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Development Of New Techniques For Behavioral Evaluation In Animals Submitted To Facial Nerve Damage.

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ABSTRACT

Three new methods for evaluating facial nerve injury were developed: Spontaneous ocular opening, vibrissae reflex and resistance to upper eyelid lifting. Nerve crush injury was performed by pressing the mice's facial nerve with forceps for 30 seconds. Scores were given based on the comparison between the behavioral assessment of the injured and non-injured sides. Facial nerve injury is currently evaluated by observing the movement of the mice's vibrissae and the ocular closure. In this study, three additional tests are proposed. Therefore, a wider evaluation can be accomplished where all the aspects of the facial mimicry can be assessed. The use of the 3 new methods described in this study, in addition to the 2 methods currently used, allows a complete behavioral assessment of facial nerve lesions as several behavioral aspects related to these injuries can be evaluated.

Keywords: Behavioral evaluation; Facial nerve; Nerve injury.

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INTRODUCTION

Peripheral nerve damage is a serious health problem that can result in significant disability as this nerve controls facial mime, swallowing, blinking, phonation and palate [1].

When a trauma occurs, the facial nerve is the most frequently injured among the nerves of the head and neck. In addition to traffic accidents, fracture of the temporal bone, lacerations of the face, removal of tumors and resections due to malignancy are common causes of facial nerve damage [2].

Facial paralysis caused by facial nerve trauma is a fairly common clinical condition. When it occurs unilaterally it causes changes in facial symmetry and incomplete ocular closure, which may cause ophthalmic problems, difficulty in swallowing and articulation of certain phonemes. Therefore, this condition brings relevant individual and social consequences, requiring great effort in an attempt to understand the various factors involved in the injury itself, as well as in the regeneration and repair processes [3].

When a peripheral nerve is lesioned, changes occur in the intersegmental site so that there is a favorable microenvironment for regeneration. This fundamental neural substrate is locally present in the injured area, being composed of Schwann cells, perineural fibroblasts, extracellular matrix components such as laminin, fibronectin, adhesion molecules, and the so-called neurotrophic factors, which are produced by the sectioned nerve that act as stimulators of regeneration [4].

In order to restore the motor and sensory integrity, the injured nerve fibers need to regenerate and to establish new connections with the target muscle or organ. When facial nerve lesions are extensive, an autologous graft is usually employed to fill the space between the stumps. In fact, the autologous nerve graft was considered for a long time as the main form of treatment for this type of lesion. However, important drawbacks are involved in this procedure, such as the need to perform incisions to remove the donor nerve and the functional loss of the site after nerve removal [1,5].

After a peripheral nerve injury, a degeneration process known as Wallerian degeneration (WD) occurs, which aims to improve the microenvironment for later regeneration. Due to the wide distribution of the peripheral nervous system (PNS), peripheral nerve lesions are relatively frequent due to crushing, ischemia or inflammation. Since neurons do not undergo mitosis, nerve injury usually leads to significant loss. However, under certain conditions, its cytoplasmic extensions have the ability to regenerate as long as favorable conditions are provided [6].

Nerve repair requires a complex interaction between a scaffold for axonal growth orientation, the support cells such as Schwann cells, growth factors and extracellular matrix. The combination of axonal scaffolds and transplanted cells provides adequate support for neural regeneration and has been investigated as a strategy to overcome the limitations of surgical repair [7].

Previous reports have shown that although the motor fibers of the facial nerve are originated in the central nervous system (CNS), they have a high regenerative capacity comparing to a peripheral motor nerve. It presents clearance of debris and myelin higher than that of the CNS. In addition, the facial nerve has a greater capacity to respond to growth factors after injury [8].

The regeneration of the facial nerve is evaluated in vivo according to the animals' behavior, where mice are submitted to observation of spontaneous facial motor function by comparing the movement of the vibrissae in the injured side with that of the non-injured side during the typical movement of the mouse when exploring the environment. In addition, regeneration of the facial nerve is evaluated through ocular closure of the animals by stimulus of an air jet, produced by the rapid compression of the plunger of a 20 mL syringe directed against the animal's eyeball [9].

The facial nerve of Wistar rats emerges from the stylomastoid foramen, located on the lateral part of the skull. The posterior auricular branch is responsible for the innervation of the auricular musculature, emerging right after this foramen. The facial nerve trunk, which runs from the posterior auricular branch to the beginning of its posterior cervical and cervical bifurcation, measures approximately 6mm and is located between the trapezius muscle and the external auditory canal [10].

However, we hypothesize that only these two parameters are not sufficient for a more in-depth analysis of facial nerve regeneration, considering that the evaluation of both sensory and motor recovery is necessary. Therefore, three other parameters were elaborated by our group in order to extend this evaluation and thus obtain more reliable results.

These novel parameters evaluate the ocular opening, the sensitivity of the vibrissae after touch and the recovery of the motor capacity of the upper eyelid. Thus, the aim of this work is to elaborate additional methods to optimize the behavioral evaluation with respect to the alterations caused after a facial nerve lesion.

MATERIALS AND METHODS

The models of crushing [9,11-13] and transection [14-17] of the facial nerve at the height of the nerve trunk or in the branches that emerge from this nerve are currently used for behavioral tests.

Surgical procedure

First, the animals were weighed for determining the correct dose of the anesthetic. The animals were anesthetized with 10% Ketamine Hydrochloride (0.1mL / 100g) (Vetnil® Injection) and 2% Xylazine Hydrochloride (0.01mL / 100g) (Kensol®) intraperitoneally. After submitting the animals to right retroauricular trichotomy, the incision marking was performed (Fig 1A) followed by a vertical retroauricular incision through the skin, subcutaneous tissue and platysma muscle (Fig 1B). After the incision, a blunt dissection and identification of the tendinous margin of the trapezius muscle, the trunk and bifurcation of the right facial nerve, as well as the parotid and external jugular veins were performed (Fig 1C) [9,11].

Once the facial nerve was located, a dissection was performed at 3 mm from its emergence (between the margin of the tendinous trapezius muscle and the external jugular vein). After the dissection of the facial nerve, a crush injury was performed by pressing the nerve with a Kelly forceps for 30 seconds (Fig 1D). It is worth to point out that partial and total transection procedures can also be performed. Finally, the skin and subcutaneous tissue were sutured using nylon 4-0 stitches (Fig 1E). In order to validate the use of the proposed scales in the investigation of the behavioral evolution of the animals submitted to facial nerve crushing, 10µl of saline was administered and a behavioral analysis was performed for 90 days. It seems reasonable to assume that animals treated with cell inoculation, tissue grafts and drugs may show more evident functional recovery when compared to that of saline administration.

Fig 1: Animal positioned in lateral decubitus, with the cervical region positioned on a mold with demarcation of the region submitted to the incision for the facial nerve damage (1A); Incision in the right retroauricular region, allowing the location of the platysma muscle (1B); Blunt dissection and location of the trapezius muscle (1C); Nerve crush injury performed by pressing the facial nerve with a Kelly forceps for 30 seconds (1D) and skin and subcutaneous tissue suturing at different sites (1E).

Behavioral observation

The animals were evaluated through observation of spontaneous facial movements such as the movement of the vibrissae of the injured side in comparison to the non-injured lateral side during the animal's typical movements while exploring the environment (Fig 2A) [9,11,13].

The position, amplitude and frequency of the movement of the vibrissae are considered where a score from 0 to 5 is given (0 characterizes no movement and 5 corresponds to effective movement) as shown in Table 1.

Table 1: Scoring scale for the observed vibrissae movement.

Score	Behavior
0	No movement
1	Moderate tremor
2	Effective movement with posterior positioning and amplitude/frequencies lower than the non-injured side
3	Effective movement with positioning similar to the non-injured side but with lower amplitude and frequency
4	Effective movement with positioning and amplitude similar to the non-injured side, but with lower frequency
5	Effective movement with positioning, amplitude and frequency similar to the non-injured side

Another parameter used to evaluate facial nerve injury is the eye closure and blinking reflex when a jet of air struck the animal's eye. This jet of air was produced by the rapid compression of the plunger of a 20 mL syringe directed against the animal's eyeball (Fig 2B).

Taking into account the closure of the eyelid, a numerical value of 0 to 5 is given, where 0 characterizes no movement and 5 a complete ocular closure, as shown in Table 2.

Fig 2: Behavioral assessment during environmental exploitation (2A); Eye closure and blinking reflex evaluation (2B); Evaluation of ocular opening using an inelastic anthropometric tape (2C); Evaluation of vibrissae reflex (2D) and evaluator lifting the animal's upper eyelid (2E).

Table 2: Scoring scale for the eyelid closure and blinking reflex evaluation

Score	Eye movement
1	No movement
2	Contraction without closure
3	50% eye closure
4	75% eye closure
5	Full eye closure

Spontaneous ocular opening

The extent of ocular opening is measured with an inelastic anthropometric tape, as shown in Figure 2C. The measurement (in millimeters) is converted into a graded score that ranges from 1 to 5 (Table 3).

Table 3: Eye opening score

Score	Eye opening
1	Eye opening of 1mm
2	Eye opening of 2mm
3	Eye opening of 3mm
4	Eye opening of 4mm
5	Eye opening of 5mm

Vibrissae reflex

The vibrissae reflex is evaluated by touching the animal's vibrissae with a cotton swab using to a Dumont # 6 forceps, where the extent of animal's reflex is assessed (Fig. 2D). In this experiment, the animal is expected to move the vibrissa in the opposite direction to the stimulus. Thus, such movement can be absent (1), at low (2), moderate (3) or normal (4) response, as shown in Table 4.

Table 4: Score for the vibrissae reflex after touch.

Score	Vibrissae reflex
1	No response after touch
2	Low response
3	Moderate response
4	Normal response

Resistance to upper eyelid lifting

In this test, the evaluator lifts the animal's upper eyelid using forceps and access the resistance against lifting, as shown in Figure 2E. In normal conditions, it is expected that the animal exert some resistance against the eyelid lifting stimulus. This resistance can be absent, mild, moderate or normal to such stimulus, yielding scores 1, 2, 3 and 4, respectively (Table 5).

Table 5: Score for the resistance to upper eyelid lifting.

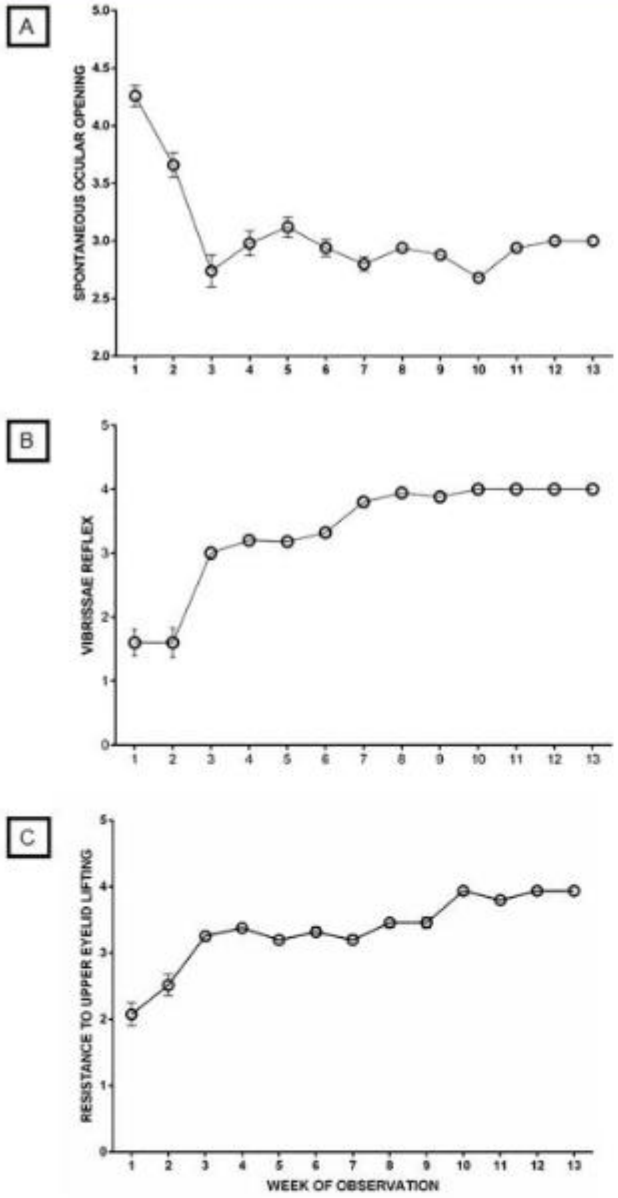
Score	Resistance to upper eyelid lifting
1	No resistance
2	Low resistance
3	Moderate resistance
4	Normal resistance

RESULTS

Behavioral evolution of the animals that received saline solution after facial nerve crushing is shown in Graph 1A. In the first week after the lesion, the animals presented ocular aperture around 5mm. However, the animals presented behavioral evolution as the time progressed, thus resuming the control of the ocular closure and keeping it around 3mm.

Graph 1B shows that in the first and second weeks after facial nerve crushing and saline administration, the animals presented a score without any response after stimulation. However, the animals presented behavioral evolution as the time went by, thus presenting normal vibrissae reflex.

Graph 1: Behavioral evolution of spontaneous ocular opening (1A); Behavioral evolution of vibrissae reflex (1B) and behavioral evolution of resistance to upper eyelid lifting(1C).



Analysis of the upper eyelid lift against resistance showed that in the first week after facial nerve crush injury and saline administration the animals showed a discrete response to the stimulus, even though the animals presented behavioral evolution over time, thus showing normal resistance to upper eyelid lifting (Graph 1C).

DISCUSSION

Although the scoring scales for eye closure and blinking reflex have been described in the literature [9,11,13], other behavioral aspects related to facial nerve injury such as ocular opening, vibrissae reflex and resistance against upper eyelid lift are important for broader behavioral assessment.

Behavioral observation studies that evaluate animals' facial nerve regeneration usually use tests that analyze facial mimics and ocular closure. Several animals have been used as experimental models, such as rabbits, hamsters, cats, dogs and rats [9].

Animals with intact facial nerves present normal blinking reflex with complete and symmetrical ocular closure, as well as regular movement of the vibrissae (anterior position) upon stimulus. On the other hand, animals with facial paralysis due to facial nerve injury usually present loss of blinking reflex and no movement of the vibrissae, which in turn assume a posterior position [9].

When evaluating the lifting force of the upper eyelid, it is possible to analyze the contraction capacity of the muscles innervated by the facial nerve, such as the temporal and orbicular muscles of the eye and upper palpebral, thus demonstrating the occurrence of nerve regeneration. This test is already performed in leprosy patients with the purpose of evaluating the severity and extent of nerve injury caused by the Hansen bacillus. Therefore, it is worth mentioning that our scale follows the same principle, considering that in leprosy an injury also occurs in the branch of the facial nerve, losing its ability to lift the upper eyelid.

Facial nerve lesions can lead to sequelae such as incomplete ocular closure, which can cause ophthalmic problems due to decreased ocular lubrication and corneal damage. It can also lead to chewing and swallowing disorders, which impart digestion and may cause malnutrition. In addition, facial nerve injury can cause speech problems due to limitations in the formation of some phonemes, thus making communication difficult. Such negative outcomes can directly influence patient's quality of life, which makes the proper evaluation of facial nerve lesion of great importance.

CONCLUSION

Currently, most evaluation studies of facial nerve regeneration take into account only the movement of the vibrissae and the ocular closure. The use of the 3 additional techniques described in this study allows a complete behavioral evaluation, considering that several behavioral aspects related to facial nerve injury can be evaluated, obtaining a broader analysis.

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REFERENCES

[1] Cao J, Xiao Z, Jin W, Chen B, Meng D, Ding W et al. Induction of Rat Facial Nerve Regeneration by Functional Collagen Scaffolds. Rev Biomaterials, 34 (2013)1302-1310.

[2] Skouras E, Angelov DN. Experimental studies on post-transectional facial nerve regrowth and functional recovery of paralyzed muscles of the face in rats and mice. Anatomy: International Journal of Experimental & Clinical Anatom, 4 (2010) 1-27.

[3] Lucena EES, Guzen FP, Cavalcanti JRL, Barboza CAG, Júnior ESN, Cavalcante JS. Experimental Considerations Concerning the Use of Stem Cells and Tissue Engineering for Facial Nerve Regeneration: A Systematic Review. J. Oral Maxillofac Surg, 72(5) (2014) 1001-12.

[4] Battiston B, Geuna S, Ferrero M, Tos P. Nerve repair by means of tubulization: literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurg, 25(4) (2005) 258-67.

[5] Cui Y, Lu C, Meng D, Xiao Z, Hou X, Ding W et al. Collagen scaffolds modified with CNTF and BFGF promote facial nerve regeneration in minipigs. Rev Biomaterials, 35(27) (2014) 7819-27.

[6] Coleman M. Axon degeneration mechanisms: commonality amid diversity. Nature Reviews, Neuroscience, 6 (2005) 889–898.

[7] Costa HJZR, Bento RF, Salomone R, Azzi-Nogueira D, Zanatta DB, Costa MP et al. Mesenchymal bone marrow stem cells within polyglycolic acid tube observed in vivo after six weeks enhance facial nerve regeneration. Brain Res, 1510 (2013) 10-21.

[8] Salomone R, Bento RF, Costa HJ, Azzi-Nogueira D, Ovando PC, Da-Slva CF et al. Bone marrow stem cells in facial nerve regeneration from isolated stumps. Muscle Nerve, 48 (2013) 423-29.

[9] Borin A, Toledo RN, Faria SD, Testa JRG, Cruz OLM. Modelo experimental comportamental e histológico da regeneração do Nervo facial em ratos. Rev Bras Otorrinolaryngol, 72(6) (2006) 776-785.

[10] Mattox DE, Felix H. Surgical anatomy of the rat facial nerve. The American Journal of Otology, 8 (1) (1987) 43-7.

[11] Terão E, Janssens S, Van Den Bosch APH, Portier MM, Klosen P. In vivo expression of the intermediate filament peripherin in rat motoneurons: modulation by inhibitory and stimulatory signals. Neuroscience, 101 (2000) 679–88.

[12] Lin H, Wang H, Chen D, Gu Y. A dose–effect relationship of ginkgo biloba extract to nerve regeneration in a rat model. Microsurgery, 27 (2007) 673–677.

[13] Chul HO, Yong BCA, Cheol HCB. Effect of ginkgo biloba extract on recovery after facial nerve crush injury in the rat. International Journal of Pediatric Otorhinolaryngology, 72 (2012) 1823–1826.

[14] Gilad WH, Tetzlaff WG, Rabey JM, Gilad GM. Accelerated recovery following polyamines and aminoguanidine treatment after facial nerve injury in rats, Brain Res, 724 (1996) 141–144.

[15] Borin A, Toledo RN, Ho PL, Testa JRG, Cruz OLM, Fukuda Y. Influence of cyclic AMP on facial nerve regeneration in rats. Braz. J. Otorhinolaryngol, 73 (2008) 675–683.

[16] Koyama Y, Fujiwara T, Kubo T, Tomita K, Yano K, Hosokawa K et al. Reduction of oligodendrocyte myelin glycoprotein expression following facial nerve transection. Journal of Chemical Neuroanatomy, 36 (2008) 209–215.

[17] Yasui G, Yamamoto Y, Shichinohe R, Funayama E, Oyama A, Hayashi T, et al. Neuregulin-1 released by biodegradable gelatin hydrogels can accelerate facial nerve regeneration and functional recovery of traumatic facial nerve palsy. Journal of Plastic, Reconstructive & Aesthetic Surgery, 69 (2016) 328–334.