8.14%, for 2W-FMD was 53.93 ± 6.26%, $P < 0.05$; modulation depth for 4W-EC group was 48.90 ± 8.49%, for 4W-FMD was 62.45 ± 8.49%, $P < 0.05$) (Fig. 2J). And the changes above increased with time. The functions recovered at different extents after removing the deflector (Fig. 2K–N).

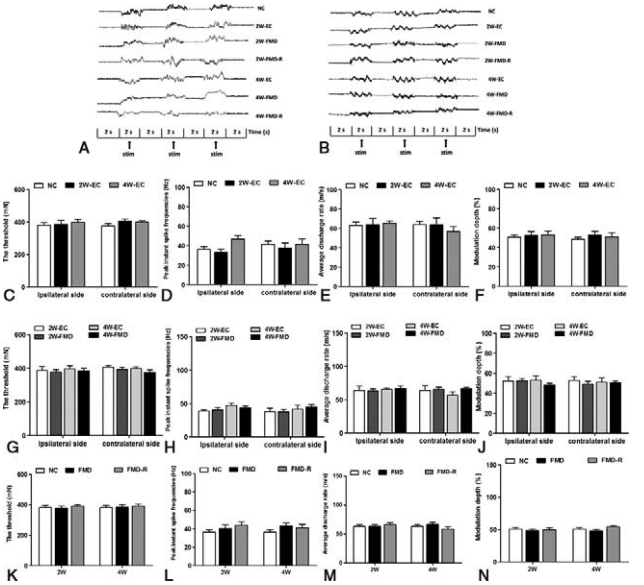

FIGURE 4. Electromyography activity of Trapezius muscle induced by functional mandibular deviation. However, trapezius in all the experimental groups and recovery groups exhibited bilateral symmetry electrophysiological responses, and no significant difference compared with the control group.

Electromyography Activity of Splenius Muscle Induced by Functional Mandibular Deviation

Changes for splenius muscle electrical activity were similar to the chewing muscle. Electromyography activity of splenius muscle was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side (Fig. 3A and B). The weight of casting deflection device had no effect on the threshold (Fig. 3C), peak instant spike frequencies (Fig. 3D), and average discharge rate (Fig. 3E) or modulation depth (Fig. 3F). After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle decreased (Fig. 3G), muscle excitement weakened (Fig. 3H), the muscle spindle afferent impulses of excitation transmission speed slowed down (Fig. 3I), and modulation depth decreased (Fig. 3J). And the changes above increased with time. The functions recovered at different extents after removing the deflector (Fig. 3K–N).

Electromyography Activity of Trapezius Muscle Induced by Functional Mandibular Deviation

However, trapezius in all the experimental groups and recovery groups exhibited bilateral symmetry electrophysiological responses, and no significant difference compared with the control group (Fig. 4).

Histidine Decarboxylase Levels in the Chewing Muscles

Histidine decarboxylase level of chewing muscles was significantly asymmetric; the abnormal muscle HDC expression was mainly detected at the ipsilateral side (Fig. 5A and B). After functional mandibular deviation, HDC protein levels (2W-FMD versus 2W-EC, 128.96 ± 6.91 ng/g versus 105.56 ± 14.70 ng/g, $P < 0.05$; 4W-FMD versus 4W-EC, 143.42 ± 6.57 ng/g versus 112.18 ± 12.71 ng/g, $P < 0.05$) on the ipsilateral sides of the

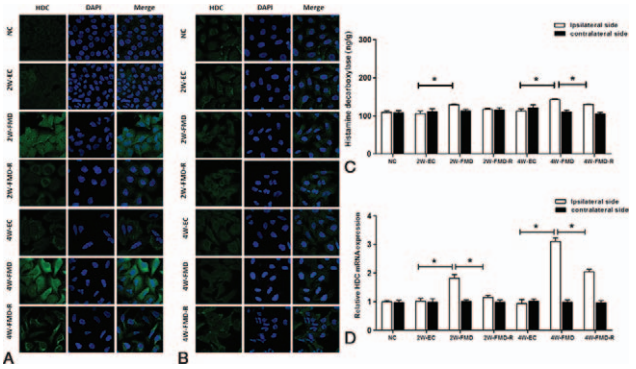

FIGURE 5. Histidine decarboxylase levels in the chewing muscles. (A, B) Histidine decarboxylase level of chewing muscles was significantly asymmetric; the abnormal muscle HDC expression was mainly detected at the ipsilateral side. (C) After functional mandibular deviation, HDC protein levels on the ipsilateral sides of the chewing muscle increased significantly. After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the chewing muscle. (D) The HDC mRNA level change was coincident with the protein change.

chewing muscle increased significantly. After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the chewing muscle (4W-FMD-R versus 4W-FMD, 129.80 $\pm$6.32 ng/g versus 143.42 $\pm$6.57 ng/g, $P < 0.05$) (Fig. 5C). The HDC mRNA level change was coincident with the protein change (Fig. 5D).

Histidine Decarboxylase Levels in the Digastric Muscle

HDC level changes for digastric muscle were contrary. Histidine decarboxylase level of digastric muscle was significantly asymmetric; the abnormal muscle HDC expression was mainly detected at the ipsilateral side (Fig. 6A and B). After functional mandibular deviation, HDC protein levels on the ipsilateral sides of the digastric muscle decreased significantly (2W-FMD versus 2W-EC, 117.50 $\pm$7.71 ng/g versus 129.36 $\pm$16.90 ng/g, $P < 0.05$; 4W-FMD versus 4W-EC, 102.77 $\pm$6.81 ng/g versus 130.38 $\pm$12.48 ng/g, $P < 0.05$). After the removal of the mandibular position deflector, HDC protein levels increased on the ipsilateral sides of the digastric muscle (4W-FMD-R versus 4W-FMD, 117.79 $\pm$9.83 ng/g versus 102.77 $\pm$6.81 ng/g, $P < 0.05$) (Fig. 6C). The HDC mRNA level change was coincident with the protein change (Fig. 6D).

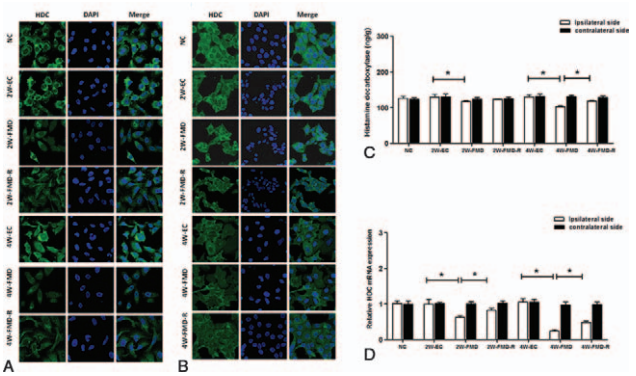

FIGURE 6. Histidine decarboxylase levels in the digastric muscle. (A, B) While HDC level changes for digastric muscle were contrary, histidine decarboxylase level of digastric muscle was significantly asymmetric; the abnormal muscle HDC expression was mainly detected at the ipsilateral side. (C) After functional mandibular deviation, HDC protein levels on the ipsilateral sides of the digastric muscle decreased significantly (2W-FMD versus 2W-EC, 117.50 $\pm$7.71 ng/g versus 129.36 $\pm$16.90 ng/g, $P < 0.05$; 4W-FMD versus 4W-EC, 102.77 $\pm$6.81 ng/g versus 130.38 $\pm$12.48 ng/g, $P < 0.05$). After the removal of the mandibular position deflector, HDC protein levels increased on the ipsilateral sides of the digastric muscle (4W-FMD-R versus 4W-FMD, 117.79 $\pm$9.83 ng/g versus 102.77 $\pm$6.81 ng/g, $P < 0.05$). (D) The HDC mRNA level change was coincident with the protein change.

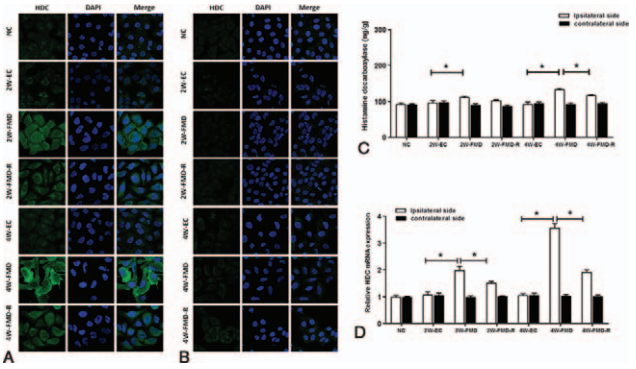

FIGURE 7. Histidine decarboxylase levels in the splenius muscle. (A, B) Histidine decarboxylase level of Splenius muscle was significantly asymmetric; the abnormal muscle HDC expression was mainly detected at the ipsilateral side. (C) After functional mandibular deviation, HDC protein levels ($P < 0.05$) on the ipsilateral sides of the Splenius muscle increased significantly. After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the Splenius muscle ($P < 0.05$). (D) The HDC mRNA level change was coincident with the protein change.

Histidine Decarboxylase Levels in the Splenius Muscle

Histidine decarboxylase level of splenius muscle was significantly asymmetric; the abnormal muscle HDC expression was mainly detected at the ipsilateral side (Fig. 7A and B). After functional mandibular deviation, HDC protein levels (2W-FMD versus 2W-EC, 110.13 $\pm$11.61 ng/g versus 91.91 $\pm$12.16 ng/g, $P < 0.05$; 4W-FMD versus 4W-EC, 132.04 $\pm$8.64 ng/g versus 91.86 $\pm$15.33 ng/g, $P < 0.05$) on the ipsilateral sides of the Splenius muscle increased significantly. After the removal of the mandibular position deflector, HDC protein levels decreased

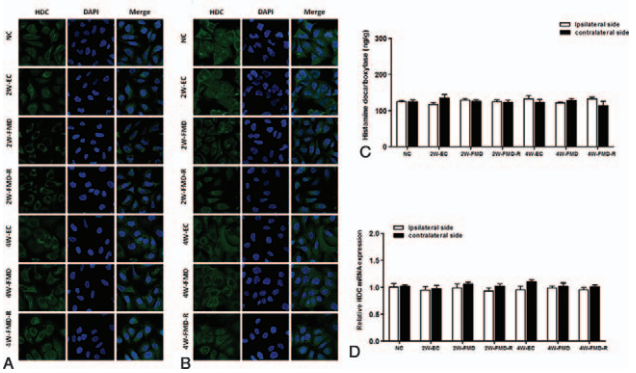

FIGURE 8. Histidine decarboxylase levels in the trapezius muscle. (A, B) The difference of histamine decarboxylase content in the bilateral trapezius in each experimental group was small. Trapezius in all the experimental groups and recovery groups exhibited bilateral symmetry HDC expression, and no significant difference. (C, D) After the removal of the mandibular position deflector, the detection indicators above decreased at different extents. After the removal of the mandibular position deflector, neither HDC protein levels nor HDC mRNA levels changed compared with the control group.

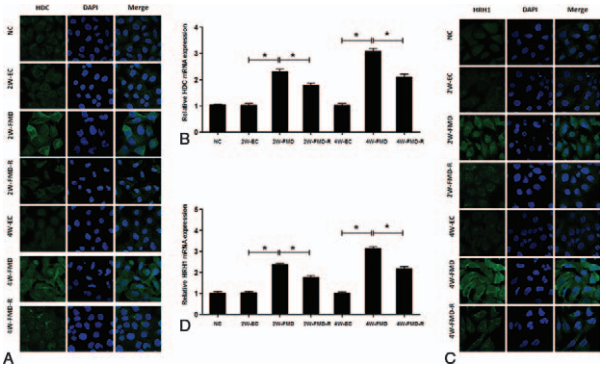

FIGURE 9. Neck monoamine neurotransmitter histamine release test results in the trigeminal nucleus. (A) After functional mandibular deviation, HDC protein levels on the ipsilateral sides of the trigeminal nucleus increased significantly. After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the trigeminal nucleu. (B) The HDC mRNA level change was coincident with the protein change. (C) After functional mandibular deviation, histamine receptor protein levels on the ipsilateral sides of the trigeminal nucleus increased significantly. After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the trigeminal nucleus. (D) And histamine receptor mRNA level change was coincident with the protein change. HAD, histidine decarboxylase.

on the ipsilateral sides of the Splenius muscle (4W-FMD-R versus 4W-FMD, 115.01 $\pm$8.22 ng/g versus 132.04 $\pm$8.64 ng/g, $P < 0.05$) (Fig. 7C). The HDC mRNA level change was coincident with the protein change (Fig. 7D).

Histidine Decarboxylase Levels in the Trapezius Muscle

The difference of histamine decarboxylase content in the bilateral trapezius in each experimental group was small. Trapezius in all the experimental groups and recovery groups exhibited bilateral symmetry HDC expression, and no significant difference (Fig. 8A and B). After the removal of the mandibular position deflector, the detection indicators above decreased at different extents. After the removal of the mandibular position deflector, neither HDC protein levels (Fig. 8C) nor HDC mRNA levels (Fig. 8D) changed compared with the control group.

Neck Monoamine Neurotransmitter Histamine Release Test Results in the Trigeminal Nucleus

After functional mandibular deviation, HDC protein levels on the ipsilateral sides of the trigeminal nucleus increased significantly (2W-FMD versus 2W-EC, 2.132 ± 0.256 versus 1.013 ± 0.060, $P < 0.05$; 4W-FMD versus 4W-EC, 2.480 ± 0.301 versus 1.007 ± 0.078, $P < 0.05$). After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the trigeminal nucleus (2W-FMD-R versus 2W-FMD, 1.543 ± 0.042 versus 2.132 ± 0.256, $P < 0.05$; 4W-FMD-R versus 4W-FMD, 1.793 ± 0.234 versus 2.480 ± 0.301, $P < 0.05$) (Fig. 9A). The HDC mRNA level change was coincident with the protein change (Fig. 9B). After functional mandibular deviation, histamine receptor protein levels on the ipsilateral sides of the trigeminal nucleus increased significantly (2W-FMD versus 2W-EC, 1.783 ± 0.103 versus 0.960 ± 0.080, $P < 0.05$; 4W-FMD versus 4W-EC, 2.311 ± 0.144 versus 1.014 ± 0.081, $P < 0.05$) (Fig. 9C). After the removal of the mandibular position deflector, HDC protein levels decreased on the ipsilateral sides of the trigeminal nucleus (2W-FMD-R versus 2W-FMD, 1.292 ± 0.193 versus 1.783 ± 0.103, $P < 0.05$; 4W-FMD-R versus 4W-FMD, 1.857 ± 0.268 versus 2.311 ± 0.144, $P < 0.05$). And histamine receptor mRNA level change was coincident with the protein change (Fig. 9D).

DISCUSSION

The control of jaw movements and position is performed by inputs from low-threshold mechanoreceptors located throughout the orofacial region. These include periodontal and mucosal mechanoreceptors and muscle spindles.[13] A change in head position was observed at the beginning of the first jaw-movement cycle, and this adjusted head position was maintained during the following cycles. In addition to the prevailing head extension, the maximal jaw-opening/-closing cycles were paralleled by head extension-flexion movements.[18] It is important to assess the morphology and function of the neck muscles and cervical spine prior to occlusal therapy in patients with asymmetric TMJ structures.[19] Functional shift of rat mandible changes the morphology of the condylar cartilage in the lateral region on the ipsilateral side.[20]

Electromyography activity of chewing muscles, digastric, and splenius muscle was significantly asymmetric; the abnormal muscle EMG activity was mainly detected at the ipsilateral side. After functional mandibular deviation, muscle sensitivity on the ipsilateral sides of the chewing muscle and splenius decreased, muscle excitement weakened, modulation depth decreased, and the muscle spindle afferent impulses of excitation transmission speed slowed down. And the changes above increased with time. Changes for digastric muscle electrical activity were contrary. The functions recovered at different extents after removing the deflector. In the trigeminal somatosensory system, there are various receptors on the orofacial structures, for example, mechanore ceptors in the tooth, periodontium, oral mucosa, and TMJ. All of them are potential candidates for modulators of the neck motor system. Recently, it was observed that periodontal mechanical stimulation could elicit reflex responses in neck muscles by.[21]During growth on the functional characteristics of TMJ mechanoreceptors, the threshold of the TMJ units was significantly lower in the experimental group than in the control group.[22] Both mandibular positions tested decreased the EMG activity of the masticatory and cervical muscles in the relaxed and fullbite positions.[23] The innervations of the TMJ by somatosensory and sympathetic fibers suggested that sympathetic nerves could be responsible for allodynia or neuropathic pain caused by temporomandibular disorders.[24]

In our study, after functional mandibular deviation, HDC protein and mRNA levels on the ipsilateral sides of the chewing muscle and splenius increased significantly. HDC level changes for digastric muscle were contrary. After the removal of the mandibular position deflector, HDC protein and mRNA levels decreased on the ipsilateral sides of the chewing muscle and splenius while they increased in the digastric muscle. The difference of histamine decarboxylase content in the bilateral trapezius in each experimental group was small. After functional mandibular deviation, HDC protein, HDC mRNA levels, histamine receptor proteins, and histamine receptor mRNA levels on the ipsilateral sides of the trigeminal nucleus increased significantly. After the removal of the mandibular position deflector, the detection indicators above decreased at different extents. A patient-control designed was used to investigate associations and interactions between muscle activities measured by surface EMG in the upper trapezius muscle and subjectively reported risk factors in workers with and without shoulder and neck pain.[25] The effects of different types of stress (water bathing, cold, restraint, and prolonged walking) on HDC activity in masseter were examined in mice. And all of these stresses elevated gastric HDC activity.[26]

In conclusion, after functional mandibular deviation, the TMJ mechanical receptors not only caused the fusimotor fiber

hypoallergenic fatigue slow response on the ipsilateral sides of splenius, but also increased the injury neurotransmitter histamine release. Our results further support the opinion that the temporomandibular joint receptors may be involved in the mechanical theory of the head and neck muscles nervous system regulation.

REFERENCES

1. Ponyi S, Szabo G, Nyilasi J. Asymmetry of mandibular dimensions in European skulls. *Proc Finn Dent Soc* 1991;87:321–327
2. Athanasiou AE, Melsen B, Mavreas D, et al. Stomatognathic function of patients who seek orthognathic surgery to correct dentofacial deformities. *Int J Adult Orthodon Orthognath Surg* 1989;4:239–254
3. Sakaguchi K, Mehta NR, Abdallah EF, et al. Examination of the relationship between mandibularposition and body posture. *Cranio* 2007;25:237–249
4. Eriksson PO, Häggman-Henrikson B, Nordh E, et al. Co-ordinated mandibular and head-neck movements during rhythmic jaw activities in man. *J Dent Res* 2000;79:1378–1384
5. Pinto AS, Buschang PH, Throckmorton GS, et al. Morphological and positional asymmetries of young children with functional unilateral posterior crossbite. *Am J Orthod Dentofacial Orthop* 2001;120:513–520
6. Akahane Y, Deguchi T, Hunt NP. Morphology of the temporomandibular joint in skeletal class III symmetrical and asymmetrical cases: a study by cephalometric laminography. *J Orthod* 2001;28:119–128
7. Goto TK, Nishida S, Nakayama E, et al. Correlation of mandibular deviation with temporomandibular joint MR dimensions, MR disk position, and clinical symptoms. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005;100:743–749
8. Kure-Hattori I, Watari I, Takei M, et al. Effect of functional shift of the mandible on lubrication of the temporomandibular joint. *Arch Oral Biol* 2012;57:987–994
9. Liu C, Kaneko S, Soma K. Effects of a mandibular lateral shift on the condyle and mandibular bone in growing rats. *Angle Orthodont* 2007;77:787–793
10. de Wijer A, Steenks MH, de Leeuw JR, et al. Symptoms of the cervical spine in temporomandibular and cervical spine disorders. *J Oral Rehabil* 1996;23:742–750
11. Hunt CC. Mammalian muscle spindle: peripheral mechanisms. *Physiol Rev* 1990;70:643–663
12. Ayada K, Tadano T, Endo Y. Gnawing behavior of a mouse in a narrow cylinder: a simple system for the study of muscle activity, fatigue, and stress. *Physiol Behav* 2002;77:161–166
13. Yabushita T, Zeredo JL, Toda K, et al. Role of occlusal vertical dimension in spindle function. *J Dent Res* 2005;84:245–249
14. Kinoshita E, Saito M. Novel histamine measurement by HPLC analysis used to assay histidine decarboxylase inhibitory activity of shoyuflavones from soy sauce. *Biosci Biotechnol Biochem* 1998;62:1488–1491
15. Bi Y, Tian M, Le J, et al. Study on the expression of PAK4 and P54 protein in breast cancer. *World J Surg Oncol* 2016;14:160
16. Jablonska K, Grzegrzolka J, Podhorska-Okolow M, et al. Prolactin-induced protein as a potential therapy response marker of adjuvant chemotherapy in breast cancer patients. *Am J Cancer Res* 2016;6:878–893
17. Kobayashi M, Yabushita T, Zeredo JL, et al. Splenius muscle activities induced by temporomandibularjoint stimulation in rats. *Brain Res Bull* 2007;72:44–48
18. Eriksson PO, Häggman-Henrikson B, Nordh E, et al. Co-ordinated mandibular and head-neck movements during rhythmic jaw activities in man. *J Dent Res* 2000;79:1378–1384
19. Kondo E. Features and treatment of skeletal class III malocclusion with severe lateral mandibular shift and asymmetric vertical dimension. *World J Orthod* 2004;5:9–24
20. Sato C, Muramoto T, Soma K, et al. Functional lateral deviation of the mandible and its positional recovery on the rat condylar cartilage during the growth period. *Angle Orthod* 2006;76:591–597
21. Widenfalk B, Wiberg M. Origin of sympathetic and sensory innervation of the temporo-mandibular joint. A retrograde axonal tracing study in the rat. *Neurosci Lett* 1990;109:30–35
22. Ishida T, Yabushita T, Soma K. Functional changes of temporomandibular joint mechanoreceptors induced by reduced masseter muscle activity in growing rats. *Angle Orthod* 2009;79:978–983
23. Ceneviz C, Mehta NR, Forgione A, et al. The immediate effect of changing mandibular position on the EMG activity of the masseter, temporalis, sternocleidomastoid, and trapezius muscles. *Cranio* 2006;24:237–244
24. Yoshino K, Kawagishi S, Amano N. Morphological characteristics of primary sensory and post-synaptic sympathetic neurones supplying the temporomandibular joint in the cat. *Arch Oral Biol* 1998;43:679–686
25. Vasseljen OJ, Westgaard RH. Can stress-related shoulder and neck pain develop independently of muscle activity? *Pain* 1996;64:221–230
26. Ayada K, Watanabe M, Endo Y. Elevation of histidine decarboxylase activity in skeletal muscles and stomach in mice by stress and exercise. *Am J Physiol Regul Integr Comp Physiol* 2000;279:R2042–R2047