**Program**

**Villa Emý B&B, Stra, Brenta Riviera (Venice, Italy), September 29th - October 1st, 2015**

Each time slot will be made of half time of data presentation followed by half time Discussion

**Tuesday September 29, 2015**

16.30 Registration

17.00 **Muscle Metrics in Aging**, Francesco Piccione, Sandra Zampieri, Chairs

17.00 Functional metrics of human muscles: decay with aging, Paolo Gava, Ugo Carraro, Padova/Venice

17.30 3D Muscle Color Computed Tomography for diagnostics and follow up of mobility impairments, Paolo Gargiulo, Andrea Marcante, Francesco Piccione, Francesco Gava, Ugo Carraro, Reykjavik/Venice

18.00 Micro morphometry for diagnostics and follow up of mobility impairments, Sandra Zampieri, et al, Vienna/Padova/Venice

18.30 Molecular adaptation of Calcium handling proteins of human skeletal muscle to FES and Leg-Press Training in aging: an in situ study, Simone Mosole et al. Padova/Vienna

19.00 Horizon 2020 Organizing Meeting, Jonathan Jarvis, Liverpool

20.30 Dinner Molini del Dolo, Brenta Riviera, Venice

**Wednesday September 30, 2015**

10.00 **MED°EL** Workshop: Functional Rejuvenation in Aging, Werner Lindenthaler, Winfried Mayr, Chairs

10.00 Functional rejuvenation in elderly: partially denervated muscle ES, selectivity and patterns for diagnostics and managements, Werner Lindenthaler, Innsbruck

10.40 Seeking economy of charge injection in activation of motor nerves, Martin Schmoll, Jonathan Jarvis, Vienna / Liverpool

11.20 MiniVStim18B: A new member in the family of FES implants for small animals goes towards denervated muscles, Manfred Bijak, Ewald Unger, Michael Haller, Martin Schmoll, Ugo Carraro, Hermann Lanmuller, Vienna/Liverpool

12.00 Chronic electrical stimulation of the RLN in rodents: Results of a feasibility study, Markus Gugatschka, Graz/Stockholm

12.40 Chronic neurostimulation of the aged larynx - first results from a large animal study, Michael Karbiener, Graz

14.20 **Muscle Rejuvenation in Aging and Neuromuscular Disorders**, Jonathan Jarvis, Stefano Masiero, Chairs

14.20 Vagus stimulation and brain ischemia, an interim report, Marco Patruno, Luca Denaro, Domenico D’Avella, Giorgio Gioffrè, Giulia Melinda Furlani, Tiziana Martinello, Laura Cavicchioli, Giovanni Caporale, Hermann Lanmuller, Jonathan Jarvis, Padova/Venice/Liverpool

15.00 Diagnostics and management of human denervated muscle: Rehabilitative approach, Andrea Marcante et al., Venice

15.40 Electrical stimulation in neuromuscular disorders: A case report, Antonio Marziali, Alfredo Musumeci, Ugo Carraro, Stefano Masiero, Padova

16.20 The Vienna and Padua strategies for stimulation of human muscles in neuromuscular disorders and aging, Winfried Mayr, Alfonc Baba, Andrea Marcante, Francesco Piccione, Ugo Carraro, Vienna/Venice

17.00 Barchesse Villa Valmarana & Villa Malcontenta 20.00 Dinner: Trattoria Ba.za.bò, Via Grandi, 34/A, Vigonza, Padova

21.00 **MED°EL** Workshop on Functional Rejuvenation, Werner Lindenthaler, Chair General Discussion 1st

**Thursday October 1, 2015**

8.00 **MED°EL** Workshop on Functional Rejuvenation, Jonathan Jarvis, Chair General Discussion 2nd, with breakfast

integrated aging of muscle may occur as the result of various track and field disciplines. Actually all track and field events are power tests and the performance of the athletes can be transformed into dimensionless parameter proportional to the power developed in carrying out the events. These are astonishingly linear in behavior and points to 0 power at 110 years of age. For some of them (running events) the processing of the athletic results into power parameters associated with the age of the master athletes is simple; for the throwing events the dimensionless parameters are calculated with a two steps process; for the jumping events the processing is more problematic. In this area we are concentrating our present work because the jumping results (high jump) are not proportional to the power developed: this is proportional to the raising of the center of gravity of the athletes.


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3D Muscle macro morphometry for diagnostics and follow up of mobility impairments

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There is something in our genome that dictates life expectancy and there is nothing that can be done to avoid this; indeed, there is not yet any record of a person who has cheated death. Our physical prowess can vacillate substantially in our lifetime according to our activity levels and nutritional status and we may fight aging, but we cannot stop it. The tissue characterization process is based on using CT data and special software tools to segment and analyze the different tissues within a region of interest. In the process of assessing muscle quality in the lower limbs we discriminate the soft tissues dividing them in: subcutaneous fat, intramuscular fat, low density muscle, normal muscle and fibrous-dense connective tissues. The first step in the segmentation process is to establish a threshold, which discriminates the tissue of interest from the rest by grey value (CT number or Hounsfield value in CT modality). The second segmentation tool which typically follows thresholding is region growing. Region growing is an image segmentation approach in which neighboring pixels of the current region’s boundaries are examined and added to the region class if no edges are detected (or more generally some inclusion criteria is met). The Muscle Color Computed Tomography allows to: 1. Quantify the percent contents of the different soft tissues (muscle, fat and other connective tissues, either loose or fibrous) and 2. the volume of anatomically defined muscles (here, rectus femoris or tibialis anterior). The methods are used to measure in a patient (male, 53 years, ASIA 1, that had suffered with a trauma to the spinal cord 15 years before the MC-CT) the residual asymmetric innervation of the leg muscles and the effects on muscles of a 6-week rehabilitation regime that allowed the patient to perform short walks without crouches after years of supported walking and many hours a day of bed resting. Further, a young active male (35 years old) and a typical sedentary senior (male of 72-years) are also analyzed.


ABSTRACTS
Molecular adaptation of Calcium handling proteins of human skeletal muscle to Functional Electrical Stimulation and Leg-Press Training in aging: an in situ study

Simone Mosole (1,2,3,4), Sandra Zampieri (1,3), Sandra Furlan (5), Hanna Fruhmann (4), Stefan Löfler (4), Michael Vogelauer(4), Helmut Kern (4), Pompeo Volpe (1,2), Alessandra Nori (1,2)

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Physical activity plays an important role in preventing chronic disease and muscle degeneration in adults and the elderly. Voluntary physical exercise is not always feasible and other therapies should be applied such as Functional Electrical Stimulation (FES) and/or Leg Press (LP) training. \(^{17}\) The key process of Calcium (Ca\(^{2+}\)) storage uptake and release is essential in muscle adaptation. This study shows the effects of physical training (LP) and FES in situ in human Vastus Lateralis (VL) muscle. Through immunofluorescence analysis of muscle cryo-sections, a huge increment of NFAT positive nuclei, was found after both treatments (from 3% to 60%); moreover after both trainings an increment of P-CaMkII was observed by western blotting analysis. These findings indicate that the muscle adaptation involves the CaM-dependent phosphatase signaling (known to be involved in muscle plasticity). Muscle total homogenates obtained from biopsies performed before and after completing a nine weeks FES treatment on a group of volunteers and Calsequestrin (CASQ), SERCA, Sarcalumenin, protein expression were determined by Western blot. After FES significant increase of SERCA2 and Sarcalumenin and decrease of CASQ1 were observed, while LP did not show any variations of proteins levels. Immunofluorescence analysis were also performed to localize in situ MHCI/SERCA2 co-expressing muscle fibers, an interesting tool to identify subpopulation of muscle fibers involved in muscle adaptation. The overall results indicate that the applied FES protocol, simulating a motoneuron slow-type firing pattern, potentiates Ca\(^{2+}\) uptake and storage in a peculiar class of skeletal muscle fibers, and further validates, at molecular level, the FES strategy.


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Functional rejuvenation in Elderly: partially denervated muscle ES, selectivity and patterns for diagnostics and managements

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Damage to the recurrent laryngeal nerve (RLN) causes: 1. severe dyspnea because of bilateral paralyzed vocal cords and 2. An impaired voice in cases of unilateral damage of vocal cords because of reduced ability to bring the vocal fold on the damaged side to the midline. The same symptom is caused by reduced firing of the recurrent laryngeal nerve in aged related to disuse atrophy. Damage of the facial nerve leads to a loss of muscle tone and the soft tissues of the face. Voluntary motor movement is lost, and mimic muscles can no longer be moved. The inability to close the eyelid indirectly leads to vision disorders and the eye may dry out. Lack of mouth movement limits speaking and eating.

Axonal collateral sprouting leads clinically to simultaneous movement of several target muscles (a condition called synkinesis) - involuntary lid closure while moving the mouth, e.g., when eating. Simultaneous movement of antagonist muscles leads to the autoparalytic syndrome: muscle forces cancel each other out and no movement is observed clinically despite innervation.

Aging in the facial region typically means a loss of muscle tone, muscle volume and/or a loss or reduction of connective and fat tissue, e.g., below the eyes and/or at the cheek. As a result of the aging muscles, facial muscles and/or other facial tissues may diminish or atrophy. FES may be an effective method of providing training therapy to human subjects weakened or denervated muscles in order to strengthen a weakened voice or face.


3. Patent No.: US 8,792,989 B2. Date of Patent: Jul. 29, 2014 System and method for facial nerve stimulation Inventors: Orlando Guntinas-Lichius, Jena (DE); Andreas Muller, Gera (DE); Werner Lindenthaler, Obergperfuss (AT)


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Investigating energy efficiency for different pulse shapes

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One of the main determinants of the overall size of neural implantable pulse generators is usually the size of the battery. Engineers are facing the challenge of designing devices that are small in volume whilst fulfilling their stimulation task as long as possible. Therefore efficient stimulation methods are crucial for their success.

Wongsarnpigoon 1 pointed out three different types of stimulation efficiency. A “charge-efficient” stimulation could have the positive effect of reducing tissue damage. As the battery size is directly proportional to the maximal instantaneous power required – a “power-efficient” stimulation could reduce battery-size and therefore the overall size of an implant. An “energy-efficient” stimulation on the other hand, could be advantageous with regard to the battery lifetime. The aim of our project is to compare different rectangular waveforms according to their “energy-efficiency”. 5 different rectangular waveforms (monophasic, biphasic, biphasic with interphase gap, asymmetric biphasic, asymmetric biphasic with interphase gap) that have shown potentially useful effects in other studies 26 have been investigated. Bipolar stainless-steel loop electrodes were placed under the common peroneal nerve of rats under buprenorphine/isoflurane anaesthesia. The isometric force produced by the extensor-digitorum-longus muscle was measured using a load-cell. Our results did not support several of the claims made in the literature. Introducing an interphase-gap generally increased the stimulating currents necessary to reach a certain force-threshold. This was also the case for asymmetric waveforms. The standard symmetric biphasic pulse was the most energy-efficient, out of all tested waveforms. The differences between this experiment and other studies reflect different stimulation methods (mostly monopolar), stimulation patterns (mostly pulse trains) or stimulation sites (e.g. cochlear stimulation, epiretinal stimulation). But they emphasise that the specific relationship between the means of the injection of stimulation energy and the measured outcome need to be considered when efficiency or efficacy of stimulation is discussed. The results from this experiments were used to propose an algorithmic approach for finding stimulation parameters that yield a particular energy-minimum for a certain targeted force-level. These parameters provide a starting point for further parameter fitting sessions in clinical settings where electrical stimulation is applied.


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MiniViStim18B: A new member in the family of FES implants for small animals goes towards denervated muscles

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According to PubMed roughly 10% of the annually added publications are describing findings from the animal model. Half of these studies are done in mice and rats. It can be assumed that there is a need for implantable electrical stimulators which are flexible, reliable and small enough (~1 cm³) that even mice can tolerate it and move freely. The MiniVStim 12A is a battery powered implant with an outer diameter of 15 mm and a volume of 1.2 cm³. It can be preprogrammed according to the experimental protocol and controlled by resetting it with a magnet. It can deliver constant current monophasic pulses up to 2 mA and 1 ms pulse width (@1 kOhm). MiniVStim 12B has the same mechanical dimensions and electrical characteristics but can be fully programmed via a wireless bidirectional data link. Both types of implants are already successfully used in studies. The latest generation of implants is the MiniVStim1BB. It is slightly larger (22 mm outer diameter) than the 12B but offers the 8 fold battery life time. Moreover, it can deliver biphasic pulses and extends the stimulation parameter range up to 8 mA at a maximum output voltage of 10 V and pulse width of 5 ms (@1 kOhm) for monophasic and 2x5 ms for biphasic pulses. This extended parameter range gives the opportunity to perform long term studies on denervated muscles in small animals.

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Chronic electrical stimulation of the RLN in rodents: Results of a feasibility study
Markus Gugatschka (1), Sophie Bachna-Rotter (1), Claus Gerstenberger (1), Jonathan Jarvis (2), Michael Karbiener (1), Gerhard Friedrich (1)

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Age related atrophy of the vocalis muscle and its adjacent structures affects the voice and may lead to presbyphonia, a condition affecting more and more people in aging western societies. So far therapy modalities comprised conservative speech therapy as well as phonosurgical approaches. Chronic electrical stimulation of the afferent nerve (recurrent laryngeal nerve, RLN) is a completely new therapeutic option that has not been tested before. 18 male Wistar rats were implanted with a unilateral nerve stimulator. One week after implantation stimulation protocol was initiated over eight weeks, twice daily. Changes were observed on the muscular level as well as on the cellular level (immuno-histochemically and qPCR). All animals tolerated the stimulation procedures well and showed normal feeding. We could not identify differences in cross-section area, number of muscle fibers, or satellite cells. We identified a trend towards increased number of neuro-muscular junctions which failed slightly statistical significance. Our pilot study proved the technical feasibility of the implantation of a RLN pacemaker system in small rodents. We could not identify significant changes after eight weeks of stimulation in any parameter. We assume that this is due to the young age of the test animals. The next step will be to test these protocols in aged rats.

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Chronic neurostimulation of the aged larynx - first results from a large animal study
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Muscle atrophy is a hallmark of the ageing process and as such also affects the larynx, where it constitutes the major cause of presbyphonia, i.e. a considerable glottic gap with massive loss of air during phonation. Patients suffer from a highly hoarse voice and rapid vocal fatigue which leads to social withdrawal. Current treatment options are mainly conservative (e.g. speech therapy) and far from being satisfactory. Thus, novel approaches for promoting hypertrophy of aged laryngeal muscles are in demand. In this respect, electrical stimulation of motor neurons constitutes a promising strategy.

Using aged sheep as an animal model, we recently completed a first efficacy study in which chronic electrical stimulation of laryngeal muscles was accomplished via a mini-electrode that targeted the right recurrent laryngeal nerve (RLN; unilateral stimulation). Based on preceding experiments, functional electrical stimulation (FES) implants were programmed to deliver a pattern able to evoke supramaximal muscle stimulation over a period of 29 days. Surgical and post-surgical interventions were well tolerated by all animals, FES implants remained functional throughout the whole study, and only relatively mild increases in the necessary threshold for muscular stimulation were recorded. At the end of the study, vocalis and posterior crico-arytenoid muscles were prepared to obtain samples for molecular and histological analyses. To quantify the expression levels of genes related to distinct muscle fiber types, a real-time PCR (RT-qPCR) analysis pipeline was newly established which will also be central to subsequent studies. First results of the analysis “at transcript level” will be discussed in the talk. Moreover, an outline of the planned further analyses will be presented.

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Vagus stimulation and brain ischemia, an interim report
Marco Patruno (1), Luca Denaro (2), Giorgio Gioffrè (2), Giulia Melinda Furlanis (2), Tiziana Martinello (1), Laura Cavicchioli (1), Giovanni Caporale (1), Hermann Lammluer (3), Jonathan Jarvis (4), Domenico D’Avella (2)

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Stroke is a leading cause of adult disability and it is constantly increasing due to growing life expectancy and unhealthy lifestyles. Fibrinolysis is the main therapy for ischemic stroke, but it has a narrow therapeutic window. Neuroprotective treatment strategies that may have the potential to reduce damage or to enhance regenerative aspects are under investigations in many experimental models. The aim of this study was to evaluate the neuromodulation effect induced by vagus nerve stimulation (VNS) in an animal model of focal cerebral ischemia, using a novel stimulator device. Ischemia was achieved by transient proximal middle cerebral arterial occlusion in XX rats with a nylon monofilament introduced in the internal carotid artery. The vagus nerve stimulator was housed subcutaneously in the abdomen and tunnelled up to the cervical part of the nerve. All rats were clinically examined and half of them were stimulated everyday for 2 weeks. At post-mortem brains were fixed to perform histological, immunohistochemical and molecular analyses. The neurological examination revealed an impairment in all treated rats. The damage seemed to be more consistent in not stimulated rats. Histological examinations did not show classic signs of CNS ischemia, whilst immunohistochemical analyses revealed higher GFAP and IBA1 expression in treated ischemic rats in comparison to sham animals. Molecular microarray analysis revealed differences in mRNA expression in many cellular pathways (neuroactive ligand-receptor interaction for example) between controls and ischaemic groups, although the investigations are still...
underway. Our preliminary data indicate that 2 weeks of VNS is feasible without any side effects, although the behavioral improvement needs to be studied deeply and with a greater number of animals. To sum up, our brain ischemia animal model should be improved since histological evidence of necrosis has not been achieved yet; however, immunohistochemical and molecular results highlight a putative neuroprotective action of the applied VNS device.

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Diagnostics and management of human denervated muscle: Rehabilitative approach
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Rehabilitation of patients suffering of muscle denervation is still a challenge for clinicians. In past years the treatment consisted essentially in prescription of orthotics and/or walking aids. This approach is still useful but recently, electrical stimulation for denervated muscle showed the possibility to evoke muscular tonic contractions and recover muscle trophism also in long term denervation. This offers the chance to include electrical stimulation in diagnostics, treatment and follow up evaluations of this condition. In this presentation, based on an example case of neuralgic amyotrophy, we discuss the rehabilitative strategies and propose our clinical approach to this kind of patients.

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Electrical stimulation in neuromuscular disorders: A case suffering with Parsonage-Turner syndrome
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The neuralgic amyotrophy (NA) or Parsonage-Turner syndrome is a rare disease of the peripheral nervous system characterized by sudden onset of severe pain in the upper limbs, followed by rapid weakness, atrophy and motor slow remission, which can last months to years. It has an idiopathic and hereditary form. The NA occurs at any age, but is most common in the 3-7 decade of life and among men. Some patients experience a relapsing/remitting course with symptom-free intervals while others have an incomplete recovery with persisting neurologic deficit. In 50% of cases, the NA is associated with a point mutation or duplication of the susceptibility gene on chromosome 17q25.3 SEPT9. Diagnosis is based on typical clinical signs and exclusion of other diseases by laboratory testing, electromyography, imaging of the cervical spine and brachial plexus. Pain control using a combination of long-acting opioids and NSAIDs associated to oral prednisone during the first weeks of an attack and accelerate recovery. Rehabilitation therapy is also important in NA. We present a case of 54 years old man with NA. When he was 49 years old, he started complaining of sudden neck pain radiating to the left shoulder and subsequent weakness of the limb. The patient presented a hypotrophy and hypotonia of flexor-extensor of the left shoulder muscles, associate a lack of tactile sensitivity of the lateral surface of the left arm, consequently the patient decide to suspend the driving. He performed laboratory tests, which were normal, an EMG of the upper limb which confirmed the presence of a peripheral nervous denervation and CT of the neck and chest that was normal. The patient was treated with oral cortisone without benefits. Due to the worsening symptoms immune globulin and cortisone were administered and the patient began physiotherapy which was later associated with muscle electrostimulation (ES), massage and physiotherapy obtaining a reduction in pain after 6-7 months. Patient had initially two and a half hours of ES once per day to muscle affected with exponential monophasic currents by “Siemens Neuroton 626 “device. One year and a half after, the patient was treated with “Miostim” device applying biphasic currents and associating massage, employs assisted exercises, exercises against resistance, and dynamic splints for finger). Currently the patient has only occasionally or no pain. He returned to work and restarted to drive. He has mild weakness bilaterally on extensors of the wrists (4/5), more to the left arm. On the left the patient has also a deficit of the flexor muscles (4/5) and the drt are hyporeflexive. The prognosis of NA is variable: usually patients recover 70-90% of their previous motor function after 1-2 years. In general, the result in our patient is satisfactory. This case shows a successful treatment including physical therapy and ES in patients with NA.


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The Vienna and Padua strategies for stimulation of human muscles in neuromuscular disorders and aging
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The Vienna Strategies for Functional Electrical Stimulation (FES) of human muscles in neuromuscular disorders and aging were inspired by conventional protocols of neuromuscular stimulation (usually called NMES, Neuro Muscular Electrical Stimulation), but succeed to be applied to the extreme case of degenerating muscles due to long term irreversible denervation when some of the dogma of NMES/FES were violated.1,4 Long term denervated muscle undergo several stage of excitatory and contraction disorders that call for specialized stimulation protocols, in particular long stimuli in the range of 100-300 msec, that is hundreds of time long impulses. However, when excitability is recovered, shortening of the impulse allow to reach the most desirable sustained contraction by train of impulses delivered for 1-2 sec at 30Hz and thus Functional
movements of the legs. That is achieved only if the inter-pulse OFF
time is shortened to 10 msec and the pulse to 30-40 msec. We
would like to stress that such a pattern of stimulation is very
different from the conventional stimulation pattern of innervated
muscle (that is, < 1 millisec at 50-100 Hz) and thus it can be used to
demonstrate that the responding muscle are NOT reinnervated.
Anyhow, protocols of progressive loading of the muscles can be
thereafter prescribed to increase muscle resistance and strength.

As to the stimulation of human muscle in aging,5–8 it is here enough
to say that the conventional NMES strategies for muscle resistance
and strength are applied, but increasing the safety measure to avoid
tendon, muscle and joint lesions.

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