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***Brainstem excitability in Hemifacial Spasm and  
Post-Facial Palsy Synkinesias and effects of  
Botulinum Toxin.***

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## Summary

Hemifacial Spasm (HFS) and Post-facial Palsy Syndrome (PFPS) are disorders of the facial nerve. HFS is characterized by involuntary intermittent contractions and synkinesis of muscles supplied by the facial nerve; PFPS is a clinical condition that follows a peripheral facial palsy (FP), often of unknown origin, and presents with synkinesis as well. The synkinesis has its electrophysiological hallmark in the lateral spread of the blink reflex.

HFS and PFPS seem to be, in one manner, two different expressions of a common neurophysiological substrate, sharing some clinical aspects but being different in others. Nevertheless, currently available data still lack direct evidence to precisely locate the site of the lateral spread that causes a widespread facial contraction.

Objectives of our study were to evaluate excitability changes in the facial and trigeminal pathways of the affected and unaffected side in HFS and PFPS in order to obtain information on the respective underlying pathophysiological mechanisms and to evaluate possible modifications in excitability of brainstem circuits after the treatment with botulinum toxin.

To these aims we assessed the Blink Reflex, Blink Reflex Recovery Cycle and Masseter Inhibitory Reflex in patients affected by HFS, PFPS, FP and in a group of healthy controls.

Data obtained revealed that, in our patients, the trigemino-facial pathway is anatomically intact on both the affected and unaffected sides. To a more detailed analysis, data suggest a central hyperexcitability pattern that occurs either in patients with HS and PFPS. However, differently from PFPS, in our HFS group, we did not

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evidence an excessive excitability on the unaffected side.

In both HFS and PFPS groups, the blink reflex recovery cycles was enhanced in the affected side, following both direct and indirect stimulation and, also on the unaffected side, both from direct and indirect evocation on the HFS, PFPS and FP groups.

Based on these data, we may conclude that the enhanced sensitivity of the blink reflex circuitry in the context of the synkinetic disorders, is not mediated specifically by a dysfunctional trigeminal afference. The absence of any significant difference between the blink reflex recovery cycles obtained from stimulation of affected and unaffected side and the normality of the masseteric inhibitory reflex arch on both the affected and unaffected sides, supports this idea.

After botulin toxin treatment, a decrease in the amplitude and duration of the R2 response, along with a longer delay, revealed the expected effects of chemodenervation on the affected side in PFPS group, but not in the HFS group. Surprisingly, the latter showed a contralateral hyperexcitability, which suggests the occurrence of central adaptative phenomena. The absence of modifications on the brainstem interneuronal pool implicated in the recovery cycle of the blink reflex after treatment with botulinum toxin, suggests that the documented effect of botulinum beyond the peripheral action is likely to occur in the central nervous system at higher level than the brainstem. Indeed, the mechanisms responsible for the inhibition of the conditioned response are considered to be segmental and thus, although mostly referred to the interneuronal net of the lateral reticular formation, are potentially influenced to a higher conditioning.

In conclusion, it is conceivable that, clinical and electrophysiological similarity patterns between PFPS and HFS, could be both interpreted as evidence for a change in brainstem hyperexcitability, which in turn can be attributed to a consequent high-ranking influence. Therefore, we can conclude that the study of trigemino-facial

reflex and, specifically, of its recovery cycle, appears to provide an objective demonstration of function abnormalities of the inhibitory brainstem interneurons in the context of the synkinetic phenomenon. This can be viewed like a central compensatory attempt in response to a facial damage, probably more serious, but temporary, in the case of PFPS and subtler, but persistent, in the case of HFS. Therefore, our data move to not exclude any of the two possibilities but admits the presence, in either case, of an excessive activation of brainstem networks, in turn inducted and/or simply amplified by an additional effect of peripheral origin.

## Introduction

Facial nerve lesions are some of the most distressing benign lesions of the peripheral nervous system, since paralysis of hemifacial muscles leads to defective spontaneous blinking, with consequent eye dryness and corneal damage, defective lip and mouth movements with consequent lack of control of food and liquids ingestion, and finally deficit in the expression of emotions which heavily impacts on nonverbal communication leading to social withdrawal. In case of non complete recovery this condition can become chronic and produce in the patient physical and social maladaptive reactions.

Idiopathic facial palsy (FP), post-facial palsy syndrome (PFPS) and essential hemifacial spasm (HFS) are the most common disorders of the facial nerve.

In the case of PFPS, patients exhibit synkinesis, myokimias and involuntary simultaneous contractions of all hemifacial muscles that may resemble those of essential HFS, a chronic condition characterized by involuntary and synchronous contractions of hemifacial muscles.

The facial nerve is one of the most complex nerves in the human body.

The facial nucleus is divided into four cell groups: dorsomedial, ventromedial, intermediate, and lateral. Cells in the intermediate group innervate the frontalis, orbicularis oculi, corrugator supercilli, and zygomaticus; cells in a small medial group innervate the stapedius muscle (Carpenter MB and Sutin J. Human Neuroanatomy. Baltimore: Williams & Wilkins; 1983: 385-386).

At its exit from the brainstem it appears to be formed by two roots:

-the facial nerve itself, which carries out fibers to all facial muscles

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-the intermediate nerve of Wrisberg, which carries parasympathetic fibers to salivary glands (submandibular and pterygopalatine ganglion).

Near the cerebellopontine angle, the facial nerve enters the petrosal bone at the internal auditory meatus. The most relevant structure in the petrosal course of the facial nerve is the geniculate ganglion, located at the genu of the facial nerve, between the labyrinthine and the tympanic segments. Inside the petrosal course the great, the small and the external petrosal nerves emerge. Then, in the mastoid segment, the nerve branches out into the stapedius nerve and the chorda tympani branch.

After emerging from the skull through the stylomastoid foramen, the facial nerve releases collaterals to the posterior auricular muscle, the posterior belly of the digastric muscle and to the stylohyoid muscle. Finally, the nervous trunk enters the parotid gland where it further divides into a frontozygomatic (upper) and cervicofacial (lower) division at the *pes anserinus* ("goose's foot").

After that division the nerve divides into its five major terminal branches: the frontal, zygomatic, buccal, marginal mandibular and cervical. These nerve branches go on to innervate the muscles of facial expression.

## **Hemifacial spasm**

### ***Clinical features***

HFS is a movement disorder of the seventh cranial nerve characterised by involuntary clonic and/or tonic contractions of the muscles of facial expression, usually unilaterally, beginning in the periorbital musculature (90%), but later

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progressing to involve the perioral, platysma and other facial muscles (Chaudhry et al., 2015). This condition, although first described by Gowers in 1888, received its current terminology by Babinski in 1905 who, at the same time, also described another characteristic feature of this disease, thereafter known as “the other Babinski sign” that is, when the orbicular oculi contracts and the eye closes, the internal part of the frontalis contracts at the same time, and the eyebrow rises during eye occlusion (Babinski, 1905). This typical feature distinguishes HFS from blepharospasm in which this sign is absent (Chaudhry et al., 2015).

Although traditionally perceived as a benign illness, HFS can lead, in severe cases, to functional blindness and disturbed sleep because, unlike other movement disorders, it persists during sleep. Furthermore symptoms increase during times of stress, daily activities like reading, speaking and sometimes eating whereas they abate with relaxation techniques and occasionally touching some parts of the face (sensory tricks) (Brissaud, 1895). Other rare features may include paroxysmal click sounds caused by the contraction of the stapedius muscle (Schwarze et al., 1995), hearing loss, neuropathic pain with the characteristic of trigeminal neuralgia (a combination known as “tic douloureux”) and subtle facial palsy (Wang and Jankovic 1998; Yalto & Jankovic, 2011). The twitching may be induced by 2-3 minutes of hyperventilation, which presumably causes respiratory alkalosis and decreases the interstitial calcium levels, triggering ectopic excitation (Nielsen 1984). In some instances there may also be long-lasting, till almost continuous muscle contractions. In rare cases (<1%) it is bilateral but not symmetrical (Jannetta & Kassam, 1999) indeed, in these cases, the side affected secondarily becomes involved after a long interval – from several months to years - and the movements on each side are usually asynchronous, typically having less severe manifestations on the side being involved later (Jannetta & Kassam, 1999).

Natural history of HFS is chronic with gradual worsening. The first symptom, classically, is a unilateral spasm in the upper face and, more precisely, an intermittent

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twitching of the eyelid muscle that can lead to involuntary forced closure of the eye (Wang, 1998). This is typically associated with the elevation of the eyebrows (“other Babinski sign”). The spasm may then gradually spread to other facial muscles on the same side. Spontaneous resolutions occur rarely: it is estimated that less than 10% of cases heal completely (Ehni & Woltman, 1945).

The diagnosis of this disorder is clinical, but it requires investigations to rule out an underlying secondary cause, such as electromyography for denervation of facial nerve and nuclear magnetic resonance of the brain for demyelination or space occupying lesions near the brainstem. Patients candidate to surgical intervention need a magnetic resonance angiography with special high resolution sequences (Nielsen, 1985).

Differential diagnosis includes facial tic, postparalytic HFS, facial myokymias, partial continuous epilepsy, essential blepharospasm, late dyskinesia, oromandibular dystonia, masticatory spasm and psychogenic disorders.

The most popular choice of treatment of HFS is botulinum toxin: a large number of trials have validated the successful outcomes of this therapy with improvements in as many as 75-100% of individuals with HFE (Defazio et al., 2002). Other options include oral pharmacotherapy (anticonvulsants such as carbamazepine, clonazepam, gabapentin, phenytoin and other drugs like baclofen, anticholinergics, haloperidol) and, in selected cases, microsurgical decompression.

## ***Epidemiology***

Epidemiological studies, usually focused on the primary form, have estimated the main prevalence of the disorder to be from 9.8 to 11 per 100,000 people with a clear predominance in women and prevalence typically for an involvement of the left side

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of the face (Auger & Whisnant, 1990; Nilsen et al., 2004).

Age of onset has a range between the fifth to sixth decades of life, with only 1-6% of presentations before the third decade (Tan & Chan, 2006); however, a presentation before the age of 40 should prompt search for an underlying secondary cause (Tan & Chan, 2006). Rare familial cases are reported, suggesting that some patients may be genetically predisposed to developing HFS (Carter et al., 1990; Micheli et al., 1994).

### ***Pathophysiology and electrophysiology***

The pathophysiological mechanism of HFS is still unclear and the results of various studies are contradictory.

The most common identified cause of HFS reported in the literature is that of an ectatic or aberrant blood vessel (mostly the anterior inferior cerebellar artery) (Soriano-Baron et al., 2015), which compresses the facial nerve at the root entry/exit zone. This area is the nerve segment between the central and peripheral nervous system where there is a transition of the cells responsible for myelination and the nerve lacks an epineurium, being protected by an arachnoid membrane only (Girard et al., 1997). Consistent with this hypothesis, patients improve after surgical intervention at the posterior fossa (Jannetta et al., 1977; Soriano-Baron et al., 2015). Nevertheless, it is interesting that clinical improvements are observed after surgical intervention in patients without any evidence of the compressing vessel. Moreover neurovascular conflicts can be identified in up to 25% of healthy controls, suggesting that compression by a vessel alone is insufficient to produce HFS (Tan et al., 1999).

Several electrophysiological studies have been put forward to explain how the compression of the facial nerve leads to hemispasm: although there is a general belief that the hyperexcitability responsible for HFS involves the facial nerve or the

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nucleus (Møller & Jannetta, 1985; Møller & Jannetta, 1984), currently available data still lack direct evidence to precisely locate the site of lateral spread that causes a widespread facial contractions (Kameyama et al., 2016).

No conduction block is usually observed in the facial nerve and no denervation signs are found in the muscles of the hemiface affected; the electrophysiological hallmark of HFS is the spread of the blink reflex to muscles other than orbicularis oculi (Kimura et al., 1975). The proposed explanation for the phenomenon is the lateral spread: an abnormal response of the orbicularis oris muscle to supraorbital nerve stimuli; unwanted synkinetic muscle activity is the most common expression of this lateral spread of excitation.

It is likely that this response is generated through ephaptic activation of neighbouring demyelinated or poorly myelinated fibers of the facial nerve innervating the perioral muscles, by those that naturally carry the reflex volley to the orbicularis oculi (Nielsen, 1984). Ephaptic transmission occurs when transmission in one axon generates action potentials in a parallel axon. This has been attributed to the passage of inputs through a demyelination zone: the slowing of conduction velocity in demyelinated fibers gives time enough for a neighbouring hyperexcitable axon to reach the firing threshold. Similarly abnormal responses have been observed in patients with peripheral facial palsy.

Alternatively, some authors have raised the hypothesis that some synkinetic hemifacial responses triggered by trigeminal nerve stimulation, may be sort of ephaptic facio-facial responses (and not a trigemino-facial reflex) generated after antidromic activation of small motor axon terminals of the facial nerve near the trigeminal afferents and innervating the orbicularis oculi and/or other facial muscles (Montero et al., 1996). To support this hypothesis, the same work group applied step by step stimuli, as in the inching technique, from the supraorbital zone towards the malar zone, following the zygomatic branch of the facial nerve. The striking observation was that the "R1-like" response recorded in the orbicularis oris

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decreased when the stimulating electrode was moved away from the site of emergence of the supraorbital branch of the trigeminal nerve to a site closer to the facial nerve. With this maneuver the length of the facio-facial axon reflex arc was shortened whereas the length of the trigemino-facial reflex arc was increased. These findings are consistent with the hypothesis of ephaptic activation of facial nerve axon terminals (Montero et al., 2007). A study conducted more recently supports the view that the generation of an abnormal muscle response requires the participation of an antidromic motor impulse (Kameyama et al., 2016).

A second possibility proposed to explain the origin of lateral spread is co-activation of many abnormally hyperexcitable motoneurons following antidromic invasion or reflexive activation of the facial nucleus (Møller & Jannetta, 1985). Specifically, Misawa and coworkers, obtained an abnormal muscle response after subthreshold stimulation of the facial nerve: this result suggested the role of aberrant cutaneous afferent volleys via the trigeminal nerve, which enhanced the reflexive excitability of the facial motoneurons, rather than antidromic activation of the facial nerve (Misawa et al., 2006).

There is some evidence that facial motoneurons are hyperexcitable in HFS. A probably extrinsic irritation of the facial nerve at the posterior fossa generates an antidromic input to facial motoneurons that causes excitability changes and spontaneous or reflex firing of motoneurons after a “kindling” effect (Møller, 1987; Martinelli et al., 1992).

To support the hypothesis of a facial motor neuron hyperexcitability, Valls-Solè and Tolosa, in 1989, studied blink reflex responses in patients with HFS to single and paired (conditioning and test) stimuli at both sides of the face. In response to single stimulus, the late responses (R2) were of larger size on the side of the spasm compared with the uninvolved side and with controls, to either ipsilateral or contralateral stimulation. With paired stimuli, the recovery curve of the blink reflex resulted enhanced on the side of the spasm and also on the contralateral side: this

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result suggests an enhanced excitability of brainstem interneurons involved on the pathway of the blink reflex and/or an enhanced excitability of facial motoneurons (Valls-Sole & Tolosa, 1989).

More recently, Eekhof et al. (1996), investigated R1 and R2 blink reflex responses to single and paired stimuli in a group of patients with HFS. To single stimulus, amplitude and area of R1 and R2 responses showed no significant differences between patients and controls, providing evidence that the blink reflex circuits are functionally intact in these patients. To paired stimulation, R2 recovery curve was significantly enhanced on the affected side at intervals from 0.21 to 0.5 seconds and R2 index (calculated as the mean recovery of peak amplitude values at interstimulus intervals of 0.5, 0.3 and 0.21 s) was enhanced in 50% of patients on the affected side and in 20% of them on the unaffected side (Eekhof et al., 1996).

These results provided additional evidence that, in patients with HFS, there is a bilateral involvement of the brainstem (facial motor neurons and/or brainstem interneurons) in at least some patients as the cause of the involuntary contractions (Eekhof et al., 1996).

In 2005, Oge and coworkers, studied the blink reflex recovery curves bilaterally (that is, from stimulus on the symptomatic and asymptomatic sides) in patients affected by HFS and PFPS to directly compare the electrophysiological excitability characteristics of the facial nucleus and related structures (Oge et al., 2005).

Perfectly in line with above mentioned "central hypothesis", the authors revealed that both HFS and PFPS groups showed an early blink reflex recovery, more prominent in PFPS patients, when stimulations and recordings were made on the symptomatic side. Interestingly, the same tendency to early recovery was also present in both groups when supraorbital nerve on the symptomatic side was stimulated and the responses from the asymptomatic side were recorded.

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## Peripheral facial palsy and post-facial palsy syndrome

### *Introduction and clinical features*

Unilateral peripheral facial palsy is one of the most common neurologic disorders affecting the cranial nerves and it is the most common cause of facial paralysis worldwide: it is thought it accounts for approximately 60-75% of cases of acute unilateral facial paralysis (Katusic et al., 1986). Although it is a benign and self-limiting inflammatory condition, it has a considerable impact on the patient because it is a disfiguring disorder (Newadkar et al., 2016) .

Peripheral facial palsy, in the majority of cases, is of unknown origin and is described as idiopathic facial paralysis or Bell's palsy (BP). Clinically it is characterized by a rapid onset, a unilateral lower motor neuron type of facial deficit, partial or complete, of muscles of facial expression for both, voluntary and emotional responses. Differently, in supranuclear lesions, although voluntary facial movements are impaired, there is some sparing of the frontalis and orbicularis oculi muscles because of the bilateral cortical representation. Depending on the site of the lesion, other defects may be associated, such as hyperacusis or loss of taste. This is due to the fact that facial nerve also contains the nerve to the stapedius muscle and the chorda tympani nerve supplying taste sensitivity to the anterior two-thirds of the tongue, and also parasympathetic secretomotor fibers to lacrimal and salivary glands. In some patients pain may be present and, sometimes, this symptom tends to precede palsy. However, if pain is significant and accompanies BP, the patient is believed to have the diagnosis of Ramsey-Hunt syndrome, probably caused by varicella zoster infection.

With the loss of facial function, patients have an incomplete eye closure, with a consequent cornea damage, and epiphora. Patients fail to control their lip and

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mouth, thereby affecting their ability to speak, eat and drink. The loss of facial muscular tone can have a significant negative impact on a person's self-image and the way he/she interacts with others in society.

BP is a clinical diagnosis and is largely a diagnosis of exclusion therefore the clinician's history and physical examination is directed toward ruling out diseases within this differential diagnosis. Many conditions can produce isolated facial nerve palsy, more frequently structural lesions in the ear or parotid gland, Guillain-Barrè syndrome, Lyme disease, otitis media, Ramsay-Hunt syndrome, sarcoidosis, central nervous system lesions and some influenza vaccines. But, although these conditions can present as isolated facial nerve palsy, they usually have additional features that distinguish them from BP.

After the clinical diagnosis, patients with BP do not require imaging studies unless they fail to recover as expected (that is if there is no recovery after 3 months from the onset) or even worse, or show atypical new dysfunctions. For those patients who have complete facial paralysis, electrophysiological testing may be considered.

Treatment of BP should be conservative and guided by severity and probable prognosis in each particular case. Studies have shown the benefit of high dose corticosteroids in the early phase but not of antiviral treatment, although it has been used in recent years (Lockhart et al., 2009) and of physical therapy to improve recovery of facial deficits. The late phase of treatment is directed toward treating and preventing synkinesis, contractures, spasms or autonomic dysfunctions such as crocodile tears. Currently, based on a lack of strong evidence, surgical decompression of the nerve is not recommended as a standard approach.

Fortunately, prognosis of BP is of a near-to-complete recovery in approximately 80% of cases; 15% experiences some kind of permanent nerve damage and only 5% remains with severe sequelae (Tate & Tollefson, 2006). Usually some recovery occurs in the first 3 weeks.

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PFPS is a condition that follows a facial nerve lesion intense enough to cause a severe paralysis, that is if a significant percentage of axons undergo wallerian degeneration. PFPS is characterized by synkinesis between facial muscles, myokimia and unintended mass contractions of hemifacial muscles (Valls-Solé & Montero, 2003). The severity of PFPS may range from a subclinical stage, only evident in electrophysiological examinations, to a complex disturbance of voluntary and automatic activation of facial muscles.

Synkinesis is a typically documented recording, with surface electrodes. It consists of bursts of electromyography activity in the orbicularis oris during spontaneous blinking. The same is seen with voluntary contraction of an individual muscle of the face, that leads to an involuntary and simultaneous contraction of other hemifacial muscles, leading to unwanted facial movements. Needle electrode recording may also show interesting phenomena indicating the complexity of synkinesia: in a few patients also movements that were not intended to activate the facial muscles at all (i.e. neck movements or accommodation of some body parts) trigger some motor unit action potentials in the orbicularis oris, which can also be seen to fire in bursts time-locked to the inhalation phase of breathing (Valls-Solé, 2002; Pavesi et al., 1994).

In some cases, very uncomfortable mass contractions are triggered by facial movements, leading to what patients describe as a spasm. In these cases, patients can experience severe episodes of muscular pain, tension and spasms induced by common maneuvers such as eating, smiling or speaking (Valls-Sole et al., 1991). This condition can mimic those observed in essential HFS, but a careful clinical and neurophysiological examination allows separation of both entities. First of all, on the basis of the clinical history, more than 65% of patients with primary HFS initially have contractions of periocular muscles alone, whereas more than 72% of patients with the post-paralytic syndrome report involvement of the upper and lower facial muscles, including platysma, simultaneously (Colosimo et al., 2006). Furthermore, in

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essential HFS, the involuntary twitches are not necessarily triggered by voluntary or automatic muscle contractions, whereas in the post-paralytic syndrome mass contractions are always brought about by facial muscles activation. The two entities, finally, differ at the electrophysiological evaluation. Some differences can be observed already with the simple recording of the compound muscle action potential, which may be smaller in patients with history of facial palsy. At the EMG analysis the two clinical entities are completely different: the muscular discharges are of lower frequency and more irregular in post-facial palsy syndrome than in essential HFS (Valls-Solé & Montero, 2003). Finally, the R2 component of the blink reflex obtained in the orbicularis oculi on stimulation of the supraorbital nerve is larger on the affected side in case of HFS, instead is larger in the non affected side in case of peripheral facial palsy (Manca et al., 2001).

## ***Epidemiology***

BP is a relatively common condition with an incidence rate of approximately 25 per 100.000, or about 1/60 people in a lifetime (Katusic et al., 1986). It represents the most common disorder affecting the facial nerve (Wolfson, 2009) and is responsible for about 80% of all facial mononeuropathies (Goroll & Mulley, 2009). BP affects the right and left sides of the face fairly equally and bilateral paralysis is rare (Vakharia & Vakharia, 2016). BP may occur at any age, although it is more frequent in adults than children, with similar incidence rates reported among males and females (Bosco et al., 2011). Particularly susceptible groups include diabetics, pregnant women, elderly and patients with hypothyroidism. Furthermore, 4% to 8% of cases have an associated family history (Wolfson, 2009).

Most patients with BP experience spontaneous resolution: a clinically important improvement occurs within 3 weeks in 85% of people and within 3 to 5 months in the

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remaining 15%. Overall, 71% of people will fully recover facial muscle function (61% of people with complete palsy, 94% of people with partial palsy). The remaining 29% are left with mild to severe residual facial muscle weakness, 17% with contracture, and 16% with hemifacial spasm or synkinesis (Peitersen, 1982; Holland & Bernstein, 2011). Incomplete recovery of facial expression may have a long-term impact on quality of life. The prognosis for children with Bell's palsy is generally good, with a high rate (>90%) of spontaneous recovery, in part because of the high frequency of partial paralysis. However, children with complete palsies may suffer poor outcomes as frequently as adults.

## ***Pathophysiology and electrophysiology***

### Early phase of damage

Controversy surrounds the etiology of BP. The cause of paresis still remains unknown even if the etiology has been discussed by several authors and herpes viruses (particularly herpes simplex virus 1, HSV-1, and herpes zoster virus, HZV) seemed to be the most plausible infective agent (Holland & Bernstein, 2011). These reports are in agreement with finding of HSV-1 DNA in the endoneural fluid of the facial nerve by polymerase chain reaction during the acute phase of BP (Murakami et al., 1996). Herpes zoster-associated facial palsy more frequently presents as *zoster sine herpette* (without vesicles), although 6% of people will subsequently develop vesicles, depicting the Ramsay-Hunt syndrome (Furuta et al., 2001). Thus, treatment plans for the management of Bell's palsy should recognize the high incidence of HZV, which is associated with worse outcomes. More recently the inactivated intranasal influenza vaccine has also been linked with BP in a case-control study: the authors reported a strong temporal and specific association between vaccine and risk of developing BP

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(Mutsch et al., 2004). There was no association of the palsy with parenteral flu vaccines. Other known documented infectious cause BP but, although the viral etiology is accepted, given the circumstantial evidence, the exact etiology still is considered to be unclear.

Histopathological correlates of a BP are inflammatory infiltrates with swollen macrophages containing demyelination debris, suggesting an inflammatory process with axonal damage (Liston & Kleid, 1989). Inflammation of the facial nerve initially results in reversible neuroapraxia, but Wallerian degeneration may occur.

Although BP is clearly a mononeuropathy of the facial nerve, some patients report pain or numbness on the paretic side of their face or the perioral area, suggesting a trigeminal nerve involvement, but little is known about this condition. There are only a few reports in literature suggesting an electrophysiological trigeminal dysfunction (Hanner et al., 1986). However, depending on the neurophysiologic test employed to study specifically the trigeminal function (like masseter inhibitory reflex, MIR), no trigeminal nerve involvement – at least of the second division - can be detected in BP (Uluduz et al., 2010).

Neurophysiological examination is helpful at all stages in patients with BP, not only because it provides cues for the prognosis and suggests the nature of the underlying pathological process, but also in order to provide the basis for a pathophysiological explanation of related abnormalities of movement control.

At the early phase (few days) the typical neurophysiological pattern is the absence of responses in the affected orbicularis oculi after electrical stimulation of the supraorbital nerve of either side (Kimura et al., 1976): this indicates that, at this stage, the lesion is certainly limited to the facial nerve and that it does not involve the trigeminal nerve, as suggested by a normal response in the orbicularis oculi of the contralateral side. At the same time, the latency and size of the compound muscle action potential of the facial nerve are completely normal, unless the lesion lies in the

extracranial segment (such as in the case of parotid tumor) or in the terminal branch (Turkof et al., 2003; Guntinas-Lichius et al., 2004). An intrapetrosal facial nerve conduction block can be confirmed with magnetic stimulation over the posterior fossa at the parieto-occipital region and at the stylomastoid foramen, but this method is not easy to apply because it induces large artifacts (Rösler et al., 1991; Glocker et al., 1994).

At about 10 days, the functional loss of axons in the affected side is revealed as a reduction of the compound muscle action potential from nasalis muscle: this is the most informative data for determination of the prognosis (Glocker et al., 1994).

A needle electromyographic (EMG) recording performed 20 days after onset allows to approximate a measure of denervation, confirmed by finding of fibrillation potentials and positive sharp waves as a consequence of the degree of axonal damage.

Subsequent evaluations provide evidences for abnormal regeneration and changes in motoneuronal excitability, that configure the PFPS (Montserrat & Benito, 1988; Valls-Sole et al., 1992).

At all these stages of the disease, it is also convenient to perform the neurophysiological examination in both paralyzed and non-paralyzed sides, not only for comparison between the damaged and the non-damaged nerve, but also to obtain information on adaptive changes occurring in the contralateral side of paralysis. Indeed, although lack of movement in the paralysed side of the face is the prominent sign of facial palsy, a few patients complain of excessive spontaneous blinking in the contralateral eye (Pastor et al., 1998). Starting from this observation, Pastor and coworkers measured the spontaneous blinking rate in 20 patients with peripheral facial palsy, between 24 and 72 hours after onset, finding a significant enhancement. This enhanced blinking rate may be an attempt of compensatory mechanism to protect the cornea of the paralysed side from dryness and it is

obviously evident on the nonparalyzed side only. Also, the EMG activity and eyelid movements, recorded simply with surface electrode during spontaneous blinking, can give interesting information. Normally this recording consists of a slow negative wave accompanied by a small burst of EMG activity on the rising phase: in patients with peripheral facial palsy, the recording of the paralysed side showed a small slow wave as a result of weakness of levator palpebrae; in the non-paralysed side the slow wave was characterized by an EMG burst of larger amplitude than normal (Valls-Solé & Montero, 2003).

Excessive blinking may also be accompanied by hemifacial hyperactivity. This observation has led to a few reports on the occurrence of contralateral blepharospasm induced by BP (Chuke et al., 1996; Baker et al., 1997). In particular, Chuke and coworkers in 1996 described a case of blepharospasm-like symptoms appearing contralaterally to an eyelid weakened by facial nerve palsy, that improved with implantation of a golden weight to assist closure of the paretic eyelid. The authors proposed that hyperexcitable blink reflex could be a maladaptive consequence of adaptive system, i.e. a physiological adaptive control mechanisms of the efficacy of the blink reflex, as suggested by the fact that aiding closure of the weak eyelid led to a reduction of the maladaptive excitability of the blink circuit. Changes in central excitability was attributed to excessive afferent input from the cornea of the paretic side and, since blinking is a conjugate behavior, the bilateral nature of the blink system-adaptive control was not unexpected (Chuke et al., 1996). The following year, Baker described 4 patients with BP and blepharospasm and, on the basis of eyelid movements kinematic study, proposed a correlation model for BP-induced blepharospasm in which a blink adaptive system may produce the maladaptive consequence of eyelid spasms (Baker et al., 1997).

To identify electrophysiological correlates for adaptive changes suggested by previous kinematic studies, Syed and coworkers, evaluated the excitability of the brainstem interneuronal circuitry in people with persistent or resolved unilateral

facial weakness (Syed et al., 1999). The finding of an enhanced recovery curve of the blink reflex in patients with residual weakness (but not in patients who recovered) suggested a role for abnormal afferent input in maintaining sensitization (Syed et al., 1999).

Further evidence in the same direction was reported simply analysing the size differences between the R2 component of the nonparalyzed side from direct (R2) and indirect (R2c) stimulation: contrary to what usually observed in healthy subjects, the mean R2c/R2 ratio was significantly larger in patients with recent peripheral facial palsy (Manca et al., 2001).

The causal relationship between sensitization of reflex pathways and abnormal motor control is supported by experimental animals studies in which rats who sustained a 6-hydroxydopamine lesion of the striatum, developed blepharospasm after peripheral facial nerve lesion. The authors postulated that, in a predisposing condition such as a lesion of the basal ganglia, an excessive trigeminal input from corneal irritation subsequent to facial weakness, would lay the grounds for the abnormal motor behavior (Schicatano et al., 1997). These data identified a crucial role of subcortical structures in the integration of sensory input, like as basal ganglia and cerebellum (Schicatano et al., 2002).

### Facial nerve regeneration phase and synkinesis

After a complete muscle denervation subsequent to facial nerve paralysis, EMG signs of regeneration can be seen at about 3 months after onset of symptoms as small amplitude polyphasic potentials (Cossu et al., 1999). These action potentials are visible during automatic or voluntary contraction, but they are elicited with difficulty by electrical stimulation of the damaged in-growing nerve, notoriously markedly hypoexcitable because poorly myelinated (Moldovan et al., 2006).

Indeed, axonal growth begins with the appearance of axon cones that emerge from

proximal stumps, elongating within the Büngner bands (a cluster of Schwann cells enclosed around a Schwann cell basement membrane). As the growing axonal cone advances, proximal segments are progressively myelinated.

However, during this regeneration processes of a motor nerve, there is a risk of two main types of error: the axon regenerates in a funiculus going to another muscle or the axon regenerates, simultaneously, into one or more funiculi going to different muscles that may have antagonistic functions or to parasympathetic axons of the intermediary nerve of Wrisberg. Lacrimation and hemifacial sweating - the so-called Fray syndrome - subsequent to muscle activation is one of the possible consequences of such reinnervation errors (Guntinas-Lichius et al., 2006).

In reality, abnormal axonal growth cannot be the sole cause of reinnervation abnormalities: a series of morphological and functional changes in the cell body, known as chromatolysis, was observed for the first time since in 1892 by Nissl and successively confirmed by other studies (Tetzlaff et al., 1996; Graeber et al., 1993). Indeed, after axonal lesion, facial motor neurons change their phenotype from a transmitter to a regenerative mode, undergoing ultrastructural changes that make them hyperexcitable (Tetzlaff et al., 1996). Anatomic evidence of the disruption of central control mechanisms was confirmed when it was discovered that early and brisk proliferation of microglia occurs when the facial nerve is cut and that these microgliaocytes remove most of the intact synapses from the neuronal surface membranes within 4 days (Blinzinger & Kreutzberg, 1968). It was later found that this synapse stripping might have greater effects on inhibitory synapses (Lux & Schubert, 1975). More recently, synaptic stripping in the human facial nucleus has been observed 3 months after a severe facial nerve injury (Graeber et al., 1993). It has also been shown that rapid genetic changes in messenger RNA with unique patterns in the facial motor neurons occur within 5 hours from axotomy (Moran & Neely, 1996).

In this context, the trigeminal stimulation would be easily induced by action potentials at level of particularly excitable facial motor neurons (Valls-Solé, 2013).

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To better understand alterations at the level of motoneuronal cells of a damaged facial nerve, Cossu and coworkers, recorded simultaneously, with needle electrode, the spontaneous, voluntary and reflex activity of orbicularis oculi and orbicularis oris muscles in patients with recent history of facial palsy (between 75 and 90 days after damage), that is, in the period before the ensuing of peripheral compensatory mechanisms, such as lateral spread. All patients showed: involuntary firing of motor unit, synkinetic activation of orbicularis oris muscle induced by spontaneous, automatic or voluntary movements of facial muscles, reflex responses in orbicularis oris and, simultaneously, in orbicularis oculi, induced by electrical stimulation of ipsilateral and contralateral trigeminal or facial nerve and also peripheral nerve (Cossu et al., 1999) .

These results revealed that, at least some of the functional abnormalities of regenerating axons, are due to a change in the excitability state of facial motoneurons and that enhanced motoneuronal activity is present precociously (from the very first connection between growing axons and denervated muscles) to offer a pathophysiological substrate for the development of PFPS (Cossu et al., 1999).

A study of blink reflex and lateral spreading in patients with synkinesia after BP has supported this possibility as an increased R1 amplitude ratio of orbicularis oris/orbicularis oculi was recorded (Eekhof et al., 2000). In physiological conditions, the amplitude of R1 response recorded at orbicularis oris muscle is smaller than R1 at orbicularis oculi. This was explained by the findings that direct cortical projections upon the lower face muscles are less so upon the upper facial muscles. In contrast to this, patients with history of BP showed an increased amplitude of R1 response in the orbicularis oris. These results were explained calling into question a loss of inhibitory cortical control (Eekhof et al., 2000) and were in line with previous results of enhanced F-waves and larger R2 responses in PFPS cases, on the symptomatic side (Valls-Sole et al., 1992).

In 2005, Oge and coworkers, studied specifically the excitability of facial nucleus and

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related brainstem reflexes in PFPS and HFS to evaluate excitability changes in the facial pathways. To this purpose they investigated F-waves, blink reflex recovery curve and magnetically elicited silent period. F-waves of facial nerve were enhanced in PFPS and HFS on the affected side. At the study of BRRRC the authors found, in both group of patients, early recruitment of R2, more prominent in PFPS patients and if recorded and/or stimulated at the symptomatic side. An early recruitment was also found, although to a lesser extent, on the asymptomatic side. Finally, duration of silent period was nearly equal on both sides. In conclusion these results revealed an exceeding hyperexcitability of blink reflex bilaterally, more prominent to stimuli and/or to recording on the affected side and it was ascribable to an increased excitability of brainstem structures, probably at facial nuclei level. Absence of differences of magnetically elicited silent periods from affected/non-affected sides implies, according to the interpretation of the authors, that cortical excitability changes do not take place in these disorders, confirming that they are confined to the brainstem level (Oge et al., 2005).

Regenerating activity, described in the abovementioned studies, may take place not only in the affected facial nerve but also in the contralateral non-affected side: recordings of EMG activity made with concentric needle electrode at the orbicularis oris muscle of the nonparalyzed side, showed a significant proportion of enhanced jitter and blocking (Valls-Solé & Montero, 2003). This data demonstrate that a regenerative process in the non injured facial nerve exists, inducted, most likely, by contralateral chemical changes and motoneuronal reorganization that has been shown to occur in distant muscles after local denervation induced by botulinum toxin injection (Olney et al., 1988; Garner et al., 1993).

## Botulinum toxin

Botulinum toxin is produced by anaerobic fermentation of the bacterium *Clostridium Botulinum*, during growth and reproduction. More precisely, *Clostridium* produces eight immunologically distinct serotypes (A-H) but the most widely used for therapeutic application is the neurotoxin type A preparation, although studies indicate that botulinum neurotoxin serotypes B and F may be useful for the treatment of selected cases (Chen et al., 1998; Brin et al., 1999).

In nature, BoNT-A is synthesized as macromolecular protein complexes that are referred to as progenitor toxins and consist of nontoxic accessory proteins (NAPs) bound to the 150-kD active neurotoxin. Currently, on the market, there are three botulinum neurotoxin type A products: onabotulinum toxin A, abobotulinum toxin A and incobotulinum toxin A.

The different products of BoNT-A vary in molecular weight (from 300 to 900 kD) depending on the composition of NAPs and the manufacturing process, whereas the toxin moiety is the same in all pharmaceutical preparations.

To exert its pharmacological effect, the 150-kD neurotoxin must dissociate from NAPs, with the exception of incobotulinum which contains only the 150-kD neurotoxin.

Therapeutical power, expressed in biological units, is related to the quantity of toxin required to achieve a median lethal dose (LD50) unit and is conditioned by many factors (mouse strain, sex, age, volume and route of injection etc.). Moreover, the LD50 units of BoNT products are not standardized across manufacturers (Frevert, 2015).

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## ***Mechanisms of action and clinical uses***

The efficacy of botulinum neurotoxin therapy is primarily due to the injection method delivery and the high affinity of cholinergic nerves for neurotoxin uptake.

Botulinum toxin acts at the neuromuscular junction to inhibit the presynaptic release of the acetylcholine neurotransmitter by the following steps:

- fast, specific and irreversible binding to acetylcholine receptors on presynaptic nerve surface
- uptake of toxin into cell vesicle
- translocation of toxin into cytosol across vesicle membrane
- toxin-activated proteolysis which, in turn, blocks the release of acetylcholine (Orsini et al., 2015).

After local injection, the toxin spreads into contiguous areas, increasing the risk of adverse effects. Although, distant spread is uncommon.

In clinical use, BoNT-A is precisely injected into specific muscles to cause a temporary chemodenervation of the skeletal muscle in those entities characterized by excessive muscle contraction.

More precisely, an injection of BoNT-A into a muscle, will reduce the alpha motoneuron activity on the extrafusal muscle fibers and thus muscle contraction. Simultaneously, muscle spindles, are also inhibited by BoNT-A through the inhibition of the gamma motor neuron control of the spindle intrafusal fibers and subsequent reduction of the Ia fiber afferent signal. This attenuated Ia signal, then, reduces the feedback to the alpha motor neurons and, subsequently, also muscle activity of non-injected muscles (the injection causes a profound reduction of spasticity in areas larger than expected and not related to the zone of diffusion) (Filippi et al., 1993).

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The overall impact of a long-term reduction of alpha, gamma and Ia neurons, may lead to secondary effects on the central nervous system. The work group of Delgado-Garcia, studying effects of BoNT in extrinsic ocular muscles of the cat, found an elimination of inhibitory postsynaptic potentials and a reduction of gephyrin-immunoreactive clusters (glycine-receptor-clustering protein): these findings indicate that the long-term paralysis of a muscle may induce the reorganization of central programs and the appearance of compensatory movements (Moreno-López et al., 1997).

Finally, BoNT therapy has been reported to alleviate pain in various conditions, with or without concomitant excess muscle contractions.

Clinical observations of antalgic effect of botulinum were made, early, in patients infiltrated for cervical dystonia in which the pain relief exceeded the motor benefit (Tsui et al., 1986; Brin et al., 1987). Successively, the antinociceptive effect was observed on headache (Binder et al., 2000), neck and back pain (Foster et al., 2001) and myofascial pain (Porta, 2000).

This antalgic effect of BoNT was explained by the in vitro and in vivo demonstration of reduction of neuropeptide release (Mustafa et al., 2013).

Local injections of BoNT have been widely used as a symptomatic treatment for movement disorders since the eighties, when it was used to correct strabismus (Scott, 1981) and dystonic blepharospasm (Fahn et al., 1985). Successively BoNT owes its effectiveness to the treatment of many motor disturbances, including upper limb dystonia and cervical dystonia (Cohen et al., 1989; Jankovic & Schwartz, 1990; Costa et al., 2005).

Since 1980, animal studies have provided evidence that the toxin inhibits, in cortical slices, the release of acetylcholine in cerebral cortex synaptosomes whereas, morphological studies, have shown that BoNT, after intramuscular injection, accumulates into spinal motoneurons (Habermann, 1974; Wiegand et al., 1976;

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Simpson, 1981). In 1993, results from animal studies, induce to supposed that the toxin also blocks the intrafusal motor end plate and provided evidence that the toxin reduces the spindle afferent discharge without altering muscular tension (Filippi et al., 1993). Patients with writer's cramp, segmental upper limb dystonia and, surprisingly, also patients with cervical dystonia not involving the limbs, have an abnormally decreased forearm reciprocal inhibition (Nakashima et al., 1989; Panizza et al., 1989; Deuschl et al., 1992). Based on these data, in 1995, Priori and Colleagues, studied reciprocal inhibition between forearm flexors and extensor muscles, before and after BoNT-A injection, in patients with upper limb dystonia, to see whether botulinum toxin treatments alters segmental motor system function (Priori et al., 1995). After treatment, the second abnormal phase of reciprocal inhibition increased and the authors postulated an indirect effect of BoNT on spinal cord circuitry, probably through changes affecting the tonic sensory inflow coming from the injected muscles (Priori et al., 1995). Similar results were found in patients with essential tremor treated with infiltration of the neurotoxin (Modugno et al., 1998).

More recently, Pauri and coworkers, investigated changes in motor evoked potentials (MEPs) in patients with leg spasticity treated with BoNT-A and found an increased MEP latency and a prolonged central conduction time. The authors attributed these findings to central changes in spinal motoneuron responsiveness to cortical descending inputs (Pauri et al., 2000).

In the same line are results of a study in which, using transcranial magnetic stimulation (TMS), the topography of the primary motor cortex projections to the upper limb muscles in patients with writer's cramp, was mapped (Byrnes et al., 1998). After treatment with BoNT the work revealed a reorganization of the cortical map, that was distorted in shape and in extension before treatment. These changes were reversible when the clinical benefit wore off. This study suggested that changes in primary motor cortex could be secondary to abnormal afferent inputs that the injected BoNT may have transiently modulated (Byrnes et al., 1998).

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The paired-pulse TMS technique, that employs a conditioning-test paradigm, demonstrated, in patients with upper limb dystonia, a normalization of intracortical inhibition, that was increased before treatment. Similarly to the abovementioned study about cortical mapping of muscles' cortical representation, three months after injection, values of cortical excitability dropped again to pre-treatment levels (Gilio et al., 2000).

Also, positron emission tomography (PET) was used to investigate possible changes in cortical activation induced by BoNT-A. PET activation studies showed, in patients with dystonia before treatment, an overactivity of the striatum and of non-primary motor areas and an hypoactivity of the primary motor cortex in patients with dystonia. After BONT-A injection, clinical benefit was accompanied by an increased activation of parietal cortex and supplementary motor areas, suggesting a cortical reorganization secondary to the temporary deafferentation (Ceballos-Baumann et al., 1997).

In 2011, the group of work of Kojovic, used TMS to study the effects of botulinum toxin on associative plasticity in patients with primary dystonia (Kojovic, 2011). Indeed, abnormal sensorimotor cortical plasticity is one of the mechanisms that contribute to the pathophysiology of dystonia (Quartarone et al., 2003; Edwards et al., 2006). The authors, hypothesizing that BoNT-A injection might normalize enhanced plasticity, studied the response to the paired associative stimulation (PAS) protocol in patients with cervical dystonia, before and after treatment. Before BoNT injection, PAS significantly facilitated MEP amplitude and, after one month, this facilitation was suppressed, whereas after 3 months it partially recovered (Kojovic, 2011). To explain these results, the authors postulated that the effect of botulinum might be secondary to changes in motor maps and, in turn, consequent to changes in afferent inputs from treated muscles. Such findings should not be surprising, since a considerable body of evidence demonstrates the importance of afferent input in modulating cortical organization and its excitability (Ridding & Rothwell, 1995; Ziemann, Corwell, et al., 1998; Ziemann, Hallett, et al., 1998).

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The altered afferent input, therefore, may be indirectly responsible for functional changes in central motor mechanisms at both spinal and supraspinal levels. Again, animal studies evidenced that intramuscular injection of BoNT-A comes from a retrograde-tracing approximately 48 hours after injecting radiolabelled BoNT into the cat gastrocnemius muscle with a distal-proximal gradient of radioactivity up to sciatic nerve, ipsilateral ventral roots and, finally, spinal cord. In a smaller amount radioactivity may spread to contralateral spinal roots and spinal cord (Wiegand et al., 1977).

Studies conducted to evaluate changes in brainstem excitability in patients with cranial dystonia, like blepharospasm, after BoNT injection, didn't show any reduction in the recovery cycle of the blink reflex: in a study by Valls-Solè and colleagues, it appears equally abnormal before and after local injection of botulinum toxin suggesting that there is little influence of muscular afferents upon the excitability of brainstem interneurons (Valls-Sole et al., 1991).

However, after botulinum treatment, patients with blepharospasm, besides a reduction of the spasm intensity, also notice a reduction of the spasm frequency and this effect is difficult to explain on the basis of muscle paresis alone.

The dissimilar effects of botulinum injection on the blink reflex, on reciprocal inhibition and cortical reorganization, might depend on the different segmental motor organization of the brainstem (for the absence of muscle spindles in facial muscles) and spinal cord.

In complete contrast with these data are the results of a study which investigated the effects of BoNT on long-term potentiation-like plasticity of the trigeminal blink reflex circuit in patients with blepharospasm. Indeed, repetitive bursts of high-frequency stimulation to the supraorbital nerve, applied to normal subjects, can induce a long-lasting facilitation of the blink reflex (Mao & Evinger, 2001): such facilitation is more pronounced if the electrical stimulation is applied on patients with dystonic disorder,

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whereas it is “normalized” by botulinum toxin treatment that reduces blinking and hence restores plasticity of the trigeminal complex toward normal values (Quartarone et al., 2006).



## Objectives of the study

HFS and PFPS are the most common disorders of the facial nerve. HFS is characterized by involuntary intermittent contractions and synkinesis of muscles supplied by the facial nerve. PFPS is a clinical condition that follows a peripheral facial palsy, often of unknown origin, and presents with synkinesis as well. Therefore, from a certain point of view, HFS and PFPS, although being two different nosological entities, can be both classified as a “synkinetic disorders”. The synkinetic phenomenon has its electrophysiological hallmark in the lateral spread of the blink reflex, that is found in both conditions (Eekhof et al., 2000; Oge et al., 2005). The presence of synkinesis and lateral spread is one of the causes of confusion in distinguishing HFS and PFPS from each other.

The pathogenesis of HFS and PFPS, despite their clinical similarity, are thought to be different.

In literature, the most common cause identified of HFS is a neuro-vascular conflict between a cerebellar artery and facial nerve at the root entry/exit zone (Soriano-Baron et al., 2015). However, it can be identified only in a part of patients and also in healthy controls, therefore, compression by vessel alone is insufficient to produce HFS (Tan et al., 1999). The typical electrophysiological observation giving support to the suspicion of chronic nerve lesion, likely a demyelination, in the proximal part of the facial nerve, is ephapsis (transmission in one axon generates action potential in a parallel axon) (Nielsen, 1984a). However, the presence of ephaptic responses, does not completely settle the question on whether the responses are generated at the peripheral side of compression or at central level. A discovery of an increased size as well as of a faster recovery curve of the R2 component of the blink reflex on the

affected side has been taken as a sign of an increased excitability, not only of facial motoneurons but also of brainstem interneurons (Valls-Sole & Tolosa, 1989; Eekhof et al., 2000). A third hypothesis implies that the extrinsic irritation of the facial nerve at the posterior fossa could generate an antidromic bombardment of inputs to facial motoneurons and, in turn, induce excitability changes at central level (Møller, 1987; Martinelli et al., 1992).

In the case of PFPS, abnormal function of facial muscles commonly occurs as a result of reinnervation errors after the palsy (Moran & Neely, 1996; Baker et al., 1997). More precisely, the most widely accepted pathogenetic mechanisms for the synkinetic phenomenon observed in PFPS, are aberrant axonal regeneration and ephaptic transmission (Kimura et al., 1975; Montserrat & Benito, 1988). Furthermore, similarly to HFS, also in case of peripheral damage of the facial nerve, some findings reveal that, at least part of the functional abnormalities of regenerating axons, are due to a change in the excitability state of facial motoneurons (Cossu et al., 1999; Eekhof et al., 2000; Manca et al., 2001). Excessive blinking and hemifacial hyperactivity revealed on the unaffected side supported the central hypothesis of adaptive changes (Pastor et al., 1998; Valls-Solé & Montero, 2003). Some authors suggested a role for abnormal afferent input from trigeminal nerve in maintaining this central sensitization (Syed et al., 1999).

HFS and PFPS seem to be, in one manner, two different expressions of a common neurophysiological substrate, sharing some clinical aspects but being different in others. Nevertheless, currently available data still lack direct evidence to precisely locate the site of the lateral spread that causes a widespread facial contraction.

Given these basis, aims of our study were:

- to evaluate excitability changes in the facial and trigeminal pathways of the affected side in HFS and PFPS to evaluate the neurophysiologic similarities and differences between these synkinetic, in order to obtain information on the respective

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underlying pathophysiological mechanisms. The results obtained will be compared to a control group of matched healthy subjects and with a small group of patients with acute facial palsy, that we have considered like a model of “pre-regenerative” state, in comparison to HFS and PFPS, both ideally expressions of “regenerative status”;

- to look for adaptive mechanisms of brainstem excitability on the unaffected side, to verify whether central mechanisms, like compensatory responses to a supposed peripheral damage may occur;

- to evaluate possible modifications in excitability of brainstem circuits after the treatment with botulinum toxin, a treatment of choice for both pathological conditions which mechanisms of action seem to go over to the direct peripheral action (Byrnes et al., 1998; Pauri et al., 2000; Quartarone et al., 2006).

## Methods

Electrophysiological tests were performed in 8 healthy subjects, 4 patients with idiopathic peripheral facial palsy (FP) in subacute phase, 7 patients with PFPS and 10 patients with HFS who referred consecutively to the neurology service of S.Maria della Misericordia Hospital (Udine). Patients affected by PFPS and HFS were enrolled from the botulinum toxin clinic between March and December 2016. None of the patients had a personal or familial history of any movement disorder nor took drugs active on the central nervous system. Local ethics committee approved the study and informed consent was obtained from each subject.

All patients were studied twice: the first time before receiving infiltrative treatment with botulinum toxin (baseline) and, the second time, about 45 days after injection, that is at the moment of maximum benefit of therapy (follow-up) (Fig.1).

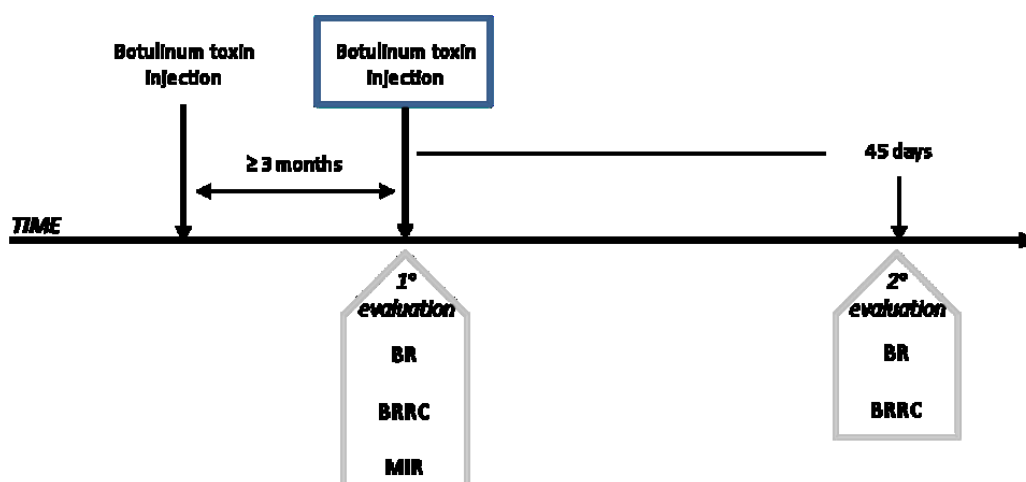


Fig.1 Experimental design of the study

*BR, Blink Reflex; BRRC, Blink Reflex Recovery Cycle; MIR, Masseter Inhibitory Reflex.*

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## ***Electrophysiological recordings***

Study of Blink Reflex (BR), Blink Reflex Recovery Cycle (BRRC) and Masseter Inhibitory Reflex (MIR) were performed bilaterally using a Dantec Keypoint® electromyography. The study was carried out while the subject was lying supine in a quiet room. The ground electrode was placed on the forehead and stimulation and recording were made using surface electrodes. Except for the study of MIR, all stimuli were delivered when the recorded muscles were at rest. In case of appearance of spasms during the electrical stimulation, complete relaxation, which could be obtained after some minutes of rest, was waited for.

### Blink reflex and its recovery cycle

Electromyographic (EMG) responses of orbicularis oculi, were recorded bilaterally using active surface cup electrodes placed on midpoint of the lower portion of the muscle and the reference electrode taped on the external *canti oculi*. The EMG signals were amplified (x5000), bandwidth filtered (2 Hz-10 KHz), and sampled (10 KHz) from 500 ms to 2000 ms after stimulus delivery.

Electrical stimulation to obtain the BR and the BRRC was applied over the supraorbital nerve in the supraorbital notch with a bipolar stimulating electrodes connected to a constant current generator. All stimuli were of 0.2 msec duration and stimulus intensity was set at 3 times the R2 threshold (lowest intensity with an R2 response with an amplitude of  $\geq 50 \mu\text{V}$  in at least 5 out of 10 trials). Subjects were studied at rest, with eyes gently closed.

### Experimental procedures

- **Blink reflex** The blink reflex to single stimulus was assessed bilaterally. Latency, area and duration of the R1 R2 ipsilateral (R2i) and R2 contralateral (R2c) responses to the stimulation were determined from 4 trials and then

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averaged. Latency was measured from the stimulus artifact to the deflection of the EMG response from the baseline, duration and area were measured from the first deflection from baseline to the final return.

- **Blink reflex recovery cycle**

The blink reflex recovery curve in response to paired stimulation of the supraorbital nerve was assessed bilaterally at interstimulus intervals of 200, 500 e 1,000 msec (4 trials each, pseudorandomized). Pairs of stimuli were separated by varying time intervals of 15–30 seconds to minimize habituation (Esteban, 1999). Trials with excessive EMG artifact were rejected online. Data were analyzed offline. The raw blink recordings were DC-corrected, and averaged. The onset latency and duration of R2 response were determined by manual cursor marking of the beginning and ending of responses. The ratio of the conditioned R2 area to the unconditioned response was calculated and plotted against the interstimulus intervals to create a blink reflex recovery curve. The upper limit of normality for R2 area recovery index was defined as the mean  $\pm 2.5$  SD of data from the age- and sex matched healthy controls.

Involuntary eyelid movements could be identified by amplified sound signal. Response with artifacts due to involuntary movements were rejected.

Masseter inhibitory reflex

Patients affected by PFPS and HFS only performed the MIR.

EMG activity was recorded simultaneously from the superficial portion of the right and the left masseter muscles by bipolar surface electrodes and computer sweeps were triggered by a synchronous output pulse of 1 ms provided by the square pulse stimulator.

The mental nerve was stimulated with single electric square pulses of 0.2 ms from a

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constant current unit and delivered by a bipolar stimulating electrode, to the skin of the lower lip directly over the corresponding mental foramina. The stimulus intensity was gradually increased from a subthreshold level up to about two times the threshold of the reflex, at a level that most subjects could bear it without considerable discomfort. The threshold was identified as the stimulus intensity for which a clear EMG suppression could be identified.

A total of 20 electrical stimulations (10 for each side) were delivered at about 60 s intervals (to prevent fatigue and habituation) during maximum clenching with the aid of auditory feedback.

Subjects were instructed to continue clenching at least 1–2 s after the stimulus delivery, in order to avoid interruption of the recording due to voluntary relaxation.

Early suppression period (SP1) and late suppression period (SP2) latencies, if present, were measured at the insertion of the rectified and superimposed signal with a line indicating 80% of the background level.

### ***Statistical analysis***

Statistical analysis of the data was performed with SPSS software for Windows (version 18.0, Chicago, Illinois, USA).

Data of the BRRC were preliminarily compared, within each group, from unilateral and contralateral stimulations and from unilateral and contralateral sides of recording, in order to rule out any interaction between sides. Consequently, for the statistical analysis, they were considered as one side of recording (direct and indirect stimulation) and calculated together for both *within-groups* and *between-groups* comparison, at baseline and when comparing baseline and follow-up performances.

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For comparison of frequencies, the chi-squared test was employed; parametric variables were analysed through Student's t-test. Comparison between non-parametric independent variables was performed with Mann-Whitney U test, for two group comparisons; in case of more than two groups, Kruskal-Wallis test was applied. Data from repeated measures were calculated with Wilcoxon signed ranks test. Finally, two-ways ANOVA was performed to determine differences in variance between groups; in case of significant differences, Tukey post-hoc test was used.



## Results

### ***Demographic and clinical data***

The FP group consisted of 3 males and 1 female with a mean age of  $54.75 \pm 17$  years (range: 39 - 76 years). Palsy was present on the left side in 3 cases and on the right side in 1 case. Onset of paresis was ascribable at 3 months before examination.

The PFPS group was made up of 6 females and 1 male with a mean age of  $42.71 \pm 14$  years (range: 33 - 71 years). The time interval between the onset of facial paralysis and the electrophysiological examination was on average of  $8.0 \pm 4.58$  years (range: 3 - 15 years) and duration of therapy was  $5.29 \pm 3.77$  years (range: 1 - 12 years). The causes of PFPS were Bell's palsy in 6 cases and injury of the petrous bone in one case. Six cases had left-sided PFPS. Neurological examination, except for the signs ascribable to PFPS, was normal in all patients.

The HFS group consisted of 7 females and 3 males aged between 54 and 77 years (mean age  $67.3 \pm 8.37$  years). Duration of symptoms before the electrophysiological examination ranged from 1 to 27 years (mean  $8.0 \pm 7.2$  years) and duration of therapy was  $6.8 \pm 6.73$  years (range 1-24 years). Five cases had left-sided and 5 right-sided spasms. There were no cases with bilateral spasms or with any history of facial paralysis. Neurological examination was unremarkable except for the findings related to HFS. Cranial magnetic resonance imaging (MRI) was performed in all patients. Signs of neurovascular conflict were detected at the posterior fossa in two patients.

The control group was composed of 8 healthy subjects (4 males and 4 females), with age ranging from 40 to 71 years.

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An overview of the demographic and clinical features of the patients is summarized in Table 1.

**Table 1** Demographic and clinical features of the population studied.

Facial nerve disorder	N° of subjects	Mean age (years)	Gender (M : F)	Mean disease duration (years)	Therapy duration (years)	Affected side (L :R)
Facial nerve palsy	4	54.75 ± 17.46	3 : 1	0.26 ± 0.16	-	3 : 1
Post-Facial Palsy Syndrome	7	42.71 ± 14.0	1 : 6	8.0 ± 4.58	5.29 ± 3.77	6 : 1
Hemifacial Spasm	10	67.3 ± 8.37	3 : 7	8.0 ± 7.2	6.8 ± 6.73	5 : 5
<i>Total</i>	21		7 : 14			14 : 7
<i>p-value</i>		<i>n.s.</i> <sup>@</sup>	<i>n.s.</i> <sup>‡</sup>	0.009 <sup>†</sup>	<i>n.s.</i> <sup>#</sup>	<i>n.s.</i> <sup>‡</sup>

M, males; F, Females; L, Left; R, Right; <sup>†</sup> *Kruskal Wallis test*; <sup>‡</sup> *Chi-squared test*; <sup>#</sup> *Mann-Whitney test*; <sup>@</sup> *Student's t-test*.

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## Blink Reflex

Data obtained from the analysis of the blink reflex responses elicited by stimulation of affected and unaffected sides, are summarized in Table 2.

**Table 2** Data of blink reflexes in the unaffected and affected sides.

Blink Reflex parameters	<i>Facial nerve palsy</i>		<i>Post-Facial Palsy Syndrome</i>		<i>Hemifacial Spasm</i>		<i>Control</i>
	<i>US</i>	<i>AS</i>	<i>US</i>	<i>AS</i>	<i>US</i>	<i>AS</i>	
<i>Baseline</i>							
<i>R1 latency</i>	10 ± 3.16	12.67 ± 3.21	10.67 ± 0.96	11.01 ± 1.21	10.4 ± 1.19	10.81 ± 0.81	12.11 ± 2.04
<i>R2i latency</i>	30 ± 2.45	33.67 ± 7.37	29.54 ± 2.32	30.2 ± 3.94	33.07 ± 3.77	33.41 ± 3.4	32.57 ± 3.19
<i>R2c latency</i>	33 ± 5.29	32 ± 4.24	30.57 ± 2.51	30.06 ± 4.16	33.39 ± 3.3	34.02 ± 2.54	32.6 ± 3.2
<i>R2i duration</i>	48.31 ± 5.91	40.92 ± 6.36	48.75 ± 7.85	51.71 ± 6.83	44.92 ± 4.52	45.95 ± 6.32	44.58 ± 9.97
<i>R2c duration</i>	43.75 ± 7.66	43.5 ± 11.83	51.8 ± 5.56	52.32 ± 7.32	48.44 ± 4.08	46.39 ± 3.97	44.59 ± 10
<i>R1 area (mV)</i>	0.68 ± 0.46	0.2 ± 0.1	0.49 ± 0.33	0.71 ± 0.62	0.45 ± 0.26	0.79 ± 0.52	1.98 ± 2.19
<i>R2i area (mV)</i>	3.57 ± 1.08	2.27 ± 0.8	3.89 ± 1.46	2.73 ± 1.14	2.89 ± 1.09	4.08 ± 2.44	4 ± 3.07
<i>R2c area (mV)</i>	1.88 ± 1.41	2.39 ± 0.91	2.29 ± 0.94	3.32 ± 1.31	2.71 ± 1.16	2.63 ± 0.88	3.6 ± 2.9
<i>Follow up</i>							
<i>R1 latency</i>	---	---	---	11 ± 1	---	11.35 ± 1.03	---
<i>R2i latency</i>	---	---	---	31.19 ± 3.85	---	31.87 ± 4.48	---
<i>R2c latency</i>	---	---	---	30.63 ± 5	---	31.62 ± 4.09	---
<i>R2i duration</i>	---	---	---	49.03 ± 6.67	---	47.79 ± 9.11	---
<i>R2c duration</i>	---	---	---	49.64 ± 8.65	---	50.97 ± 6.62	---
<i>R1 area (mV)</i>	---	---	---	0.56 ± 0.55	---	0.68 ± 0.47	---
<i>R2i area (mV)</i>	---	---	---	2.42 ± 1.44	---	3.48 ± 2.24	---
<i>R2c area (mV)</i>	---	---	---	3.15 ± 1.94	---	3.51 ± 1.49	---

*US, unaffected side; AS, affected side. R1, R1 component of the blink reflex; R2, R2 component of the blink reflex; i, ipsilateral; c, contralateral. Comparison between blink reflex responses in facial nerve palsy, post-facial palsy syndrome and hemifacial spasm groups and a control group, at baseline and after treatment with Botulin Neurotoxin A. was made through Mann-Whitney U-test. Comparison within group and between baseline and follow-up was made through Wilcoxon Signed Ranks test.*

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At baseline, data obtained from affected and unaffected sides were first compared within each group: in FP patients, on the paretic side, there was a general increase of latency and decrease in area and duration of all the components of the blink reflex, both from direct and indirect stimulations. Particularly, R2 area and duration obtained from direct stimulation of the affected side, reached significance, if compared with the unaffected side ( $p=0.005$  and  $p=0.04$  respectively).

As for the PFPS group, R2 areas of the affected side, obtained from both direct and indirect stimulation, were significantly smaller than those of the unaffected side ( $p=0.0005$  and  $p=0.0001$  respectively). There was also a significant increase in duration of the R2 component of the affected side from direct stimulation ( $p=0.04$ ).

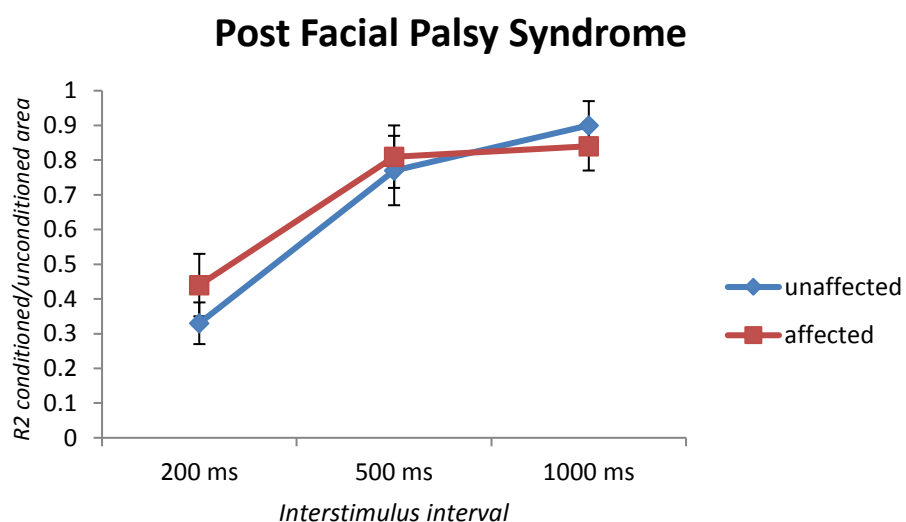
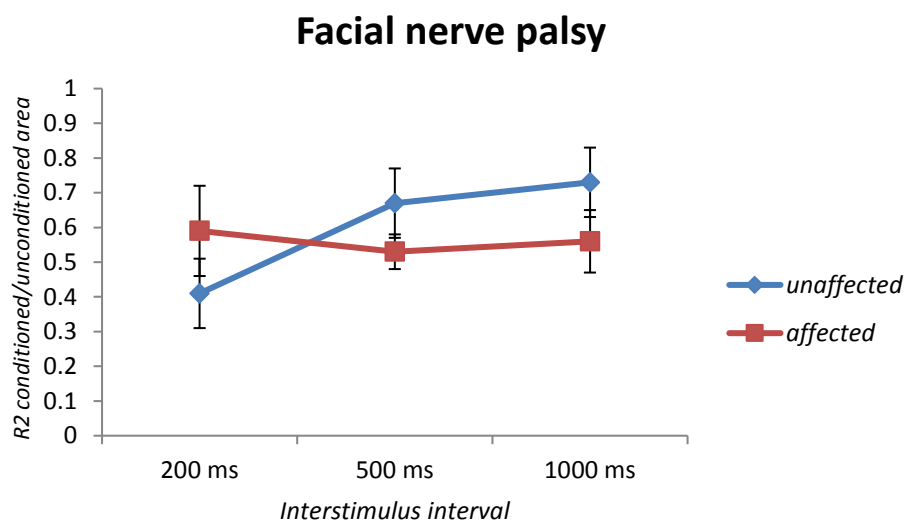
On the contrary, in the affected side of the HFS group, the area of the R2 component obtained from ipsilateral stimulation was significantly larger compared to the contralateral one ( $p=0.001$ ). Also the R1 component was larger than that obtained from the unaffected side, but this did not reach significance. The duration of the R2 component obtained in the affected side following indirect stimulation was also longer ( $p=0.03$ ) than that obtained in the unaffected side.

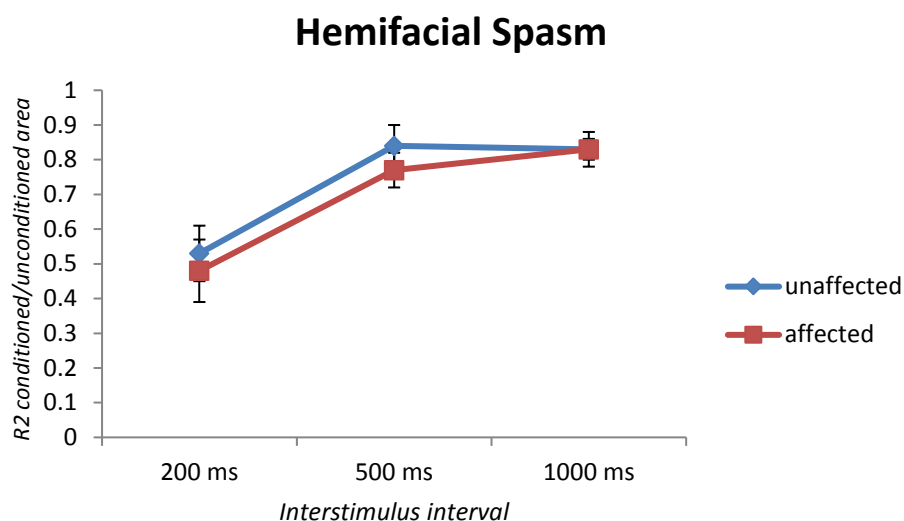
Secondly, patients and controls were compared with each other. A significant increase was found in PFPS patients, when duration of the R2 components, obtained both for direct and indirect stimulation on the affected side, was compared with the control group ( $p=0.03$  and  $p=0.02$ , respectively). The same was seen for the unaffected side, but limitedly to the indirect stimulation ( $p=0.01$ ). On the contrary, controls and HFS patients did not differ for the parameters studied.

At follow up, in comparison with baseline results, PFPS patients showed a shortening in duration of R2 on the unaffected side ( $p=0.03$ ) with a trend towards significance contralaterally. After BoNT-A, from indirect stimulation, HFS the R2 latency of the unaffected side shortened significantly ( $p=0.04$ ), the area increased ( $p=0.0002$ ) and the duration prolonged ( $p=0.002$ ).

## Blink Reflex Recovery Cycle

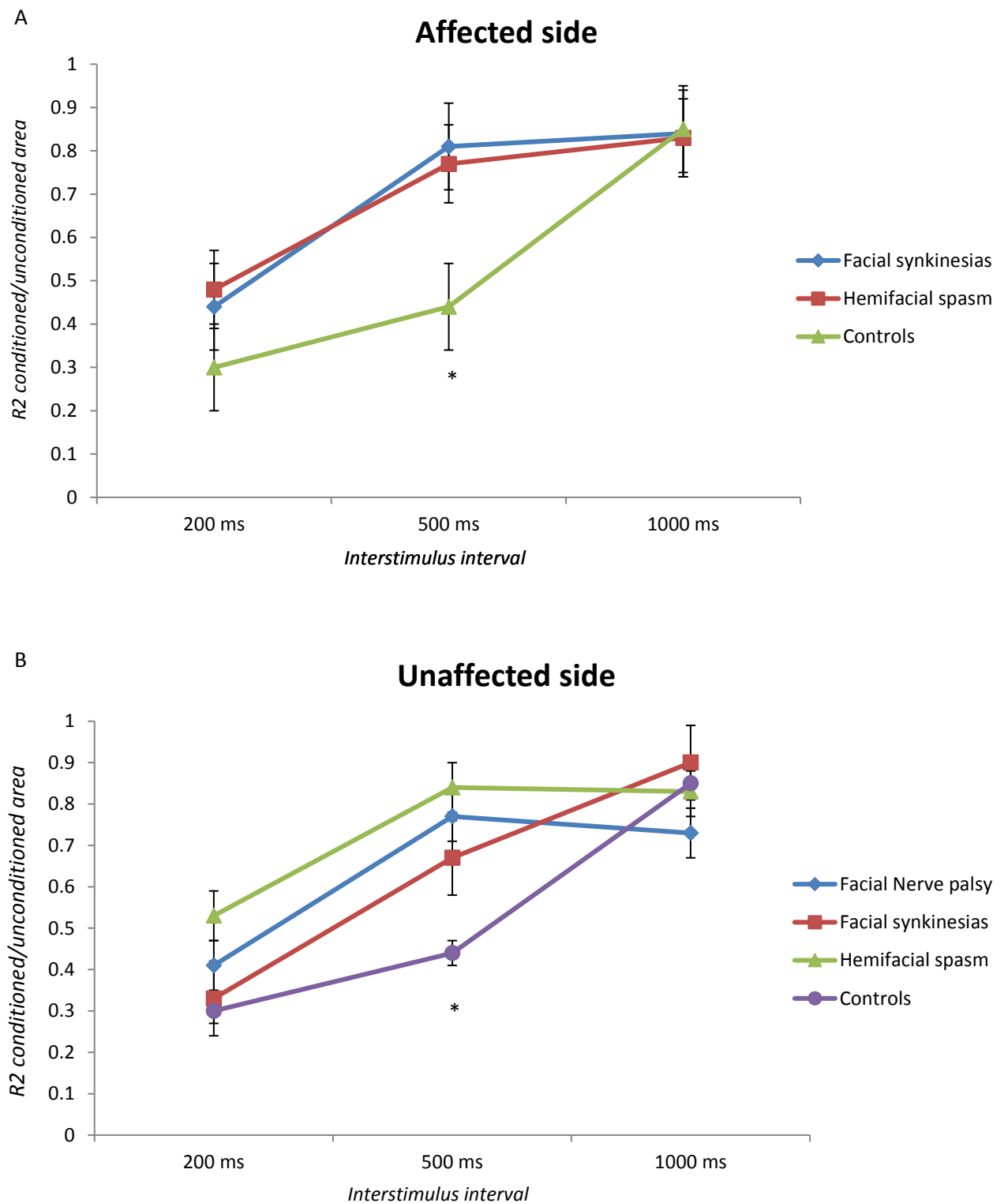
By analyzing each group at baseline, no significant differences emerged in the comparison between the affected and the unaffected side within FP, PFPS and HFS patients (Fig.2).





**Fig. 2** Intra-group comparison of the recovery cycle of the R2 conditioned/unconditioned areas between affected and unaffected side in each group of patients at baseline. Affected and unaffected sides are expressed as total of direct and indirect stimulation. Two ways ANOVA.

Conversely, comparing the groups, R2 conditioned response showed a significantly earlier recruitment in PFPS and in HFS in respect with the control group (Fig.3). The recruitment was bilaterally enhanced in both groups when responses were obtained from stimulation of the affected side (Fig.3A,  $p=0.00007$ ). Also when the asymptomatic side was stimulated, responses obtained omolaterally and contralaterally were enhanced in a similar manner (Fig.3B,  $p=0.0007$ ). Post-hoc analysis allowed to detect a significant difference at 500 ms ISI between PF, PFPS and HFS patients and controls, from both the unaffected ( $p=0.01$ ,  $p=0.04$  and  $p=0.000004$ , respectively) and the affected side ( $p=0.00006$  for HPFP and  $p=0.000003$  for HFS).

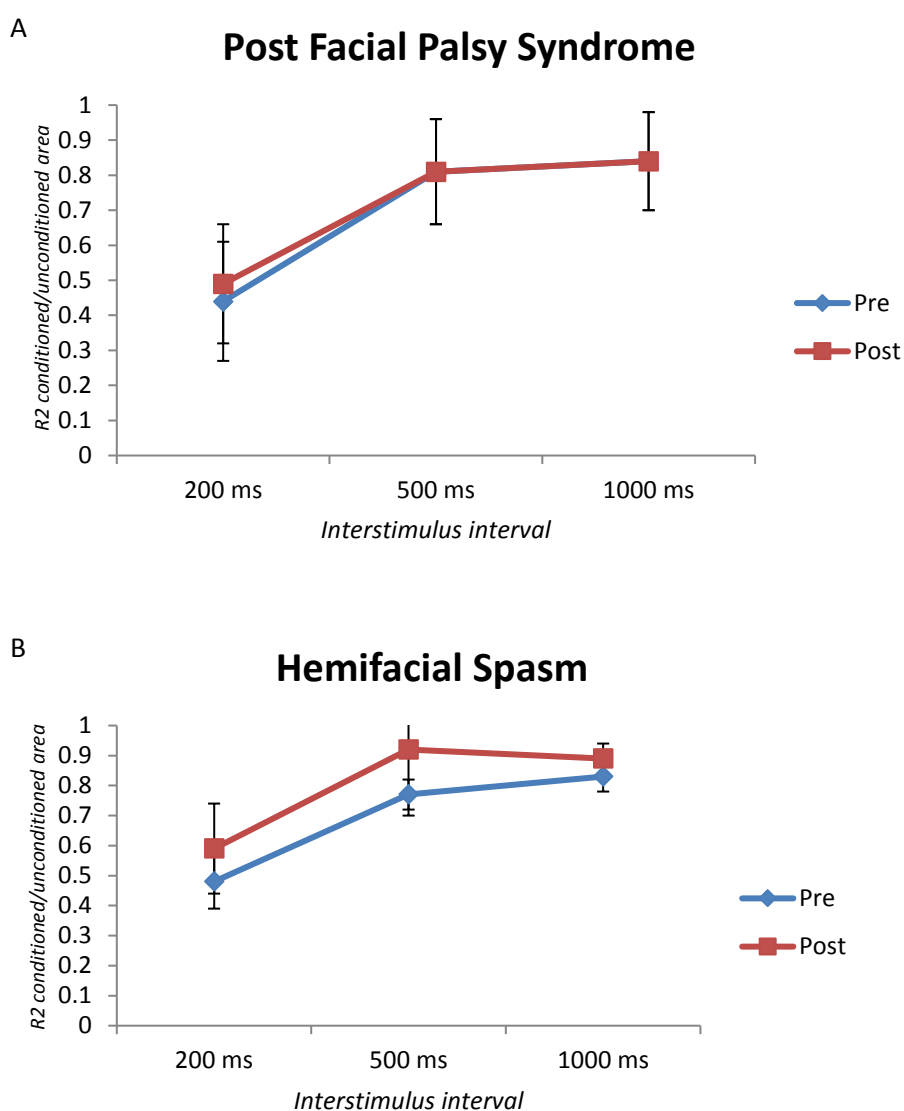


**Fig. 3** Inter-group comparison (with SE) at baseline of the recovery cycle of R2

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conditioned/unconditioned areas between groups of affected (A) and unaffected (B) side of recording. Two ways ANOVA with Tukey post-hoc test ( $*= p < 0.05$ ).

No statistically significant differences emerged in the study of BRRC on the affected side of PFPS and HFS patients after treatment with BoNTa, compared with baseline (Fig.4A and 4B).



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**Fig. 4** Comparison of Blink Reflex Recovery Cycle curves of affected side from Post Facial Palsy Syndrome (A) and Hemifacial Spasm (B) patients before (PRE) and after (POST) BoNT-A treatment. Two ways ANOVA.

### ***Masseter inhibitory reflex***

At the study of MIR, latencies of the early and the late silent periods from direct and indirect stimulation overlapped between the PFPS and HFS groups, without differences between sides of each group Table 3).

**Table 3** Comparison of MIR between patients with Post-Facial Palsy Syndrome and Hemifacial Spasm for both unaffected and affected side stimulation and recording.

<i>Stim.</i>		<i>Post-Facial Palsy</i>	<i>Hemifacial spasm</i>	<i>P value*</i>
<i>Unaffected side</i>	<i>SP1i (ms)</i>	13.88±0.99	14.35±1.14	<i>n.s.</i>
	<i>SP1c (ms)</i>	13.85±1.01	15.28±2.53	<i>n.s.</i>
	<i>SP2i (ms)</i>	51.33±6.82	53.86±9.24	<i>n.s.</i>
	<i>SP2c (ms)</i>	51.9±10.6	53.77±8.4	<i>n.s.</i>
<i>Affected side</i>	<i>SP1i (ms)</i>	14.33±0.75	14.13±0.91	<i>n.s.</i>
	<i>SP1c (ms)</i>	14.32±0.84	13.99±0.88	<i>n.s.</i>
	<i>SP2i (ms)</i>	52.8±11.6	54.66±9.53	<i>n.s.</i>
	<i>SP2c (ms)</i>	51.3±9.47	53.54±9.66	<i>n.s.</i>
	<i>P value†</i>	<i>n.s.</i>	<i>n.s.</i>	

SP1, early silent period; SP2, late silent period; I, ipsilateral; c, contralateral. Mann-Whitney U-test. \* = intergroup comparison; † = intragroup comparison.

## Discussion

The synkinetic phenomenon is a neurologic dysfunction that occurs in various clinical entities and whose pathogenetic mechanism has not been clarified yet. The most widely accepted explanations are ephaptic transmission and/or aberrant axonal regeneration in case of synkinesias consequent to peripheral damage of the facial nerve (Montserrat & Benito, 1988). In the context of hemifacial spasm, it has been hypothesized that an hyperexcitability of motoneurons of the facial nucleus (Møller & Jannetta, 1984) or a vascular compression and resulting cross-talk between demyelinated nerve fibers (ephaptic transmission) at extra-axial level (Nielsen, 1985) can be accounted for the synkinetic phenomenon.

All components of the Blink Reflex were observed in all categories of patients studied, both on the affected and unaffected side. This finding indicates that the trigemino-facial pathway underlying this reflex is anatomically intact in our patients (Pauletti et al., 1993). In particular, the circuit for the early blink reflex (or R1), that is relatively well known in humans, has the whole reflex circuit in the pons and it is constituted of non-nociceptive A $\beta$  trigeminal afferents, principal nucleus of trigeminal nerve, facial nucleus and efferent motor fibers (Kimura et al., 1994). The anatomical-functional organization of the late R2 blink reflex, starts from trigeminal afferents, entry into the pons, descend along the trigeminal spinal complex to the level of the inferior olive and finally projects, by relay station, to ipsilateral and contralateral motoneurons of facial nuclei. When ascending from the medulla to the pons, the two sets of interneurons run close to each other (Cruccu et al., 2005).

Also, the integrity of the connections underlining the MIR indicates that an involvement of the trigeminal nerve, at least of the second division, can be excluded (Uluduz et al., 2010). Early masseter inhibitory reflex (or SP1) is mediated by  $\beta$ -

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myelinated afferents and its circuit is thought to be restricted to the mid-pons, where fibers run through the rostral portion of the trigeminal spinal tract. Interneurons, in turn, project to bilateral trigeminal motor neurons. The late period (or SP2) is thought to emerge via fibers descending with the spinal trigeminal nerve to the level of ponto-medullary junction and then project to neurons within bulbar reticular formation (Ongerboer de Visser et al., 1990; Cruccu et al., 2005).

To a more detailed analysis within preserved reflexes, an increase emerges in the latency and a decrease in the area and duration of the R2 wave in the FP group. These data should not surprise because they may represent the direct consequence of the previous neuropraxic and axonal damage of the facial nerve at peripheral level (Liston & Kleid, 1989). In the group of patients who, consequently to a facial palsy, developed a post-facial palsy syndrome (PFPS group), there is a persistence of the decrease in area but, at the same time, an increase occurs in the duration of the R2 component, furthermore evident also in the unaffected side following contralateral stimulation.

We argue that, in the PFPS group, the increase of R2 duration could be a central compensatory attempt to optimize muscle contraction in a condition of reduction of motor units by increasing their central firing (Kimura et al., 1976). Accordingly, the observation of longer R2 also from indirect stimulation of the unaffected side suggests a bilateral sensitization of the blink reflex polysynaptic pathways to inputs from the damaged side only.

Data suggest that the same central hyperexcitability occurs in patients with hemifacial spasm in whom, however, the compensatory mechanism is exerted through an increase in the R2 area and, partially, in its duration. Differently from PFPS, in our HFS group, we did not evidence an excessive excitability on the unaffected side. Indeed, in contrast with the pathogenesis of PFPS, in HFS patients there is, by definition, no history of facial palsy, and hence there lacks the requirement of a compensatory attempt. This interpretation is perfectly in line with

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our knowledge about the “adaptive control system”: an adaptive gain mechanism, efficient for any motor system, that is operative to ensure that there is a precise match between the intended and effectively realized movement (Chuke et al., 1996). The gain control mechanisms operate to maintain the efficacy of the protective blink reflex as well, that is in fact widely used in the study of motor learning. However, previously studies, established the participation of adaptive systems in human eyelid movement disorders by mean the development of a maladaptive plasticity, not only from a quantitative point of view but also qualitatively unsuitable (i.e. extended in site in which, at best, may serve no purpose and, at worse, may have maladaptive consequences) (Baker et al., 1997).

In both HFS and PFPS groups, the blink reflex recovery cycles was enhanced in the affected side, following both direct and indirect stimulation. These findings reveal that a sensitization of blink reflex polysynaptic pathways exists bilaterally. This result is coherent with previous data reported in literature that were explained assuming an hyperexcitability of the facial nucleus in case of HFS (Valls-Sole & Tolosa, 1989) and a sensitization of polysynaptic pathways of the blink reflex induced by the trigeminal input from the affected side in case of PFPS (Syed et al., 1999).

However, in our study, we demonstrated the same early pattern of blink reflex recovery curve also on the unaffected side, both from direct and indirect evocation, on the HFS, PFPS and FP groups. Based on these data, we may conclude that the enhanced sensitivity of the blink reflex circuitry the synkinetic disorders, is not mediated specifically by a dysfunctional trigeminal afferent. A two-fold consideration supports this idea: the absence of any significant difference between the blink reflex recovery cycles obtained from stimulation of affected and unaffected side and the normality of the masseteric inhibitory reflex arch on both the affected and unaffected sides.

Our finding of an increased brainstem excitability since the early phase of facial palsy, and thus before the onset of peripheral compensatory mechanisms, allows to

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hypothesize that this represents a likely pathophysiological substrate for the development of PFPS. In support to this, the findings of a previous study in which an enhanced motoneuronal activity was present from the very first connections between growing axons and denervated muscles, at about 3 months after a facial nerve insult (Cossu et al., 1999).

Modifications of parameters of Blink Reflex after treatment with botulinum toxin, and specifically a reduction in duration of the R2 component observed in PFPS group, may be explained referring to the well-known peripheral effect of chemodenervation. By contrast, in the HFS group, botulinum toxin does not seem to carry out an apparent direct effect on the side undergoing treatment. In fact, the only observed effect consisted of an increase in both duration and area as well as in a latency decrease of the R2 component of the blink reflex in the unaffected side, which might be interpreted as a reactive hyperexcitability secondary to the contralateral chemodenervation.

The absence of modifications on the brainstem interneuronal pool implicated in the recovery cycle of the blink reflex after treatment with botulinum toxin, suggests that the documented effect of botulinum beyond the peripheral action (Filippi et al., 1993) is likely to occur in the central nervous system at higher level than the brainstem. Indeed, the mechanisms responsible for the inhibition of the conditioned response are considered to be segmental and thus, although mostly referred to the interneuronal net of the lateral reticular formation (Kimura, 1973a), are potentially influenced to an higher conditioning.

In this regard, plastic changes on shape and size of cortical representation of muscles infiltrated with BoNT-A in patients with dystonia are revealed both at morphological and functional investigations (Byrnes et al., 1998; Kojovic, 2011), as well as it was shown a reorganization of central programs with the appearance of compensatory movements (Moreno-López et al., 1997). Also the discovery of indirect effects of BoNT on spinal cord circuitry (Priori et al., 1995), was attributed to central changes in

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spinal motoneuron responsiveness to cortical descending inputs (Pauri et al., 2000). These dissimilar effects of botulinum injection on the blink reflex, on reciprocal inhibition and cortical reorganization, might depend on the different segmental motor organization of the brainstem and spinal cord.

## Conclusions

In conclusion, it is conceivable that, clinical and electrophysiological similarity patterns between PFPS and HFS, could be both interpreted as evidence for a change in motoneuronal hyperexcitability, which in turn can be attributed to a consequent high-ranking influence. In fact, the influence of this network on the recovery cycle is supported by the observation that, in disorders involving basal ganglia, such as dystonia, Parkinson's disease or Huntington's disease, the R2 component of the blink reflex has an abnormal excitability (Agostino et al., 1987, 1988; Nakashima et al., 1990). In the case of PFPS, a persistent facial weakness sustains the central compensatory attempt. Indeed, a previous study demonstrated that enhancement of recovery of the blink reflex is directly dependent on entity of facial weakness (Cossu et al., 1999). However, the "lateralization" of compensatory effort, highlighted at level of affected side at BR study, fails when we move to observe a more extended and complex circuit like those subtended to BRRC, that imply an interneuronal widespread network (Kimura, 1973). The same explanation is enforceable at HFS category, in which hyperexcitability is mainly confined to the study of the blink reflex recovery cycle, and thus implies the involvement of not only brainstem structures but also, potentially, all structures that exercise an influence on its excitability.

We can conclude that the study of trigemino-facial reflex and, specifically, of its recovery cycle, appears to provide an objective demonstration of function abnormalities of the inhibitory brainstem interneurons in the context of the synkinetic phenomenon. This can be interpreted like a central compensatory attempt in response to a facial damage, probably more serious, but temporary, in the case of PFPS and more subtle, but persistent, in the case of HFS (Ruby & Jannetta, 1975).

Our interpretation of data, in one context of literature that is split principally and

categorically between peripheral and central origin of synkinetic phenomena, move to not exclude neither of the two possibilities but admits the presence, in either case, of an excessive activation of brainstem networks, in turn inducted and/or simply amplified by an additional effect of peripheral origin.



## Bibliography

Agostino R, Berardelli A, Cruccu G, Pauletti G, Stocchi F, Manfredi M. Correlation between facial involuntary movements and abnormalities of blink and corneal reflexes in Huntington's chorea. *Mov. Disord.* 1988 Jan 5;3(4):281–9.

Agostino R, Berardelli A, Cruccu G, Stocchi F, Manfredi M. Corneal and blink reflexes in Parkinson's disease with "on-off" fluctuations. *Mov. Disord.* 1987 Jan 4;2(4):227–35.

Auger RG, Whisnant JP. Hemifacial spasm in Rochester and Olmsted County, Minnesota, 1960 to 1984. *Arch. Neurol.* 1990 Nov 4;47(11):1233–4.

Babinski J. Hémispasme facial périphérique. *Nouv Iconographie de la Salpêtrière.* Hémispasme facial périphérique. *Nouv Iconographie de la Salpêtrière.* Hémispasme facial périphérique. *Nouv Iconographie de la Salpêtrière;* 1905;18:418–23.

Baker R, Sun W, Hasan S, Rouholiman B, Chuke J, Cowen D, et al. Maladaptive neural compensatory mechanisms in Bell's palsy-induced blepharospasm. *Neurology.* 1997;49(1):223–9.

Binder WJ, Brin MF, Blitzer A, Schoenrock LD. Botulinum toxin type A (BOTOX) for treatment of migraine headaches: an open-label study. ... -Head and Neck Surgery [Internet]. 2000; Available from: <http://www.sciencedirect.com/science/article/pii/S0194599800758530>

Blinzinger K, Kreutzberg G. Displacement of synaptic terminals from regenerating

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin.* Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

motoneurons by microglial cells. *Zeitschrift für Zellforschung. Zeitschrift für Zellforschung*; 1968;85:145–57.

Bosco D, Plastino F, Bosco F. Bell's palsy: a manifestation of prediabetes? . *Acta Neurol Scand. Acta Neurol Scand*; 2011;123(1):68–72.

Brin MF, Fahn S, Moskowitz C, Friedman A, Shale HM, Greene PE, et al. Localized injections of botulinum toxin for the treatment of focal dystonia and hemifacial spasm. *Mov. Disord.* 1987 Jan 4;2(4):237–54.

Brissaud E. Vingt-quatrième leçon, Tics and spasms cloniques de la face. *Lecçons sur les maladies nerveuses. Paris: Masson . Lecçons sur les maladies nerveuses. Paris: Masson ; 1895;1893– 1894.*

Byrnes ML, Thickbroom GW, Wilson SA, Sacco P. The corticomotor representation of upper limb muscles in writer's cramp and changes following botulinum toxin injection. *Brain.* *Brain*; 1998;121:977–88.

Carter JB, Patrinely JR, Jankovic J, McCrary JA, Boniuk M. Familial hemifacial spasm. *Arch. Ophthalmol.* 1990 Feb 4;108(2):249–50.

Ceballos-Baumann AO, Sheean G, Passingham RE, Marsden CD, Brooks DJ. Botulinum toxin does not reverse the cortical dysfunction associated with writer's cramp. A PET study. *Brain.* 1997 Apr 2;120 ( Pt 4):571–82.

Chaudhry N, Srivastava A, Joshi L. Hemifacial spasm: The past, present and future. *J Neurol Sci.* 2015;356(1-2):27–31.

Chuke J, Baker R, Porter J. Bell's palsy-associated blepharospasm relieved by aiding

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin.* Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

eyelid closure. *Ann Neurol*. 1996;39(2):263–8.

Cohen LG, Hallett M, Geller BD. Treatment of focal dystonias of the hand with botulinum toxin injections. *Journal of Neurology* [Internet]. 1989; Available from: <http://jnnp.bmj.com/content/52/3/355.short>

Colosimo C, Bologna M, Lamberti S, Avanzino L, Avanzino L, Marinelli L, et al. A comparative study of primary and secondary hemifacial spasm. *Arch. Neurol*. 2006 Mar 3;63(3):441–4.

Cossu G, Valls-Solé J, Valdeoriola F, Muñoz E, Benítez P, Aguilar F. Reflex excitability of facial motoneurons at onset of muscle reinnervation after facial nerve palsy. *Muscle Nerve*. 1999;22(5):614–20.

Costa J, Espírito-Santo CC, Borges AA. Botulinum toxin type A therapy for cervical dystonia. *The Cochrane* ...[Internet]. 2005; Available from: <http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD003633.pub2/full>

Cruccu G, Iannetti GD, Marx JJ, Thoemke F, Truini A, Fitzek S, et al. Brainstem reflex circuits revisited. *Brain*. 2005 Feb 2;128(Pt 2):386–94.

Defazio G, Abbruzzese G, Girlanda P, Vacca L, Currà A, De Salvia R, et al. Botulinum toxin A treatment for primary hemifacial spasm: a 10-year multicenter study. *Arch. Neurol*. 2002 Mar 5;59(3):418–20.

Deuschl G, Seifert C, Heinen F, Illert M, Lücking CH. Reciprocal inhibition of forearm flexor muscles in spasmodic torticollis. *J. Neurol. Sci*. 1992 Nov;113(1):85–90.

Edwards MJ, Huang YZ, Mir P, Rothwell JC. Abnormalities in motor cortical

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

plasticity differentiate manifesting and nonmanifesting DYT1 carriers. Movement ... [Internet]. 2006; Available from: <http://onlinelibrary.wiley.com/doi/10.1002/mds.21160/full>

Eekhof J, Aramideh M, Bour L, Hilgevoord A, Speelman H, Visser\* B. Blink reflex recovery curves in blepharospasm, torticollis spasmodica, and hemifacial spasm. *Muscle Nerve*. 1996;19(1):10–5.

Eekhof JL, Aramideh M, Speelman JD, Devriese PP, Ongerboer De Visser BW. Blink reflexes and lateral spreading in patients with synkinesia after Bell's palsy and in hemifacial spasm. *Eur. Neurol*. 2000 Jan 6;43(3):141–6.

Ehni G, Woltman H. Hemifacial spasm. *Arch. Neurol. Psychiatr. Arch. Neurol. Psychiatr.*; 1945;53:205–11.

Esteban A. A neurophysiological approach to brainstem reflexes. Blink reflex. *Neurophysiol Clin*. 1999 Feb 1;29(1):7–38.

Fahn S, List T, Moskowitz C, Brin M, Bressman S. Double-blind controlled study of botulinum toxin for blepharospasm. *Neurology* [Internet]. 1985; Available from: <http://insights.ovid.com/neurology/neur/1985/04/001/double-blind-controlled-study-botulinum-toxin/639/00006114>

Filippi GM, Errico P, Santarelli R, Bagolini B, Manni E. Botulinum A toxin effects on rat jaw muscle spindles. *Acta Otolaryngol*. 1993 May 6;113(3):400–4.

Foster L, Clapp L, Erickson M, Jabbari B. Botulinum toxin A and chronic low back pain a randomized, double-blind study. *Neurology* [Internet]. 2001; Available from: <http://www.neurology.org/content/56/10/1290.short>

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Frevert J. Pharmaceutical, biological, and clinical properties of botulinum neurotoxin type A products. *Drugs R D*. 2015 Mar;15(1):1–9.

Furuta Y, Ohtani F, Sawa H, Fukuda S, Inuyama Y. Quantitation of varicella-zoster virus DNA in patients with Ramsay Hunt syndrome and zoster sine herpette. *J. Clin. Microbiol*. 2001 Aug 3;39(8):2856–9.

Garner CG, Straube A, Witt TN, Gasser T, Oertel WH. Time course of distant effects of local injections of botulinum toxin. *Movement disorders* [Internet]. Wiley Online Library; 1993;8(1):33–7. Available from: <http://onlinelibrary.wiley.com/doi/10.1002/mds.870080106/abstract>

Gilio F, Currà A, Lorenzano C, Modugno N, Manfredi M, Berardelli A. Effects of botulinum toxin type A on intracortical inhibition in patients with dystonia. *Ann. Neurol*. 2000 Jul 6;48(1):20–6.

Girard N, Poncet M, Caces F, Tallon Y, Chays A, Martin-Bouyer P, et al. Three-dimensional MRI of hemifacial spasm with surgical correlation. *Neuroradiology*. 1997 Jan 3;39(1):46–51.

Goroll A, Mulley A. *Primary care medicine: office evaluation and management of the adult patient*, 6th ed. Philadelphia (PA) Lippincott Williams & Williams. 2009;

Graeber MB, Bise K, Mehraein P. Synaptic stripping in the human facial nucleus. *Acta Neuropathol*. 1993 Jan 5;86(2):179–81.

Guntinas-Lichius O, Gabriel B, Klusmann JP. Risk of facial palsy and severe Frey's syndrome after conservative parotidectomy for benign disease: analysis of 610 operations. *Acta Otolaryngol*. 2006 Oct;126(10):1104–9.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Guntinas-Lichius O, Klusmann JP, Schroeder U, Quante G, Jungehueling M, Stennert E. Primary parotid malignoma surgery in patients with normal preoperative facial nerve function: outcome and long-term postoperative facial nerve function. *Laryngoscope*. 2004 May 6;114(5):949–56.

Habermann E. 125 I-labeled neurotoxin from *Clostridium botulinum* A: preparation, binding to synaptosomes and ascent to the spinal cord. *Naunyn-Schmiedeberg's archives of pharmacology* [Internet]. 1974; Available from: <http://www.springerlink.com/index/Q82Q0W376MG22632.pdf>

Hanner P, Badr G, Rosenhall U, Edström S. Trigeminal dysfunction in patients with Bell's palsy. *Acta Otolaryngol*. 1986 Jan 3;101(3-4):224–30.

Holland J, Bernstein J. Bell palsy. *Am Fam Physician*. 2011;84(8):947–8.

Jankovic J, Schwartz K. Botulinum toxin injections for cervical dystonia. *Neurology* [Internet]. 1990; Available from: <http://www.neurology.org/content/40/2/277.short>

Jannetta PJ, Abbasy M, Maroon JC, Ramos FM, Albin MS. Etiology and definitive microsurgical treatment of hemifacial spasm. Operative techniques and results in 47 patients. *J. Neurosurg*. 1977 Sep 4;47(3):321–8.

Jannetta PJ, Kassam A. Hemifacial spasm. *J. Neurol. Neurosurg. Psychiatr*. 1999 Feb 1;66(2):255–6.

Kameyama S, Masuda H, Shirozu H, Ito Y, Sonoda M, Kimura J. Ephaptic transmission is the origin of the abnormal muscle response seen in hemifacial spasm. *Clin Neurophysiol*. 2016;127(5):2240–5.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Katusic SK, Beard CM, Wiederholt WC, Bergstralh EJ, Kurland LT. Incidence, clinical features, and prognosis in Bell's palsy, Rochester, Minnesota, 1968-1982. *Ann. Neurol.* 1986 Nov 6;20(5):622-7.

Kimura J. Disorder of interneurons in Parkinsonism. The orbicularis oculi reflex to paired stimuli. *Brain.* 1973 Jan 1;96(1):87-96.

Kimura J. Disorder of interneurons in Parkinsonism. The orbicularis oculi reflex to paired stimuli. *Brain.* 1973 Jan 1;96(1):87-96.

Kimura J, Daube J, Burke D, Hallett M, Cruccu G, Ongerboer de Visser BW, et al. Human reflexes and late responses. Report of an IFCN committee. *Electroencephalogr Clin Neurophysiol.* 1994 Jun 3;90(6):393-403.

Kimura J, Giron LT, Young SM. Electrophysiological study of Bell palsy: electrically elicited blink reflex in assessment of prognosis. *Arch Otolaryngol.* 1976 Mar 1;102(3):140-3.

Kimura J, Rodnitzky RL, Okawara SH. Electrophysiologic analysis of aberrant regeneration after facial nerve paralysis. *Neurology.* 1975 Oct 3;25(10):989-93.

Kojovic M. Botulinum Toxin Injections Reduce Associative Plasticity in Patients with Primary Dystonia. *Mov Disord. Mov Disord.*; 2011 May 2;26(7):1282-9.

Liston SL, Kleid MS. Histopathology of Bell's palsy. *Laryngoscope.* 1989 Jan;99(1):23-6.

Lockhart P, Daly F, Pitkethly M, Comerford N, Sullivan F. Antiviral treatment for Bell's palsy (idiopathic facial paralysis). *Cochrane Database Syst Rev.* 2009 Oct

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin.* Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

3;(4):CD001869.

Lux H, Schubert P. Some aspects of the electroanatomy of dendrites. *Adv Neurol. Adv Neurol*; 1975;12:29–44.

Manca D, Muñoz E, Pastor P, Valldeoriola F, Valls-Solé J. Enhanced gain of blink reflex responses to ipsilateral supraorbital nerve afferent inputs in patients with facial nerve palsy. *Clin Neurophysiol*. 2001 Jan 1;112(1):153–6.

Mao J-B, Evinger C. Long-term potentiation of the human blink reflex. *J Neurosci* [Internet]. 2001;21(12):RC151. Available from: [http://www.hsc.stonybrook.edu/som/neurobiology/pdf\\_files/Evinger3.pdf](http://www.hsc.stonybrook.edu/som/neurobiology/pdf_files/Evinger3.pdf)

Martinelli P, Giuliani S, Ippoliti M. Hemifacial spasm due to peripheral injury of facial nerve: a nuclear syndrome? *Mov. Disord*. 1992 Jan 3;7(2):181–4.

Micheli F, Scorticati MC, Gatto E, Cersosimo G, Adi J. Familial hemifacial spasm. *Mov. Disord*. 1994 May;9(3):330–2.

Misawa S, Kuwabara S, Ogawara K, Hattori T. Abnormal muscle responses in hemifacial spasm: F waves or trigeminal reflexes? *J. Neurol. Neurosurg. Psychiatr*. 2006 Feb 3;77(2):216–8.

Modugno N, Priori A, Berardelli A, Vacca L, Mercuri B, Manfredi M. Botulinum toxin restores presynaptic inhibition of group Ia afferents in patients with essential tremor. *Muscle Nerve*. 1998 Dec 2;21(12):1701–5.

Moldovan M, Sørensen J, Krarup C. Comparison of the fastest regenerating motor and sensory myelinated axons in the same peripheral nerve. *Brain*. 2006 Sep 5;129(Pt

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.



9):2471–83.

Montero J, Junyent J, Calopa M, Povedano M, Valls-Sole J. Electrophysiological study of ephaptic axono-axonal responses in hemifacial spasm. *Muscle Nerve*. 2007 Feb 4;35(2):184–8.

Montero J, Serra J, Montserrat L. Axon reflexes or ephaptic responses simulating blink reflex R1 after XII-VII nerve anastomosis. *Muscle Nerve*. 1996 Jul 1;19(7):848–52.

Montserrat L, Benito M. Facial synkinesis and aberrant regeneration of facial nerve. *Adv Neurol*. 1988 Jan 5;49:211–24.

Moran C, Neely G. Pattern of facial nerve synkinesis. *Laryngoscope*. *Laryngoscope*; 1996;106:1491–6.

Moreno-López B, Pastor AM, de la Cruz RR, Delgado-García JM. Dose-dependent, central effects of botulinum neurotoxin type A: a pilot study in the alert behaving cat. *Neurology*. 1997 Feb 6;48(2):456–64.

Murakami S, Mizobuchi M, Nakashiro Y, Doi T, Hato N, Yanagihara N. Bell palsy and herpes simplex virus: identification of viral DNA in endoneurial fluid and muscle. *Ann. Intern. Med*. 1996 Jan 1;124(1 Pt 1):27–30.

Mustafa G, Anderson EM, Bokrand-Donatelli Y, Neubert JK, Caudle RM. Antinociceptive effect of a conjugate of substance P and light chain of botulinum neurotoxin type A. *Pain*. 2013 Nov 5;154(11):2547–53.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Mutsch M, Zhou W, Rhodes P, Bopp M, Chen RT, Linder T, et al. Use of the inactivated intranasal influenza vaccine and the risk of Bell's palsy in Switzerland. *N. Engl. J. Med.* 2004 Feb 4;350(9):896–903.

Møller AR. Hemifacial spasm: ephaptic transmission or hyperexcitability of the facial motor nucleus? *Exp. Neurol.* 1987 Oct 4;98(1):110–9.

Møller AR, Jannetta PJ. On the origin of synkinesis in hemifacial spasm: results of intracranial recordings. *J. Neurosurg.* 1984 Sep 6;61(3):569–76.

Møller AR, Jannetta PJ. Microvascular decompression in hemifacial spasm: intraoperative electrophysiological observations. *Neurosurgery.* 1985 May 3;16(5):612–8.

Nakashima K, Rothwell JC, Day BL, Thompson PD. Reciprocal inhibition between forearm muscles in patients with writer's cramp and other occupational cramps, symptomatic hemidystonia and hemiparesis due to .... *Brain* [Internet]. 1989; Available from: <http://brain.oxfordjournals.org/content/112/3/681.short>

Nakashima K, Rothwell JC, Thompson PD, Day BL, Berardelli A, Agostino R, et al. The blink reflex in patients with idiopathic torsion dystonia. *Arch. Neurol.* 1990 Apr;47(4):413–6.

Newadkar UR, Chaudhari L, Khalekar YK. Facial Palsy, a Disorder Belonging to Influential Neurological Dynasty: Review of Literature. *N Am J Med Sci.* 2016 Jul 5;8(7):263–7.

Nielsen VK. Pathophysiology of hemifacial spasm: I. Ephaptic transmission and ectopic excitation. *Neurology.* 1984 Apr;34(4):418–26.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin.* Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Nielsen VK. Pathophysiology of hemifacial spasm: II. Lateral spread of the supraorbital nerve reflex. *Neurology*. 1984 Apr;34(4):427–31.

Nielsen VK. Electrophysiology of the facial nerve in hemifacial spasm: ectopic/ephaptic excitation. *Muscle Nerve*. 1985 Sep;8(7):545–55.

Nilsen B, Le K-DD, Dietrichs E. Prevalence of hemifacial spasm in Oslo, Norway. *Neurology*. 2004 Oct 2;63(8):1532–3.

Oge AE, Yayla V, Demir GAA, Eraksoy M. Excitability of facial nucleus and related brain-stem reflexes in hemifacial spasm, post-facial palsy synkinesis and facial myokymia. *Clin Neurophysiol*. 2005 Jul 5;116(7):1542–54.

Olney RK, Aminoff MJ, Gelb DJ, Lowenstein DH. Neuromuscular effects distant from the site of botulinum neurotoxin injection. *Neurology*. 1988 Nov 2;38(11):1780–3.

Ongerboer de Visser BW, Cruccu G, Manfredi M, Koelman JH. Effects of brainstem lesions on the masseter inhibitory reflex. Functional mechanisms of reflex pathways. *Brain*. 1990 Jun 5;113 ( Pt 3):781–92.

Orsini M, Leite M, Chung T, Bocca W, Souza J, Souza O, et al. Botulinum neurotoxin type A in neurology: update. *Neurology Int*. 2015;7(2).

Panizza ME, Hallett M, Nilsson J. Reciprocal inhibition in patients with hand cramps. *Neurology* [Internet]. 1989; Available from: <http://www.neurology.org/content/39/1/85.short>

Pastor P, Munoz E, Valledoria F, Marti M, Tolosa E, Valls-Solè J. Enhanced blink rate and involuntary contralateral eye closure in patients with Bell's palsy. *Muscle Nerve*.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Muscle Nerve; 1998;21:1596.

Pauletti G, Berardelli A, Cruccu G, Agostino R, Manfredi M. Blink reflex and the masseter inhibitory reflex in patients with dystonia. *Mov. Disord.* 1993 Oct 5;8(4):495–500.

Pauri F, Boffa L, Cassetta E, Pasqualetti P, Rossini PM. Botulinum toxin type-A treatment in spastic paraparesis: a neurophysiological study. *J. Neurol. Sci.* 2000 Dec 5;181(1-2):89–97.

Pavesi G, Medici D, Macaluso GM, Ventura P, Allegri I, Gemignani F. Unusual synkinetic movements between facial muscles and respiration in hemifacial spasm. *Mov. Disord.* 1994 Jul 5;9(4):451–4.

Peitersen E. The natural history of Bell's palsy. *Am J Otol.* 1982 Oct 5;4(2):107–11.

Porta M. A comparative trial of botulinum toxin type A and methylprednisolone for the treatment of myofascial pain syndrome and pain from chronic muscle spasm. *Pain.* 2000 Mar 3;85(1-2):101–5.

Priori A, Berardelli A, Mercuri B, Manfredi M. Physiological effects produced by botulinum toxin treatment of upper limb dystonia. Changes in reciprocal inhibition between forearm muscles. *Brain.* 1995 Jun 4;118 ( Pt 3):801–7.

Quartarone A, Sant'Angelo A, Battaglia F. Enhanced long-term potentiation-like plasticity of the trigeminal blink reflex circuit in blepharospasm. *Journal of ...* [Internet]. 2006; Available from: <http://www.jneurosci.org/content/26/2/716.short>

Quartarone A, Bagnato S, Rizzo V, Siebner HR, Dattola V, Scalfari A, et al. Abnormal

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin.* Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

associative plasticity of the human motor cortex in writer's cramp. *Brain*. 2003 Dec 1;126(Pt 12):2586–96.

Ridding MC, Rothwell JC. Reorganisation in human motor cortex. *Can. J. Physiol. Pharmacol.* 1995 Feb 3;73(2):218–22.

Ruby JR, Jannetta PJ. Hemifacial spasm: ultrastructural changes in the facial nerve induced by neurovascular compression. *Surg Neurol.* 1975 Oct 3;4(4):369–70.

Schicatano EJ, Basso MA, Evinger C. Animal model explains the origins of the cranial dystonia benign essential blepharospasm. *J. Neurophysiol.* 1997 May 4;77(5):2842–6.

Schicatano EJ, Mantzouranis J, Peshori KR, Partin J, Evinger C. Lid restraint evokes two types of motor adaptation. *J. Neurosci.* 2002 Jan 2;22(2):569–76.

Schwarze HP, Hirsch BE, Johnson PC. Oculostapedial synkinesis. *Otolaryngol Head Neck Surg.* 1995 Dec 5;113(6):802–6.

Scott AB. Botulinum toxin injection of eye muscles to correct strabismus. *Trans Am Ophthalmol Soc.* 1981 Jan 4;79:734–70.

Simpson LL. The origin, structure, and pharmacological activity of botulinum toxin. *Pharmacological Reviews* [Internet]. 1981; Available from: <http://pharmrev.aspetjournals.org/content/33/3/155.short>

Soriano-Baron H, Vales-Hidalgo O, Arvizu-Saldana E, Moreno-Jimenez S, Revuelta-Gutierrez R. Hemifacial spasm: 20-year surgical experience, lesson learned. *Surg Neurol Int.* 2015 Jan 4;6:83.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Syed NA, Delgado A, Sandbrink F, Schulman AE, Hallett M, Floeter MK. Blink reflex recovery in facial weakness: an electrophysiologic study of adaptive changes. *Neurology*. 1999 Mar 3;52(4):834–8.

Tan E-KK, Chan L-LL. Primary vs postparalytic hemifacial spasm. *Arch. Neurol*. 2006 Aug 2;63(8):1204; author reply 1204.

Tan EK, Chan LL, Lim SH, Lim WE, Khoo JB, Tan KP. Role of magnetic resonance imaging and magnetic resonance angiography in patients with hemifacial spasm. *Ann. Acad. Med. Singap*. 1999 Mar 1;28(2):169–73.

Tate JR, Tollefson TT. Advances in facial reanimation. *Curr Opin Otolaryngol Head Neck Surg*. 2006 Aug 2;14(4):242–8.

Tetzlaff W, Leonard C, Krekoski CA, Parhad IM, Bisby MA. Reductions in motoneuronal neurofilament synthesis by successive axotomies: a possible explanation for the conditioning lesion effect on axon regeneration. *Exp. Neurol*. 1996 May 3;139(1):95–106.

Tsui JC, Stoessl JA, Eisen A, Calne S, Calne D. Double-blind study of botulinum toxin in spasmodic torticollis. *The Lancet* [Internet]. Elsevier; 1986;328(8501):245–7. Available from: <http://www.sciencedirect.com/science/article/pii/S0140673686920702>

Turkof E, Richard B, Assadian O, Khatri B, Knolle E, Lucas S. Leprosy affects facial nerves in a scattered distribution from the main trunk to all peripheral branches and neurolysis improves muscle function of the face. *Am. J. Trop. Med. Hyg*. 2003 Jan 3;68(1):81–8.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Uluduz D, Kiziltan ME, Akalin MA. Acute peripheral facial palsy: is there a trigeminal nerve involvement? *Neurosci. Lett.* 2010 Jul 1;479(2):171–4.

Vakharia K, Vakharia K. Bell's Palsy. *Facial Plast Surg Clin North Am.* 2016 Feb 1;24(1):1–10.

Valls-Sole J, Tolosa ES. Blink reflex excitability cycle in hemifacial spasm. *Neurology.* 1989 Aug 2;39(8):1061–6.

Valls-Sole J, Tolosa ES, Pujol M. Myokymic discharges and enhanced facial nerve reflex responses after recovery from idiopathic facial palsy. *Muscle Nerve.* 1992 Jan 3;15(1):37–42.

Valls-Sole J, Tolosa ES, Ribera G. Neurophysiological observations on the effects of botulinum toxin treatment in patients with dystonic blepharospasm. *J. Neurol. Neurosurg. Psychiatr.* 1991 Apr 1;54(4):310–3.

Valls-Solé J. Facial palsy, postparalytic facial syndrome, and hemifacial spasm. *Mov. Disord.* 2002 Jan 2;17 Suppl 2:S49–52.

Valls-Solé J. Facial nerve palsy and hemifacial spasm. *Handb Clin Neurol.* 2013 Jan 2;115:367–80.

Valls-Solé J, Montero J. Movement disorders in patients with peripheral facial palsy. *Movement Disord.* 2003;18(12):1424–35.

Wiegand H, Erdmann G, Wellhöner HH. 125 I-labelled botulinum A neurotoxin: pharmacokinetics in cats after intramuscular injection. ... -Schmiedeberg's archives of ... [Internet]. 1976; Available from: <http://www.springerlink.com/index/F578130426185061.pdf>

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin.* Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.

Wiegand H, Hilbig G, Wellhöner HH. Early local tetanus: does tetanus toxin change the stimulus evoked discharge in afferents from the injected muscle? *Naunyn Schmiedebergs Arch. Pharmacol.* 1977 Jun 3;298(2):189–91.

Wolfson A. *Narwood-Nuss' Clinical Practice of Emergency Medicine*, 5th ed. Philadelphia (PA): Lippincott Williams & Williams. 2009.

Yalto T, Jankovic J. The many faces of hemifacial spasm: differential diagnosis of unilateral facial spasms. *Mov. Disord. Mov. Disord.*; 2011;26 (9)(Aug 1):1582–92.

Ziemann U, Corwell B, Cohen LG. Modulation of plasticity in human motor cortex after forearm ischemic nerve block. *J. Neurosci.* 1998 Feb;18(3):1115–23.

Ziemann U, Hallett M, Cohen LG. Mechanisms of deafferentation-induced plasticity in human motor cortex. *J. Neurosci.* 1998 Sep 2;18(17):7000–7.

Maria Paola Cabboi. *Brainstem excitability in Hemifacial Spasm and Post-Facial Palsy Synkinesias and effects of Botulinum Toxin*. Tesi di dottorato in Scienze Biomediche, Università degli Studi di Sassari.