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ORIGINAL ARTICLE

Time Dependent Electrophysiological Changes in Nerve-Crush Traumatic Facial Nerve Injury in Rats

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Objective: To investigate experimentally the time dependent changes of latency, amplitude, threshold of neural response in injured rat facial nerve in a nerve-crush trauma model.

Materials and Methods: Thirty Wistar rats weighing 220-280 g (12-16 week), were grouped for permanent and transient nerve injury during time course analysis of electrophysiological changes at 1st week, and 1st, 3rd and 6th months. For the nerve-crush group, facial nerve was pressed by vascular clamps for 40 minutes without disturbing the nerve integrity (10 rats). For the nerve-cut group, the facial nerve was cut and a 5-mm part was removed (10 rats). 10 rats with intact facial nerve served as control. CMAP (compound muscle action potantial) recordings were obtained at each time interval. Measurements were compared with Variance-analysis.

Results: In the nerve-crush group, recovery of the latency from 1st week to 1st month (0.029, $p \leq 0.05$) and excitability thresholds in 1st week was statistically significant ($p \leq 0.05$) as compared to the control. Recovery of the reduced amplitude at consecutive intervals was not statistically significant. In the nerve-cut group, no electrical response was obtained throughout the follow-up.

Conclusion: In the follow-up of electrophysiological changes of traumatic injury of the facial nerve in rats, the results appear to indicate that parameters "latency and threshold" may reflect the different aspect of injured nerve condition from the parameter "amplitude". Amplitude comparison alone could bring some problems in the clinical setting. These findings suggest complete measurement of all parameters to outline the prognosis of traumatic facial paralysis.

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Introduction

Traumatic facial nerve paralysis usually occurs as a result of temporal bone trauma or otological and neurotological surgery. Medical or surgical measures are taken according to the severity of the injury. [1] When post-traumatic facial paralysis does not spontaneously resolve or the injury proceeds to peripheral denervation as measured by facial ENoG or EMG, it is a common sense that exploration and nerve repair should be performed as soon as possible.[2] However, estimation of extension of the facial nerve damage in advance whether it is permanent or transient, the critical time when the surgery provides better prognosis than medical follow-up based on electrophysiological testing of the nerve still remain controversial. Estimation of an extent of injury can be followed by serial electrophysiological investigation.[3,4] However, experimental and comparative analysis of the predictive role of time dependent follow-up of latency, amplitude of the action potentials of the damaged nerve and the threshold of the neural response is lacking. Electrophysiological involvement of the injury can be properly examined in an experimental model. However, development of such model is difficult since the physiopathological process is different and depends on the type of the trauma. Traumatic facial nerve injuries can be classified into 5 types as follows; laceration/avulsion, traction/stretch, compression ischemia/nerve crush, thermal/electrical injury and nerve cut/interruption.[5] Nerve-crush and nerve-cut models are suitable in which more or less stable condition could be obtained.

The aim of this study is to compare the reliability of the electrophysiological parameters and to document the progression of the impairment or recovery of the facial paralysis in a time dependent electrophysiological follow-up in nerve-cut and nerve-crush injury model of traumatic facial paralysis in rats.

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Materials and Methods

Thirty male Wistar rats weighing 220-280 g (12-16 week), which were housed individually, were selected for the study. The study was approved by local ethical committee on animal rights (15.06.2005/05-56). Right facial nerve was used for the dissection in all rats which were anesthetized by intramuscular injection (Ketamine 150mg/kg, Xylocaine 4 mg/kg) for the dissection under sterile condition. Right side of their face was shaved and cleaned with antiseptic solutions. Then the facial nerve was exposed by transecting the skin from post auricular area to the angle of the mouth. After delineating the facial nerve, the configuration and the angularity of the bifurcations were noted and the length of the main trunks was measured. Average length of the main trunks and SDs were noted. All dissections were also recorded on video to be precise not to injure the nerve during dissection. 10 rats were served as control and the rest of the animals were classified in 2 groups, 10 rats in each, for acute permanent (nerve-cut) and transient (nerve-crush) nerve injury. The electrophysiological studies were done for each group at 1st week, and 1st, 3rd and 6th months.

For the nerve-crush group, main trunk of the facial nerve was pressed by similar vascular clamps for 40 minutes without disturbing the nerve integrity. For the nerve-cut group, a-5-mm piece of the main trunk of the facial nerve was measured and removed. Then the incision was closed by 4/0 interrupted silk sutures at the end of each procedure. The rats were given 100 mg im penicillin for prophylaxis. Control group underwent Sham operation. (Main trunk of the facial nerve was dissected and after was closed)

None of the rats had any problem during follow-up. At 1st week, and 1st, 3rd and 6th months, rats were anesthetized and operated again for electrophysiological analysis of the nerve. During CMAP (compound muscle action potantial) recordings, the recording electrode (Medtronics; 0.45 mm diameter, 20 mm long, concentric fine needle electrodes, Catalog; 9013-L-0512) was placed in oris muscle and the ground electrode was placed in sternocleidomastoid muscle (Medtronics; 0.7 mm diameter, 35 mm long, catalog; 9013-L-0611). Custom made and insulated bipolar needle electrodes are used for neural stimulation. Proximal part of the main trunk of the facial nerve was freed from the surrounding tissues, slightly suspended and prepared for stimulation. Motor unit action potentials were obtained from orbicularis oris muscles after stepwise increasing of stimulus intensity starting from 0.1 mA (Dantec, Keypoint, Denmark). Stimulus duration was 50 msec with 0.1 or 0.2 msec square wave pulses. Latency, amplitude and thresholds of the evoked potentials were measured. Latency was defined as the elapsed time from the beginning of the stimulation to the appearance of first deflection from the isoelectrical line. Amplitude was measured from negative to positive peaks of the compound muscle potential. Excitability threshold was defined as the level of stimulation which can produce an identifiable potential (usually at 50 μV) (Figures 1A and B). No specific therapy was given to the rats and only spontaneous progression of the neural injury was followed.

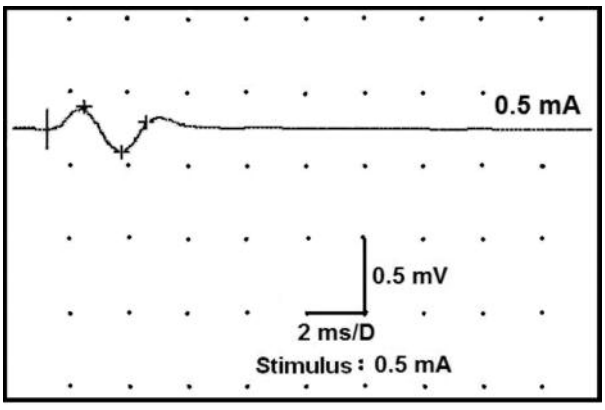

Figure 1-A.

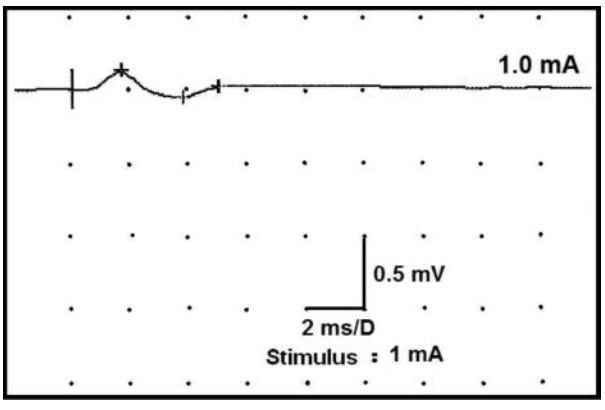

Figure 1-B.

Figure 1- (A) CMAP recordings of the facial nerve from control and **(B)** paralytic rat a week after injury. (Note: that stimulus threshold is increased, latency is prolonged and amplitude is decreased)

SPSS 14.0 (SPSS Inc,.IL, USA) software program was used for statistical comparison. Average ± standard deviations were measured. Variance-analysis test was used to analyze the difference between the consecutive measurements. LSD test (least significant difference) was used for post-hoc test. Level of inaccuracy was kept at =0.05. P values equal or smaller than this were accepted as statistically significant.

Results

None of the rats with acute permanent nerve injury had any response to nerve stimulation at all intervals (Figure 2). Prolongation of the latency in rats with transient nerve injury was highest in the first week. Recovery of the latency (change in latency value) from the first week to the first month as compared to the control was statistically significant. However, the difference between the average delayed latency values at consecutive intervals in 1st, 3rd and 6th months and the difference between the controls and the values at these intervals was not statistically significant. (Figure 3, Tables 1 and 2). Decrease in the amplitude was highest in the first week. However, there was no statistically significant recovery (change in amplitude values) at consecutive intervals from 1st week through the 6th months and the amplitude never returned to normal level as compared to the control (Figure 4, Tables 3 and 4). Excitability threshold was highest for the 1st week. There was statistically significant recovery from the first week to the first month. However, there was no statistically significant change at consecutive intervals from 1st month to the 6th months and excitability threshold never returned to normal level at the end of 6th months (Figure 5, Tables 5 and 6). Loss of function according to the excitation threshold of the nerve as compared to control was 80% (0.58 v 2.43) for the 1st week, 50% (0.58 v 1.09) for the 1st month, 35% (0.58 v 0.89) for the 3rd months and 29% (0.58 v 0.81) for the 6th months after injury.

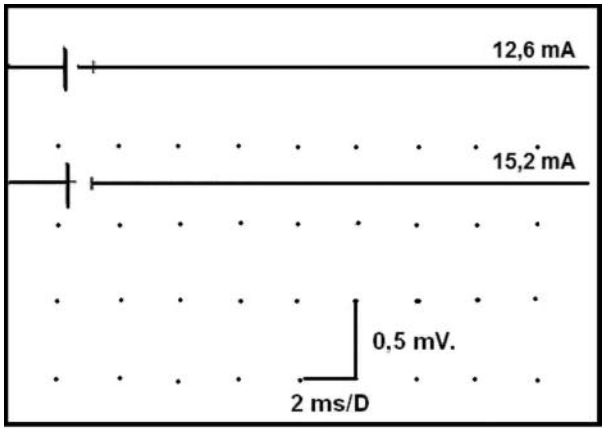

Figure 2- CMAP recordings demonstrate no electrical response in rats with nerve-cut

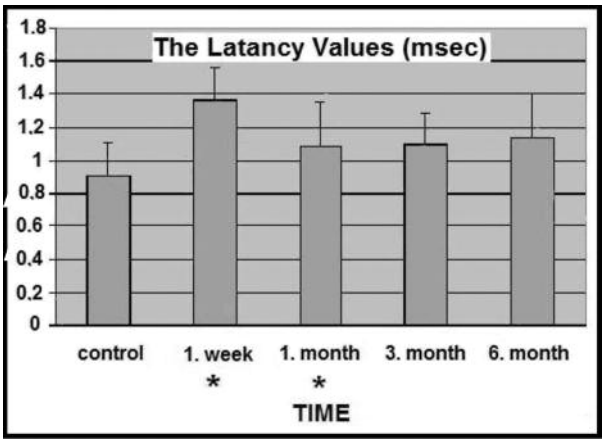

Figure 3- Mean latency values (msec) and SDs of the CMAP in rats with nerve-crush (*: statistically significant) ($p < 0.05$)

Discussion

Variability of waveform morphology of action potentials is low in facial nerve electrophysiological studies.[6,7] Prognostic predictability of facial electroneurography is crucial to propose appropriate therapy since there is usually no correlation between the severity of trauma and severity of the neural damage. However, controversial points still exist. Electroneurography was first proposed by Esslen and then was popularized by Fisch in which it has been theorized that the amplitude of the compound action potential of the facial muscle is proportional to the number of neuropraxic nerve fibers.[8] Fisch proposes urgent total exploration of the facial nerve within 6 days if paralytic nerve has more than 90% degeneration by comparing the amplitudes of the compound muscle action potential evoked in the paralyzed and normal side of the face.[8,9] Quaranta reported 13 patients undergoing late surgery for facial paralysis with greater than 90% degeneration on electroneurography due to temporal bone fracture resulting with successful recovery.[10] However, Sinha et al reported that out of 15 patients with Bell's palsy who had CAP reduction greater than 90% only 3 (20%) had severe dysfunction during follow-up without surgery.[11] Latency studies related with the paralytic facial nerve are very few. Skevas et al have reviewed 80 patients with idiopathic facial paralysis and demonstrated that as the latency time prolonged or there was no response, functional recovery of the nerve was incomplete and recovery time was long.[12] Time course of electrical reaction in facial paralysis can vary which is probably related to the pathogenesis. Sillman et al compared the prognostic value of evoked EMG in idiopathic and traumatic facial paralysis in terms of CAP decline of greater than 90% in patients who did not undergo surgery and demonstrated that the test is more predictive in idiopathic form than the traumatic one.[2]

Table 1. The latancy values (msec) of the rats with acute traumatic temporary lesion at different time intervals.

Rats	The Latancy Values (msec)				
	Control	***1. Week***	***1. Month***	***3. Month***	***6. Month***
1	0.8	1.6	1.2	1.17	1.16
2	1.42	1.2	0.8	0.88	0.9
3	0.8	1.2	1.25	0.8	1.2
4	0.7	1.2	1.2	0.9	1.25
5	0.9	1.2	1.6	1.12	1.23
6	0.95	1.2	0.8	1.25	1.04
7	0.8	1.6	1.2	1.12	1.12
8	0.9	1.6	1.2	1.3	1.19
9	1	1.2	0.8	1.4	1.13
10	0.8	1.6	0.8	1	1.14
Mean	**0.907**	**1.360**	**1.085**	**1.094**	**1.136**
SDs	**0.201**	**0.207**	**0.273**	**0.196**	**0.103**

Table 2. Comparison of the latancy values (msec) of the rats with acute traumatic temporary lesion (*:Statistically significant) ($p < 0.05$)

P value	The Latancy Values (msec)			
	1. Week (1.360±0.207)	***1. Month*** (1.085±0.273)	***3. Month*** (1.094±0.196)	***6. Month*** (1.136±0.103)
Control (0.907±0.201)	***0.002**	0.199	0.066	0.056
1. Week (1.360±0.207)	-	***0.029**	***0.008**	***0.010**
1. Month (1.085±0.273)	***0.029**	-	0.938	0.472
3. Month (1.094±0.196)	0.066	0.938	-	0.557

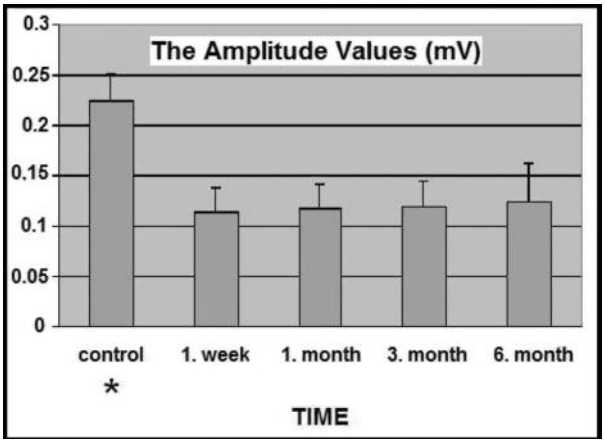

Figure 4- Mean amplitude values (mV) and SDs of the CMAP in rats with nerve-crush (*: statistically significant) ($p < 0.05$)

Translation of presented experimental results into clinical utility in humans is limited which just gives an insight into electrical recovery of the injured facial nerve. However, presented data indicate that significant recovery of the latency is seen within first month in transient injury group and there is no significant electrophysiological improvement after 1 month. This result indicates that if latency recovery is not seen in the early course of the paralysis one should consider a more severe problem. However, the difference in recovery of the amplitude was not statistically significant from the beginning of the injury when each consecutive time interval, from 1st week through the 6th months, was compared. This result indicated slow recovery in terms of amplitude change and it was difficult to designate a specific time range to

Table 3. The amplitude values (mV) of the rats with acute traumatic temporary lesion at different time intervals.

Rats	The Amplitude Values (mV)				
	Control	***1. Week***	***1. Month***	***3. Month***	***6. Month***
1	0.25	0.1	0.15	0.15	0.15
2	0.25	0.1	0.1	0.1	0.09
3	0.25	0.1	0.15	0.15	0.1
4	0.2	0.15	0.1	0.1	0.1
5	0.24	0.1	0.1	0.1	0.2
6	0.2	0.1	0.1	0.1	0.15
7	0.2	0.1	0.13	0.1	0.13
8	0.2	0.15	0.15	0.15	0.15
9	0.26	0.1	0.1	0.15	0.09
10	0.2	0.15	0.1	0.1	0.09
Mean	**0.225**	**0.115**	**0.118**	**0.120**	**0.125**
SDs	**0.027**	**0.024**	**0.024**	**0.026**	**0.037**

Table 4. Comparison of the amplitude values (mV) of the rats with acute traumatic temporary lesion (*:Statistically significant) ($p < 0.05$)

P value	The Amplitude Values (mV)			
	1. Week (0.115±0.024)	***1. Month*** (0.118±0.024)	***3. Month*** (0.120±0.026)	***6. Month*** (0.125±0.037)
Control (0.225±0.027)	***<0.001**	***<0.001**	***<0.001**	***<0.001**
1. Week (0.115±0.024)	-	0.790	0.678	0.531
1. Month (0.118±0.024)	0.790	-	0.751	0.599
3. Month (0.120±0.026)	0.678	0.751	-	0.742

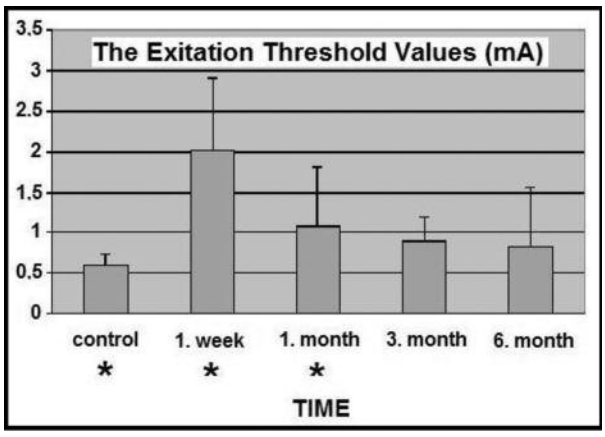

Figure 5- Mean exitability threshold of the facial nerve (mA) in rats with nerve-crush at different time intervals (*: statistically significant) ($p < 0.05$)

differentiate an accelerated healing contrary to the latency follow-up. There was statistically significant difference between the excitability thresholds of 1st week and that of other each interval. There was no statistically significant change at consecutive intervals from 1st month to the 6th months. Recovery of excitability threshold at early interval was matching with the latency recovery.

According to recent studies, prognostic role of the electroneurography based on comparison of the amplitude on the paralytic side to that of the healthy side in unilateral facial paralysis could lead to misleading conclusions. In 2002, Psillas and Daniilidis clearly demonstrated an increase in the amplitude on the normal side of the paralytic patients. They stated that as long as facial paralysis clinically persisted, the amplitude of the action potential did not alter and once facial function

Table 5. The exitation threshold values (mA) of the rats with acute traumatic temporary lesion at different time intervals.

Rats	The Exitation Threshold Values (mA)				
	Control	***1. Week***	***1. Month***	***3. Month***	***6. Month***
1	0.5	4	0.5	0.5	0.5
2	0.7	3	1	0.7	0.6
3	0.5	2	0.7	0.7	0.8
4	0.7	1	1	1	1
5	0.3	1.5	1	1	1
6	0.5	1.7	0.5	0.5	0.75
7	0.5	2	0.7	1	0.9
8	0.7	2	1.5	1	1
9	0.7	1.9	3	1	0.75
10	0.7	1.2	1	1.5	0.8
Mean	**0.580**	**2.030**	**1.090**	**0.890**	**0.810**
SDs	**0.140**	**0.880**	**0.734**	**0.300**	**0.171**

Table 6. Comparison of the exitation threshold values (mA) of the rats with acute traumatic temporary lesion (*:Statistically significant) ($p < 0.05$)

P value	The Exitation Threshold Values (mA)			
	1. Week (2.430±1.799)	***1. Month*** (1.090±0.734)	***3. Month*** (0.890±0.300)	***6. Month*** (0.810±0.171)
Control (0.580±0.140)	***0.001**	***0.042**	***0.007**	***0.012**
1. Week (2.430±1.799)	-	***0.041**	***0.009**	***0.004**
1. Month (1.090±0.734)	***0.041**	-	0.383	0.258
3. Month (0.890±0.300)	***0.009**	0.383	-	0.345

begun to recover, action potential subsequently presented a rapid rise in amplitude. They attributed this finding to the central compensatory process and they assumed that increased amplitude reflects a state of improved stimulation of the facial nerve in which more neural axons and their innervated muscles fibers become activated.[13] Contralateral motor cortex changes as a result of the cortical reorganization that follows peripheral facial nerve transection were described in adult rats.[14] Plasticity of the central nervous system could affect the amplitude comparison used as the prognostic indicator. On the other hand, Sittel have found significant left-right differences in healthy subjects and amplitude ratio was not constant in every individual at repeated measurements.[15]

Many studies have been conducted on experimental morphological changes after traumatic facial nerve injury. However, time dependent electrophysiological follow-up is lacking. Cai et al studied many types of trauma on rabbits and reported that myelin lesion was more severe than the axonal lesion. [5] Wang et al demonstrated retrograde degeneration of the facial injury after acute traction on parotid gland in cats and proposed total surgical exploration.[16] Glial derivated Schwann cells of the facial nerve are known to be involved in the maintenance of the axonal growth after facial injury. In case of a facial cut, axon will regenerate to bridge the gap through the small tunnels with the guidance of the Schwann cells which normally envelop the axons. Schwann cell proliferation after neural discontinuity is an initial indicative of an effort of the damaged nerve to regenerate. If this does not occur in a certain period neurotubules will be plugged with the fibrous tissue eventually resulting with the loss of neurofibrillary

elements which will never interface the other cut end. Actually myelin degeneration begins very early. Ge et al reported ultrastructural degeneration and demyelination process in guinea pig facial nerves a week after crush injury.[17] Presence of CMAP in response to ENoG stimulation in distal segment in 2-3 days after the facial nerve transaction can be explained in this manner. In case of Bell's palsy in which the strangulation of the nerve at the meatal foramen has been concerned, Fisch et al proposed that distal progression of the degeneration takes at least 2 days to traverse the 3.5-4 cm distance up to a point distal to the stylomastoid foramen.[8] They brought the concept of surgery if more than 90% degeneration is present 3 days after onset of complete facial paralysis. For mild cases re-myelination of axons occurs by mitogenic activity of Schwann cells and finally axonal growth through the tunnels reaches to the terminal motor end plaque at the body of muscle cell.[18-19] Felix et al presented long-term findings of facial nerve biopsies in 12 patients in whom repair of the facial nerve after temporal bone trauma required end-to-end anastomosis or cable grafting. They demonstrated 95% to 100% demyelination, areas of Schwann cell proliferation representing traumatic neuroma and regenerating myelinated fibers blocked by endoneural fibrosis either in the distal and proximal segments.[20]

Conclusion

Interpretation of electrophysiological test results in case of facial nerve injury should be cautious. In the follow-up of electrophysiological changes of transient injury of the facial nerve in rats, excitability threshold and latency were found to demonstrate significant recovery in the early period as compared the amplitude in which there is no recovery at 6th month. We should re-evaluate our policy on decision making for surgery in traumatic facial nerve injury if it is based on amplitude comparison only. Complete neurological working is necessary before having final decision for surgery in patients with traumatic facial nerve paralysis.

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