ANNUAL REPORT

First year - PhD Program in Sciences & Medical Biotechnology
University of Piemonte Orientale "Amedeo Avogadro", Novara

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PART 1: Scientific activity

PART 2: Didactic and educational activities

PART 1: Scientific activity

Project title:
MULTIMODAL STUDY OF EPILEPTOGENIC NETWORKS IN EPILEPSY

Scientific background

Epilepsy is one of the most common serious brain disorders characterized by recurrent seizures. In Italy 30000 new cases are diagnosed each year, with an incidence higher in infants and elderly people. Around 500000 people are affected. Epilepsy has negative effects on quality of life, function, and increases risk of mortality despite available treatments (Duncan et al., 2006). It is refractory to the treatment in about one-third of cases and the mechanisms underlying this drug resistance are not understood.

The current understanding of the pathophysiology of epilepsies relies on the "system hypothesis" that goes beyond the classical dichotomy between focal and generalized epilepsy: some types of epilepsy may depend on the susceptibility to epileptogenic factors within a specific brain system (Avanzini et al., 2012). Unravelling how the epileptogenic process is affecting the brain networks will invaluably advance our understanding of drug resistant epilepsy and will lead to development of improved therapeutic perspectives. The epileptogenesis is a complex dynamic process that progressively alters neuronal excitability, establishes critical interconnections and requires intricate structural changes before the first spontaneous seizure occurs (Pitkanen and Lukasiuk, 2011). In addition, during epileptogenesis may occur maladaptive plastic processes ('pathological plasticity'). It begins after the occurrence of an insult (eg. traumatic brain injury or stroke), or even during the insult (prolonged febrile seizure, status epilepticus, or encephalitis). Changes at the molecular level ultimately translate into an unbalance between excitatory and inhibitory neural circuits leading to cortical hyperexcitability in specific networks.

Transcranial magnetic stimulation (TMS) is a well-established, safe, painless and non-expensive neurophysiologic method for non-invasive measurement of cortical excitability (Badawy et al., 2014). This relies on several physiologic variables, which, over the past 25 years, proved much informative in terms of both physiology and disease in epilepsy (Badawy et al., 2014). Furthermore, a variety of TMS protocols have been developed to probe mechanisms of synaptic plasticity in the intact human brain. Plasticity induced by TMS can be modulated by pharmacological interventions, targeting ion channels, or neurotransmitters (Hamada et al., 2012; Strigaro et al., 2014).

On the other hand, molecular changes can include alterations in gene-expression patterns, intracellular signalling and synaptic function (Pitkanen and Lukasiuk, 2011). The latter is regulated by different neuropeptides along with classic neurotransmitters (Kovac and Walker, 2013). Ghrelin is a novel neuropeptide (Kojima et al., 1999) which has a primary role in metabolism and stimulates the release of growth hormone by the pituitary gland (Kovac and Walker, 2013). Ghrelin circulates in the bloodstream in two forms: acylated ghrelin (AG) and unacylated ghrelin (UAG). Interestingly, recent studies performed in vitro or in animal models of epilepsy demonstrated a clear anticonvulsant and neuroprotective action of ghrelin (Portelli et al., 2012). Thus, an impairment of ghrelin signalling could be involved in the

pathophysiology of some forms of epilepsy, drug resistance and pathological plasticity, opening new treatment strategies.

Rationale of the study

To increase understanding into the specific networks involved in the pathophysiology of different types of epilepsy, we propose a multimodal approach involving both extensive neurophysiological studies and molecular studies on patients with epilepsy. First, we will study patients with photosensitive epilepsy since they represent a "model" of system epilepsy. Later, we will focus on patients with drug-resistant epilepsy to unravel the neurophysiological and molecular basis of the epileptogenic networks refractory to the pharmacological treatment. From this, we aim to define specific neurophysiological phenotypes which may be the expression of unique molecular alterations and translate the findings into clinically useful parameters. Further investigation into the pathophysiology of these diseases would increase understanding into the ictogenesis of human epilepsies and the neural networks involved and eventually open new therapeutic targets.

Subjects and Methods

Thirty patients with epilepsy will be recruited from the Epilepsy Clinic at the Department of Neurology, ″Maggiore della Carità″ Hospital of Novara. Patients with a diagnosis of photosensitive epilepsy and drug-resistant epilepsy will be selected. The diagnosis of epilepsy will be made on the basis of the clinical history, imaging, and electroencephalography (EEG) findings by two experienced epileptologists not involved in the present study, according to the ILAE criteria.

Thirty healthy volunteers matched for age, sex and BMI will be recruited as controls. All neurophysiological tests will take place in the afternoon, between 14.00 and 17.00 and will be performed according to the published safety guidelines (Rossi et al., 2009).

Neurophysiological study

Cortical excitability pattern of patients with epilepsy: patients and controls will be tested with single and paired pulse TMS to investigate motor cortex excitability. A set of abnormal measures will be provided for each subjects and later will be investigated a correlation between the neurophysiological data and the molecular data.

Several TMS measures will be used to investigate different epileptogenic networks. The brain functional connectivity will be assessed through a multimodal approach involving single pulse and repetitive TMS. I have already developed a novel TMS protocol to study the visuomotor functional connectivity with milliseconds resolution as part of my year of fellowship at the Sobell Department of Motor Neuroscience at the Institute of Neurology, University College London supervised by Prof. J. Rothwell (Strigaro et al., *in revision*). Other tests will include the evaluation of the interhemispheric connectivity (interhemispheric inhibition - IHI) (Ferbert et al., 1992) and the excitability of the visual network by means of paired flash visual evoked potentials (PP-VEPs), recently described in healthy subjects (Cantello et al., 2012). Finally,

the synaptic plasticity will be studied by means of repetitive TMS paired associate stimulation (PAS) (Hamada et al., 2012; Strigaro et al., 2014).

Molecular study

Fasting AG and UAG levels in all subjects and circulating drug levels in patients will be evaluated at 8.00-8.30 a.m. following an overnight fasting. Blood plasma samples for AG e UAG measures will be collected in tubes containing EDTA and a protease inhibitor (pepstatin A 10µM), centrifuged at 3000 rpm for 15 min (temperature = 4°C), then supernatans will be stored at -20°C. Samples for AG determination will be also added of 1mM of p-hydroxymercuribenzoic acid (PHMB) to prevent AG degradation by protease, acidified by the addition of 1N HCl, then stored at -80°C. AG and UAG levels, expressed in pg/ml, will be measured separately by ELISA kits in according to the manufacturer's instructions (BioVendor - Laboratorni Medicina a.s., Brno, Czech Republic). Sensitivity will be 0.8 pg/ml and 0.6 pg/ml for AG and UAG, respectively. This part will be performed in collaboration with the Laboratory of Pediatrics, University of Piemonte Orientale "A. Avogadro".

Preliminary results

Neurophysiological study

The preliminary results indicate that a widespread epileptogenic network beyond the visual system is affected in photosensitive epilepsy. The visual system itself showed an intrinsic defective inhibition (Strigaro et al., 2012). Additionally, a distant area, i.e. the motor cortex, showed an abnormal synaptic plasticity, revealed with the paired associative protocol, supporting the role of sensorimotor networks in the genesis of motor symptoms (myoclonic jerks) in photosensitive epilepsy (preliminary data). However, since most of the patients were undergoing a successful antiepileptic treatment, it is not possible to rule out a potential confounding effect of the drugs on these parameters.

Although there are no direct anatomical connections between visual and motor cortex, we described an inhibitory functional visuomotor connectivity in normal subjects (Strigaro et al., in revision) studied by means of paired TMS. This novel protocol, applied to the patients, showed a defective inhibition in the visuomotor connectivity that may be involved in the pathophysiology of photosensitivity (Strigaro et al., in preparation). These abnormalities survived the drug regimen. Consequently, paired TMS, applied to the study of visuomotor connectivity, may represent a potential neurophysiologic test in preclinical/clinical studies of photosensitive epilepsy.

Recently, non-invasive brain stimulation techniques (i.e. transcranial direct current stimulation, tDCS) were shown to modulate cortical excitability and are currently explored as therapeutic tools in several neurological disorders, including migraine and epilepsy (Di Lazzaro et al., 2014). If impaired neural inhibition is a pathophysiologic factor of photosensitive epilepsy, an effective therapeutic tool would be expected to normalize this defect. Thus, we studied the effects of occipital tDCS on PP-VEPs in healthy subjects (Strigaro et al., 2014). Although tDCS is transiently modulating the visual cortical excitability and auditory mismatch negativity as recently demonstrated (Chen et al., 2014), the pilot study on normal subjects failed to

demonstrate significant inhibitory effects which could be used later in patients to revert the excitability to normal levels (Strigaro et al., 2014). Further methodological studies are needed to improve polarization efficacy.

Molecular study

Fifty-six patients were recruited: 19 with idiopathic generalized epilepsy, 18 with cryptogenic focal epilepsy and 19 with symptomatic focal epilepsy. Twenty-six healthy subjects of similar age, sex and body mass index (BMI) acted as controls. AG and UAG levels were measured following an overnight fasting and contrasted to the clinical and biometric features.

AG and UAG levels were similar between patients and controls. The AG/UAG ratio was higher in patients, also when weighted for covariates (age, BMI, gender, drugs). Splitting patients according to their epileptic syndrome, drug-resistance or AED number/type resulted in no significant difference in AG, UAG or their ratio. Yet, AG and UAG levels were positively predicted by disease duration, independently by confounders. In conclusion, in adult patients with epilepsy, interictal ghrelin levels did not differ from controls, though the AG/UAG ratio was imbalanced. Interpretation of the latter phenomenon is uncertain. Further, levels of AG and UAG were in direct proportion to disease duration, which may represent a long-term compensatory mechanism, antagonistic to the epileptic process (Varrasi et al., 2014).

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CONGRESSES AND TRAVEL GRANTS

- Riunione policentrica in Epilettologia, January 30-31, 2014, Roma, Italy
- 59° National Congress of the Italian Society of Clinical Neurophysiology, May 14-17, 2014, Milano, Italy.
- 37° National Congress of the Italian League Against Epilepsy, June 4-6, 2014, Trieste, Italy. Travel grant for authors of the best selected orals
- XLV Meeting of the Italian Neurological Society, October 11-14, 2014, Cagliari, Italy. Travel grant for authors of the best selected orals

ORAL COMMUNICATIONS

- "*Transcranial direct current stimulation effects on single and paired flash visual evoked potentials*". 59° National Congress of the Italian Society of Clinical Neurophysiology, May 14-17, 2014, Milano, Italy
- "*Connettivita' visuo-motoria nelle epilessie fotosensibili*". 37° National Congress of the Italian League Against Epilepsy, June 4-6, 2014, Trieste, Italy.
- "*Transcranial direct current stimulation effects on single and paired flash visual evoked potentials*". XLV Meeting of the Italian Neurological Society, October 11-14, 2014, Cagliari, Italy.
- "*Visuo-motor connectivity in photosensitive epilepsies*". XLV Meeting of the Italian Neurological Society, October 11-14, 2014, Cagliari, Italy.

PUBLICATIONS (2014)

1. **Strigaro G**, Cerino A, Falletta L, Mittino D, Comi C, Varrasi C, Cantello R. Impaired visual inhibition in migraine with aura. *Submitted. In revision.*
2. **Strigaro G**, Ruge D, Chen JC, Marshall L, Desikan M, Cantello R, Rothwell JC. Visuomotor functional connectivity: a TMS study. *Submitted. In revision.*
3. **Strigaro G**, Mayer I, Chen JC, Cantello R, Rothwell JC. Transcranial Direct Current Stimulation Effects on Single and Paired Flash Visual Evoked Potentials. *Clin EEG Neurosci.* 2014 Sep 23.
4. **Strigaro G**, Hamada M, Murase N, Cantello R, Rothwell JC. Interaction between different interneuron networks involved in human associative plasticity. *Brain Stimul.* 2014 Sep-Oct;7(5):658-64.
5. Varrasi C, **Strigaro G**, Sola M, Falletta L, Moia S, Prodam F, Cantello R. Interictal ghrelin levels in adult patients with epilepsy. *Seizure.* 2014 Jul 19.
6. Lochner P, Cantello R, Brigo F, Coppo L, Nardone R, Tezzon F, Raymkulova O, **Strigaro G**, Comi C, Leone MA. Transorbital Sonography in Acute Optic Neuritis: A Case-Control Study. *AJNR Am J Neuroradiol.* 2014 Jul 17.

7. Chen JC, Hämmerer D, **Strigaro G**, Liou LM, Tsai CH, Rothwell JC, Edwards MJ. Domain-specific suppression of auditory mismatch negativity with transcranial direct current stimulation. *Clin Neurophysiol.* 2014 Mar;125(3):585-92.
8. Badawy RA, **Strigaro G**, Cantello R. TMS, cortical excitability and epilepsy: The clinical impact. *Epilepsy Res.* 2014 Feb;108(2):153-161.

PART 2: Didactic and educational activities

- Seminars and lessons of the PhD Program at the University of Piemonte Orientale, Novara
- Seminars at the Department of Neurology, University of Piemonte Orientale, Novara
- Course on "*HORIZON 2020, Programma Quadro Europeo per la Ricerca e l'Innovazione (2014 - 2020)*", March 25, 2014.
- Meetings "*Bando Cariplo 2014*", April 2014, University of Piemonte Orientale, Novara
- *"Aggiornamenti in Neuroriabilitazione".* April 8, 2014, Fondazione Maugeri, Veruno, Italy
- *"Course on advanced neuroimaging: clinical application"*. XLV Meeting of the Italian Neurological Society, October 11-14, 2014, Cagliari, Italy.
- From October 2014, teaching appointments at the "Neurology course" of the School of Physiotherapy and School of Medicine, University of Piemonte Orientale, Novara
- Contribution to the application to the call for proposals "Fondazione Cariplo 2014, biomedical research conducted by young researchers" funding program – Call 2014" with a project titled "ERMES: Epilepsy Research and MEtabolism Study".

October 24, 2014 Gionata Strigaro