PhD Day

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PhD Program in Applied Biotechnologies
Flavonoids are secondary metabolites that play important roles in determining grape and wine quality. They are synthesized by the phenylpropanoid biosynthetic pathway that is finely regulated by a conserved MBW complex, including a MYB and a bHLH transcription factor, and a WD40 regulatory protein (Holton and Cornish, 1995). The MYB proteins are responsible for the specificity of the complex to activate different branches of the pathway. In grapevine the direct role of some MYB factors in flavonoid synthesis regulation was functionally demonstrated (Kobayashi et al., 2002; Kobayashi et al., 2004; Walker et al., 2007; Bogs et al., 2007; Terrier et al., 2009; Czemmell et al., 2009). VvMYB5a and VvMYB5b, identified as general regulators of the early flavonoid structural genes (Deluc et al., 2006; Deluc et al., 2008), have been recently studied in Petunia heterologous system demonstrating their role in anthocyanin synthesis regulation and in other cellular processes involving vacuolar acidification (Cavallini et al., 2014). Both of these MYBs belong to a cluster that includes AtMYB5 and PhPH4 that are involved in the control of different processes through the activation of a WRKY transcription factor, AtTTG2 and PhPH3 respectively (Gonzalez et al., 2009; Li et al., 2009; Quattrocchio et al., 2006). The closer homolog of PhPH3 and AtTTG2 in grapevine is VvWRKY26, and complementation analysis in petunia ph3 mutants demonstrated that VvWRKY26 can fulfil the function of the endogenous WRKY in the regulation of vacuolar pH (Cavallini, 2012). Moreover, transgenic grapevine with an altered expression of VvMYB5a, VvMYB5b and VvWRKY26 were obtained and in the VvMYB5a/VvMYB5b silencing plants VvWRKY26 expression resulted significantly down-regulated, suggesting that this WRKY gene acts downstream of VvMYB5a/5b (Cavallini, 2012).

This project aims to functionally characterize the regulatory network involving VvMYB5a, VvMYB5b and VvWRKY26. The final goal will be the dissection of the putative linkage between phenylpropanoid biosynthetic pathway and vacuolar acidification pathway in grapevine that is probably reserved to VvWRKY26. Consistently with this working hypothesis a preliminary microarrays analysis on petunia ph3/35S::VvWRKY26 revealed that VvWRKY26 regulates genes involved in both flavonoids synthesis and in vacuolar homeostasis. During next years, I will better characterize the transgenic grapevine with an altered expression of VvMYB5a, VvMYB5b and VvWRKY26 in order to identify target genes of the regulatory network. These putative targets will be analyzed by a transient petunia leaves protoplast co-transfection and Dual-Luciferase assay.
Grapevine (*Vitis vinifera*) is one of the world’s largest fruit crop with a production of more than 69 million tons (Statistical report on World Vitiviniculture 2012, OIV) and relevant economic impact in different sectors. Unfortunately all cultivars currently used for grape or wine production are susceptible to pathogens. Although several natural resistant sources are available, breeding effort to obtain a resistant cultivar with good organoleptic quality did not lead to significant achievements (Cadle-Davidson L., 2008).

Our laboratory is focused on the study of the interaction between *Vitis* spp. and *Plasmopara viticola*, the causative agent of Downy mildew, with the aim to increase grapevine resistance toward this pathogen.

In a previous microarray experiment we analyzed the differentially modulated genes after the infection of both a susceptible (*Vitis vinifera* cv. Pinot Noir) and a resistant species (*Vitis riparia* cv. Gloire de Montpellier) with *P. viticola*. The results showed many differentially modulated genes; among them, a small group of 8 genes belonging to the ATL family appeared to be particularly interesting since highly induced only in the resistant species.

On the basis of these results, and according to the information present in the literature, we selected a candidate gene to be used in the stable transformation of *V. vinifera* cv. Shiraz. During this year we have been able to regenerate 25 grapevines overexpressing the putative ATL2 derived from *V. riparia*, the resistant species. Plants are now ready for the molecular characterization and for the phenotyping of their resistance towards *P. viticola* and other pathogens or stress conditions.

In the meantime, trying to clarify the differential response of the putative ATL2 in *V. vinifera* and *V. riparia* to *P. viticola*, we started studying the regulative regions of the candidate gene in both species by functional studies in model plants: transient transformation of *Nicotiana benthamiana* by agroinfiltration and stable transformation of *Arabidopsis thaliana* by floral dipping. Preliminary results showed the ability of both regulative regions to promote the transcription of reporter genes (GUS and GFP) in a heterologous system.
RNA-Sequencing (RNA-Seq), an approach based on deep sequencing technology, allowed many advances in transcriptome characterization. First step in RNA-Seq workflow is library preparation. Standard methods do not preserve information about which strand is originally transcribed. Because there are a lot of antisense transcripts and many genes are overlapping, it became necessary to distinguish strands in order to define gene boundaries, determine exactly gene expression levels and above all, do a good genome annotation. Several methods were developed to perform directional libraries but only two are functional: 2nd strand marking method and adapter ligation based method. Here we want to demonstrate that is necessary to have stranded libraries to determine genes orientation and we wanted to test different strategies to perform directional libraries to find the more suitable for each situations. So we compared standard and directional Illumina protocols using a sample of RNA of *Arabidopsis thaliana* and then we compared Illumina directional protocol (based on 2nd strand marking method) and Lexogen protocol (based on adapters ligation method). The results indicate that only with directional library is possible to discriminate gene orientation and to resolve overlapping genes, so it is necessary to use a directional protocol to do a good genome annotation. Also it seems that for our kind of sample is better to use a directional protocol based on 2nd strand marking method, like Illumina protocol.
Development of a cyanate-based wheat allergoid for the treatment of baker’s asthma

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Wheat is a staple food for world population due to its agronomic adaptability, easy conservation of grains, nutritional quality and technological versatility of the products obtained by grinding. In spite of these positive traits, it is well known that several proteins expressed in modern wheat \textit{(Triticum aestivum, Ta)} are capable of inducing allergic reactions such as food allergy or inhalant disease. The most occupational inhalant allergy is known as “Baker’s asthma” (BA), caused by several proteins and, in particular, by a groups of water/salt soluble proteins called α-amylase inhibitors. There are different solutions to treat wheat allergy: the easiest way is to avoid the ingestion of wheat-based foods. A second possibility could be the intake, where possible, of hypoallergenic substitutes. To this purpose, ancestral forms \textit{(Triticum monococcum, Tm)} seem to be promising, since not expressing specific allergenic proteins (e.g. omega gliadins). A third approach could be specific immunotherapy, that consists in the administration to patients by intradermic or sublingual via of increasing doses of modified allergens. The aim of this project is the production and the characterization of a cyanate-based allergoid to be employed in the future Specific Immunotherapy (SIT) for the treatment of BA, since from our experiments the use of \textit{Tm} did not behave as hypoallergenic for this patients population. The flour extract (FE) and the allergoid (K-MFE) were analyzed by IgE immunoblotting and ELISA inhibition in order to evaluate the residual IgE-binding capacity of treated proteins (using 14 patients’ sera affected by BA). The modification of lysine with Potassium Cyanate strongly affected linear epitopes harbored in wheat proteins and ELISA gave a reduction of the IgE-binding capacity of K-MFE of nearly 50% of the naïve FE proteins. To verify whether the K-MFE could still raise a specific immune response, this was used to immunize a group of mice. The immunization with FE or K-MFE developed antibodies specific for different wheat proteins, but only the mice group treated with K-MFE presented antibodies capable of binding the major allergens α-amylase inhibitors: probably the modification induced by Potassium Cyanate had somehow made these proteins differently immunogenic (in mouse). The immunized mouse serum was used for an immunoblotting inhibition experiment co-incubating a pool of patients’ sera. The experiment showed that mice IgG competed with human IgE, indicating that the epitopes recognized are similar. Since one of the goals of SIT is the induction in the host of IgG that compete with IgE for allergen binding, these results could be considered the “proof of concept” that immunization via KOCN-modified flour extract produce protective IgG capable of interfering with the IgE-recognition of allergens, among which, the α-amylase inhibitors.
Towards the optimization of an automated pipeline for genome functional annotation

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The last decade has seen the beginning of the post-genomic era, when the revolution in sequencing technologies has led to the accumulation of a large amount of genomic sequences in public databases. Genome annotation is a crucial step for downstream data analyses and experimental studies, as it is the process of identifying the locations of biological “features” and determining their biological meaning, from both a physical and functional standpoint. Indeed it is divided in structural annotation, that is the identification of genomic elements on the genome, such as coding regions, and functional annotation, the process of assigning to protein coding genes a biological function, a metabolic role or structural features. At the moment, even considering the most well-studied organisms many genes are still uncharacterized, since the process of experimentally determining the role of a protein is a complex and time consuming task. As the number of sequenced genomes rapidly grows, the large amount of data produced can be processed computationally in order to achieve higher speed and throughput. Over the past two decades, many computational methods for predicting function have been developed, based on information coming from sequence similarity, structural features, genomic-context or a combination of different algorithms and multiple data sources and then modeling of the results.

Given this plethora of annotation tools, the aim of this work is to assess their performance in order to set up an optimal pipeline exploiting a fully automated approach. The evaluation of prediction accuracy will rely on a experimentally annotated dataset from Arabidopsis thaliana. No methodology for assessing the accuracy of function prediction programs has yet been developed, therefore the here presented assessment will hopefully provide insight not only into the ability of selected tools to characterize proteins functionally, understanding the strengths and weaknesses of any individual tool, but also for the field in general and might guide future biological experiments.
The hypersensitive response (HR) is a plant defense mechanism against pathogens which leads to the activation of a programmed cell death at the attempted sites of infection, aiming to restrict pathogen infection and spread. During the HR a set of defense related genes is induced and a rapid accumulation of reactive oxygen species (ROS) and nitric oxide (NO) have been shown, which have both been previously demonstrated to be involved in triggering the localized HR-cell death (Delledonne M, 2005). Nevertheless the signaling mechanism underlying this process is still largely unknown. To identify genes involved in this signaling mechanism a forward genetic screening is being performed in our Lab. Mutant lines impaired in NO-induced cell death are first identified by NO fumigation. Then, as a second step, mutant lines impaired also in HR-cell death induced by avirulent pathogen are being identified among pre-selected lines. Once mutant lines impaired in NO-mediated cell death in plant defense will be selected, they will be further characterized at biochemical, molecular and physiological level, in processes related to HR development, to finally confirm that mutation specifically affect NO signaling during HR-cell death. Identification of genetic location of causal mutation will be undertaken for best candidates to identify genes involved in NO signaling during HR-cell death.
Physiological and molecular characterization of magnesium deficiency in grapevine

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Magnesium (Mg) is an essential mineral macro-nutrient for plants. Despite its relevant biochemical and physiological role for plant little information is available regarding the effects of its deficiency on crop species and the uptake from soil. In grapevine, one of the most economically important fruit crop in the world, Mg deficiency often occurs in acidic and sandy soil and in the presence of high quantity of exchangeable potassium, with a negative effect on quantity and quality of the final products. The aim of this work is the comparison of the physiological and molecular responses to Mg starvation of two grapevine rootstocks, 1103 Paulsen (Vitis berlandieri x Vitis rupestris) and SO4 (Vitis berlandieri x Vitis riparia). The two rootstocks are reported as tolerant and susceptible to the Mg deficiency in field respectively. Analyses of growth and physiological parameters performed after 4 weeks of starvation confirmed field observations regarding the different tolerance of the two grapevine genotypes. The Mg deficiency caused a reduction in SPAD index only in the SO4 genotype. The increase of the shoot/root ratio under starvation was recorded only in the susceptible rootstock as previously observed in other plant species (e.g. common bean and sugar beet).

Focusing on the effects of Mg deficiency on the levels of phosynthates in leaf and root tissues, considered to be an early symptom of Mg-deficiency, we observed a statistically significant difference only in sucrose concentration in basal leaves of Mg-deprived plants of both rootstocks. Sucrose accumulation in basal leaves was then correlated to expression analysis in these tissues of four genes encoding putative sucrose transporters (transporters SUC11, SUT1, SUC12, SUC 27 and SUT2). Real-time RT-PCR experiments showed that only SUC12 and SUC27 were expressed in basal leaves and that SUC27 was downregulated under Mg deficiency. This expression trend could suggest a possible role of this gene in phloem loading and sugar retrieval during long-distance transport as previously reported.

Seven putative Mg transporters belonging to the CORA/MRS2/ALR gene family were identified in grapevine genome. Further molecular and functional characterization will be performed in order to clarify the possible involvement of these genes in Mg uptake and transport in grapevine plants.
GSH and Trx system are involved in the adaptation of *O. oeni* into wine

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*Oenococcus oeni* is the species of lactic acid bacteria (LAB) more adapted to wine and it is mostly used for induction of malolactic fermentation (MLF). *O. oeni* faces many adverse conditions which limit its ability to survive in wine: ethanol, SO₂, phenolics, low amount of nutrients, low pH, suboptimal temperature, etc.

The redox mechanisms of response to oxidative stress, caused by ethanol above all, are little known in *O. oeni*. These mechanisms are basically the systems glutathione / glutathione reductase and thioredoxin / thioredoxin reductase, which are few known in *O. oeni* and its adaptation to the wine. Glutathione (Glu-Cys-Gly) is a nonproteic tripeptide almost universal, that in its reduced form (GSH) acts as an antioxidant, through the thiol group of cysteine, which neutralizes the ROS. Thioredoxin (TRX) is a small protein (12 kD) also present in almost all organisms. It is an oxidoreductase, which acts, such as glutathione, as an antioxidant neutralizing ROS through its active site Cys-Gly-Pro-Cys, and it also maintains many intracellular proteins in a reduced state by thiol-disulfide exchange in the cysteines.

This thesis proposes to deepen the study of these redox systems in *O. oeni* in order to elucidate and obtain information about the response of the cell studying them from different approaches.

The uptake of the GSH into the cell has been determined and the presence of this antioxidant in the media produces a higher growth of the strain even if there is some stress factor (low pH or presence of ethanol). So, GSH could be used in the preadaptation before wine’s inoculation.

This work analyzed the changes of *O. oeni* transcriptome during adaptation to wine-like conditions. The analysis of microarray results revealed new data showing the activation of the transcription of several peptidase genes. Since peptides account for the largest proportion of total nitrogen in wine, these results suggest the relevance between wine nitrogen composition and the ability of *O. oeni* to cope with its environment.

Referring to the thioredoxin / thioredoxin reductase system, it has been measured the different expression between strains and trx genes during a normal growth and during the MLF. Moreover, the presence of these genes depends on the strain studied so it could mean a higher resistance in the wine.
Probiotics, functional pasta and the gut microbiota

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Functional foods consist in products enriched with particular ingredients, such as prebiotics, probiotics, vitamins and minerals which have a peculiar role in ensuring a state of wellness and prevention of certain diseases. Since grain-derived foods, and in particular pasta, are very popular foods, they could be used as potential vehicles to introduce functional ingredients with the diet, reaching a large part of the population.

The general aims of this research project are related to the development of a probiotic-enriched pasta and the evaluation of its impact on human gut microbiota when administered to healthy overweight subjects. It is crucial that the viability and stability of the probiotic strain is maintained during processing, storage, and delivery of the final product, as well as its survival and bio-persistence during the transit through the gastrointestinal tract. Thus the goal of this study is to set up methods suitable for the specific quantification of the probiotic ingredient, i.e. the spore former Bacillus coagulans GBI-30, 6086, GanedenBC30® (BC30), both in the pasta and in the fecal samples.

A batch of experimental pasta was prepared in a pilot plant (Rustichella d’Abruzzo, Italy) using semolina enriched with a BC30 freeze-dried preparation to concentrate CFU/g. Plate count analysis of the dried and cooked pasta showed that the probiotic strain resists to the pasta-making and cooking processes, resulting in about 10⁶ CFU/g. Furthermore, PCR-based methods (conventional PCR and Real-Time qPCR) were applied for detecting the probiotic strain directly in the cooked pasta, without a preliminary cultivation step. The obtained results were consistent with those achieved from plate counts.

To study the gut microbiota of the volunteers that will receive the probiotic pasta in a randomized, parallel trial, a preliminary evaluation of the primer pairs to be used in Real-Time qPCR analysis of the fecal microbiota was carried out. The primer pairs available for any interesting bacterial genus related to the most important gut phyla (Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, Verrucomicrobia) and targeting the 16S rRNA gene, were searched in literature. Then, to select the proper primer pair for each bacterial group, a bioinformatic tool for taxonomic analysis, TestPrime, was used. This approach revealed often an ambiguous or incorrect primer specificity definition. Thus a more clear and rigorous primer specificity assessment in Real-Time qPCR studies is strongly recommended to guarantee a correct results interpretation and to allow the comparison between results from different studies.
Ammonium transport in *Zea Mays* roots:

* a physiological and molecular characterization

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In most soils, NH$_4^+$ and NO$_3^-$ are the predominant sources of N that are available for plant nutrition. Although the average NH$_4^+$ concentrations of soils are often 10-1000 times lower than those of NO$_3^-$, the difference in soil concentrations does not necessarily reflect the uptake ratio of each N source. Many plant species show a preference towards the absorption of NH$_4^+$, as the process of assimilation does not require a reduction reaction, like the case of NO$_3^-$. Nitrogen nutrition based solely on NH$_4^+$ on the other hand has deleterious effects on vegetative growth (reduction of above-ground and below-ground biomass) and the absorption of other cations. Optimal plant growth is usually achieved when N is supplied in both forms. Previously works performed in rice and Arabidopsis have been shown that the rate of absorption of NH$_4^+$ as a function of the external concentration of substrate is biphasic, following Michaelis-Menten kinetics at low concentrations (< 1 mM) and linear kinetics at higher concentrations, revealing the existence of two transport systems with high affinity and one low affinity respectively. Members of ammonium transporter/methylamine permease/rhesus (AMT/MEP/Rh) family are involved in NH$_4^+$ transport at concentration below 1 mM. In *Arabidopsis thaliana* 5 members of the AMT1 subfamily were identified. The genes *AtAMT1;1*, *AtAMT1;3* and *AtAMT1;5* are mainly expressed in the root of N-deficient plants and their activity contributes predominantly to the high-affinity transport of NH$_4^+$. The purpose of this work is the biochemical and molecular characterization of NH$_4^+$ transport in seedlings of a maize inbred line (T250), hydroponically grown. A high-affinity transport system and a low-affinity transport system were characterized using a $^{15}$NH$_4^+$ as tracer and its detection through IRMS analysis. The influence of H$^+$ concentration of the uptake solution both on HATS and LATS was also evaluated showing that the transport rate is not dependent from H$^+$ availability. Furthermore, homology sequence analyses allowed the identification of 11 putative AMT genes in maize genome. A phylogenetic analysis suggested that three of these genes belong to the AMT1 subfamily. The analysis of the expression of these three AMT1 genes in maize roots in response to different N conditions is in progress.
Grape berry maturation can be described as a succession of physiological and biochemical changes reflecting the transcriptional modulation of many genes. Nonetheless, little is known about these transcriptional changes and their regulation. The aim of this research project is to find common transcriptomic traits in order to establish the grape berry transcriptomic route over its development, as well as to understand the physiological and biochemical differences among ten Italian cultivars by analyzing and comparing all the transcriptomes.

Ten varieties, five red and five white, were chosen among hundreds of Italian varieties for their diversity in agronomical and oenological traits, and for adaptation to different growing locations. The ten varieties were grown in the same experimental vineyard (Conegliano, Italy) and grape berry samples were collected during the same growing season (2011) at four different phenological stages, two pre- and two post-véraison, in triplicate. In total, 120 RNA samples were sequenced using Illumina HiSeq 1000 sequencer. An average of 33.4 million 100-base-long reads for each sample was obtained. The raw reads were aligned onto the 12X version 1 sequence of the Pinot Noir 40024 reference genome, resulting in a mapping quality of 85.3%.

By analyzing the dataset, we determined that 23,079 of the annotated genes are expressed at least in one condition, and 7,760 genes are differentially expressed in all ten varieties over berry development and ripening. A Principal Component Analysis was realized on the whole dataset. The analysis showed that during berry development, the ten varieties group, independently on their skin-color, in pre- and post-véraison phases corresponding to a high gene expression modulation during véraison. In order to establish the berry transcriptomic route over its development, a gene clustering was realized by comparing the expression level between pre- and post-véraison samples for all the 10 varieties: 975 and 182 genes were associated to the herbaceous and the maturation phase respectively. However, the Principal Component Analysis also revealed that the red and white varieties can be separated, at a transcriptomic level, at the post-véraison stages indicating skin-color-specific transcriptional programs during maturation.

In conclusion, this first RNA-Seq assay done for ten grapevine varieties helps to deeper describe the molecular route towards berry ripening. It will be also useful to find transcriptomic traits specific to either red or white varieties and to characterize the particular typicity of Italian grapevine varieties.
Genome assembly: Experimental design optimization considering the genome characteristics and the quality of a priori information

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The advent of Next Generation Sequencing (NGS) technologies opened up the possibility for researchers of sequencing genomes at a lower cost and higher throughput but none of them is able to sequence an entire chromosome, thus it is necessary to assemble the sequenced sequences to obtain the complete genome information. The methods to assemble a genome can be divided in two major categories: the de novo assembly methods, that rely only on the sequencing results as source of information, and the reference based assembly methods, which along with sequencing results take advantage of a reference genome sequences as a priori information. The results obtained with the reconstruction de novo of Pyrenochaeta lycopersici genome, a phytopathogenetic fungus, shows that for simple genomes the use of only short reads sequencing can produce results suitable for describing the gene content and the peculiarities of a species. In contrast, the same approach applied to more complex genomes, such as plant genomes, results in a highly fragmented assembly due to the higher repetitive content and a different approach must be accomplished.

The results obtained for Vitis vinifera cv. Tannat clearly illustrate this aspect. In fact, the de novo assembled genome of this variety consisted only for 10% of sequences longer than an average grape gene and thus unsuitable for any downstream genetic analysis. Since a complete assembly is available for a different cultivar of Vitis vinifera species, a reference-guided approach was followed as for Arabidopsis thaliana in Gan et al. 2011. The pipeline identifies the differences among the reference genome and the one of interest by iteratively mapping the sequenced reads on the genome and validating the results with the de novo assembled sequences. As expected, the Tannat genome was assembled in chromosomes, thus resolving the fragmentation problem, but the analysis of the gene space represented in the sequence revealed that 3035 (~10%) genes were missing from the assembly, of which 62% cultivar specific and 38% in common with the reference cultivar but not represented in the reference genome. This last result highlights two weaknesses of this approach, one due to the limited power of the algorithm in resolving long inserted regions, the other is that errors and limitations of the reference genome are also introduced as a priori information and thus affect the final genome assembly.
Dietary intake of tomatoes and tomato products has been shown to be associated with a decreased risk of chronic diseases. Tomato fruits contain several bioactive molecules, such as β-arotene and lycopene. Recently two cystine-knot miniproteins present in tomato fruit have been identified as compound bearing potential biological activity on endothelial cells at submicromolar concentrations. These tomato miniproteins (TCMP) showed an in vitro anti-angiogenic activity on human umbilical vein endothelial cells (HUVECs) without inhibitory effects on cell proliferation and viability (Cavallini C et al, BJP, vol. 162, n. 6, 2011, pp. 1261-1273). This research project is aimed at characterizing TCMPs in tomato fruits and tomato-based products and also investigating their biological action on endothelial cells in vitro and in vivo.

First we developed a protocol for the extraction and purification of TCMPs from tomato fruit and tomato paste applying in succession different chromatographic techniques. TCMPs purified from fruit was subjected to simulated in vitro digestion assays proving that TCMPs are resistant to gastric peptidases (pepsin 40 μg/ml and pancreatin 24 μg/ml). Furthermore we developed an inhibition ELISA assay to quantify TCMPs in fruit extracts and in processed products. Considering the potential of TCMPs as a nutraceutical agent, we estimated by ELISA and Western-blotting analysis the TCMPs content in the mature fruits of several tomato varieties. Only limited variations in the content of TCMP were detected in the varieties analysed.

Regarding the characterization of the biological activity of TCMPs on endothelial cells, in a previous qRT-PCR expression analysis of genes involved in angiogenesis, we found an up-regulation of VEGF-A gene in TCMP-treated HUVEC. Based on this evidence, we investigated the effects of TCMPs on endothelial cell migration, an important step in the process of angiogenesis controlled by VEGFA. The scratch wound healing assay was employed for studying HUVEC migration. We observed that TCMP inhibits by approximately 50% the increase in cell migration induced by VEGF-A and by EGF plus VEGF-A (BPS winter conference 2012). In order to further elucidate the inhibitory effect of TCMP on migration, we have focused our attention on nitric oxide (NO) an important cellular mediator involved in migration and angiogenesis. To study the influence of TCMP on NO production in endothelial cells, we used the DAF-FM method. A statistically significant decrease in fluorescent intensity of DAF-FM was revealed when HUVECs were stimulated for 30 minutes with 200nM TCMP and 10ng/ml VEGF as compared with cells treated with VEGF alone. This finding suggests that TCMP might affect nitric oxide production. To understand the possible mechanism underlying TCMPs inhibition of angiogenesis, we are testing the hypothesis that TCMPs could affect VEGF-Akt pathway that is one of the main pathways involved in endothelial cell migration. The biological activity of TCMPs will be evaluating in vivo using zebrafish-based models.
Biophysical and biochemical characterization of Arabidopsis thaliana Calmodulin 1 (CaM1) and the study of its interaction with functional targets

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Calmodulin (CaM) is an ubiquitous ~17 kDa calcium (Ca\textsuperscript{2+})-binding cytosolic protein and is one of the most conserved proteins in eukaryotes. CaM consists of two globular lobes joined by a short central linker. The binding of Ca\textsuperscript{2+} to CaM induces conformational changes in both lobes causing the increased exposure of hydrophobic Met side chains that enable CaM to associate with target proteins. CaM's central linker acts as a flexible tether to allow for the correct orientation of the two globular lobes to associate to its targets. Interestingly, in mammals a single isoform of CaM is encoded by three genes. In plants, there is evidence for 7 CaM isoforms and more than 50 CaM-like proteins (CMLs) reflecting the functional diversity of plant CaM binding proteins and suggesting their involvement in important processes for plants. Aim of our study is the biophysical and biochemical characterization of three important regions of Arabidopsis thaliana CaM1, namely the linker region, calcium binding sites, and Met rich region, in the binding and activation of A. thaliana pyridoxal 5'-phosphate (PLP)-dependent glutamate decarboxylase 1 (Gad1), which is regulated by Ca\textsuperscript{2+}/CaM1. Here, we report the protein expression and purification of 3 CaM mutants in the linker region, 2 charge CaM mutants (T80D/S82D and E85R/E88K) and 1 deleted CaM mutant (ΔE83-85). To understand how a shorter/different linker region influences CaM1 interactions and activation of the target, we will study the effect of mutations on CaM1 conformational changes upon Ca\textsuperscript{2+}-binding, thermal stability and activation of Gad1.
PhD Program in Computer Science
We consider the coalition structure generation (CSG) problem on synergy graphs, which arises in many practical applications where communication constraints, social or trust relationships must be taken into account when forming coalitions. We propose a novel representation of this problem based on the concept of edge contraction, and an innovative branch and bound approach (CFSS), which is particularly efficient when applied to a general class of characteristic functions. This new model provides a non-redundant partition of the search space, hence allowing an effective parallelisation. We evaluate CFSS on two benchmark functions, the edge sum with coordination cost and the collective energy purchasing functions, comparing its performance with the best algorithm for CSG on synergy graphs: DyCE. The latter approach is centralised and cannot be efficiently parallelised due to the exponential memory requirements in the number of agents, which limits its scalability (while CFSS memory requirements are only polynomial). Our results show that, when the graphs are very sparse, CFSS is 4 orders of magnitude faster than DyCE. Moreover, CFSS is the first approach to provide anytime approximate solutions with quality guarantees for very large systems (i.e., with more than 2700 agents).
Dictionary based approaches became commonly used in the bioinformatics field for a wide range of applications, like indexing techniques, statistical analysis or similarity metrics.

Biological sequences are described as a composition of words, called k-mers. Presence, distribution or correlation of these small pieces are used to analyse genomic sequences from local to wide behaviours. The InfoGenomics approach, by the use of dictionaries, aims to represent genome sequences by their words composition (presence/absence, multiplicities, and so on). Extending such techniques and performing their computational costs can play an important role for the development of these technologies. New distributions are considered for describing genomes. Novel k-mers spectrum typologies and distal co-appearance of words lead to a better understanding of genomes composition and help to identify peculiar elements (i.e. sparse long repeat regions). Decrease in computational costs allow to present new graphical visualization of sequences, their dictionaries and distributions. Moreover, a theoretical study on reconstructability of sequences through small factors, with application to NGS data, is addressed. Lastly, a new pipeline for the extraction of statistical over-represented k-mers, both from single sequence or a set of sequences, is applied to de novo transcription factors detection.

Based on a similar concept, the extraction of words of fixed length, Graph-Grep and its derivatives use dictionaries for indexing of labeled graphs, databases or stand alone instances. A parallel version for SMP architectures, GRAPES, is presented showing significant speed-up. Moreover, preliminary studies on distribution and statistical correlation of network entities, such as genes and their targets inside biological pathways, show possible application in the field of drug discovery.
Model-Based Security Testing

Automated Generation of Vulnerability-driven Test Cases

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Model-Based Testing (MBT) casts the problem of generating test cases as a model-checking problem. Each attack trace returned by the model-checker is interpreted as a test case. The main advantage of MBT is that it provides formal methods to design and generate test cases. However, a considerable effort has to be put in concretizing the obtained test cases as they are too abstract to be executed on the implementation of the System Under Test (SUT).

The aim of my Ph.D. thesis is the definition of formal methodologies for the automated generation of test suites for testing the security of Web Applications and/or protocols specified in ASLan/ASLan++ (the formal specification languages of the AVANTSSAR Platform, a state-of-the-art tool for the analysis of security protocols and services).

In the first three years of my Ph.D., I have developed semantic mutation operators to generate vulnerability-driven test cases and the ASLan Mutation Tool allowing one to automate the mutation process for ASLan models. Upon selecting from a library which mutation operators to apply and providing part of the semantics of the original model, the tool generates all the mutants resulting from the application of the selected operators. Since each mutation operator injects a specific vulnerability into the original model, the attack traces generated from the mutants represent test cases for those vulnerabilities.

Another technique I developed is the prioritization of test cases through weighted mutation operators. Such operators combine results from Risk Analysis (RA) with MBT to obtain an ordered set of test cases. Each mutation operator is assigned with a weight, given by RA, which represents the likelihood of having the associated vulnerability in the SUT. This weight is then injected along with the vulnerability so to obtain weighted attack traces allowing the tester to prioritize the concretization and execution of the most promising traces.
Spectator Crowd: a Social Signal Processing Perspective

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Social signal processing and crowd behavior analysis are new research fields that are spreading a lot in the last few years, involving several disciplines such as computer vision, psychology, sociology, cognitive science and many others. What we propose with our research project is a new type of crowd analysis in computer vision, focused on the spectator crowd, that is, people “interested in watching something specific that they came to see”.

As preliminary investigation we focused on three main aspects

• **Spectators segmentation**: finding diverse groups of people among the spectators;
• **Excitement calculation**: in a given time interval, quantizing the level of excitement of some parts or of the entire crowd;
• **Event segmentation**: segmenting diverse activities of the crowd, and studying how these activities are related with the observed event;

For the spectators segmentation and the excitement calculation issues, we use local flow information (position, flow intensity and direction), as input of a Gaussian clustering framework operating on the single frame. The spatial segmentations are then joined together along the temporal axis by a hierarchical clustering, including also Lempel-Ziv complexity. After this, with the adoption of entropic measures, the degree of excitement of such groups can be quantified. The results are impressive, since it becomes possible to distinguish the different fan groups, even when they are merged; regions of activities indicating how much lively some supporters are can also be automatically found. In the event segmentation problem, we calculate global flow measures at each frame, obtaining a 2D signal which is subsequently quantized by Mean Shift segmentation. This way, important events (goals, shots on goal) can be easily discovered. In the future we will work on more sophisticated models: dynamic Bayesian networks may embed spatial and temporal reasoning in a unique model; gesture recognition, face detection and expression recognition may provide detailed cues to better understand the nature of the spectators activities, allowing the discrimination between supporting, heckling or just watching.
Improving ABV by automatic generation and abstraction of assertions

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Assertion-based Verification (ABV) aims at providing verification engineers with a way to formally capture the intended specifications and check their compliance with the implemented system.

Several approaches exist in the literature for automatic extraction of model behaviors (specifications) represented in the form of formal properties. Some of them, rely on static analysis of the source code, other dynamically mine specifications by analyzing simulation traces. In both cases, most of them work at bit level and generate assertions in the form of combinational or temporal relationships among Boolean expressions. Such techniques are suited only for gate level or RTL HW models. There are also approaches working on system-level descriptions and SW programs, but they generate assertions to express only the sequential ordering of communication function calls and events, while the functional part of the implementation is ignored. Also, none of them have the capability of estimating assertions quality.

To fill in the gap, this thesis is intended to define a dynamic methodology that works on gate-level, RTL and system-level HW descriptions as well as embedded SW, to find a way to automatically generate and evaluate temporal assertions capturing their behavior.

On the other hand, the recent trend towards the use of abstraction levels higher than RTL to manage the complexity of modern systems, has risen the problem of integrating ex-novo transaction level model (TLM) components with existing RTL IPs, that often come with a correspondent verification environment. While techniques to either reuse or abstract RTL IP-cores into a TLM design have begun to appear, the problem of reusing at TLM a verification environment originally intended for an RTL IP-core is still underexplored, particularly when ABV is used. Some papers propose techniques and frameworks to deal with ABV at TLM, but they assume a top-down design and verification flow where assertions are defined ex-novo at TLM level. On the contrary, the abstraction of existing assertions in an RTL-to-TLM bottom-up design flow has not been analyzed yet. Thus, the other purpose of this thesis is to fill in the gap by proposing an automatic methodology to abstract assertions originally defined for an RTL IP-core towards assertions suited for a corresponding TLM model.
MP Grammars, Reactive Systems and Electric Circuits

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The cell, one of the most basics unity of life, is a small dimension but complex and dynamical system that suggests, in its internal machinery, the existence of a computational process with I/O operations on molecules; this architecture stimulated the development of a series of computational models, including the Vincenzo Manca’s Metabolic P system which has been successfully applied in the deterministic mathematical modeling of discrete dynamical systems, in particular from biological origin, based solely on observed (experimental) behaviours.

In a similar way, control and electrical engineers use system and electrical circuits theory to model (electrical) signals and their transformations seeking to understand and interact with the physical environment. For this purpose, they employ models of analog and discrete nature plus a wide range of theoretical knowledge, including graph theory, dynamical systems, ordinary differential equations and many others.

Inspired by the work on long-term potentiation done by Terje Lømo in neuroscience, the current proposal seeks to discover a possible symmetric equivalence relation between metabolic P systems and electric circuits—either analog or digital ones—in a way it is possible to convert a model described in one of these languages in another, in a lossless way. A similar to the approach for gene regulatory networks was subject of Marchetti in, in which he defines a table of equivalence among elements of this kind of graphs and the MP ones.

After the theoretical and experimental verification of the existence of such equivalence, the general procedure for the transformation is defined through the study of the underlying (mathematical) structures of both systems—such as graphs, grammars and dynamics—and formalizations of the common patterns found are performed.
Formal Methods for 
Web Application Security Testing

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Web Application Security is a current and newsworthy subject: more and more companies are relying on Web-based applications to support e-commerce sales and many services are now available on the Internet.

Alongside with the benefits, new opportunities arise for criminals who can steal user’s sensitive personal data or cause service downtime. In order to prevent such attacks we need to test web applications for security flaws.

In the literature exists three main methodologies for use for the testing of software programs that could be adapted for, or could be used to guide, the security testing of web application: program analysis, information flow and model-based testing.

Program analysis and information flow techniques usually require complete access to the web application’s source code but it may not be available during penetration testing. Moreover these two methodologies do not perform well when the architecture of a web application is very complex.

Model-based testing can be applied without the knowledge of the web application’s source code since the security analysis relies on the web application’s abstract model. This high level view can provide a better understanding of the web application resulting in more complete security tests.

Since model-based testing can provide a better view of the web application under test, resulting in a better test coverage, it is best suited to guide the penetration testing activities.

In my first year I started to develop a formal and flexible model-based security testing framework that supports a security analyst in carrying out security testing on web applications. The main idea underlying this framework is that the use of model-checking techniques can automate the research of possible vulnerable entry points in the web application, i.e., it permits an analyst to perform security testing without missing important checks. Moreover, the framework also allows for reuse: the analyst can collect her expertise into the framework and (re)use it during future tests on possibly different web applications.
Modeling and Verification Techniques for Cyber-Physical Systems Design

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Embedded systems are constantly growing in complexity and heterogeneity. Moreover, they are everyday more and more immersed in, and interacting with, the physical environment that must now be taken into account to preserve a correct design flow. The notion of Cyber-Physical System (CPS), where Cyber stands for the computational part and Physical for the environment, emphasizes this aspect.

Typically, a CPS consists of a set of highly heterogeneous components and domains, such as digital hardware, analog components, HW-dependent software and physical processes. Each of such domains presents an already extensive literature on specification methods and computational models that are used in the specific domain and many approaches tried to tackle CPS heterogeneity. These approaches are divided in two main categories. Top-down design flows start from a high level specification of the system to produce the final implementation through a set of refinement steps. Conversely, bottom-up approaches are based on reuse and integration of pre-defined and pre-verified components.

Even if bottom-up approaches proved to be very effective for classical embedded systems, heterogeneity in CPS makes integration and reuse of already existing components a very complex task. This work exploits a recently introduced Model of Computation, called UNIVERCM, to reduce the heterogeneity of CPS into homogeneous models.

On top of this computational model, a set of methodologies working on the homogeneous UNIVERCM models are going to be proposed, covering simulation, system manipulation and automatic verification for CPS. Efficient simulation techniques for UNIVERCM models are crucial in order to support efficient and effective dynamic techniques for performance estimation and system validation. Correct-by-construction model manipulation techniques will allow to tune the systems in order to optimize it and target functional and non-functional design constraints. Finally, automatic verification techniques, both dynamic (i.e., simulation-based) and static (i.e., based on formal methods) will be proposed to prove the correctness for CPS.
Count representation and models for protein homology detection

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In recent years, several Pattern Recognition and Computer Vision problems have been successfully faced by techniques based on the count representation - a representation which characterizes a pattern with a vector of counts. However, in the bioinformatics field, the potentialities of these tools and representation have not completely been exploited, due to the methodological and applicative challenges derived from the peculiar context. Only very recently, they have been employed for microarray sample classification [2] or HIV regression [3]. The count representation is pervasive also in structural bioinformatics, where fragments of protein backbones can be counted and grouped into functionally related elements [1].

Here we propose an application to protein remote homology detection, which combine this count representation proposed for 3D structures with a natural count description of sequences, namely k-mers count. Since both worlds now are represented in the same way, we can employ a probabilistic model to combine this information and solve the protein remote homology task (which is usually performed using only the sequence information) in an effective way. Quantitative experiments proved the suitability of our approach.

References


Semantic Gap Reduction in Image Understanding Using Ontology-Based Approaches

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Image Understanding (IU) is one of the most challenging and interesting problems in the area of computer vision. It is the process of interpreting an image to figure out what it represents in the real world in terms of text, natural language, etc. IU is a multidisciplinary topic that requires the application and combination of information from various fields like image processing, machine learning, and pattern recognition. Up to date, the traditional recognition methods heavily rely on the simple visual features extracted from still images to recognize objects and ignore to integrate therein the high-level concepts formulated by users. In addition, recognition accuracy is highly dependent on the quality of training sets. Although the use of machine learning and computer vision techniques has extensively been improved, the semantic gap, which is the lack of the coincidence between low-level features visual content and high-level semantic in object recognition, remains an open issue. The research focuses on a specific sub-topic of IU that is object recognition, aiming to automatize the process of learning object classes. Two specific goals are identified: first, to improve object recognition methods by means of ontologies; second, to formally and automatically build an ontology using computer vision techniques and big data. As preliminary studies, we utilized the semantic technology together with the ontological analysis to overcome the problems associated with the conventional methods and to automatically build training sets. The proposed method exploited one of the popular lexical databases that is WordNet to search for related terms to a target word, used the ontological analysis such as DOLCE to remove visually irrelevant terms accompanying the target word, and then automatically crawled training images using the composed words from Google image search engine. The style bag of words model is adopted using single class support vector machine. The preliminary results showed a constant increment in the recognition accuracy and outperformed the traditional methods of about 10%.
A Logic for Topological Quantum Computation

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Topological Quantum Computation (TQC) is a paradigm for quantum computation that permits to construct a quantum computer that resists to decoherence, i.e. the destroying interaction between the elaboration system and the environment. This feature is based on the use of special particles called anyons, that live in a bi-dimensional space.

A physical scheme to describe the computation in TQC is summarized in two steps. First, encoding the information in the splitting space generated during the creation of an anyon system. Second, imposing an evolution of the system by a sequence of rotations of neighboring anyons. In the first step we note that the computational space of qubits increases as a base of two, instead the splitting space increase with a function that increase faster. So we simulate the a register of n qubits with a splitting space with more than n states. This lead to the leakage error: after a computation some results are inserted in the in the states that not encode the qubits, this produce the lost of that information.

In literature to correct the leakage error the researchers increase the number of rotations to avoid the use of the extra states. This process is hard to compute, since the behaviour of the anyon system is described by the F and R matrices with complex numbers as entries and the research is a brute force attack on all the possible sequence of fixed length of these matrices. The result we produced during my period abroad with prof. Pachos is an alternative way to correct the leakage error for the Fibonacci anyon model. At the end of the computation we insert a pair of ancillar anyons and impose the interaction of them with the anyons of the system. After we measure the ancillar anyons and reading the result we know that with a certain probability we have corrected the leakage error.

To study more in general this problem and also to study from a computer science viewpoint the characteristics of TQC, we have developed an formal calculus for TQC and its operational semantics.
Irreflexive temporal frames and instant propositions

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Irreflexivity is a desired property of structures used to represent temporal reasoning according to the opposition earlier-later. Indeed, such property corresponds to the intuition that no instant precedes or follows itself. In first-order logic irreflexivity can be represented by means of the formula $\forall t \neg (t < t)$ where $t$ is an instant of time and $<$ is the relation of temporal precedence: one may define the class of irreflexive temporal structures as the class of frames on which this formula is valid. However, in the case of propositional tense logic, i.e. a logic with propositional variables, truth-functional connectives and the modal operators $H$ (always in the past) and $G$ (always in the future), there is no formula corresponding to this property that can be used as an axiom to define the relevant class of frames.

Many solutions have been considered to force irreflexivity in an indirect way. The most common is Gabbay’s rule IRR which allows to build, from the canonical model of a given system $S$, a sub-model where $<$ is irreflexive and all theorems of $S$ are preserved. Another idea is to have operators for inaccessible points, as in some logics studied by Humberstone.

The approach I would like to focus on adopts a language enriched with “instant propositions” or nominals. Blackburn develops the minimal system of nominal tense logic $Ktn$ adding to the basic set of axioms for tense logic the infinitary schema $M(c \land \alpha) \rightarrow L(c \rightarrow \alpha)$, where $c$ is a metavariable for nominals, $L$ is an unbroken sequence of $H$ and $G$ and $M$ is an unbroken sequence of $P$ and $F$. To force irreflexivity, he adds also the axiom $c \rightarrow G\neg c$ and uses the technique of “bulldozing” to eliminate reflexive points from the canonical frame of the system obtained. A related solution is offered by Garkov and Goranko, who use the rule COV to build, from the canonical model of a system $S$, a sub-model where all points are named by some instant proposition and all theorems of $S$ are valid. A way to improve this approach is to consider an axiom that defines the relevant frames for a given system as only those where each point is associated with a nominal. If this turns out to be possible, then no complication would arise for the proof of completeness: indeed there would be no need of ad hoc techniques.
A Model-based Testing Approach for Web Applications

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Penetration testing is the most common approach for testing the security of web applications, but model-based testing has been steadily maturing into a viable alternative/complementary approach. Penetration testing is very cost-efficient, in the sense that little pen-testing time usually is enough to reveal several bugs, but the experience of the security analyst is crucial; model-based testing relies on formal methods but the security analyst has to first create a suitable model of the application under test.

My research aims at developing a methodology for model-based testing of web applications that takes into consideration the methodologies used in penetration testing. This is possible through the use of a database of components and a definition of low-level actions that ties concrete actions, performed through a web browser, to components present in the model. I have formalize the modeling of web applications in our framework, define the concretization methodology, and discuss a realistic case study allowing for the possibility of concatenated attacks.

Regarding the concretization methodology, in the literature we can find an abundant number of tools for aiding developers and penetration testers to spot common software security vulnerabilities. However, testers are often confronted with situations where existing tools are of little help because a) they do not account for a particular configuration of the application and b) they do not include tests for certain vulnerabilities. To cope with this I use the VERA-tool, that allows users to define attacker models where the payloads and the behavior are cleanly separated and that abstract away from low-level implementation details such as HTTP requests.

From these premises, the main idea is to use the Model Checker in order to find a counterexample (representing which sequence of components we have to use in order to leads the application into a probable insecure state), and then use the VERA-tool in order to test the application.
Using Interpolation for the Verification of Security Protocols

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The formal analysis of security protocols is one of the most delicate and interesting problem areas of information security. This is because devising protocols that indeed guarantee the security properties that they have been conceived for is an inherently difficult problem and experience over the last few decades has shown that the development of such protocols is a highly error-prone activity. Only formal analysis can provide the level of assurance required by both the developers and the users of such protocols. On the other hand, formal analysis is a very time consuming task, e.g. it usually consider an infinite state space or an attacker that can lead to state explosion.

The aim of my PhD is to create new methods to speed-up the search of security flaws via formal methods. The main result has been achieved using Craig interpolation. In fact, interpolation has been successfully applied in formal methods for model checking and test-case generation for sequential programs. Security protocols, however, exhibit such idiosyncrasies that make them unsuitable to the direct application of such methods. In this thesis, I address this problem and present an interpolation-based method for security protocol verification, which ultimately also allows me to extract test cases from discovered attack traces. This method starts from a formal protocol specification and combines Craig interpolation, symbolic execution and the standard Dolev-Yao intruder model to search for goals (possible attacks on the protocol). Interpolants are generated as a response to search failure in order to prune possible useless traces and speed up the exploration.

I have implemented a Java prototype called SPiM (Security Protocol interpolation Method) based on Z3 and iZ3 for satisfiability checking and interpolant generation, respectively. In order to show that the method concretely speeds up the validation, I have tested SPiM with and without the interpolation part on Needham-Schroeder public key protocol (NSPK) and NSPK with Lowe fix (NSL). The total execution time on a general purpose computer ranges from 8s for NSPK to 83s with Lowe fix. While for NSPK there are no pruned paths and the two versions of the algorithm perform with the same time, on NSL SPiM is 1.5-3.5% faster when using interpolation. This experiment shows that the time of validation decreases when using interpolant-based annotations. This is also confirmed by the fact that the average time needed to calculate and propagate an interpolant is 9.1-27.3% lower than the average time used to perform a step of symbolic execution together with the corresponding satisfiability checking.
Temporal Data Warehousing, OLAP, and Mining in Pharmacology

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The amount of clinical data available is increasing every day as a result of technology advancements in computer performance and storage capacity. Data warehousing satisfies the need of organization in all fields to perform sophisticated data analysis to support their decision-making process. The intrinsic temporal nature of clinical information leads to non trivial issues that need to be addressed.

In this thesis, we study and propose data warehousing techniques for addressing temporal aspects in modeling, analyzing, and mining data. Pharmacovigilance, the activity related to the analysis of unexpected Adverse Drug Reactions, will be the medical domain motivating several aspects and providing applications for the proposed framework. Research on temporal multidimensional data model is mainly focused on changes in the structure of dimension through time, e.g., split of a term into two or changes in the grouping relation. Little attention has been given to models for exploring temporal data. We are developing a temporal multidimensional model, based on an existing one, namely MultiDimER. We are investigating, through a collaboration with professor Esteban Zimányi from ULB Belgium, the discovery and analysis of temporal trends on different time granularities in an OLAP Business Intelligence context. The model will allow users to analyze several time dimensions at once, which will lead to the issue of grouping on temporal dimensions called Temporal Aggregation. Many applications involve bi-temporal, or n-temporal data. Instead of dealing with time intervals, it becomes necessary to deal with time regions on multiple time dimensions, which need to be defined. The application of data mining techniques would enhance the analysis capability of Pharmacovigilance analysts. We want to extend the model proposed by Sacchi et al., by letting pharmacovigilance analysts define temporal rules of their interest, and also let them define these rules using several hierarchical structures of the data defined for this domain. Finally, we will address the visualization of trends and mined temporal rules.
Unveiling the Multimedia Unconscious:
*Implicit Cognitive Processes and Multimedia Content Analysis*

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One of the main findings of cognitive sciences is that automatic processes of which we are unaware shape, to a significant extent, our perception of the environment. The phenomenon applies not only to the real world, but also to multimedia data we consume every day. Whenever we look at pictures, watch a video or listen to audio recordings, our conscious attention efforts focus on the observable content, but our cognition spontaneously perceives intentions, beliefs, values, attitudes and other constructs that, while being outside of our conscious awareness, still shape our reactions and behavior. So far, multimedia technologies have neglected such a phenomenon to a large extent. This paper argues that taking into account cognitive effects is possible and it can also improve multimedia approaches. As a supporting proof-of-concept, the paper shows not only that there are visual patterns correlated with the personality traits of 300 Flickr users to a statistically significant extent, but also that the personality traits (both self-assessed and attributed by others) of those users can be inferred from the images these latter post as “favourite”.

Sensing Capabilities for Soft Robots

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Soft robotics is a new trend that is emerging from the bio-inspired robotic research field. Its main aim is the development of machine that not only exhibit more natural movements by mimicking biological systems, but also that are made by soft material such as silicon rubbers. The use of this type of deformable mater is crucial for systems that deal with uncertain and dynamic tasks/environments e.g., grasping and manipulation of unknown objects, locomotion in rough terrains, and physical contacts with living cells and human bodies.

The use of soft materials has the potential to leads to robots that are more adaptable, capable, and safer than the existing machines. By doing so, it enables us to automate tasks that are beyond the capabilities of current robotic technology. However, introducing softness into the mechanics introduces design issues that differ completely from the classical ones known from “hard” type engineering. In fact, when compared to their rigid counterparts, robots made with soft matter are less precise and controllable. Thus, a novel set of design and control principles – that can act as a bridge between hard and soft type engineering – has to be elaborated.

Since its young age, this research field has still many open problems that have been only partially solved. Despite this, various examples of soft machines have been developed. Most of them have not yet proven their applicability in different situations that were not limited to the laboratory test-bed. To let this new technology spreads into the market, it is important to provide reliable methods to model and retrace robot’s configuration and position in space, predict and control their motion, and provide the correct driving force through actuators.

The main aim of my Ph.D. research is developing a novel type of technology that can act as a soft encoder to retrace information related to the spacial configuration of a soft deformable manipulator. Having such technology will be beneficial for the development of adaptable human-robot interfaces and provide sensorial feedback for future minimally invasive medical devices.
Towards Brain Tissue Microstructure Characterization using Diffusion MRI

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Diffusion Magnetic Resonance Imaging (Diffusion MRI) is an evolution of standard MRI which, using an additional sequence of radio frequency pulses, permits to measure the signal attenuation due to the diffusion of water molecules in different directions. Although in normal MRI images there is a clear contrast between white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF), nothing can be said about the inner structure of these regions. WM in particular is composed principally of bundles of axons, also called fibers, linking the different regions of the brain. The characterization of these fibers is the goal of diffusion imaging techniques. Diffusion MRI quantifies the diffusion of the water molecules in the examined biological tissue. The use of Diffusion MRI for characterizing the brain white matter microstructure is now a clinical standard for pre-surgical planning and in vivo neuroanatomy research. From the raw signal it is possible to calculate the corresponding ensemble average propagator (EAP), representing the probability density function of the average displacement of the population of water molecules in the physical space. Nowadays different reconstruction techniques are used to model the diffusion signal starting from complex acquisition schemes in Fourier space in order to reconstruct the propagator. Numerous features can be then derived from the EAP in order to extract information about brain tissues characterization, white matter fibers orientation and fibers volume. The overall goal of this work is to improve the SOA by facing the following issues: optimal sampling in the q-space, optimal basis for signal reconstruction, definition of new scalar indices (features) that are anatomically and biophysically plausible besides being objectively measurable in a robust, accurate and stable manner. This will open new perspectives in both the scientific and clinical framework for the assessment of structural properties of tissues in both healthy subjects and patients besides supporting and improving cortical connectivity modelling.
PhD Program in Multimodal Imaging in Biomedicine
Electroclinical, neuroradiological and neuropsychological study in 68 subjects affected by Pervasive Developmental Disorder

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PDD refers to a group of disorders characterized by deficits in the development of multiple basic functions including socialization and communication. The etiology of autism is largely unknown, but widely accepted by the scientific community that it is a neurobiological disorder with a genetic basis. This study considers the data of 68 subjects with Pervasive Developmental Disorders received at Child Neurology and Psychiatry Unit of University Hospital of Verona from January 2008 to November 2010 and have joined the ITAN project. We extracted from the clinical database of the ITAN project the following data: sex, age, personal history, gestational age, birth weight, Apgar, head circumference at birth, prenatal, perinatal and postnatal complications, psychomotor development, ADI (Autism Diagnostic Interview-Revised), ADOS (Autism Diagnostic Observation Schedule), IQ and GQ (WISC-III, WPPSI, Leiter-R, Griffiths scales), neurological examination, head circumference at visit time, dimorphisms, malformations, discolorations, head circumference at visit time, lateralization, EEG and MRI.

The data obtained in this study allow us to conclude as follows:

a) Pervasive Developmental Disorders associated with Mental Retardation and/or language impairment have a higher risk to have brain abnormalities detectable by MRI and paroxysmal EEG abnormalities.

b) Pervasive Developmental Disorders with regression history have a higher probability of detecting paroxysmal abnormalities.

c) The clinical and instrumental investigations utilized in clinical practice, in particular MRI, do not allow, in patients with High Functioning PDD, to collect data that can help us in understanding the etiology of the disease.
Magnetic Nanoparticles as theranostic agents for MRI

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Theranostics is an innovative method that aims to eliminate the multi-step procedures, reduce the delays in treatment and develop personalized medicine. Nano-theranostics allows the simultaneous imaging and effective treatment of disease through the application of nanoparticles. Among the different types of nanoparticles, iron-oxide nanoparticles are considered to be promising candidates in cancer theranostics due to their superparamagnetic behavior. Magnetosomes (MS), iron oxide nanoparticle produced by magnetotactic bacteria, are here proposed as theranostic agents for imaging and thermo therapy of tumors. MS are monodomain, well-crystallized nanoparticles surrounded by a lipidic membrane, organized in chains that are used by bacteria as a compass for geomagnetic navigation. Magnetic nanoparticle extracted from magnetotactic bacteria could be employ both Magnetic Resonance Imaging (MRI) contrast agent and therapeutic agent in Magnetic Fluid Hyperthermia (MFH). Indeed, MS release heat under the application of an alternating magnetic field. In this work, several techniques have been used to characterize MS. Magnetic nanoparticles were extracted from M. gryphiswaldense strain MSR-1, purified, lyophilized, irradiated with γ-rays and stored at -20°C; MR images were acquired using a Bruker tomograph operating at 4.7 T and magnetic measurements were carried out on powders and suspensions using a Quantum Design SQUID MPMS XL-7 magnetometer. Hyperthermic properties of MS was investigated by recording the temperature kinetics of the sample dispersed in a gel matrix during the exposition to an alternate magnetic field. Preliminary results indicate that the transversal relaxivity of MS, measured in agarose gel (0.25%) at 4.7 T by using imaging techniques was comparable to the relaxivity of Endorem and T2w images of HT-29 xenografts show the presence of MS as a dark region thanks to high transversal relaxivity. Finally, we confirmed that MS have a high hyperthermic efficiency: Specific Absorption Rate (SAR) of 806 ± 85 W per grams of iron, in line with data reported in the literature, was measured. In this paper we investigated the magnetic properties of MS and we demonstrated that magnetic nanoparticle extracted from magnetotactic bacteria have high transversal relaxivity and high hyperthermal efficiency. Furthermore, biological origin and lipid coating should guarantee low toxicity. For all this reasons MS are potential contrast and therapeutic agents.
The Ice Figure Skate: Clinical Implications Related to Its Use and Biomchanical Evaluation of New Boot Designs for the Reduction of Landing Impact

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Several studies reported an alarming increase of figure skaters suffering from injuries in the past 15 years, and suspected that most of these injuries are the indirect consequence of the increased demand for technical difficulties in the programs, like triple-triple combinations and quadruple jumps, which resulted in an increased time spent on the ice and to an increased stiffness of the skating boots. Both these factors may be determinant for the development of injuries in the skaters.

The present project aims to provide new insights into the relationship between injuries and the ice figure skate. In particular, the first aim of this study is to investigate the biomechanical and clinical condition of 100 Italian ice figure skaters during an entire training season, to determine the most common type of injuries among the skaters and their relation with the skates they are using. The second aim of the study is to quantify the effect of some design variables of the ice figure skate on the reduction of the landing impact which has been addressed as the main responsible for the development of overuse injuries.

To address the first aim of the study, we performed the first of three evaluations on a population of 100 skaters in the middle of the training season. Preliminary results showed that the most common clinical problem is bursitis in the heel area, which was found on 44% of the skaters. However, many of the clinical problems commonly described for skaters have not been found consistently in this population. To address the second aim of the study, several skate prototypes have been designed to test the effect of many design variables (such as 1) heel heights 2) tongue stiffness, 3) lacing techniques and 4) shock absorbing solutions) on the range of motion of the ankle and on the magnitude of the impact. A motion analysis system, force platform and an accelerometer have been used on 4 skaters while performing a drop jump on a 2 cm thick artificial ice surface to determine the kinematic and dynamic response of the skaters to the new skate design. The post processing of the data still need to be performed so any preliminary result is unavailable.

The scientific impacts of this study are far-reaching in term of understanding the relationship between the skaters’ injuries and the ice figure skates, and in translation of clinical and biomechanical results into an improved skate design that can reduce the rate of injuries which affects this fascinating sport.
Magnetosome as nanotechnology platform for thermotherapy of tumor

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Magnetosomes are specialized intracellular organelles, synthesized by magnetotactic bacteria (MTB), that comprise membrane–enveloped, nano-sized crystals of a magnetic iron material. Bacterial magnetosomes (BMs) are used as therapeutic agents to obtain an increment of temperature in tumor mass. This method based on the increment of temperature to suppress tumor growth is called magnetic hyperthermia. Here, BMs extracted by Magnetospirillum gryphiswaldense, strain MSR-1, are used. Cytotoxicity of BMs and their interaction with cellular elements are evaluated in vitro with human colorectal adenocarcinoma cells, HT-29, and in vivo with the aim to develop new therapeutic approaches for neoplastic diseases. The in vitro studies show low toxicity of BMs and a strong cellular uptake. SEM analysis show that BMs are localized in the cytoplasm of HT-29 cells, TEM images reveal that the process of internalization of BMs occurs by a process divided in three steps: adherence to the cell membrane, transport to the cytoplasm and accumulation in the Golgi apparatus. The in vivo studies are performed on male nude mice in which one million of HT-29 cells are injected subcutaneously in the right flank. BMs are injected directly in the tumor volume followed by three cycles of application of alternating magnetic fields (AMF), at alternate days, at 187 kHz and 23 kA/m. Magnetic Resonance Imaging (MRI) detects the localization of the site of injection of BMs as well as an evaluation of the development of tumor mass. Histological analysis performed on the tumor mass show that mice treated with both BMs and magnetic hyperthermia are characterized by the presence of fibrotic and necrotic areas close to the site of injection of BMs. In conclusion, BMs could represent a powerful tool for magnetic hyperthermia in the treatment of HT-29 tumors. Further studies on glioblastoma cell line, U-87 MG, are ongoing.

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Body composition assessment in sports

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In the frame of my doctoral research project, the effect of different sports on body composition was assessed in specific populations (pre-menarcheal girls and men with neurologic disorders) using dual energy X-ray absorptiometry (DXA). During the second year of my PhD project a total of forty-five subjects were recruited: 1. Thirty pre-menarcheal girls (11.1±1.33[SD]y) participating in volleyball (VOLLEY, n=10) and artistic gymnastics at high (HGYM, n=10) and low (LGYM, n=10) intensity training. 2. Nineteen wheeled athletes (WA) participating in wheelchair basketball (n=12) and wheelchair rugby (n=7). All subjects underwent DXA analysis for bone mineral content [BMC], fat-free soft tissue mass [FFST], fat mass [FM], and %FM at the total body and regional level. A battery of 5 reliable and valid field tests were also used to evaluate the performance outcomes in WA. Results showed that: 1. In pre-menarcheal girls, VOLLEY had lower BMC and FFST, and greater FM and %FM vs. both HGYM and LGYM. The main difference between LGYM and HGYM was greater %FM in the former. Similar results were found at the regional level (but for similar BMC in the legs) and at lumbar spine and pelvis. 2. In WA, no significant correlation between FM and sport-specific performance parameters was present. Significant correlation was found between total body- (BMC as well as subtotal FFST) and regional (trunk, right and left arm FFST) body composition parameters, and several field tests. The findings of this study provide evidence that: 1. Greater bone mineral accrual takes place in pre-menarcheal impact loading athletes participating in artistic gymnastics vs. volleyball and the dose of impact loading activity mainly affect %FM; moreover, results suggest that the amount of impact activity has major impact on BMC accrual. 2. FFST is a key to positive responses in WA field test performance. These results are important for better activities planning and adequate training monitoring, in addition to being used as athlete-motivating features.
Obesity and type 2 diabetes disease in Zucker Fa/Fa rat intestine and pancreas

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Introduction: Obesity is the major risk factor for certain diseases, it can develop adipose tissue stores in the body, and with an inappropriate food intake present a major risk factors for the onset of type 2 diabetes. During the prediabetes phase, that precedes the onset of type 2 diabetes, The hyperinsulinemia compensates for insulin resistance, the body makes enough insulin produced from beta cells but the cells in the body have become resistant to the salutary action of insulin.

Zucker rats were bred to be a genetic model for research on obesity and diabetes. There are two types: a lean Zucker rat, denoted as the dominant trait (Fa/Fa) or (Fa/fa); and the characteristically obese (or fatty) Zucker rat, which is a recessive trait (fa/fa) of the leptin receptor. Obese Zucker rats have high levels of lipids and cholesterol in their bloodstream and are resistant to insulin, they develop hyperglycemia at 10-12 weeks and diabetes at 12-14 weeks of age due to a decreased secretion of insulin by the pancreatic cells and peripheral insulin sensitivity.

Aims: To find out the expression levels of various antibodies identified diabetes and obesity in the pancreas like an important organ in these diseases and in the intestine that is poorly explored in this field. To evaluate the morphological and phenotypic aspects of these organs, using the same markers in order to determine any effects of insulin resistance and lipotoxicity on their structure and function.

Materials and methods: We use Zucker rats-Leprфа/фа (with obesity associated with mellitus diabetes, non-insulin dependent) of 8-10 weeks and 16 weeks of age, Zucker rats-Leprфа/фа or FA/FA (control animals, non-obese, non-diabetic) 8-10 weeks and 16 weeks of age, Wistar rats, matched for sex and age with the Zucker rats. The sample of intestine and pancreas will be performed with the use of techniques of light and electron microscopy, immunohistochemistry for various markers like Insulin, Insulin receptor alpha, Ghrelin, Leptin receptor,

Preliminary results: We find an important presence of insulin receptor alpha in the duodenum glands of the Zucker rat control and less to absent in the obese Zucker rat. Then we observe for the first time the presence of Leptin receptors in the pancreas of Zucker rat control and nothing to report in obese Zucker rats. Finally, the amount and appearance of Ghrelin are more important in the duodenum villus of the Zucker rats control in comparison with the obese Zucker.

Prospective: Proceed with other immunohistochemistry and also molecular biology studies to complete our preliminary data. Then we have to use other markers of obesity and diabetes, and eventually amplify our studies on other organs that may be interesting to pull out new approach.
Tracking nanoparticles and monitoring drug delivery in *in vitro* neuronal cells

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Chitosan nanoparticles (ChiNPs) are biocompatible drug carriers able to protect the encapsulated molecules and/or improve their bioavailability by modifying their pharmacokinetics. In particular, ChiNPs proved to be suitable for delivering molecules characterised by low stability, such as peptides, proteins, oligonucleotides and plasmids [1]. In addition, ChiNPs can cross the blood brain barrier [2,3], thus representing a promising carrier to central nervous system.

In our studies, diaminobenzidine (DAB) photoconversion was applied to correlate fluorescence and transmission electron microscopy for investigating the intracellular fate of ChiNPs in a neuronal cell line [4,5]. ChiNPs were mostly found within electron-lucent vacuoles, and were ubiquitously distributed in the cytoplasm, from the cell periphery to the perinuclear region; some NPs were also found to be free in the cytosol. After long incubation times (8 to 24 hours) the ChiNPs were observed to accumulate in perinuclear position, but never inside the cell nucleus. Moreover, many NPs were found inside multivesicular or residual bodies: their morphology was often severely altered in either organelle, so they were only recognizable from the dark reaction product.

Subsequently, neuronal cells were administered ChiNPs loaded with D-Ala2-D-Leu5-enkephalin (DADLE), a sytethic opioid able to induce reversible hypometabolizing effects [6] that has been extensively studied for its potential use in biomedicine, i.e., for preservation of explanted organs [7], neuroprotection [9] and anti-tumour treatments [9]. However, DADLE has a short plasmatic half-life (a few minutes) and it is unable to cross the blood brain barrier, thereby making systemic administration inefficient.

Ultrastructural immunocytochemistry and morphology were used to test the efficacy of ChiNPs in DADLE delivery and to evaluate the distribution of DADLE molecules in the various cellular compartments as well as their effects on transcriptional activity and cellular organelles. Our results demonstrated that DADLE-loaded ChiNPs internalized by neuronal cells still contain and release DADLE 24h after their withdrawal from the culture medium, thus maintaining low transcriptional activity [10]. In conclusion, our data demonstrate that ChiNPs are valuable tools to deliver the hypometabolizing opioid DADLE to neuronal cells, paving the way to *in vivo* experiments aimed at elucidating whether DADLE-loaded ChiNPs may efficiently deliver the opioid to the central nervous system.

“Histological and ultrastructural changes of skin and subcutaneous layer after use of fat, stromal vascular fraction-enriched fat and adipose-derived stem cells: Anti-aging treatment”

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The regenerative and rejuvenating properties of the adipose tissue’s graft have been described in the last years. Despite the potential interest for therapy, it is not clear if the clinical results are due to the stem cell population or are linked to other components of the adipose tissue. This doctoral project is aimed at the analysis of the histological and ultrastructural changes of aged facial skin after injections in the skin using 3 methods: fat graft; fat graft added with stromal vascular fraction of the fat obtained by centrifugation; expanded adipose-derived stem cells. The study will be performed in 20 consecutive patients, candidates for facial rejuvenation surgery, with age ranged between 45 and 65 years. The patients will be subject to the sampling of 20 cc of fat by liposuction from the abdominal region. The injection of expanded cells in the preauricular area will be performed 5 weeks after in vitro cultivation. Before injections, a fragment of skin will be removed from the preauricular and post-auricular areas and analyzed by optical and electron microscopy (SEM and TEM), which will be repeated after 3 months from the injections. The ultrastructural examination will focus on modifications in tridimensional architecture of the reticular and papillary dermis considering elastic fibers and collagen network, besides changes in cutaneous vascularization or microvascular bed, which may represent a rejuvenation effect. This study is currently in progress to collect the material and define the cutaneous alterations.

Key words: aging process, regenerative medicine, fat graft, adipose-derived stem cell, elastic fibers, microvascularization.
Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). During the last 15 years functional Magnetic Resonance Imaging (fMRI) studies have shown that cortical reorganization consistently occurs both in acute and clinically stable MS patients. These studies suggest that cortical reorganization might play a role in limiting the impact of structural tissue damage along with disease progression. Several Magnetic Resonance Imaging (MRI) techniques have so far been applied to Experimental Autoimmune Encephalomyelitis (EAE), as model of MS both in small rodents and non-human primates. In the present work we have applied fMRI techniques with somatosensory stimulation of the right forepaw and investigated the alterations in functional response that occur in rats with spinal cord homogenate (SCH)-induced chronic EAE.

We demonstrate that the evoked cortical activation is strongly altered in EAE rat brain, compared to controls. As early as 30 days post-EAE induction (dpi), we have observed a strong decrease in the Laterality Index (LI), which later on showed a slight tendency towards pre-induction values at 60 dpi. At both 30 and 60 dpi, fMRI showed a strong increase in the activated volume compared to the pre-induction value involving also the cortex ipsilateral to the stimulated forelimb and some extra-cortical areas. Moreover co-registration of functional images on a MRI rat brain template allowed quantitative investigation of the distribution of activated pixels among relevant brain regions.

Thus, brain cortex remodelling occurs in rat EAE and reproduces a remarkable feature of MS making our model a relevant tool in preclinical MS studies.

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Background
Blood glucose control in the postprandial phase plays a key role for the development of micro- and macro-vascular damage in insulin dependent diabetes (T1D). It is necessary to improve the available knowledge on the substrate fluxes, in particular glucose, and its metabolic disposal (oxidative and non-oxidative), in the postprandial phase.

Study design
Twenty prepubertal children with T1D in a non-randomized, cross-sectional study for repeated measures. Baseline blood sample were obtained for biochemical and hormonal measurements. Continuous subcutaneous insulin infusion was administrated by insulin pump. A meal composed by carbohydrates naturally enriched with stable isotopes was administered.

Measurements of energy expenditure
Respiratory exchange measurements were determined using an open circuit computerized indirect calorimeter (Deltatrac, Datex, Inc., Finland). The resting energy expenditure (REE) was calculated from oxygen production (VO2) and carbon dioxide production (VCO2).

Macronutrient oxidation rate
The macronutrient oxidation rate will be calculated from VO2 and VCO2 using the following formulas: Fox (g/min) = 1.67 VO2 (L/min) - 1.67 VCO2 (L/min) - 0.307 Pox; and Gox (g/min) = 4.55 VCO2 (L/min) - 3.21 VO2 (L/min) - 0.459 Pox, where Fox is fat oxidation, Gox is glucose oxidation and Pox is protein oxidation.

Assessment of exogenous carbohydrate oxidation
A breath collection was taken every 30’ from the baseline period for 3 h after the ingestion of the naturally labeled [13C]carbohydrate. This was done to assess the isotopic ratio of 13C to 12C in breath carbon dioxide and to obtain an estimate of the exogenous carbohydrate oxidation in the postprandial period.

Biochemical analyses
Plasma glucose, triglyceride and insulin levels were measured.

Results
The analyses of collected data are ongoing. The results will show the differences between subjects with higher oxidative/not-oxidative glucose ratio and subjects with lower ones and the differences between subjects with higher exogenous/endogenous glucose oxidation ratio and subjects with lower ones. This will be assessed in order to better understand the behavior of glucose substrates in the post-prandial phase.
Design of RT Biosusceptometer by multiphysics simulation

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This research was carried out at the laboratories of U.O.C. of physics for biomedical technologies. The purpose of this study is to improve the design of a room-temperature biosusceptometer (RTBS). An RTBS is an instrument to measure liver iron concentration safely and comfortably, and is a low-cost diagnostic technique. An RTBS comprises principally: a primary circuit (first order gradiometer) that generates a specular magnetic field (mf), variable in time, and a secondary circuit (second order gradiometer) used to evaluate the asymmetries of the mf. During measuring, the instrument induces the magnetization of two tissues: the overlayer (mainly fat tissue that is present between the skin and the liver) and the liver. To increase the signal / noise ratio, the signal of the secondary circuit is modulated in amplitude, by moving the instrument with a natural harmonic motion along its axis. We have developed a simulation method able to assess changes of output signal due to a variation in secondary circuit geometry. The method has two phases: first the finite element method is used to calculate the mf in the presence of a 2-layer material. Depending on the composition of the material, 4 different simulations are carried out: 1) both layers comprise the same homogeneous medium which contains the RTBS (mf generated by the primary circuit), 2) the upper layer (layer A) comprises fat tissue and the lower layer (layer B) contains the homogeneous medium, 3) layer A contains the homogeneous medium and layer B contains liver tissue; 4) layer A contains fat tissue and layer B contains liver tissue. To avoid the eddy current effect, the $\varepsilon_r$ and $\sigma_r$ of the medium and tissues are set respectively to 1 and 0. Assume that every point where it is possible to construct the lower sensing coil of the secondary circuit is crossed by a turn (with the same axis of the gradiometer and infinitesimal wire section). In the second phase for every turn the contributions of liver magnetization and fat magnetization to the magnetic flux concatenated by the turn are calculated separately (respectively: $lc$ and $fc$). This method was applied to different simulations: testing two different types of primary circuits and two different types of homogeneous medium (empty and water), varying the thicknesses of the overlayer and the liver, and varying the RTBS-material distance. The preliminary results suggest some useful guidelines for the design of a new RTBS: it is possible to analyze the signal produced from a material comprising both fat and liver tissue, by studying the effects of the two tissues separately; different RTBS-material distances imply different $|lc|/|fc|$ ratios; a secondary circuit with the highest sensitivity to the liver’s signal requires a baseline that is as short as possible and a radius that is as large as possible. This study shows that multiphysics simulation is able to verify the effectiveness of improvements of the instrument in the design phase resulting in obvious cost savings in terms of time and resources.
PhD Program in Nanotechnologies and Nanostructured Materials for Biomedical Applications
Sulfated TiO$_2$-added nanocomposite Nafion membranes for fuel cell applications: a spectroscopic characterization

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Fuel cells are a promising technology for clean and efficient power generation. They basically work by converting chemical energy from a fuel into electricity through a chemical reaction with oxygen. If H$_2$ is used as fuel, the cell waste emission is pure water. Among the fuel cells, proton exchange membrane fuel cells (PEMFC) are characterized by low start-up time and low working temperature, since they use a proton exchange membrane, usually Nafion, as electrolyte, which is permeable to proton but electrically insulating.

Nafion is a sulfonated fluoropolymer, consisting of the same backbone of Teflon and terminal sulfonic groups. The mandatory condition to get good proton conduction is a high Nafion membrane hydration level. As a matter of fact, one of the main problems with pure Nafion membranes is the loss of net proton conductivity above 100 °C due to decrease of water content following the evaporation.

In order to improve the electrical, thermic and mechanical properties of the electrolyte, a large variety of different hydrophilic fillers has been studied. Among them, hydrophilic inorganic fillers proved to be a cost-effective solution to improve the physical properties of this membrane.

This presentation reports on the vibrational dynamics investigations of pure Nafion membrane and Nafion composite membranes, filled with TiO$_2$/S nanopowders (TiO$_2$ functionalized with sulphate groups) in different percentage (2, 5 and 7 weight %) by means of FTIR spectroscopy (in Attenuated Total Reflection mode) and Raman microspectroscopy.

The nano-powders were analysed in order to assess the purity degree of the TiO$_2$, the titania crystalline phase and the nature of the TiO$_2$-SO$_4^{2-}$ bonds. Raman spectra did not reveal any appreciable impurity content within the powder, the titania resulted to be in anatase phase with a small amount of rutile phase. From FT-IR was possible to assess that the TiO$_2$-SO$_4^{2-}$ bonds are bidentate at low ambient humidity but they change to monodentate with high ambient humidity.

Concerning the polymer samples, both our FTIR and Raman vibrational spectra of pure Nafion are in fair agreement with the literature data. As for the composite membranes, a clear increase in the TiO$_2$ peaks intensity was seen increasing the amount of filler, and the peak at about 1000 cm$^{-1}$ suggest that the sulphate groups are still present after the filler incorporation. Finally, a Raman study of the shift of the 1060 cm$^{-1}$ peak (SO$_3^-$ vibration) at different ambient humidity was performed in order to better understand the filler effects on the membrane and the water distribution inside the Nafion.
Diffusion Tensor Imaging and Voxel Based Morphometry in an experimental model of MS in rats

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Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Experimental autoimmune encephalomyelitis (EAE) represents a reliable animal model of chronic-progressive variant of multiple sclerosis and has been extensively characterized by conventional magnetic resonance imaging (MRI) techniques. Modern MRI techniques, as DTI, has been extensively used to assess brain or spinal cord lesions in mice, but few groups have used rats as study subjects. The major aim of this work is to define a reliable preclinical model as a basis for the evaluation of the efficacy of therapeutic agents in the difficult environment of the brain diseases, particularly related to the blood-brain barrier (BBB) issue. Delivering drugs across the blood–brain barrier is one of the most promising applications of nanotechnology in clinical neuroscience. Nanoparticles could potentially carry out multiple tasks in a predefined sequence, which is very important in the delivery of drugs across the BBB.

DTI experiments were planned in order to characterize the correlation between brain damages and the diffusion parameters. Fractional Anisotropy (FA), Axial Diffusivity (AD), Radial Diffusivity (RD) and Apparent Diffusion Coefficient (ADC) are the main indexes used in literature to detect and characterize EAE brain lesions. DTI experiments were executed in eight subject before, 30 and 60 days post immunization (dpi). For each subjects, DTI data acquired before immunization were considered as control reference. In addition, acquisition of high resolution T2 weighted anatomical images, were planned at each time point for each subject. These data have been used for a Voxel Based Morphometry (VBM) analysis of the treated versus control rats.

DTI experiments showed a significative decrease (p<0.05) in FA (CTRL 0.31 ± 0.02, 30 dpi 0.26 ± 0.02, 60 dpi 0.27 ± 0.02) and AD (CTRL 0.00133 ± 0.00008 mm^2/s, 30 dpi 0.00105 ± 0.00009 mm^2/s and 60 dpi 0.00116 ± 0.00009 mm^2/s) when evaluating the whole brain. When analyzing RD and ADC we found no significative differences between the pre and post induction acquisitions. VBM analysis, based on the T2 weighted high resolution images, showed statistically significative decrease of the grey matter volume as the disease progresses.
Lanthanide doped fluorides are very interesting hosts for their efficient luminescence in the visible and infrared regions. Therefore, they are promising probes in biomedical diagnostics. Er\(^{3+}\)/Yb\(^{3+}\) and Tm\(^{3+}\)/Yb\(^{3+}\) doped MF\(_2\) (M=Ca, Sr) nanoparticles (NPs) have recently received attention, due to their strong upconversion (UC) emission [1-3]. It is here presented an easy hydrothermal one-step procedure to prepare citrate capped CaF\(_2\) NPs triply doped with Yb\(^{3+}\), Er\(^{3+}\) (or Tm\(^{3+}\)) and Gd\(^{3+}\) ions. The present NPs are easily dispersible in saline solutions, essential properties for their potential use in biological fluids. The obtained NPs are cubic single phase and are well size monodispersed with average sizes that can be easily tuned by changing the preparation conditions. The obtained transparent colloidal dispersions show strong UC emission in the red (around 650 nm) and in the NIR (around 800 nm) for the Er\(^{3+}\) doped and Tm\(^{3+}\) doped NPs, respectively, upon laser excitation at 980 nm in the \(^{2}F_{5/2}\) level of Yb\(^{3+}\). Both the excitation and the emission radiations are close to or inside the biological window, suggesting their possible use for in-vitro and in-vivo biological optical imaging. Results of spin echo measurements on saline colloidal dispersions of the NPs have also shown significant proton relaxivities, indicating that the NPs are interesting MRI contrast agents.

References
Bio-imaging by Luminescent Porous Silicon Particles

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Among the different nano materials employed in the medicine field, silicon-based ones have some advantages due to their intrinsic biocompatibility. This research focused on bio-imaging of silicon nanostructures. To achieve this propose, we synthesised light emitting of porous silicon (pSi) particles by anodization technique. By controlling synthesis conditions, nano and macro particles were obtained. In addition, surface functionalizations were carried out by carboxyl or amine groups, in order to favour cell penetration, and also stabilization from optical ageing.

In aspects of biocompatibility and cell penetration, the micro-pSi particles had better performances in comparison with the nano particles. In view of morphology, micro-pSi particles are in the range of 2-10 µm, and about 30 nm porosity (Figure 1a). After functionalization with carboxyl (COOH-pSi), there is a broad bond at 600 nm which is typical for pSi due to quantum confinement (Figure 1b). By further functionalization to achieve amine group (NH$_2$-pSi), another peak appeared at 420 nm. To the best of our knowledge, having two emission bonds from Si, has not reported before. Then, penetration and light emission of the NH$_2$-pSi in dendritic human cells were demonstrated by confocal microscopy (Figures 1C and D), which is promising for imaging and drug delivery.

Figure 1. pSi SEM (a); PL Emission of NH$_2$- and COOH-pSi by excitation at 350nm (b); light emission of the NH$_2$-pSi in human dendrite cell by confocal microscopy, excitation 405nm, emission at red (c) and green (d).

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PhD Program in Neuroscience
Leptin-controlled diurnal activity of periaqueductal gray neurons affects nociception during obesity

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Leptin-deficient ob/ob mice display altered innervation on hypothalamic orexin-expressing neurons (OX-N) in favor of inhibitory inputs. Sufficient excitatory inputs remain for on-demand endocannabinoid (eCB) synthesis. The eCBs act on presynaptic cannabinoid receptor type 1 (CB1R), located on pre-synaptic terminals, thereby, reducing the neurotransmitter release. Thus, OX-N of ob/ob mice are disinhibited by an eCB-mediated reduction of inhibitory transmitter release, putatively causing a net activity increase. Consistent with this finding, the periaqueductal gray (PAG), one of the terminal projection fields of OX-N, displays increased orexin release. The antinociceptive effect of orexin administered to PAG neurons projecting to the rostroventral medulla (RVM) is accompanied by an eCB-mediated depression of postsynaptic currents. Blocking CB1R and orexin receptors in PAG sensitizes nociception in wild type (wt) and ob/ob mice, co-occurring with increasing ON and decreasing OFF cell activity in RVM. However, the alterations of functional PAG projection neurons’ properties in ob/ob mice, promoting analgesia, are unclear.

I show that PAG projection neurons are more depolarized in ob/ob mice than in wt mice. Furthermore, I describe that the firing activity of these neurons is higher in wt than in ob/ob mice when the patch-clamp recordings were made during the animals’ light phase. I also verified, that the observed firing rates are biologically relevant and not artificially caused. Currently, I am investigating resting membrane potential and firing activity of PAG projection neurons of wt and ob/ob mice during the dark phase and their dependence on orexin signaling both in light and dark phases. Initial results show a strongly increased firing activity in ob/ob mice compared to wt mice.
Nanovesicles from mesenchymal stem cells: experimental assessment of an innovative therapeutic approach for ALS

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Therapeutic strategies for the fatal neurodegenerative disease amyotrophic lateral sclerosis (ALS) are actually minimally effective on patients' survival and quality of life. Stem cells are becoming more and more important in the treatment of neurodegenerative disease but information on the involved molecular mechanisms and on its efficacy is still limited.

Here we suppose a paracrine effect of stem cell and assess the efficacy of a novel non-cell approach based on the use of nanovesicles (NV) obtained from mesenchymal adipose stem cells (ASC).

For evaluate the neuroprotective effect of ASC-NV in vitro, we used \( \text{H}_2\text{O}_2 \) (as oxidative stress) with or without ASC-NV and we evaluated cell death and phenotypic cell changes.

We used naïve NSC-34 motoneuronal cell line and NSC-34 cells transiently transfected with mutant SOD1 human gene. In both case we showed that the administration of ASC-NV in the culture medium protect cells from oxidative damage, with a 30-40% increase of cell viability.

Therefore, ASC-NV have neuroprotective effect in vitro.

For in vivo experiment, we use transgenic mice overexpressing mutant human SOD1(G93A) gene. We injected intravenously ASC-NV on mice at clinical onset once a week, until terminal stage, to assess whether systemic administration of ASC-NV can ameliorate the disease course and duration. We did not find any differences in terms of motor performance and in life spam between ASC-NV and control animals; so we plan to do other experiment in which we inject ASC-NV at presymptomatic stage and in different concentrations.

Moreover, we want process lombar spinal cord for stereological cell counting and immunohistochemical phenotyping of motoneurons (Nissl staining), microglia (anti-CD11b antibodies) and astrocytes (anti-glial fibrilary acid protein).
THE CAPSAICIN MODEL TO EXPLORE THE VARIABILITY OF PAIN SENSORY PROFILES IN HUMANS.

A COMBINED PSYCHOPHYSICAL AND fMRI STUDY IN NORMAL CONTROLS AND POSSIBLE CLINICAL APPLICATIONS IN NEUROPATHIC PAIN PATIENTS

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Background. Neuropathic pain (NP) is defined by the International Association for the Study of Pain (IASP) as pain arising as a direct consequence of a lesion or disease affecting the somatosensory system and it frequently may include allodynia and hyperalgesia. Animal models offer a bulk of experimental data, which are difficult to translate in the clinical setting. As a result, the neurophysiological mechanism underlying NP in humans are not well understood and drugs are often ineffective. The capsaicin model is widely used to explore NP sensory profiles in humans. Topical capsaicin, the active ingredient of chili peppers, is a transient receptor potential vanilloid 1 (TRPV1) agonist that causes activation of TRPV1 followed by degeneration of C fibers. Capsaicin allows the study of both gain-of-function (hyperalgesia and secondary allodynia) and loss-of-function (hypalgesia) phenomena. In the present research project, high-concentration capsaicin (8%, patch) is used in normal controls and patients affected by different types of NP.

Aims. (1) to investigate the variability of experimental pain perception in normal controls by studying somatosensory profiles before and during an experimental pain condition using standardized psychophysical testing: mechanical quantitative sensory testing (QST; with a set of von Frey filaments and of pinprick stimulators) and thermal QST (with the Medoc Thermosensory analyzer); (2) to investigate the changes and the connectivity in the resting state networks (RSNs) such as attentive parietal-frontal, sensory-motor, visual and default mode networks, using blood oxygen level dependent (BOLD) functional magnetic resonance imaging (fMRI) before and during experimental pain condition; (3) investigate the somatosensory profiles by QST and the connectivity in the RSNs by fMRI in chronic NP patients.

Study design. (1) The QST protocol, proposed by the German Research Network on Neuropathic Pain (DFNS) in 2006, comprises 13 psychophysical parameters. The QST protocol is performed in a group of normal controls prior to (baseline, T0) and after topical capsaicin application (3 x 3 cm, 60’) on the forearm (T1: early primary and secondary hyperalgesia/allodynia; T2: late hypalgesia). To date, I studied a control group of 18 normal subjects to define the range of normal baseline QST values and I acquired experimental pain condition tests in 2 volunteers.

(2) Healthy volunteers undergo two fMRI sessions using a 1.5T MR scanner before and after topical capsaicin application, at rest without any task. The functional data are processed using Multivariate Exploratory Linear Optimized Decomposition into Dependent Component (MELODIC) FSL toolbox and Probabilistic Independent Component Analysis.

We have analyzed the RSNs maps of a control group of 10 partecipants and we have acquired data from experimental pain condition sessions in 2 subjects.

Preliminary results.

(1) The results coming from the control group are in line with those reported in the literature. Regarding the preliminary results coming from the pain model, we noticed a decrease of heat pain threshold on the application site at T1 and an increase of the heat pain threshold and of the cold pain threshold at T2.

(2) The results coming from the control group are in line with those reported in the literature. Regarding the pain model we noticed an increased of extension of the sensory-motor network (in particular on the secondary somatosensory cortex bilaterally and left supplementary motor cortex) and also of the posterior cingulate cortex and the parietal association cortex bilaterally.
Manipulating spatial selective attention.
A behavioral, psychophysiological and reward-based study.

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Visual scenes in every-day life contain a vast amount of information, exceeding what the human visual system can process at a given time. Therefore different visual elements compete for privileged access to processing resources and spatial selective attention is enacted to focus perception onto a subset of the incoming input. The notion of a limited attentional processing capacity is at the core of the attentional bottleneck theory, stating that the attentional system can attend to a limited number of stimuli. Control mechanisms of spatial attention reside in a coordinated network of interconnected cortical (frontal and parietal) and subcortical brain structures (e.g. the superior colliculus). Within this control network, spatial priority maps are encoded, namely real-time representations of the behavioral salience of locations in the visual field, resulting from the combined influence of stimulus-driven activity and top-down signals related to the current goals of the individual. They arbitrate which of a number of (potential) targets in the visual field will win the competition for attentional resources. In a first phase of this behavioral research, performed on healthy human volunteers, we implemented a visual search task, in which one or multiple targets were presented among distractors, in combination with the non invasive scalp recording of event-related potentials (ERPs), in order to directly reveal the attentional bottleneck and its neural manifestations (Exp. 1). In a second phase we tested whether by means of a suitable reward-based training regime, we could produce enduring changes in priority maps that are responsible for directing spatial attention and arbitrating selection under conditions of cross-stimulus competition (Exp. 2). The first experiment allowed us to unveil major determinants for target report, with a strong influence of the position (or relative position) of the target(s) within the array. Higher accuracy was assessed for single targets presented in the most lateralized positions, and for double targets presented in opposite (vs. same) hemifields, suggesting some degree of parallel processing between hemispheres. In addition, neural correlates of the attentional bottleneck (N2pc waveform component) were measured. The N2pc was modulated by the number of targets identified, as well as by their relative position, but was not affected by the actual number of targets in the array, therefore solely reflecting top-down visual selective attention. In the second experiment, we demonstrated that spatial priority maps can be shaped via reward-based learning, reflecting long-lasting alterations (biases) in the behavioral salience of specific spatial locations. These biases exert an especially strong influence on performance under conditions of cross-target competition, conferring competitive advantage to targets presented in spatial locations associated with higher reward during training. The acquired biases of spatial attention were persistent, non-strategic in nature and generalized across stimuli and task contexts. These results suggest that reward-based attentional learning can induce plastic changes in spatial priority maps, endowing these representations with the “intelligent” capacity to learn from experience.
The Role of Tumor Microenvironment in Cerebral Glioma Progression

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**Background**: Gliomas are the most common brain tumors, accounting for more than 30% of all central nervous system malignancies. Glioblastoma Multiforme (GBM, WHO grade IV) is the most aggressive subtype. Despite notable achievements reached in neuroimaging and surgical techniques along with multimodal radio- and chemotherapy, GBM patients have a median survival of only 14.6 months. The tumor microenvironment contains a key population of tumor cells named Glioma Stem Cells (GSCs) which are able to self-renew, proliferate and they are chemo/radioresistant. Among the non-tumor cells, the tumor-associated macrophages (TAMs) which are functionally subclassified into M1 and M2. M1 are pro-inflammatory and have an anti-tumor effect, while, M2 are immunopermisive and promote tumor growth. GBM studies revealed a cross talk between GSCs and TAMs in an immunosuppressive milieu. Through multiple paracrine loops, GSCs polarize TAMs into M2 and in turn, M2 promote GSCs proliferation, angiogenesis and invasion. Thus, this symbiotic relationship is skewed in favor to glioma growth. The low grade gliomas (LGGs) "grade II" have a longer survival compared to GBM; however, finally they progress to the high grade gliomas (HGGs). For reasons only in part known (biomolecular markers), the time to progression (TTP) is extremely variable, ranging from few months to several years. To date, LGGs lack any treatment guidelines and usually a wait-and-see approach is followed. **Objectives**: to analyze the role of M2 in LGGs by calculating M1/M2 ratio (higher M2 ratio might correlate with a shorter TTP from LGGs to HGGs) and to understand the contribution of soluble factors released by M1 and M2 on glioma cells. **In vivo Experiments**: Database search was performed on clinical and radiological records from 500 glioma patients. We recruited 18 patients who were surgically resected twice and did not receive chemo/ radiotherapy. We performed immunohistochemistry for CD163 to evaluate the presence of M2 cells in 22 LGG; CD163+ cells were detected in all examined specimens. We performed qRT-PCR to investigate the mRNA expression of IL-8, IL-10, Macrophage-Colony Stimulating Factor (M-CSF), Tumor Growth Factor-b (TGF-b) in the same LGG cases; the mRNA levels of these cytokines were highly expressed relative to actin mRNA; pointing to an immunopermisive milieu. **In vitro Experiments**: U937 macrophage cells were exposed to IFN-g and IL-4 to be polarized into M1 and M2 respectively. After 16 hours, macrophages-conditioned media (M-CM) was collected then applied on U251 glioma cells. Next to this step, we performed MTT assay to measure the metabolic activity of glioma cells and BrdU incorporation test to evaluate glioma proliferation. U251 glioma cells showed significant decrease in their activity following IFN-g exposure. Our preliminary data showed that M1 and M2 can contribute to either glioma inhibition or proliferation respectively.
Pisa Syndrome in Parkinson’s Disease: An Electrophysiological, Imaging and Biomechanical Study

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Pisa syndrome (PS) is clinically defined as the sustained lateral bending of the trunk worsened by a prolonged sitting position or by walking. It was first described by Ekbom and colleagues as the consequence of acute axial dystonia related to neuroleptics administration. In recent years, cases of PS have been described in patients affected by neurodegenerative disorders such as Multiple System Atrophy and Parkinson’s Disease (PD).

The pathophysiological underpinnings of PS are still not fully elucidated and different mechanisms have been taken into account. The few studies investigating the pattern of muscular activation on electromyography (EMG) yielded conflicting results. In 20 PD patients with PS, Bonanni et al. first reported that EMG of lower paraspinal muscles (though they did not detail the level of EMG exploration) ipsilateral to the bending side showed a pattern of continuous muscle activity while standing compatible with a dystonic contraction. Di Matteo et al. recorded EMG from paraspinal muscles at the thoracic–lumbar level (longissimus thoracis at T12–L1) in ten PS patients and confirmed in only a minority of them a dystonic activity characterized by a continuous muscle activity ipsilateral to the bending side. By contrast, in most patients a continuous activity in muscles contralateral to the leaning side was detected. Very recently, Tassorelli et al. reported an ipsilateral EMG hyperactivity in the abdominal oblique and paraspinal thoracic muscles.

The discrepancies of these studies may rely on methodological differences, particularly on the EMG testing paradigm (e.g., static vs. dynamic assessment of muscular activation) and, more importantly, limited and different assessed muscles. Indeed, it is known that the lateral bending of the trunk is produced by several muscles, including lumbar and thoracic paraspinal muscles, abdominal oblique as well as iliopsoas muscles.

In order to clarify the role of dystonia and muscle weakness in the pathophysiology of PS, we are going to assess with qualitative and quantitative EMG analysis, the activity of muscles involved in lateral bending in three groups of subjects: 1) patients with PD and PS, 2) patients with only PD and 3) age-matched normal subjects. The EMG data will be correlated with demographic, clinical and magnetic resonance imaging findings.
Interdigit tactile perception in Parkinson’s disease

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Alterations in tactile perception have been found in different movement disorders, such as Parkinson's disease (PD) and dystonia. The occurrence of similar sensory deficits in different movement disorders seems to be an epiphenomenon of a common underlying factor: a basal ganglia dysfunction. Indeed, these subcortical structures are involved both in motor control and somatosensory processing. However, by means of Aristotle’s illusion, we recently found an alteration in tactile perception in the affected hand of focal hand dystonia patients but not in those with other focal dystonia (cervical dystonia and blepharospasm). This specific alteration in tactile domain might be a specific feature of focal hand dystonia, probably related to an abnormal somatosensory cortical activation.

The aim of the current study was to investigate whether this tactile function is compromised also in PD patients and whether it is related to the severity of motor symptoms.

Aristotle’s illusion consists in the illusory doubling perception of a single object touching two crossed fingers. This tactile illusion derives from the interaction between two functionally unrelated skin areas and allows to investigate on the interdigit functional relation in tactile perception. We applied a specific designed Aristotle’s illusion paradigm in 15 PD patients, with motor symptoms mainly localised on one body side, and in 15 healthy controls. We tested three pairs of fingers in crossed (evoking the illusion) or parallel position (not evoking the illusion). We applied on participants' fingers one or two spheres. The blindfolded participants had to refer whether they felt one or two stimuli. The affected and less/non-affected side were tested.

The amount of illusion didn’t differ between patients and controls. We also found no significant difference between the affected and less/non-affected side.

Despite the alterations in other types of tactile discrimination tasks, the Aristotle’s illusion is preserved in Parkinson's disease. This suggests that basal ganglia, which are dysfunctional both in PD and dystonia, may not be causally involved in this function. Furthermore, the fact that the illusion similarly occurred in both affected and less/non-affected side, suggests that the motor symptoms do not influence this kind of tactile perception.
Characterization of pathophysiology in cellular models of different forms of neuronal ceroid lipofuscinosis

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The neuronal ceroid lipofuscinosis (NCL) are a heterogeneous group of progressive neurodegenerative disorders affecting children and adults, characterized by the endolysosomal storage of autofluorescent material and lipofuscin within neurons as well as peripheral tissues, such as skin fibroblasts and blood lymphocytes. The CLN1 disease is caused by mutations in CLN1, which encodes the lysosomal enzyme palmitoyl protein thioesterase 1 (PPT1). The majority of patients are diagnosed as infantile-onset NCL. The exact physiological function of PPT1 still remains elusive. A recent study has outlined the spatial relationship between lysosomal engulfment and damaged mitochondrial reticulum in CLN1 fibroblasts, and has demonstrated that the mitochondrial compartment is affected in these cells in vitro. Kufs disease (KD), the most common form of adult NCL, can be subgrouped in type A and B depending on the presence of myoclonus epilepsy. Mutations in the Cathepsin F gene (CTSF) have recently been discovered in autosomal recessive type B KD families of French-Canadian, Australian, and Italian origin. CTSF is a widely expressed lysosomal cysteine protease whose role in vivo is unknown. Over-expression of N-terminus truncated forms of human cathepsin F in HEK 293T cells have recently been associated with features suggestive of aggresome-like inclusions, inducing autophagic features.

Aim 1. To study the cell pathology caused by Mediterranean CLN1 mutations using in vitro cellular models, we planned to use CLN1 human fibroblasts and derived cultured neuroblastoma cells. We aimed to test mitochondrial structure and functions in these cells, aiming at the identification of biomarkers of CLN1 disease. Aim 2. To perform the cellular characterization of a novel mutation in CTSF associated with type B KD. We planned to investigate the role of a N-terminally truncated form of Cathepsin F in human fibroblasts.

In the first part of this study, we evaluated the effects of lysosomal storage on the mitochondrial compartment in primary cells (skin fibroblasts) harboring Mediterranean CLN1 mutations (namely, c.124+1215_235-102del3.6Kb/p.A43_G145del, and c.665T>C/p.L222P). We observed a fragmentation of the mitochondrial network, and a significative reduction of oxidative ATP production. Also, we analyzed the mitochondrial functions in differentiated SHSY5Y wild-type and stably transfected cells harboring mutations in CLN1 (p.A43_G145del and p.L222P). The oxidative ATP production was comparable in the different cells lines whereas the complex I and complex IV activities, evaluated by BN-IGA, showed an increase in the mutant clones when compared with SHSY5Y cells. Western blotting using antibodies against different subunits of respiratory chain complexes revealed normal steady state levels of all subunits. We also observed mitochondrial fragmentation in the p.L222P clone. In the second part of the study, we identified an additional type B KD family with pseudo-dominant transmission novella of a new homozygous c.213+1G>C mutation in CTSF. In cultured skin fibroblasts from two patients, we observed incorrect splicing removing exon 1 and predicting a mutant protein with a chopped N-terminus. Western blotting showed low cathepsin F expression in cultured cells. We also observed ultrastructural features resembling aggresome-like structures in cultured primary cells together with enhanced expression of polyubiquitinated proteins and higher levels of Lamp2 and p62/sqstm1. Also, we demonstrated high expression of LC3II protein, suggestive of dysregulated autophagy.
Synaptic plasticity in the hypothalamic nuclei is coordinated by hormonal signals response to the physiologic status of feeding and is strictly regulated by the fine balance between phosphorylated/unphosphorylated Tau protein, mainly controlled by the glycogen synthase kinase-3 beta (GSK-3β) activity. GSK-3β is constitutively active under resting condition and is inactivated by extracellular signals like leptin through phosphorylation of Ser-9 residue (Greco et al., 2009). In opposition, GSK-3β is activated by lysophosphatidic acid (LPA) which, in turn, leads its phosphorylation in Tyr-216 and subsequent Tau phosphorylation through tyrosine kinase Pyk2 (Sayas et al., 2006). LPA is a bioactive lipid precursor of the endocannabinoid 2-arachidonylglycerol 2-AG (Bisogno et al., 1999). In the brain of leptin defective signalling obese ob/ob mice we found an increase of both 2-AG levels, in the lateral hypothalamus, and orexin-A/hypocretin-1 (OX-A) expression in the arcuate nucleus (ARC) (Cristino et al., 2013). In the same condition, we found in the ARC a strong increase of pTau/Tau ratio by immunohistochemical and WBs analysis; this condition was reverted 60 min after acute i.p. leptin injection. On this basis we hypothesized a functional orexin/endocannabinoid/leptin interaction as upstream signalling for the fast hypothalamic synaptic plasticity in the ARC. In order to test this hypothesis we examined how leptin and OX-A can affect both GSK-3β phosphorylation, by activation of p-Akt or p-Pyk2, and pTau/Tau ratio in the ARC of wt and ob/ob adult male mice matched to controls (untreated mice) after in vivo leptin, OX-A, SB336847 (the antagonist of OX-A) treatments.
Alzheimer’s Disease and Rac1 alteration: a possible interesting correlation

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Alzheimer’s Disease (AD) is a neurodegenerative disorder characterized by a dramatic synaptic degeneration and dendritic spine loss, and by two types of protein deposits in the brain, the intracellular neurofibrillary tangles, due to hyperphosphorylated tau protein and extracellular amyloid plaques, containing predominantly the β-amyloid peptide (Aβ). Dendritic spine stability depends on actin dynamics, which is mainly regulated by Rho GTPases. Rac1, one of the best characterized members, is able to promote spine formation growth and stabilization. Moreover, recent evidence indicates an interesting link between Rac1 and AD, suggesting its involvement in the processing of Aβ from its precursor APP.

The aim of this work is to investigate the effects of Aβ exogenous administration on Rac1 expression, localization and activity in different in vitro models: a neuroblastoma cell line and primary cortical neurons.

The project started with the preparation and characterization of Aβ synthetic oligomers. Then, cells were treated with Aβ and the toxicity of the preparation was verified with a colorimetric assay (MTT). The activation of Rac1 protein after administration of Aβ oligomers was studied with immunofluorescence.

Our preliminary results show that, after 1 µM Aβ treatment, there is an increase in Rac1 activation in both cell types. Since it is known that Rho GTPases are tightly regulated at spatio-temporal level, and classical methods used to study their complex signalling network are focused only in one dimension, we decided to perform a FRET (fluorescence resonance energy transfer) experiment. This imaging technique allows the study of the proteins of interest on a spatio-temporal level. We have started the optimization of FRET experiments and we are currently investigating whether Rac1 alteration might be directly connected to Aβ metabolism.
Testing nanoparticles for intracerebral drug delivery

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The blood-brain barrier (BBB) controls the passage of molecules and cells into the brain. This is crucial for the delivery of drugs in the treatment of central nervous system (CNS) diseases. Nanotechnologies applied to the drug delivery can offer many advantages over conventional therapeutic methods.

The drug suramin, used in the treatment of the peripheral infection caused by the parasites African trypanosomes, does not cross the BBB and therefore cannot be used to cure the encephalitic stage of disease. This doctoral project is ultimately aimed at achieving the transport of suramin into the CNS using NPs as carriers. During the second year of the project it was determined in preliminary studies that direct delivery of suramin into the brain does not cause overt cell damage or inflammatory response in the brain, both in naïve mice and in African trypanosome-infected mice. The experiments of the second year focused especially on the screening of potential NP carriers, assessing the capability of metal-based NPs and polymer-based NPs to cross the BBB.

Concerning metal-based NPs, after 24h from iv injection in mice, CaF$_2$ and SrF$_2$ NPs were observed mostly in the liver and spleen, and were also found in the brain parenchyma. Cerium oxide (CeO$_2$) NPs are of special interest since they can exert neuroprotective effects. Their short-term biodistribution was investigated with different techniques. The findings pointed out that the largest accumulation of CeO$_2$ NPs occurs in the liver and spleen, and also these NPs were found in the brain parenchyma.

Concerning polymeric NPs, PLGA NPs conjugated with a peptide derived from Apolypoprotein E and Prostaglandin-D-synthase were tested. These NPS were found to enter the brain parenchyma at 2h after peripheral administration, despite their relative high dimension due to the polymeric structure.

The present data indicate that metal-based NPs can enter the CNS, probably due to their small dimension (~10nm), despite their accumulation in peripheral organs. Possible surface modifications are currently considered to overcome macrophage recognition and to improve the blood circulation time. PLGA NPs can also reach the CNS, albeit in limited amount, and targeting with a peptide seems to improve their BBB crossing.

In parallel, the investigation of CeO$_2$ NPs and PLGA NPs effect on brain cells was initiated. Glial cell phenotyping was pursued to reveal astrocytes using glial fibrillary acidic protein (GFAP) as marker, and CD11b antibodies were used to visualize microglia. Both glial cell types reacted to the penetration of the tested NPs into the brain. It remains to be investigated whether this represents a detrimental or a beneficial response.
Alzheimer’s disease (AD) is characterized by cognitive impairment and neuropathological hallmarks. Deficits, however, are late signs of disease progression, while altered rhythms of cortical activity and sleep abnormalities can precede memory deficits. The identification of reliable, early biomarkers of Alzheimer’s disease (AD) is crucial in unraveling its pathogenesis and in developing therapeutic strategies based on early intervention. Since data in humans indicate that electroencephalographic (EEG) and sleep abnormalities can precede overt disease, we examined these parameters in a transgenic (Tg) mouse model of AD, the TASTPM mice. Wild-type (Wt) and Tg mice of 5 and 21 months of age (4 mice per group) were subjected to continuous EEG and EMG recording (1 fronto-parietal bipolar derivation) with telemetry, under a 12h/12h light-dark cycle, according to EU ethical guidelines. Data were analyzed with MATLAB 8, using the eeglab toolbox and custom scripts. Vigilance stages (active QW and quiet wakefulness QW, NREM, and REM sleep) were manually scored over a 12-hour time window. Also, EEG power spectra were computed on at least 8 epochs (4 seconds long) for each vigilance stage. The EEG power spectra (1-20 Hz) were averaged for each genotype/age group considering specific spectral bands (delta: 0.5-5Hz, theta: 5-9Hz, alpha: 9-14Hz, and beta:14-20 Hz). Preliminary results: TASTPM mice showed an increase in wakefulness amount and reduction in NREM and REM sleep amount during the light phase at 20 months old of age. The amount of wakefulness episodes after sleep onset (WEASO) was increased in TASTPM group at age of 20 months old of age. Also, the total number of transitions between the different vigilance stages was increased in Tg old mice. Multifactorial analysis detected an Age-Genotype interaction for NREM-QW transitions (p<0.039). Preliminary EEG power spectra analyses during the light phase show that, at 5 months, TASTPM mice tend to have increased EEG power during wakefulness (in alpha and gamma band: peak between 25-30 Hz, p<0.001), in REM sleep (peak in alpha band, p<0.001), and NREM sleep (with peak in theta band, p<0.001) with respect to Wt. Moreover, at 20 months old, TASTPM mice exhibited persistent increase in REM sleep EEG power in delta, theta y alpha bands. Quantitative EEG analysis is a cost-effective tool used for several decades in AD study and can provide useful information related with cortical activity changes in Tg mice models of AD at early stages. Our preliminary findings indicate that EEG changes could precede Aβ deposition, thereby pointing to cortical activity as a potential early biomarker with diagnostic/predictive value in AD. Further analyses, including comparisons of sleep/wake cycles between strain and ages, are on-going.
Administration of deoxyribonucleosides or inhibition of their catabolism as a pharmacological approach for mitochondrial DNA depletion syndrome

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Defects in some of the enzymes involved in the homeostasis of mitochondrial deoxyribonucleoside triphosphate (dNTP) pool have been associated to mitochondrial DNA (mtDNA) depletion and deletion syndromes (MDDSs), such as mitochondrial neurogastrointestinal encephalomyopathy (MNGIE) or deoxyguanosine kinase (dGK)-deficiency. Despite their severity there are hitherto no effective therapeutic options for MDDSs.

We show that treatment with tetrahydrouridine (THU), an inhibitor of cytidine deaminase (CDA), increases deoxycytidine (dCtd) concentration and partially prevents the thymidine-induced mtDNA depletion cellular model of MNGIE. Furthermore, we demonstrate that 100 mg/kg THU intravenous acute administration to a murine model of MNGIE, significantly increases systemic dCtd concentration and avoids the dUrd accumulation observed in this murine model. When THU and dCtd are co-administered, a positive correlation between circulating dCtd and mitochondrial dCTP is evidenced in targeted tissues such as brain and liver, indicating that mitochondrial dCTP pool size can be manipulated in vivo. Additionally, we provide evidence that deoxyguanosine (dGuo) supplementation prevents mtDNA depletion in dGK-deficient fibroblasts. Remarkably, we observe that the specific inhibition of purine nucleoside phosphorylase in fibroblasts by immucillin H prevents dGuo degradation and allows for mtDNA copy number stabilization at lower doses of the administered nucleoside.

In light of the present results, we propose the use of deoxyribonucleosides and/or the specific inhibitors of their catabolism, as a potential pharmacological approach for treating MDDSs due to defects in dNTP homeostasis.
Sleep and infections

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The present experimental doctoral project deals with the pathogenesis of sleep disturbances in two infections: infection with influenza virus (experimental series A), that has been recently associated with narcolepsy; infections with the parasites African trypanosomes, during which narcolepsy-like changes in sleep pattern appear (experimental series B). Several rodent strains and treatment regimens were used. Electroncephalogram (EEG) and actimetry were recorded with the NeuroLogger device. Under ethical approval, infected mice were sacrificed following the onset of sickness signs and brain samples were collected.

A. Rag1-/- (lacking B and T cells) and wild-type mice infected with a mouse-neuroadapted or a 2009 H1N1 influenza A virus strain were used. In Rag1-/- mice, immunohistochemistry for antigens of the mouse-neuroadapted influenza strain showed hypothalamic and upper brainstem viral localization, including neurons involved in sleep-wakefulness regulation. Moreover, preliminary EEG analyses showed severe changes in the sleep-wake pattern in the infected Rag1-/- mice. Levels of viral RNA and inflammatory markers were analysed by real-time RT-PCR. Interestingly, preliminary data showed the presence of transcripts encoding the non-structural viral protein (NS1) in the brain of mice infected with either viral strain was transient in wild-type mice, but persisted in Rag1-/- mice. Up-regulation of transcripts of inducible nitric oxide synthase (iNOS) was seen in infected wild-type mice.

B. iNOS-/- and wild-type mice infected with African trypanosomes (the parasites Trypanosoma brucei brucei, T.b.b.). Moreover, T.b.b.-infected rats were used to understand if the chemokine CXCL-10 can represent a candidate marker for the late stage of African trypanosomiasis. ELISA analysis showed that CXCL-10 concentration increases in both serum and cerebral spinal fluid (CSF) of T.b.b.-infected rats during the progression of the disease. In particular, a steep increase of CXCL-10 levels was observed between 6-14 days post infection (dpi) in serum while in CSF a progressive increase was observed from 14 dpi. The analysis of EEG recordings from iNOS-/- and wild-type infected mice is currently ongoing.

In conclusion, the data suggest that infection of wild-type and Rag1-/- mice may provide useful models to study changes in sleep patterns caused by H1N1 influenza A virus strain, and may increase our understanding of the role of direct viral versus innate and adaptive immune response effects. Also, ELISA data analysis suggests that the increase of CXCL-10 levels follows the evolution of the disease in an African trypanosomiasis rodent model.
Orexin in inflammation and aging-related neurodegenerative diseases

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The neuropeptides orexins (OX) A and B, also called hypocretin-1 and 2, are expressed by neurons of the lateral hypothalamus, and largely co-localized in the same neurons. The orexinergic system has been implicated in a variety of mechanisms including food intake and energy homeostasis, reward mechanisms, arousal and the maintenance of consolidated periods of wakefulness, as well as in synaptic plasticity. Data obtained in the sleep disorder narcolepsy suggest that OX neurons could be vulnerable to inflammatory signaling. On the other hand, progressive sleep disturbances are widely documented in aging-related neurodegenerative diseases, and in particular in Alzheimer disease (AD), whose histopathological hallmarks include neuroinflammatory features. On this basis, the present doctoral project is aimed at assessing changes in the orexinergic system after inflammatory challenges and in aging-related neurodegenerative diseases.

In the second year of the project the study focused on the analysis of OX-A changes in: A) mice of 12 and 20 months of age treated with central (intracerebroventricular) or systemic (ip) injections of the endotoxin lipopolysaccharide (LPS) and examined 24 h or 5 days after LPS exposure; B) transgenic mice which provide murine models of AD (triple mutant B6 tau 152 mice of 8 and 24 months of age), and in the murine model of the motoneuron disease amyotrophic lateral sclerosis (ALS), represented by mutant SOD1(G93A) mice at disease onset and end stage. The studies were based on immunophenotyping of OX-A neurons and quantitative analyses (cell counts) performed with unbiased design-based stereology.

The findings indicate a progressive decrease of OX-A neurons after LPS exposure. Significant loss of OX-A neurons was also documented in the transgenic murine models of AD and ALS during disease progression. In all these data sets, marked activation of glial cells (astrocytes and microglia) was documented in the lateral hypothalamus. Furthermore, amyloid-β deposition was found in the hypothalamus of the aged triple mutant mice which provide a model of AD.

Taken together, the findings point to vulnerability of OX neurons to neuroinflammatory signalling in paradigms which do not implicate neurodegeneration (LPS exposure), or associated with the degeneration of distinct neuronal populations in different regions of the central nervous system.

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PhD Program in Psychological and Psychiatric Sciences
Developing evidence-based guidelines with the GRADE methodology.

A project of knowledge translation in the Verona Department of Mental Health

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Background: Evidence-based treatment guidelines are considered a valuable link between primary research and daily clinical practice, as they allow clinicians to take research findings into account when making decisions under ordinary circumstances.

Objectives: This project aims to develop a set of evidence-based guidelines in the local context of the Verona Department of Mental Health (DMH); to build up a shared strong methodology for choosing and managing pharmacological treatments; to assess guideline impact on clinical practice.

Methods: Psychiatrists with a specific interest in the rational use of psychotropic drugs were identified and appointed as members of a Guideline Development Group (GDG). The GDG identified controversial areas in the use of psychotropic drugs, defining scoping questions and outcomes of interest. The GDG was supported by a scientific committee (Unit of Clinical Psychopharmacology of the University of Verona), which searched the evidence and provided scientific support with the GRADE (Grading of Recommendations Assessment, Development and Evaluation) methodology for summarizing the evidence base and grading its quality. On each topic identified a recommendation was drafted and discussed among GDG members, and then in two plenary sessions involving all medical staff of the DMH in order to reach a consensus and a formal agreement.

Results: Recommendations were formulated on 12 topics, including use of medicines in particular populations (pregnant women, patients with comorbidities). According to GRADE methodology, the quality of evidence was assessed making a judgement on studies’ Limitations, Inconsistency, Indirectness, Imprecision and Reporting Bias.

Conclusions: This project of guidelines development included several peculiar aspects, such as a bottom-up approach, which means that physicians were involved since the very initial steps of guideline production, including the choice of topics. Hopefully, the monitoring phase that is currently ongoing will clarify whether this process is likely to be an effective implementation strategy.
The involvement of breast cancer patient and family members during oncological consultation

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Background: the interest in communication concerning the information needs of oncology patients has steadily increased. Studies on patient involvement show that patients who participate in the decision making process show greater treatment adherence and have better health outcomes. Different methods to promote an active participation have been described, however similar studies in Italy are lacking.

Aims: The aims are: 1) assess the effects of a pre-consultation intervention (QPS) to increase the involvement of breast cancer patients by determining an increase in questioning, and 2) explore the role of the family member in the information exchange.

Methods: all patients with breast cancer who attend the Oncology Out-patient Services for the first time are randomly assigned to the intervention or to the control group. The intervention consists of the presentation of a list of relevant illness-related questions (QPS) before the consultation. Standardised questionnaires are administered at baseline (before the randomisation) and immediately after the consultation.

The main outcome measures are: 1) the number of questions asked by patients during the consultation, 2) the patient’s involvement by the oncologist, 3) patient’s perceived achievement of her information needs, and 4) the quality of the doctor-patient relationship

Preliminary results: the recruitment phase has been concluded. Among all patients (537), 143 (26.63%) were excluded according to our exclusion criteria. Of 394 (73.37%) eligible patients, 70 (17.77%) patients refused to participate in the study, so that 324 (82.23%) patients were assigned, according to the randomization. 16 (4.94%) audiotapes were lost due to technical reasons so that the final sample comprised 308 patients with complete data: 158 cases and 150 controls. Concerning the main outcome, what emerges from the preliminary analysis is that the prompt-sheet has not improved the number of patient’s questions in the experimental group, compared to the control group. Furthermore patients tend to ask more questions with a mean of 15 (SD = 10.94) compare to what is reported in the literature, but patients in the control group seem to ask more questions (mean 16, SD = 12.50, range 0-74) compared to the experimental one (mean 13, SD = 9.04, range 0-55), with a significant difference (t = -2.14, p = 0.03).

Discussion: the intervention of the prompt-sheet has not increased the number of questions made by patients as we hoped. So we are trying to understand what happened and which are the reasons behind this phenomenon. Multilevel analyzes will be done to determine the weight of possible confounding variables.
Pharmacological treatment of Panic Disorder: a systematic review and meta-analysis of antidepressants versus placebo studies

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A panic attack is a discrete period of fear or anxiety that has a rapid onset, reaches a peak within 10 minutes and in which at least four of thirteen characteristic symptoms are experienced. Many of these symptoms involve bodily systems, such as racing heart, chest pain, sweating, shaking, dizziness, flushing, stomach churning, faintness and breathlessness. Further recognised panic attack symptoms involve fearful cognitions, such as the fear of collapse, going mad or dying, and derealisation. Panic disorder, with or without agoraphobia, is highly co-morbid with other psychiatric disorders.

The treatment of panic disorder includes psychological and pharmacological interventions, often used in combination. Given the complexity of the condition and the lack of recent data from systematic reviews in panic disorder it would be very important to carry out a comprehensive review of available drug options, especially in the framework of network meta-analysis (NMA), to assess which treatments, if any, are the most effective and safe.

The objectives of the review are: (1) to determine the efficacy of antidepressants in alleviating symptoms of panic disorder, with or without agoraphobia, in comparison to placebo; (2) to review the acceptability of antidepressants in panic disorder, with or without agoraphobia, in comparison with placebo. (3) to investigate the adverse effects of antidepressants in panic disorder, with or without agoraphobia, including general prevalence of adverse effects, compared to placebo.

My work is part of a bigger project involving other international members of the Cochrane Collaboration Neurosis and Anxiety group. I am involved in the “Antidepressants versus placebo review and meta-analysis”, which will be conducted according to Cochrane recommendations, as first reviewer in cooperation with Dr. Andrea Cipriani, as second reviewer. To date I have carried out the first selection of the abstracts and references provided by the CCDAN search, highlighting 101 out of 1383 titles which could be potentially relevant for our purpose. This has been done according to the inclusion and exclusion criteria mentioned in the protocol. I have also begun to retrieve the hard copies, as the next steps of my contribute to this project will be: reading the full text, defining the studies to be included, extracting data, performing the statistical analysis and discussing our findings.
The challenges of measuring quality in social care:

*Social care and its interfaces with mental health care*

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The issue of quality in social care has differed from that in the health sector due to the specific challenges that measuring and assessing quality in social care presents. In the last decade, with the diffusion of the concept of “recovery-oriented” treatments in mental health, the relevance of social care provided to users with a diagnosis of psychiatric disorders has become crucial. Since social care interventions play a key role in supporting personal recovery it is important to develop a methodology to assess the quality derived from the two fields involved: mental health care, on one side, and social care on the other side.

The project presents five core scientific objectives: (1) to map and describe the characteristics of social care services in nine European countries; (2) to investigate and describe the quality of social care using a measure of social care-related quality of life as rated by service users; (3) to investigate and describe the quality of social care using a measure of service users’ continuity of care; (4) to assess the relationships between social care-related quality of life, continuity of care and organizational and staff characteristics; (5) to assess the relationships between social care-related quality of life, continuity of care and health and social outcomes.

The first objective is developed in the framework of the REFINEMENT (Research on FINancing systems’ Effect on the quality of MENTal health care) project. The Refinement Mapping Services Toolkit (REMAST) was used aiming at standardizing description and classification of mental health and social services. Objectives from 2 to 5 are developed in the Italian context. A multicentre study is being carried out involving three Community Psychiatric Services (CPS) in Italy: Lecco (North West of Italy), South Verona (North East of Italy), and Legnago (North East of Italy). Both staff and service users have been involved in the study.

Within the REFINEMENT project we were able to distinguish services that are typically related to the health sector from those that are not. The total number of mapped mental health services in the eight REFINEMENT study areas was 745, with 470 (63%) services in the health sector and 275 (37%) in the non-health sector. Moreover, a package of instruments has been identified to assess quality of mental health and social services at both staff and patient level. A participatory approach was used to pre-test the instruments as well as investigate service users perspectives on quality and continuity of care.
Illusions of reality: The effects of brightness on behavior and perception

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Simple reaction times (RT) are inversely related to the luminance of a visual stimulus, with RT increasing as luminance decreases, and decreasing as luminance increases. A potential discrepancy in the link between luminance and RT, however, stems from the perception of luminance itself. Luminance, the measured amount of light coming from a stimulus, is different from brightness, the perceived amount of light coming from a stimulus. Two stimuli with the same luminance can be perceived as having different brightness. This disparity creates a flaw in the relationship between luminance and RT: is RT modulated by a stimulus’ objective luminance or its subjective brightness? In the present experiment, we tested whether illusory brightness yields an effect similar to actual luminance by using the well-known simultaneous brightness contrast (SBC) illusion: a stimulus appears darker when viewed over a light background and lighter when viewed over a dark background.

In Experiment 1, participants were asked to respond as quickly as possible to light and dark stimuli presented over backgrounds with an actual difference in luminance (control condition) and a perceptual difference in brightness (illusory condition). The results demonstrated that RTs were modulated in both the control and illusory conditions, indicating that in addition to actual luminance, brightness can also influence behavior.

In Experiment 2, we tested the hypothesis that an illusion of brightness could also modulate the frequency and/or RTs to artificial visual stimuli. Using the same conditions as Experiment 1, we applied TMS over the primary visual cortex in order to induce sensations of light (phosphenes) and darkness (scotomas) in the visual field. Participants were asked to react as quickly as possible to the percepts and to report the percepts’ luminance on a gray-scale bar. The results revealed that for both the control and illusory conditions, the frequency of percepts was influenced by the background. This effect, however, did not extend to RT.

These data lend support to our conclusion that an illusion of brightness, as well as actual luminance, has a robust effect on behavior to real stimuli and perception of artificial percepts.
Recovery of sight in the blind field of hemianopics as a result of visual imagery

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Hemianopia is a deficit that affects visual perception in a visual hemifield; despite this visual loss, it has been shown that some unconscious visual abilities (“blindsight”) could be present in the blind field. Visual imagery represents the ability to produce a mental visual image in the absence of a retinal input. A growing number of studies showing that visual imagery and perception recruit similar neural pathways as well as cognitive and structural representations have suggested that visual imagery might be a good candidate for trying to shift vision from blindsight to full perceptual awareness by acting on retinotopically organized striate cortex. Thus, one of the aims of this project is to try and restore visual function in hemianopia by means of visual imagery.

Up to now we have developed the first part of the project, devoted to establish which stimulus features are better suited to lead to a strong response in the visual cortex, as assessed by behavioral experiments. Healthy participants have been tested in different behavioral visual tasks to assess the effect on speed and accuracy of response, of variables such as retinal eccentricity and orientation of the presented stimuli, in both perception and imagery conditions. In the perception condition participants were asked to detect the presence of a stimulus or perform a go-no go task, while in the imagery condition they were required to imagine a specific stimulus in a given position and a specific orientation while keeping eyes open and fixating on a central point. We found a similar behavioral effect on perception and imagery of stimuli at different eccentricities or with different orientations. These results suggest the use of these and other tasks for electrophysiological recordings (ERP) and subsequently for brain imaging techniques (fMRI and MEG) in order to evaluate the recruitment of retinotopically organized visual cortex during visual imagery in both normal sighted participants and in the affected and intact cortex of hemianopic patients.
Listening to the subjective experience. 
Functioning, disability and subjective perception of the illness in first episode psychotic patients

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Background: Psychotic disorders are among the most severely disabling mental illnesses, leading to great personal suffering for patients and their families. They are associated with multiple social disabilities, in work, study, independent living, interpersonal relations and self-care, limiting the individual’s ability to achieve the expected social roles and tasks. Most clinical and psychosocial deterioration in psychosis occurs within 5 years after the illness onset, and this timeframe is a crucial period for diagnosis and initiating treatment. International treatment guidelines and research for first episode psychosis recommend a prompt and integrated approach, based on a multi-element perspective (psychotherapy, pharmacological treatment, case management etc.).

An important target for the evaluation of the efficacy of this perspective is confirmed by the view of service users. Subjective appraisal of having a mental illness determines behavioral and emotional consequences. The meaning that patient attaches to having a psychiatric problem depends on two appraisal processes, how the illness is conceptualized and what that means about the person experiencing it. Subjective and objective assessments can offer distinct but complementary outcome variables.

The aim of this PhD thesis is to test whether a multi-element approach can produce effect on subjective appraisal of symptoms, disability and functioning in early psychosis.

Methods: Four hundred forty-four patients with first episode psychosis took part in The Psychosis early Intervention and Assessment of Needs and Outcome (PIANO), that is part of a larger research program (Genetics, Endophenotypes and Treatment: Understanding early Psychosis - GET UP) which aims to compare, at 9 months, the effectiveness of a multi-component psychosocial intervention versus treatment as usual (TAU) in a large epidemiologically based cohort of patients with first episode psychosis and their family members recruited from all public community mental health centers (CMHCs) located in two entire regions of Italy (Veneto and Emilia Romagna) and in the cities of Florence, Milan and Bolzano. 272 patients were randomized to experimental treatment and 172 to usual treatment. Subjective appraisal of symptoms (measured by PSYRATS), global functioning (measured by GAF) and disability (measured by WHO-DAS II) variables were taken both at baseline and at 9-month follow-up.

Results: Preliminary results seem to highlight significant post-treatment improvements on the PSYRATS and DAS scales. Experimental treatment seems to play a crucial role for “total delusion score”, for the subscales regarding “cognitive” and “distress” levels and for all items concerning the different aspects of delusional beliefs. Significant improvements on disability, in the area concerning relationship with partner, is also observed. Further analyses are now in progress.

Conclusion: a structured multi-element approach seems to play an important role in disability and subjective appraisal of delusional beliefs for early psychotic patients.