

# Contents

<b>1 Introduction</b>	<b>1</b>
<b>2 Materials and Methods</b>	<b>3</b>
2.1 First Major Set: Mild Indirect Stimulation of the Trigeminal Afferents After Combined Surgery on the Infraorbital and Facial Nerves by Removal (Clipping) of the Contralateral Vibrissal Hairs	3
2.1.1 Animal Groups and Overview of the Specific Methods Used in the First Experimental Set	3
2.1.2 Combined Nerve Surgery (FFA + ION-S)	5
2.1.3 Increased Ipsilateral Vibrissal Use (Vibrissal Stimulation, VS) After Combined Surgery in Group 2	8
2.1.4 Manual Stimulation of Vibrissal Muscles After Combined Surgery in Groups 3 and 4	8
2.1.5 Observations on Whisking Behavior	9
2.1.6 Analysis of Vibrissae Motor Performance During Exploration	9
2.1.7 Fixation	10
2.1.8 Analysis of Target Muscle Reinnervation	10
2.1.9 Synaptic Input to the Facial Motoneurons	12
2.1.10 Number of Retrogradely Labeled Trigeminal Ganglion Cells	16
2.1.11 Statistics	17
2.2 Second Major Set: Intensive Indirect Stimulation of the Trigeminal Afferents After Facial Nerve Surgery by Excision of the Contralateral Infraorbital Nerve	18
2.2.1 Experiments to Determine the Degree of Collateral Axonal Branching by Application of Fluorescent Dyes on the Transected Superior and Inferior Buccolabial Rami of the Buccal Facial Branch	18
2.2.2 Experiments to Determine the Accuracy of Reinnervation by Means of Intramuscular Injections of Fluorescent Dyes	23
2.3 Third Major Set: Direct Stimulation of the Trigeminal and Facial Nerves After Facial Nerve Surgery by Massage of the Vibrissal Muscles	28
2.3.1 Animal Groups and Overview of Experiments	28
2.3.2 Surgery	33
2.3.3 Standard Housing/Enriched Environment	33
2.3.4 Mechanical Stimulation of the Vibrissal Muscles	34
2.3.5 Handling of the Animals	34
2.3.6 Analysis of Vibrissae Motor Performance During Exploration	34
2.3.7 Analysis of the Synaptic Input to the Facial Motoneurons	34

2.3.8 Estimation of Axonal Branching by Triple Retrograde Labeling .....	35
2.3.9 Analysis of Target Muscle Reinnervation .....	37
2.3.10 Statistical Evaluation .....	37
2.4 Fourth Major Set: Direct Stimulation of the Trigeminal and Facial Nerves After Facial Nerve Surgery by Application of Electric Current to the Vibrissal Muscles .....	38
2.4.1 Animal Groups and Overview of Experiments .....	38
2.4.2 Surgical Procedures .....	38
2.4.3 Electrical Stimulation .....	39
2.4.4 Analysis of Vibrissal Motor Performance .....	40
2.4.5 Estimation of Axonal Branching by Triple Retrograde Labeling .....	42
2.4.6 Analysis of Target Muscle Reinnervation .....	42
2.4.7 Statistical Evaluation .....	42
<b>3 Results .....</b>	<b>43</b>
3.1 Mild Indirect Stimulation of the Trigeminal Afferents After Combined Surgery on the Infraorbital and Facial Nerves by Removal of the Contralateral Vibrissal Hairs Improves Vibrissal Function .....	43
3.1.1 Observations on Restoration of Vibrissal Whisking .....	43
3.1.2 All Three Interventions (Sensory, Mechanical, and Sensory + Mechanical Stimulation) Improved Vibrissal Function After Combined Facial and Infraorbital Nerve Injury .....	43
3.1.3 For All Treatments (Sensory, Mechanical, and Sensory + Mechanical Stimulation) Functional Outcome Correlates with Quality of Target Muscle Reinnervation .....	44
3.1.4 Numbers of Synaptophysin-Positive Axon Boutons in the Facial Nucleus Are Unaffected, Regardless of the Treatment .....	46
3.1.5 No Neuronal Loss in the Trigeminal Ganglion After ION Lesion .....	47
3.2 Intensive Indirect Stimulation of the Trigeminal Afferents by Excision of the Contralateral ION Attenuates the Degree of Collateral Axonal Branching and Improves the Accuracy of Muscle Reinnervation .....	48
3.2.1 Reduced Degree of Collateral Axonal Branching as Determined by Application of Two Fluorescent Dyes on the Transected Superior and Inferior Buccolabial Rami of the Buccal Facial Branch .....	48
3.2.2 Improved Accuracy of Reinnervation as Established by Means of Intramuscular Injections of Fluorescent Dyes and Electrophysiological Measurements .....	54
3.3 Direct Stimulation of the Trigeminal and Facial Nerves by Massage of the Vibrissal Muscles Improves the Quality of Target Reinnervation and Promotes Full Recovery of Whisking Function .....	60
3.3.1 Analysis of Vibrissae Motor Performance During Exploration .....	60
3.3.2 Manual Stimulation Counteracts Posttraumatic Loss of Synaptophysin-Positive Axon Terminals in the Facial Nucleus .....	62
3.3.3 Degree of Collateral Axonal Branching Remains Elevated Regardless of Stimulation .....	62
3.3.4 Mechanical Stimulation Reduces the Degree of Motor End Plate Polyinnervation .....	64
3.3.5 Manually Stimulated Recovery of Motor Function After Facial Nerve Injury Requires Intact Sensory Input .....	64

3.4 Direct Stimulation of the Trigeminal and Facial Nerves by Electric Current to the Vibrissal Muscles Fails to Improve Quality of Target Reinnervation and Does Not Promote Recovery of Vibrissal Function .....	65
3.4.1 Electrical Stimulation of the Vibrissal Muscles Does Not Promote Recovery of Whisking .....	65
3.4.2 A High Degree of Collateral Axonal Branching Occurs Regardless of ES .....	65
3.4.3 ES Does Not Reduce Polyinnervation of the Motor End Plates .....	66
3.4.4 ES Reduces the Number of Motor End Plates .....	67
<b>4 Discussion .....</b>	<b>69</b>
4.1 Mild Indirect Stimulation of the Trigeminal Afferents by Removal of the Contralateral Vibrissal Hairs Has a Beneficial Effect on Motor Recovery .....	69
4.1.1 Importance of Sensory Fiber Regeneration for Motor Axonal Regrowth .....	69
4.1.2 Influence of Synaptic Coverage on Axonal Regrowth and Quality of Target Reinnervation .....	70
4.1.3 Clinical Application .....	71
4.2 Beneficial Effect of the Intensive Indirect Stimulation of the Trigeminal Afferents by Excision of the Contralateral Infraorbital Nerve .....	72
4.2.1 Removal of the Contralateral Trigeminal (ION) Input Attenuates the Degree of Collateral Axonal Branching Within the Transected Buccal Branch of the Facial Nerve .....	72
4.2.2 Observations on the Recovering Vibrissal Function .....	74
4.2.3 Removal of the Contralateral Trigeminal (ION) Input Improves Quality of Whisker Pad Musculature Reinnervation .....	76
4.3 Complete Recovery of Motor Function After Direct Stimulation of the Trigeminal and Facial Nerves by Massage of the Vibrissal Muscles .....	79
4.3.1 Methodological Considerations .....	79
4.3.2 Importance of the Stimulation Type .....	80
4.3.3 Possible Mechanisms of the Beneficial Effects .....	80
4.3.4 Adverse Effect of Trigeminal Nerve Ablation on Functional Recovery After FFA .....	85
4.3.5 The Effect of Manual Stimulation Depends on the Integrity of the Trigeminal Sensory System .....	85
4.4 Deleterious Effect of the Direct Stimulation of the Trigeminal and Facial Nerves by Application of Electric Current to the Vibrissal Muscles .....	86
4.4.1 Rationale to Use Electrical Stimulation for Treatment of Denervated Muscles .....	87
4.4.2 Effect of Electrical Stimulation on the Quality of Muscle Reinnervation .....	88
<b>5 Conclusion .....</b>	<b>91</b>
<b>References .....</b>	<b>93</b>
<b>Subject Index .....</b>	<b>107</b>

Stimulation of Trigeminal Afferents Improves Motor  
Recovery After Facial Nerve Injury  
Functional, Electrophysiological and Morphological  
Proofs

Skouras, E.; Pavlov, S.; Bendella, H.; Angelov, D.N.  
2013, XV, 110 p., Softcover  
ISBN: 978-3-642-33310-1