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## Method Article

# A method package for electrophysiological evaluation of reconstructed or regenerated facial nerves in rodents



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## A B S T R A C T

Compound muscle action potential (CMAP) recording via reconstructed or regenerated motor axons is a critical examination to evaluate newly developed surgical and regeneration techniques. However, there is currently no documentation on technical aspects of CMAP recordings via reconstructed or regenerated facial nerves. We have studied new techniques of plastic surgery and nerve regeneration using a rat facial nerve defect model for years, standardizing an evaluation pipeline using CMAP recordings. Here we describe our CMAP recording procedure in detail as a package including surgical preparation, data acquisition, analysis and troubleshooting. Each resource is available in public repositories and is maintained as a version control system. In addition, we demonstrate that our analytical pipeline can not only be applied to rats, but also mice. Finally, we show that CMAP recordings can be practically combined with other behavioral and anatomical examinations. For example, retrograde motor neuron labeling provides anatomical evidence for physical routes between the facial motor nucleus and its periphery through reconstructed or regenerated facial nerves, in addition to electrophysiological evidence by CMAP recordings from the same animal.

- Standardized surgical, recording and analytical procedures for the functional evaluation of reconstructed or regenerated facial nerves of rats, extended to mice.
- The functional evaluation can be combined with anatomical evaluations.
- The methods described here are maintained in public repositories as version control systems.

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<https://doi.org/10.1016/j.mex.2018.03.007>

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## ARTICLE INFO

*Method name:* Compound muscle action potential recordings via reconstructed or regenerated facial nerves

*Keywords:* Facial nerve, Reconstruction, Regeneration, Compound muscle action potential, Retrograde tracer, Rat, Mouse, Analysis, Igor Pro, MATLAB, Version control system

*Article history:* Received 17 October 2017; Accepted 28 March 2018; Available online 30 March 2018

## Specifications Table

Subject area	Medicine and Dentistry
More specific subject area	Reconstruction and regeneration of the facial nerves
Method name	Compound muscle action potential recordings via reconstructed or regenerated facial nerves
Name and reference of original method	Reconstruction or regeneration of the facial nerves of rats. <ul style="list-style-type: none"> <li>* R. Sasaki, S. Aoki, M. Yamato, H. Uchiyama, K. Wada, T. Okano, H. Ogiuchi, Tubulation with dental pulp cells promotes facial nerve regeneration in rats, <i>Tissue Eng. Part A</i> 14(7) (2008) 1141-7.</li> <li>* H. Matsumine, R. Sasaki, M. Takeuchi, M. Yamato, H. Sakurai, Surgical procedure for transplanting artificial nerve conduits for peripheral nerve regeneration, <i>Plast. Reconstr. Surg.</i> 128(2) (2011) 95e-97e.</li> <li>* H. Matsumine, R. Sasaki, Y. Takeuchi, M. Miyata, M. Yamato, T. Okano, H. Sakurai, Vascularized versus nonvascularized island median nerve grafts in the facial nerve regeneration and functional recovery of rats for facial nerve reconstruction study, <i>J. Reconstr. Microsurg.</i> 30(2) (2014) 127-136.</li> <li>* H. Matsumine, Y. Takeuchi, R. Sasaki, T. Kazama, K. Kano, T. Matsumoto, H. Sakurai, M. Miyata, M. Yamato, Adipocyte-derived and dedifferentiated fat cells promoting facial nerve regeneration in a rat model, <i>Plast. Reconstr. Surg.</i> 134(4) (2014) 686-97.</li> <li>* Y. Niimi, H. Matsumine, Y. Takeuchi, R. Sasaki, Y. Watanabe, M. Yamato, M. Miyata, H. Sakurai, Effectively axonal-supercharged interpositional jump-graft with an artificial nerve conduit for rat facial nerve paralysis, <i>Plast. Reconstr. Surg. Glob. Open</i> 3 (2015) e416.</li> </ul>
Resource availability	CMAPMethods ( <a href="https://doi.org/10.17632/9g5n35fd3f.1">https://doi.org/10.17632/9g5n35fd3f.1</a> ) CMAPAnalysis ( <a href="https://github.com/yuichi-takeuchi/CMAPAnalysis">https://github.com/yuichi-takeuchi/CMAPAnalysis</a> ) CMAPAnalysisMATLAB ( <a href="https://github.com/hironobu-osaki/CMAPAnalysisMATLAB">https://github.com/hironobu-osaki/CMAPAnalysisMATLAB</a> ) RetrogradeMotorNeuronLabeling ( <a href="https://doi.org/10.6084/m9.figshare.5445199">https://doi.org/10.6084/m9.figshare.5445199</a> )

## Method details

## Animals

LEW/Crl rats (200–300 g; RRID:RGD\_737932) have been employed in our research on the reconstruction or regeneration of the buccal branch of facial nerves [1–3]. C57BL/6 mice (20–40 g; RRID:IMSR\_JAX:000664) were introduced to our research in this study. All experiments conducted were approved by the Animal Care and Use Committee of the Tokyo Women's Medical University and performed according to the institutional guidelines.

## Surgical procedures for reconstruction or regeneration of the buccal branch of rat facial nerves

Briefly, rats are anesthetized with 4% isoflurane via a nasal mask connected to a Univentor 400 Anesthesia Unit (Univentor, Zejtun, Malta) [4]. A periauricular incision is made on the left side of the face to expose the buccal and marginal mandibular branches of the facial nerves and the parotid gland [1]. The marginal mandibular branch is cut using microsurgical scissors and ligated with 7–0 nylon sutures. After that, the buccal branch is exfoliated from surrounding connective tissues (as

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