NEW EXPERIMENTAL MODEL OF FACIAL NERVE FUNCTION AND INTEGRITY TO EVALUATE REGENERATION

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ABSTRACT

Introduction: The ideal animal model for nerve regeneration studies is the object of controversy, because all models described by the literature have advantages and disadvantages. Small animals such as rats have been repeatedly used in experimental procedures because of their easy caretaking and handling, resistance to manipulation and infections and low cost. In this case, rats have the additional benefit of tolerating bilateral facial paralysis. **Objective:** To describe the histological and functional (electroneuromyographical) patterns of the mandibular branch of the facial nerve of Wistar rats, in order to create a new experimental model of facial nerve regeneration. **Methods:** Forty two male Wistar rats were submitted to neurophysiological evaluation by a nerve conduction test of the mandibular branch of the facial nerve to obtain the *compound* muscle *action potential* (CMAP). Twelve of these rats had the mandibular branch of the facial nerve surgically removed, and submitted to histological analysis (total axon number, partial axonal density and axonal diameter) of the proximal and distal nerve segments. **Results:** There was no statistical difference in the functional and histological variables studied. **Conclusion:** These new histological and functional standards of the mandibular branch of the facial nerve of rats establish an objective, easy and greatly reproducible model for future facial nerve regeneration studies. **Keywords:** facial nerve, electromyography, nerve regeneration, histological analysis.

INTRODUCTION

There is no ideal model of facial nerve regeneration in animals[1].

For this purpose, several kinds of animals have been studied: pigs, dogs, rabbits, cats, horses, mice and others, but all of them have their advantages and disadvantages[1-2-3].

OBJECTIVE

To establish a new experimental model for evaluating facial nerve regeneration through nerve conduction studies and histologic analyses of the mandibular branch of the facial nerve (MBFN) of *Wistar* rats.

MATERIALS AND METHODS

This research was approved by the institution´s Ethics Review Board (protocol number 0291/07) with financial support from FAPESP and CNPq.

For functional evaluation 42 healthy *Wistar* male rats, weighing between 250-300g, were subjected to the method described by and Salomone *et al.* (2012)[2], in which amplitude, duration and latency of the compound muscle action potential (CMAP) were evaluated on the right and left sides by electroneuromyography (ENMG).

For histological analyses, 12 rats with the same features were used after ENMG. The right MBFN was dissected and the neural segment extracted and used to generate two normal histological groups (P- proximal; D- distal). Qualitative and quantitative histological analyses were performed in P and D groups.

The values of the variables obtained in this study were compared by *Wilcoxon* signaled posts test (p> 0,05).

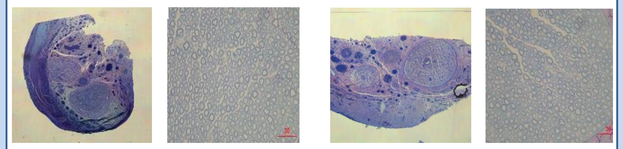
RESULTS

The CMAP results obtained from the MBFN of either the right or left sides did not differ among groups (p> 0,05).

CMAP Variable	Side	n	Mean value	Standard deviation	Lower Limit	High Limit	Significance (p)
Amplitude (mV)	Right	42	5,8	1,89	2,52	10,67	0,871
	Left	42	5,76	1,9	1,59	9,08	
Latency (ms)	Right	42	1,17	0,28	0,58	2,05	0,613
	Left	42	1,15	0,31	0,6	2,66	
Duration (ms)	Right	42	4,46	1,08	2,06	6,83	0,990
	Left	42	4,41	0,83	2,62	6,08	
Stimulus (mA)	Right	42	2,73	0,65	1,7	4,5	0,514
	Left	42	2,46	0,52	1,5	4	

The morphological qualitative analyses of both groups showed 2 or 3 fascicles of similar morphology with rounded axons, regular diameters and even distribution.

Group P Group D



The quantitative morphological analysis did not show any differences in either axonal number, density or diameter between groups P and D (p<0,005).

Axonal	Segment	n	Mean value	Standard deviation	Minimum	Maximum	Median	Significance (p)
Number	Proximal	12	1662	131,03	1336	1847	1669,5	0,666
	Distal	12	1646	115,96	1382	1789	1663	
Density (number/µm²)	Proximal	12	0,18	0,01	0,15	0,21	0,19	0,666
	Distal	12	0,18	0,01	0,15	0,2	0,18	
Diameter (µm)	Proximal	12	4,5	0,21	4,2	5,01	4,48	0,937
	Distal	12	4,49	0,27	4,09	5,06	4,51	

DISCUSSION

The sciatic nerve of rats is one of the most widely used models in studies of neural regeneration, mainly due to quite extensive and easy exposure[2-3]. However, this nerve splits soon after emerging from below the sacrum, potentially invalidating the electrophysiological test results[2]. Furthermore, the facial nerve presents important differences in regenerative responses due to the long distance it travels through a bony canal[1-2-3].

The choice of creating an experimental model with the MBFN of rats was due to several factors:

- easy access compared to the other branches
- less complex anatomy compared to the temporal and zygomatic branches
- long pathway (10- 12mm) before bifurcation
- visualization by transparency [2-3-4].

Regarding functional results, there was no significant statistical difference in the values of the variables among groups (sides).

Such results showed values within the range established by Salomone[2] and in accordance with those described by Salomone[5] and Costa[6], by proving that this technique is for objective evaluation of high reproducibility.

Regarding results obtained in the quantitative histological study, there was no difference between the results previously described by Mattox and Felix[3], Salomone[2-5] and Costa[4-6] which showed that the mandibular branch of the facial nerve of rats provides a good model of qualitative and quantitative histological study of neural regeneration of the facial nerve.

CONCLUSION

With the standardization of histological analyses (qualitative/quantitative) and the nerve conduction study (ENMG) parameters of the mandibular branch of rats, we established a new and easy experimental model of assessment of the facial nerve regeneration, which will help future studies in this area.

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