

研究者：Guan Hao 管 浩

(所属：東京医科歯科大学大学院 歯学総合研究科 咬合機能矯正学分野)

研究題目：The effect of mandibular lateral shift and its recovery on masseter and temporalis muscles in growing rats.

目 的：

Unilateral posterior crossbite (UPXB) is a frequent malocclusion in the early dentition stage and it is usually associated with mandibular lateral shift (MLS). As one of potential side-effects that MLS may lead to, asymmetric masticatory muscles activity was revealed in clinical studies. However, at present, more detailed explanation for muscle regeneration concerning MLS or possible cellular mechanism are still not fully understood and there is no animal experiment evidence about the effect of MLS and its recovery on masticatory muscle. In order to clarify the potential mechanism and provide the evidence of early treatment for MLS, our research is attempted to establish rat mandibular lateral shift model and investigate whether fiber size and cellular signaling related factors of masseter and temporalis muscle recover to control level after recovery of MLS in growing rats.

対象および方法：

Animal experiments were performed under a protocol approved by the Animal Welfare Committee of Tokyo Medical and Dental University. Male Wistar rats (Sankyo Labo, Tokyo, Japan) were housed two per cage in a climate-controlled environment with a 12-hour dark and 12-hour light cycle, with powder food and water ad libitum. Animals were rested for 7 days to acclimatize them to the facility. Thirty-five rats were divided randomly into MLS, control, and recovery groups (n=7 each). To detect whether the MLS or treatment reversal affects ipsilateral and contralateral muscles differently, we observed both sides of the masseter and temporalis muscles in experimental groups.

Rats were euthanized according to a standard CO₂/O₂ protocol at the age of 7-week-old and 9-week-old (n=7 per group per time point). After euthanasia, the bilateral masseter and temporalis muscles were dissected moderately and completely. After collection, wet weight of muscles was measured immediately. Samples obtained from the muscle belly submersed in optimal cutting temperature embedding compound (Sakura Finetek Japan, Tokyo, Japan) were directly frozen in a super-cooled bath of 2-methyl butane by liquid nitrogen. For each muscle type, transverse sections were cut using a cryomicrotome (CM3500; Leica Microsystems, Nussloch, Germany). Then, sections are subjected to staining with hematoxylin and eosin to assess the mean cross-sectional area (CSA) using ImageJ software, to adenosine triphosphatase (ATPase) activity staining (protocol from Neuromuscular Lab of School of Medicine of Washington University) to assess MHC

composition, in addition to immunohistochemical staining analyses of IGF-1 and GDF-8. Finally, the total RNA of samples will be performed Real-Time Polymerase Chain Reaction of each target gene (IGF-1 and GDF-8).

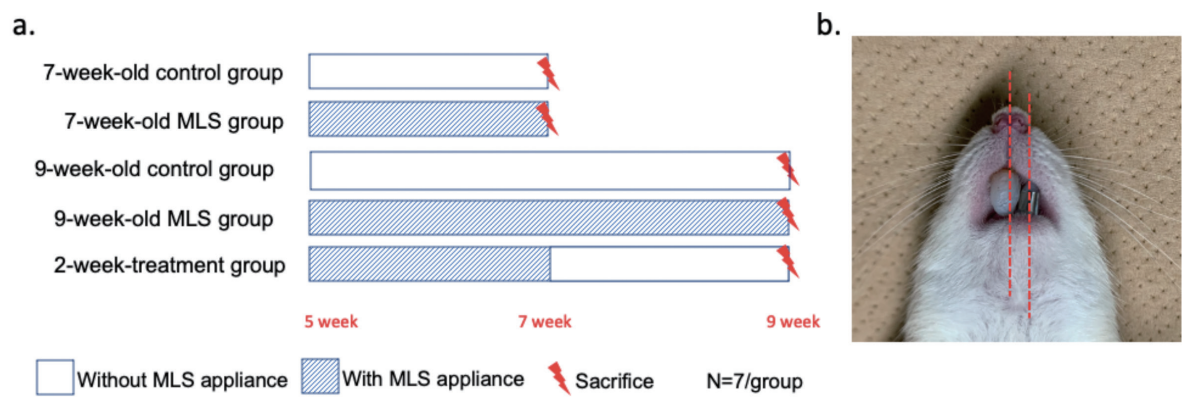


Figure a. Grouping schemes and time schedules : Thirty-five 5-week-old male Wistar rats were randomly assigned to 5 groups : 7-week-old control group ; 7-week-old MLS group ; 9-week-old control group ; 9-week-old MLS group ; 2-weektreatment group. In the experimental groups, the MLS appliances were attached at the age of 5-week-old. Following 2-weeks of MLS, the appliances in the 2-week-treatment group were removed until 9-week-old.

Figure b. The MLS appliance : The appliance is composed of two incisor guiding plates (made by band materials (Shofu, Kyoto, Japan) and light cure resin (TOMY International, Tokyo, Japan), which were able to shift the mandible to the left side by ~2 mm when rats closed their mouth.

結果および考察：

(Only part of results is available till now, it’s expected to complete all analysis within this year.)

[Body weight and daily food intake]

There was a slight decrease of the daily food intake immediately on the first day of appliance attachment. But it returned to normal condition and started to increase again after second day of application. The body weight of all rats increased in a normal manner throughout the experimental period. There were no significant differences in body weight and food intake at final stage between the rats in each group. (Figure c.)

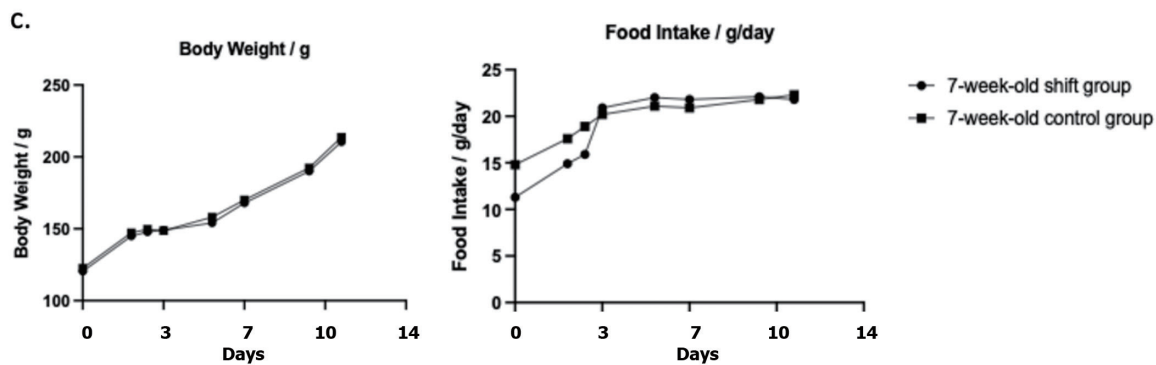


Figure c. Between shift group and control group, there were no significant differences when sacrificed in body weight and daily food intake as well.

[Muscle wet weight]

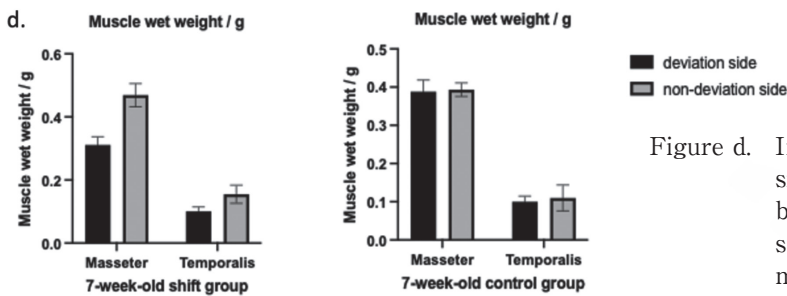


Figure d. In the 7-week-old shift group, there is significant difference of muscle wet weight between deviation side and non-deviation side both in masseter and temporalis muscles. Muscle mass on the deviation side was significantly lighter than that on the non-deviation side.

[ATPase Activity Staining and Immunohistochemistry]

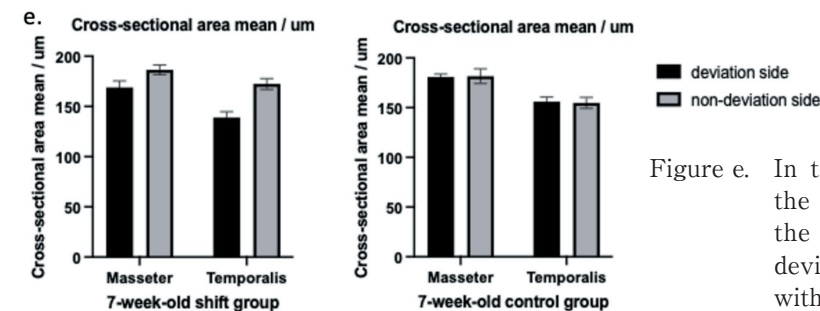


Figure e. In the masseter and temporalis muscles, the mean CSA was significantly lower on the deviation side than that on the non-deviation side in the shift group compared with the control group.

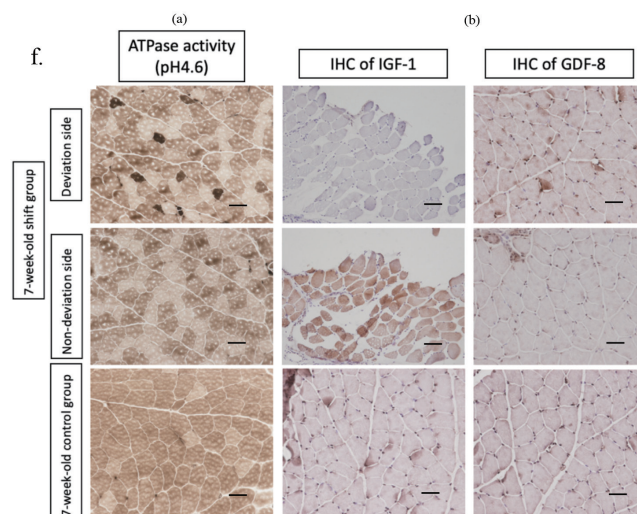


Figure f. ATPase activity staining and IHC of masseter muscle in 7-week-old shift group and 7-week-old control group. Scale bar, 100 μ m. (a) The ATPase activity staining procedure stained type-1 myofibers dark brown, type-2a myofibers white, and type-2b myofibers medium brown. At 7 weeks of age, the percentage of type-2b fibers on the deviation side in the shift group was decreased compared with that on the non-deviation side and those of the control group as well. (b) IHC staining of IGF-1 and GDF-8 : In the 7-week-old shift group, it was shown that enhanced GDF-8 immunoreactivity and optically lower IGF-1 immunoreactivity was found in sections of the deviation side compared with those of nondeviation side, which indicated that mandibular lateral shift may affect the expression of certain positive or negative regulator to regenerate the muscle mass and fiber size. Further analysis to be continued will be operated to complete all the groups.

成果発表：(予定を含めて口頭発表、学術雑誌など)

1. 2022 年 10 月、第 81 回日本矯正歯科学会学術大会（大阪国際会議場）、発表予定。