

European Meeting of Neuroscience by PhD students

)nd Edition

At the Grenoble Institute of Neuroscience http://neurophdmeeting.sciencesconf.org/

Friday 29th April 2016



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Organized by Neurodocs PhD students

































Acknowledgments

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We would also like to thanks the following sponsors for their support in the making of the meeting: Vigny Musset, VWR and Dutscher.

The committee wants to thank all particularly Alan SANFEY and Laura SOUCEK for accepting our invitation to come share their works.

This 2nd edition would not have been possible without the support of Frederic SAUDOU director of the GIN, the advice of Nicolas MAURIN and the precious help of Amélie DANFOSSY for the financial management of the event as well as the traveling organization. We are grateful to them and we thank them.

The organization committee

Welcome

Dear colleagues,

It is with pleasure that we welcome you within the Grenoble Institute of Neuroscience for this 2nd edition of the European Meeting of Neuroscience by PhD students. We hope that during this day you will discover current work led in Europe in the field of the Neuroscience. We also hope that it will be an opportunity for you to exchange and to establish links.

This volume contains the program as well as the abstract of all communications realized during this meeting. It is a part of the welcome pack which will be distributed to you upon your arrival.

Many thanks to all the people who contributed to the preparation of this day and we wish you an excellent congress

The organization committee

Lucie Dardevet, Julie-Anne Rodier, Brice Poreau, Anne-Cécile Chiollaz, Julie Jonckheere, Muriel Sébastien, Maëlle Gueguen, Benoit Boulan, Chrystelle Aillaud, Anthony Bosson, Astrid Kibleur, Lena Trebaul, Amandine Virlogeux.

European Meeting of Neuroscience, 2nd Edition, Grenoble Institute of Neuroscience

European Meeting of Neuroscience by PhD students

Friday, April 29, 2016

Grenoble Institute of Neuroscience

WELCOME		
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09:40 - 10:05	Suicus-based linear mapping of sensorimotor integration in the hand motor area - <i>Raffaele DUBBIOSO</i>	
10:05 - 10:35	Social motivations: insights from decision neuroscience - <i>Alan SANFEY</i>	
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ORAL COMMUNICATIONS

SEARCHING FOR CAUSAL NETWORKS IN THE BRAIN

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Over the course of the last fifteen years, functional Magnetic Resonance Imaging (fMRI) research highly contributed to cognitive science and and new therapeutic strategies such as Deep Brain Stimulation. Today, this field is further developing, and has been recently pivoting around concepts such as spatiotemporal processes (Shen et al, NeuroImage 2014) and reverse inference (Poldrack, TiCS 2006).

In the last few years however, the communication in large-scale brain networks became of interest. As spatial ICA reveals, the brain functionally divides into groups or regions who share common activity in the resting state, and have a biological meaning (Smith et al, PNAS 2009). The term 'resting state networks' (RSNs) was coined for clusters of these co-active areas. In fact, RSNs can be further divided into smaller regions with novel hierarchical ICA protocols (van Oort et al, in prep), however so far, the causal patterns of communication between those regions have not been determined.

Determining causal links between brain regions on the basis of BOLD fMRI encounters a few serious issues (Smith et al, NeuroImage 2011). Firstly, human haemodynamics is slow and therefore acts like a lowpass filter to a neuronal activity. Secondly, collecting volumes happens with a frequency much lower than the intrinsic brain dynamics. Thirdly, the signal to noise ratio is very low in general. These are the main obstacles to overcome on the way to the first human directed connectome on the basis of BOLD fMRI.

In my PhD, I am developing a new causal inference method which aims at solving these issues. I do not include regression in time into the analysis, and concentrate on systematic differences in the shape of the distribution of BOLD values between upstream and downstream regions in the brain. In order to create a data-informed but paramameter free method, I simulate simple two-node networks using Dynamical Causal Modeling (Friston et al, NeuroImage 2003) forward model which emulates generation of BOLD fMRI from neuronal

networks. Furthermore, I am using a range of computational tricks to overcome the issue of high noise levels. I am also testing the method on very high quality Human Connetome Project resting-state data which most probably gives the upper bound on the possible signal to noise ratios in fMRI research. The resulting method outperforms the competitive methods both in terms of causal inference on synthetic datasets, and test-retest reliability on the true HCP datasets.

The first causal maps of communication in the resting state networks of the human brain have biological relevance and give new insights into communication in the human brain. So far, certain patterns of functional (undirected) connectivity were found in multiple cognitive disorders, including schizophrenia (Lynall et al, J Neurosci 2014) or Major Depression (Greicius et al, Biol Psychiatry 2007). Now, this research can be extended to investigating directed connectivity and causal links between brain regions. For certain cognitive disabilities such as ADHD, reliable biomarkers still have not been found; therefore our results open a promising new field of research in psychiatry.

Key words: connectivity, networks, causal research, biomarkers, fMRI

NOTES :

OC2: 9:40 - 10:05

SULCUS-BASED LINEAR MAPPING OF SENSORIMOTOR INTEGRATION IN THE HAND MOTOR AREA

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Introduction: We have recently introduced neuronavigated linear transcranial magnetic stimulation (TMS) mapping as a method to capture motor somatotopy in the hand motor area (M1HAND). In contrast to other mapping methods, linear TMS mapping adjusts the TMS coil position and orientation to the individual shape of the central sulcus (CS).1 Here we used this technique to map the spatial representation of short-latency afferent inhibition (SAI) in M1HAND. SAI refers to a suppression of the motor evoked potential (MEP) amplitude by preceding peripheral electrical nerve stimulation of the contralateral hand.2 SAI is somatotopically specific: it's stronger when electrical stimulation is applied close to the TMS-target muscle (homotopic stimulation) and weaker when not (heterotopic stimulation).3

Aim: We hypothesized a somatotopic expression of SAI in M1HAND for homotopic as opposed to heterotopic stimulation.

Methods: Electrical stimulation of the right index finger or little finger was applied 23 ms before TMS of left M1HAND. MEPs were recorded from right first dorsal interosseous (FDI) and abductor digiti minimi (ADM) muscles. SAI was applied randomly at seven M1 target sites following the individual shape of the left CS.

Results: We found a clear somatotopic representation of SAI. Homotopic SAI of the ADM muscle was expressed more medially than homotopic SAI of the FDI muscle along the M1HAND. We also found somatotopy for heterotopic stimulation. Here SAI was replaced by a "surrounding" facilitation of the heterotopic muscle.

Conclusions: Linear sulcal TMS mapping revealed a somatotopically defined centre-surround organization of sensorimotor integration in the human M1HAND.

Key words: Sensorimotor integration, TMS, electrical stimulation, mapping, surround facilitation.

OC3 : 10:05 - 10:35 SOCIAL MOTIVATIONS: INSIGHTS FROM DECISION NEUROSCIENCE

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Our lives consist of a constant stream of decisions and choices, from the mundane to the highly consequential. The standard approach to experimentally examining decision-making has been to examine choices with clearly defined probabilities and outcomes, however it is an open question as to whether decision models describing these situations can be extended to choices that must be made by assessing the intentions and preferences both of oneself and of another social partner. This class of social decision-making offers a useful approach to examine more complex forms of decisions, which may in fact better approximate many of our real-life choices. I will present data from several experiments where we use economic games to observe how players decide in real, consequential, social contexts, and will discuss how we can use these brain insights to build better models of human social preferences, incorporating both psychological and neurobiological constructs.

Key words: Emotion and cognition in decision, making, decision conict, risk and uncertainty, human, judgment

PERFORMANCE MONITORING AND PERFECTIONISM IN ADOLESCENTS WITH FIRST-EPISODE ANOREXIA NERVOSA AND ADOLESCENTS RECOVERED FROM ANOREXIA NERVOSA

<u>Tine Pedersen</u>^{1,2,3}, Estelle Raffin^{2,4,5}, Ayna Nejad^{1,2}, Alessandro Calamuneri², Kasper Andersen², Norbert Brüggemann², Kristoffer Madsen², Mette Bentz^{1,3}, Hartwig Siebner², and Kerstin Plessen^{1,3}

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Background: Anorexia nervosa (AN) is a life-threatening psychiatric disorder with high rates of relapse but knowledge regarding factors that may predict treatment outcome is scarce. Perfectionistic behavior, such as an excessive control of behavior, may play an important role in the development and maintenance of AN. Few studies have examined the neurobiological correlates of perfectionism. It is unclear whether abnormalities related to perfectionism are present in the first phase of AN in adolescents and whether they persist after recovery. We hypothesize that perfectionism, as a behavioral trait will correlate with increased neural activity related to performance monitoring.

Methods: In this study, we examined performance monitoring to assess the association between perfectionistic traits and neurobiological measures, using functional magnetic resonance imaging (fMRI). Neurobiological factors can be difficult to discriminate from physiological changes that are due to weight loss. We thus compared a group of 23 adolescent girls recovered from AN (BMI=21.3), 33 adolescent girls with first-episode AN (BMI=16.6), and 56 female control subjects (BMI=22.0). The control subjects were split into a younger group that was age-matched to the girls with current AN, and an older healthy group, which was age-matched to the recovered group. The subjects performed a modified go/no-go task, and they evaluated their performance every minute in a subjective manner. We used fMRI to investigate whether activation in the cingulo-opercular system - the core network for performance monitoring - differs on a trial-by-trial basis, especially in individuals exerting perfectionistic behavior.

Results: Preliminary behavioral data show that adolescents recovered from AN (N=23) were significantly faster on go-trials than adolescents with AN (N=33) and the control subjects (N=44). The error rates did not differ across groups. Moreover, self-evaluations were not significantly different between the recovered adolescents and the adolescents with first-episode AN but the recovered adolescents evaluated themselves significantly more negatively than the age-matched controls, despite a better performance.

Discussion: Preliminary data of behavioral performance suggest that perfectionism, as a behavioral trait, is present during first-episode AN and persists after recovery. The study design allows us to discriminate individual features in the subjects, which are due to current illness and underweight with recent onset, from more stable traits. To our knowledge, this is the first study to examine perfectionism and performance monitoring in both adolescents with first-episode AN and recovered adolescents by using brain mapping. If subgroups of adolescents with AN and recovered adolescents have heightened levels of perfectionism, it may open new avenues of how to individually adjust treatment and thus minimize the number of patients who respond poorly to treatment.

Key words: anorexia nervosa, adolescents, perfectionism, fMRI

OC5: 11:45 - 12:10

NEURAL CORRELATES OF MOTOR FATIGUE AND FATIGABILITY IN MULTIPLE SCLEROSIS - A FUNCTIONAL MRI STUDY

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Introduction: The neural mechanisms causing motor fatigue and fatigability in multiple sclerosis (MS) is poorly understood and neural correlates of fatigue and fatigability in MS is highly needed.

Methods: We enrolled 46 right-handed relapsing-remitting MS patients and 25 age- and sex-matched healthy controls (HC). Fatigue was evaluated with Fatigue Scale for Motor and Cognitive Functions (FSMC), and disability with Expanded Disability Status Scale (EDSS), Nine-Hole Peg Test and Jebsen Taylor Hand Function Test. Subjects underwent whole-brain 3 Tesla functional magnetic resonance imaging while performing a visual guided three-phased precision-griptask. In the first (pre-fatigue) and final phase (post-fatigue) the subjects altered between resting and pressing on a force transducer with right thumb and index finger applying 15% of their individual maximal grip-force. In the second phase (fatiguing phase) the subjects performed a tonic contraction until motor fatigue. Family-wise error (FWE) correction was performed at cluster level, applying a cluster-forming threshold of P uncorrected< 0.001. Significance threshold was set at PFWE< 0.05.

Results: The MS group had mean EDSS 2.3 (range 0-3.5) and no major functional impairment of the right upper extremity. We found a significant group-difference in the post-pre fatigue contrast in anterior putamen bilaterally. In HC, the activation decreased, whereas MS patients showed a relative increase. In MS patients, the motor FSMC score correlated negatively with the post-pre activity change in left putamen (r = -0.31, P = 0.04): The higher the individual fatigue score, the smaller was the fatigue-induced activity increase in the putamen.

Conclusion: The changed neural responses to a fatiguing task suggest a key role of the putamen in the development of motor fatigue in MS.

Key words: Multiple Sclerosis, Motor Fatigue, fMRI, Functional Magnetic Resonance Imaging.

NOTES :	

OC6: 12:10 - 12:25

IN THE PURSUIT OF THE FEAR ENGRAM: IDENTIFICATION OF NEURONAL CIRCUITS UNDERLYING THE TREATMENT OF ANXIETY DISORDER

Ossama Khalaf¹

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Fear and other anxiety disorders are extraordinarily robust and difficult to treat. Among the most effective treatments for anxiety disorders are exposurebased therapies, during which a patient is repeatedly confronted with the originally fear-eliciting stimulus in a safe environment so that the once fearful stimulus can be newly interpreted as neutral or safe. A fundamental element for successful exposure-based therapies is the reactivation/recall of the traumatic memory, which initiates a time-limited process called memory reconsolidation, during which a memory becomes susceptible to disruption. Presently, the neuronal subpopulations underlying successful fear memory extinction remain completely unknown, which represents a big gap in memory research. Therefore, we aim to identify these neuronal subpopulations that are causally implicated in effective attenuation of remote fear memories in order to determine whether the original traumatic memory trace has been permanently modified or a new memory trace of safety has been superimposed over the original one. Using exposure-based therapy in transgenic mice, which allows for a time-limited activation of neurons upon remote memory recall, making it not only possible to visualize those neurons but also to experimentally isolate them from the rest of the neurons for further molecular investigations.

Key words: Remote traumatic memory, Fear Extinction, Memory Reconsolidation

OC7: 14:00 - 14:25

UNRAVELING THE DIFFERENTIAL CONTRIBUTION OF NEURONS AND ASTROCYTES TO PHYSIOPATHOLOGICAL MECHANISMS OF HUNTINGTON'S DISEASE

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Neurons in a neurodegenerative context are considered the most vulnerable cells and their dysfunctions are the key events triggering neurological alterations. In Huntington's disease (HD), a specific degeneration of Spiny Projection Neurons (SPN) of the striatum is observed. However, dysfunctions or functional modifications of surrounding glial cells, in particular astrocytes, are frequently described in HD, suggesting a more active role of these cells in this complex neurodegenerative process. In this study, we aimed to characterize the respective contribution of striatal neurons and astrocytes to the physiopathology of Huntington's disease (HD). We used viral vectors to develop three new mouse models, which express the mutant huntingtin (mHTT) either in neurons, astrocytes, or both cell populations in parallel in the basal ganglia circuit. We observed that the expression of mHTT in neurons is sufficient to induce motor coordination deficits, anxiety and weight loss, in parallel with progressive neuronal dysfunction. In contrast, the selective expression in astrocytes only leads to mild motor deficits. The co-expression of mHTT in both cell populations does not only exacerbate neuronal symptoms but induces a distinct phenotype with additional hyperactivity, aggressiveness and abnormal clasping. Interestingly, we found that several physiopathological hallmarks such as astrogliosis require the co-expression of mHTT in both cell populations. Finally, we showed that mechanisms linked to glutamatergic excitotoxicity such as glutamate re-uptake by astrocytes could be altered by either astrocytic or neuronal mHTT expression. This is the first time that comparable in vivo cell-type specific models are used in HD. These results highlight the importance of considering neuron-astrocyte interactions for the development of new therapeutic approaches in HD, and potentially in other neurodegenerative diseases.

Key words: Huntington's disease, Neuron, Astrocyte interactions, Viral vectors, Striatum

NOTES :	

OC8: 14:25 - 14:50

MITOCHONDRIAL DYSFUNCTION IN PARKINSON'S DISEASE: ROLE OF THE MITOCHONDRIAL QUALITY CONTROL

<u>Sandra Franco-Iborra</u>¹, Annabelle Parent¹, Jordi Bove¹, Miquel Vila^{1,2}, and Céline Perier¹

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Mitochondrial dysfunction has long been implicated in the pathogenesis of Parkinson's disease (PD). However, the molecular mechanisms linking mitochondrial dysfunction with dopaminergic cell death are still under investigation. Mitochondria contain approximately 1,500 different proteins, 99% of which are encoded by the nuclear genome. Therefore, the import, sorting and assembly of nuclearly encoded mitochondrial proteins are essential for normal mitochondrial function. As only 13 proteins of the respiratory chain are encoded by the mitochondrial genome and synthesized in mitochondria, nuclearly encoded mitochondrial membrane translocases: translocase of the outer membrane (TOM) and translocase of the inner membrane (TIM). Protein import represents a dynamically regulated process that varies in response to oxidative stress or aging. In turn, mitochondrial protein import regulates mitochondrial protein response (mtUPR).

Here we have investigated in vitro and in vivo whether complex I inhibition impair the mitochondrial protein import systems and leads to modifications of the mtUPR response.

In our dopaminergic cell line model, complex I inhibition leads to a concentration-dependent cell death with no changes in the overall mitochondrial mass. The sole inhibition of complex I decreased the expression levels of translocases such as Tom20 and Tim23 by 50%. Confirming those results, the study of functional mitochondrial import in isolated mitochondria showed that complex I inhibition partially blocked protein import into the mitochondria. In the vivo MPTP-induced mouse model of PD, Tim23 expression levels are decreased by 50% as early after the last MPTP injection and maintained until 7 days after the last injection. Tom20 protein levels are also decreased but at later time points. These results suggest that complex-I inhibition leads to a reduction

in the activity of the mitochondrial protein import system, probably by decreasing translocases protein levels.

It was previously showed that impairment of mitochondrial protein import might result in UPRmt induction, which can be measured by changes of expression of various mitochondrial proteases and chaperones. In our in vitro setting, we would have expected an activation of mtUPR associated with complex I inhibition. Interestingly, no activation was observed, suggesting that this pro-survival pathway is somehow altered in in vivo models of PD-related complex-I inhibition.

Interestingly enough, complex I inhibition in vitro induces an enrichment in detergent insoluble mitochondrial proteins, that could indicate an increased presence of misfolded proteins when complex I is inhibited with a specific increase of a ATPaseV-subunit, suggesting that complex I inhibition, maybe through a deactivation of mitochondrial proteases, impairs the proper folding or degradation of mitochondrial proteins, resulting in its accumulation.

All together, these results suggest that complex I inhibition negatively modulates mitochondrial protein import which is associated to mitochondrial quality control dysfunction and subsequently to an increase of aggregates inside the mitochondria; all of which might represent a critical pathogenic event contributing to dopaminergic neurodegeneration in PD.

Key words: Parkinson's disease, mitochondria, mitochondrial quality control

OC9 : 14:50 - 15:20 TARGETING THE "UNDRUGGABLE" MYC IN GLIOBLASTOMA

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Gliomas are the most common primary tumors affecting the adult central nervous system and respond poorly to standard therapy. Myc is causally implicated in most human tumors and the majority of glioblastomas have elevated Myc levels. Using the Myc dominant negative Omomyc, we previously showed that Myc inhibition is a promising strategy for cancer therapy. Recently, we pre-clinically validated Myc inhibition as a therapeutic strategy in mouse and human glioma, using a mouse model of spontaneous multifocal invasive astrocytoma and its derived neuroprogenitors, human glioblastoma cell lines, and patient-derived tumors both in vitro and in orthotopic xenografts. Across all these experimental models we find that Myc inhibition reduces proliferation, increases apoptosis, and remarkably, elicits the formation of multinucleated cells that then arrest or die by mitotic catastrophe, revealing a new role for Myc in the proficient division of glioma cells. Now, we present our current efforts in translating this genetic approach into a clinically viable therapeutic option for glioma patients. Indeed, we will discuss the exciting results showing that Omomyc-based Cell Penetrating Peptides (CPPs) can be used as novel, state-ofthe-art direct in vivo Myc inhibitors for treating tumors in the lung and brain, where the peptides biodistribute after intranasal administration. This offers an unprecedented therapeutic opportunity to target Myc in cancer.

Key words: Omomyc, Myc, Gliomas, cancer

THE INTERACTION BETWEEN MUTANT PRION PROTEIN AND GLUTAMATE RECEPTORS: A NOVEL MECHANISM FOR NEURONAL DYSFUNCTION IN GENETIC PRION DISEASES

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Genetic prion diseases are rare, invariably fatal neurodegenerative disorders linked to mutations in the PRNP gene encoding the cellular prion protein (PrPC). PRNP mutations favor the conformational conversion of PrPC into a pathogenic, misfolded isoform that accumulates in the central nervous system of affected individuals and kills neurons through an unknown mechanism.

Evidence is emerging that neuronal loss in inherited prion diseases is preceded and possibly caused by synaptic dysfunctions. However, the ultimate link between synaptic dysfunction and neurodegeneration is yet to be found.

We previously demonstrated that mutant PrP is retained in the endoplasmic reticulum where it interacts with the alpha2delta subunits of voltage-gated calcium channels. This impairs the correct delivery of the channel complex to the cell surface, impacting synaptic transmission (Senatore et al., Neuron 2012). Nevertheless, this phenomenon alone does not account for neurodegeneration. It has been shown that PrPC engages functional interactions with other proteins that are important for synaptic function such as glutamate receptors. We hypothesize that intracellular retention of mutant PrP may also alter the trafficking of glutamate receptors, thereby producing adverse effects on neuronal function and survival. We started to address this possibility by carrying out biochemical and morphological analyses, electrophysiological recordings and functional imaging in neurons from transgenic mouse models of genetic prion diseases.

We found that mutant PrP impairs the membrane delivery of specific AMPA and NMDA receptor subunits. This is associated to a reduction in dendritic spines and basal glutamatergic transmission. Moreover, retention of the GluA2 subunit of AMPA receptor results in exposure of GluA2-lacking, calcium-permeable AMPA receptors, leading to increased calcium permeability and enhanced sensitivity to excitotoxic cell death.

Our results demonstrate that mutant PrP impairs the trafficking of glutamate receptors, leading to a significant alteration in glutamatergic neurotransmission and increased predisposition to excitotoxicity. These findings identify a new pathological mechanism for genetic prion diseases and may lead to novel therapeutic approaches for such incurable conditions.

Key words: Prion diseases, dendritic spines, glutamate receptors, excitotoxicity

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OC11: 16:30 - 16:55

PLATIPUS: A NEW METHOD FOR TRANSCRIPTOME-WIDE ANALYSIS OF DISTINCT CELL POPULATIONS IN THE ADULT BRAIN

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Neurons are evolving in an environment composed of multiple cell populations with close relationships to ensure an adapted brain functioning. In particular, neuron-glia interactions are crucial for numerous processes ranging from brain homeostasis to synapse maintenance and their role in the neuronal vulnerability observed in neurodegenerative diseases is now recognized. Precise characterization of the contributions of these subpopulations in the central nervous system is essential to improve our comprehension of the brain complexity in normal and pathological contexts. Actually, cell-type specific characterization is facing a major challenge in the isolation of cellular populations from adult animals with techniques compatible with highthroughput analysis. Here we describe PLATIPUS, a highly efficient and flexible method for dissection and isolation of thousands of cells, compatible with highthroughput -OMICS analysis. We used this tool to generate a transcriptome-wide profile of coding (mRNA) and non-coding RNA (miRNA) of striatal projecting neurons (Drd1 and Drd2 neurons), astrocytes and microglia. We identified specific molecular signatures revealing the implication of each cell-type in specialized functions but also in multi-cellular pathways. Co-analysis of mRNA and miRNA profiles highlighted differential regulation off gene expression across cell populations. Furthermore, comparison of our database with a previous transcriptomic study in Huntington's disease patients show the potential of this method for discovering new cell-type specific transcriptional dysregulations in a neurodegenerative state. This resource provides a powerful database unravelling specific roles of neurons and glial cells in the striatum with a great potential to improve our comprehension of the central nervous system.

Key words: Transcriptomic, cell, type specific method, neurodegenerative context, striatum

OC12: 16:55 - 17:20 MOLECULAR MECHANISMS UNDERLYING TUBULINOPATHIES: A COMBINED BIOCHEMICAL AND STRUCTURAL APPROACH

Luca Signorile¹

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The cytoskeleton, represented by intermediate filaments, actin filaments and microtubules (MTs), is of primary importance for cells. MTs are important during mitotis and cell migration, but also for conferring specific morphologies to cells and for long range intracellular transport. MTs are made of alpha/beta tubulin dimers that associate in a head-to-tail fashion to build a polar protofilament, with the beta tubulin subunit exposed at the fast-growing end of the MT, the plus end (+ end). About 13 protofilaments associate laterally to generate a hollow cylinder, the MT, of approximately 25 nm in diameter. MTs display dynamic instability, meaning that they can inter-convert between polymerization and depolymerization phases. This property is essential for MT function and needs to be tightly regulated. This is largely done by by so-called MT plus end tracking proteins (+TIPs), whose core components are the EB1-like proteins (EB1-3), that can directly bind the MT +end by recognizing specific features. Previous studies by other groups have identified several tubulin mutations which give rise to malformations of the brain (polymicrogyria, lissencephaly and others). We wanted to gain further understanding of the molecular basis for these brain malformations and developed a protein purification strategy to isolate functional, specific isoforms of GFP-tagged tubulin, either wild-type or mutant. In this way we could test the properties of these proteins in the regulation of MT dynamics and of +TIP interactions with MTs in a cell-free in vitro polymerization assay, using Total Internal Reection Fluorescence (TIRF) microscopy. We also modelled the structure of these mutant tubulins and performed molecular dynamics (MD) simulations to study their behaviour, either as dimers or when incorporated into MTs.

Key words: tubulin, microtubules, +TIPs, brain, TIRF, molecular dynamics

POSTERS

BEHAVIOURAL ANOMALIES IN NEURODEGENERATIVE DISEASES: ROLE OF THE IMMUNE RESPONSE

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Alzheimer's Disease (AD) is the most common form of dementia. Regarding physiopathology, the key event is currently considered the aggregation and deposition of the beta-amyloid peptide (AB). However, neuroinflammation is postulated to play an essential role. Besides altered cognition, one of the main causes of distress in caregivers of AD patients is represented by Behavioural and Psychological Symptoms of Dementia (BPSD). Among them, aggression and agitation (A/A) represent a serious clinical problem, requiring qualified people and specialized structures. BPSD biological bases are still unknown and no definitive pharmacological treatments are available. Translocator protein 18-kDa (TSPO-18) is a receptor located on the outer mitochondrial membrane, formerly known as the Peripheral Benzodiazepine Receptor due to its pharmacological properties. TSPO is up-regulated in microglia in AD and is considered a biomarker of neuroinflammation, employed as a target for PET tracers (e.g., PK11195). The endogenous ligand is DBI (Diazepam Binding Inhibitor) that acts as an inverse agonist on GABAA receptors as well (i.e., as an anxiogenic peptide). TSPO transports cholesterol from cytoplasm to mitochondria in order to start the biosynthesis of neurosteroids. A reduction in TSPO expression in PBMCs and changes in serum neurosteroids levels have been shown in patients with anxiety disorder. Moreover, exon 4 of the TSPO gene contains a SNP called rs6971 (Ala147Thr substitution) that has been associated to separation anxiety. Finally, TSPO is also expressed on the plasma membrane of peripheral blood monocytes, where regulates chemotaxis and the passage through the blood-brain barrier (BBB) for contributing to the inflammatory milieu. Given TSPO involvement in neuroinflammation, steroids' biosynthesis and anxiety genesis, this receptor seems an interesting target of study for understanding BPSD.

At the moment, we genotyped 40 AD patients for rs6971 and we measured TSPO mRNA levels in their PBMC. Moreover, we assessed neurotrophins and DBI serum concentrations in the same patients. All the patients were screened for BPSD with the NPI and disease severity was evaluated by the MMSE. Moreover, we performed chemotaxis assays using THP-1 cells (acute myeloid leukaemia cell line) and MCP-1/A β peptide as chemoattractants, in order to get preliminary results about cellular migration in the presence of A β . We plan to run the same experiments on monocytes obtained from our patients. Further step will be including a model of BBB (transmigration assay). Our final goal is to find out whether these biological aspects could be linked to the presence of more or less severe BPSD, in order to cast light on the real determinants of these serious dementia-associated symptoms.

Key words: Alzheimer's Disease (AD), Behavioural and Psychological Symptoms of Dementia (BPSD), Translocator protein 18kDa (TSPO18), neuroinflammation, chemotaxis, neurosteroids, rs6971, THP1

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HIBISCUS SABDARIFFA AND BORTEZOMIB COMBINATION AGAINST HUMAN MULTIPLE MYELOMA CELLS IN VITRO

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Background: Hibiscus Sabdariffa is a plant of the Malvaceae family commonly used in Asian and African folk medicine and for the preparation of a cold drink called Karkadè. Thanks to its high polyphenol content, antioxidant and antiinammatory properties Hibiscus Sabdariffa has gained interest as a possible chemopreventive agent. In our laboratory we demonstrated the in vitro antitumor effect of a Hibiscus Sabdariffa Extract (HSE) against RPMI 8226 human multiple myeloma cells. Bortezomib (BTZ) is a proteasome inhibitor and one of the most used chemotherapy agent for multiple myeloma. But, even if BTZ is very effective, it has a lot of different adverse effects, among which peripheral neuropathy is the most frequent. The aims of our project is to treat RPMI 8226 human multiple myeloma cells with both HSE and BTZ in order to identify the modality and the concentrations at which the two compounds could exert a synergic effect, allowing a dose reduction of BTZ and consequently its side effects (especially its peripheral neuropathy).

Materials and methods: Human multiple myeloma cells RPMI 8226 were cultured and treated with different HSE and BTZ concentrations and combinations (simultaneous administration, not-simultaneous administration, pre-treatment). Cell viability was evaluated by MTT assay and Trypan blue vital count. Cells migration and invasion was assessed using Boyden Chamber assay and a gelatine coated polycarbonate membrane. Rat embryo dorsal root ganglia (DRG) cultures were used to assess HSE and BTZ combinations neurotoxicity in vitro. The effect of the combinations was compared to RPMI cells treated with HSE or BTZ.

Results: HSE and BTZ simultaneous and not-simultaneous administration were not more effective against RPMI 8226 cells than HSE-only treatment and BTZ effect was probably inhibited by HSE. 24h pre-treatment with BTZ, followed by HSE 24-48-72h treatment was the most effective assessed combination and it

was more effective when compared with the administration of two compounds given separately. Moreover, HSE, that usually has a cytostatic effect against RPMI 8226 cells, became more cytotoxic after BTZ pretreatment. BTZ pretreatment also enhanced HSE ability to impair cell migration and invasion and it was not neurotoxic in a DRG model of neurotoxicity in vitro.

Conclusions: In this study we evaluated in human multiple myeloma cells RPMI 8226, three different modalities of administration for BTZ and HSE. Our results demonstrate that only the pre-treatment for 24h with BTZ followed by HSE treatment showed an increased effectiveness and no neurotoxicity compared to BTZ or HSE treatment. Our results suggest that this combination is promising for multiple myeloma treatment and further analysis will be necessary to elucidate the molecular basis of the synergic action of BTZ and HSE.

Key words: Multiple myeloma, Bortezomib, Hibiscus sabdariffa, neurotoxicity, in vitro

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NATURE OF CHANGE IN CREATINE KINASE AND CA2+ ATPASE ACTIVITY UNDER STRESS CAUSED ISOLATION AND VIOLATION OF NATURAL DIURNAL CYCLE

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We have studied the influence prolonged isolation and violation of diurnal cycle has on behavioral activity and hormonal status among animals. It has been showed that such conditions result in development of stress. In this state, changes are evident in the activity of rat brain creatine kinase isoforms: activity of both isoforms of the enzyme decreases. We have established kinetic parameters (Vmax, Km) of the brain enzyme and nature of their modification. It has been suggested that prolonged isolation and violation of natural diurnal cycle among animals may result in lower CK-BB activity caused by structural changes in the enzyme and quantitative decrease in ATP and creatine substrates. Lowered enzyme activity is accompanied by accumulation of Ca²⁺- ions in the cell. This may be considered the reason for decreased activity of plasma membrane and mitochondrial Ca-ATPase. Prolonged isolation and violation of diurnal cycle has been also accompanied a worsened functional state of mitochondrial transitional permeability pore (MPTP). Such deterioration suggests presence of intensified apoptotic process in cells and may lead to various neurodegenerative pathologies.

Key words: Stress, Ca²⁺ATPase, Creatine kinase, MPTP

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MEF2D AND MEF2C PATHWAYS DISRUPTION IN SPORADIC AND FAMILIAL ALS PATIENTS

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Amyotrophic lateral sclerosis (ALS) is a progressive neuro-muscular disease characterized by motor neuron loss. MEF2D and MEF2C are members of the myocyte enhancer factor 2 family (MEF2), a group of transcription factors playing crucial roles both in muscle and in neural development and maintenance; for this reason, a possible involvement of MEF2 in ALS context has been investigated.

Since the transcriptional activity of each tissue specific MEF2 isoform is conserved in different cell types, we chose to assess our parameters in an easily accessible and widely used experimental tool such as peripheral blood mononuclear cells (PBMCs) obtained from 30 sporadic ALS patients (sALS), 9 ALS patients with mutations in SOD1 gene (SOD1+) and 30 healthy controls. Gene expression analysis showed a significant up-regulation of MEF2D and MEF2C mRNA levels in both sporadic and SOD1+ ALS patients. Although protein levels were unchanged, a different pattern of distribution for MEF2D and MEF2C proteins was evidenced by immunohistochemistry in patients. Moreover, a significant down-regulation of MEF2 downstream targets BDNF, KLF6 and RUFY3 was reported in both sporadic and SOD1+ ALS patients, consistent with an altered MEF2 transcriptional activity. Furthermore, the potential regulatory effect of histone deacetylase 4 and 5 (HDAC4 and HDAC5) on MEF2D and MEF2C activity was also investigated. We found that MEF2D and HDAC4 colocalize in PBMC nuclei, while HDAC5 was localized in the cytoplasm. However, the unchanged HDACs localization and protein levels between sALS and controls seem to exclude their involvement in MEF2 altered function. In conclusion, our results show a systemic alteration of MEF2D and MEF2C pathways in both sporadic and SOD1+ ALS patients, underlying a possible common feature between the sporadic and the familial form of disease. Although further analyses in other neuromuscular diseases are needed to determine the specificity of changes in these pathways in ALS, measuring MEF2 alterations in accessible biofluids may be useful as biomarkers for disease diagnosis and progression.

Key words: amyotrophic lateral sclerosis (ALS); MEF2D; MEF2C; BDNF; KLF6; RUFY3; HDAC4; HDAC5; SOD1.

STUDY OF CHAPERONE MEDIATED AUTOPHAGY IN LYMPHOMONOCYTES OF SPORADIC AND FAMILIAL ALS PATIENTS EVIDENCED HSC70 REDUCTION AND TDP-43 AGGREGATION

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the selective degeneration of both upper and lower motor neurons. Causes of disease remain still unknown, although protein aggregate formation in motor neurons is a neuropathological hallmark. These aggregates contain several different proteins, but transactivation response (TAR) DNAbinding protein (TDP-43) is considered the major component. Recent evidences showed that TDP-43 is degraded not only by the ubiquitin proteasome system (UPS) and macroautophagy, but also by the chaperone-mediated autophagy (CMA) through an interaction between the cytosolic chaperone heat shock cognate 70 (Hsc70, also known as HSPA8) and ubiguitylated TDP-43. We assessed the two principal parameters of CMA in easily accessible cells like peripheral blood mononuclear cells (PBMCs) obtained from 30 sporadic ALS patients (sALS), 9 ALS patients carrying mutations in SOD1 gene (SOD1+) and 30 healthy controls. Our results showed a significant reduction of hsc70 in sALS and SOD1+ patients with respect to controls, while no changes were observed for the lysososme-associated membrane protein 2A (lamp2A), the rate limiting protein of CMA. Moreover, to estimate the degradative activity of this pathway we evaluated the levels of specific and nonspecific CMA substrates: although myocyte enhancer factor 2D (MEF2D) levels were unchanged, we showed increased TDP-43 in sALS patients. TDP-43 is physiologically degraded through a caspase-3-mediated cleavage to generate two fragments (35 and 25 kDa, respectively). We here reported an increased TDP-43/TDP-35 ratio in sporadic ALS patients with respect to controls, suggesting a possible reduction of efficiency of the normal proteolytic cleavage. Immunohistochemistry analysis, performed in 3 sALS and 3 healthy controls, revealed that TDP-43 was aggregated in patients cells outside nuclei, confirming that PBMCs are able to recapitulate this pathological feature previously identified in affected tissues of ALS patients. Furthermore, we also investigated parameters related to other degradative mechanisms, reporting a significant reduction of mRNA levels of two macroautophagy-related parameters, SQSTM1/p62 and LC3, in both sALS and SOD1+ patients with respect to controls. Lastly, we assessed mRNA expression of two co-chaperones BAG1 and BAG3, which regulate trafficking of substrates to ubiquitin-proteasome system (UPS) and macroautophagy respectively, and we showed decreased levels of BAG1 in both sporadic and familial ALS patients. In conclusion, our results evidenced reduced hsc70 levels without alteration of lamp2A and accumulation of CMA substrates, excluding an impairment of this catabolic pathway in ALS patients. Anyway, the accumulation of TDP-43 suggests that other degradative pathways could be altered in ALS, further investigations are needed to investigate UPS and macroautophagy in patients cells to explore this hypothesis.

Key words: amyotrophic lateral sclerosis (ALS), chaperone, mediated autophagy (CMA), hsc70, lamp2A, TDP, 43, SQSTM1/p62, LC3, BAG1, BAG3, SOD1

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THE SEVERITY OF OXALIPLATIN NEUROTOXICITY IS RELATED TO DIFFERENTIAL EXPRESSION OF A SOLUTE TRANSPORTER IN MICE

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Oxaliplatin (OHP) is one of the most effective anticancer drug in the treatment of advanced and metastatic colorectal cancer, but peripheral neurotoxicity is an important limitation in its clinical use. This side effect has a high incidence and may be long lasting or permanent. Unfortunately still today there are no therapies available to treat or reduce this complication that could strongly compromise the life quality, so understanding the pathogenesis of OHP-induced peripheral neurotoxicity (OIPN) could lead to new treatments development.

On this basis we evaluated OIPN differences in 6 mice strains (A/J, BALB/c, C57BL6, DBA, FVB, CD1) with different genetic background and, in order to assess if OHP treatment could affect these mice strains more or less severely, we compared the different manifestations of the neurotoxicity through a multimodal characterization. Hence mice responses to mechanical stimuli were detected through the Dynamic Aesthesiometer test while the cold threshold was identified bv the Cold Plate test. Nerve conduction velocitv and morphological/morphometrical analysis were performed to assess OHP-induced functional and structural changes in peripheral nerves and dorsal root ganglia (DRG). Finally cutaneous innervation was assessed through quantification of intraepidermal nerve fibers (IENF) in the mouse footpad. Our data evidence that all the strains show signs of OIPN but with different severity degree, supporting the hypothesis that genetic modification might have a role in the OIPN type and severity. In this sense a crucial role may be played by membrane transporter super-families that are implicated in transport and accumulation of platinum in cells and in DRG neurons too. Preliminary molecular studies have focused on some of the main membrane transporters and in particular on the solute carrier SLC4A1 that seems to be differentially up-regulated in DRG of BALB/c and C57BL6 mice, that are the most and least affected above the investigated strains.

Taken together our results suggest that different genetic backgrounds cause different drug toxicity response and that the solute carrier SLC4A1 may play an important role in OIPN severity degree. However the role of the identified transporter in OHP accumulation and toxicity will be further investigated.

Key words: oxaliplatin, neurotoxicity, genetic variability, membrane transporters

<u>NOTES</u> .		

STUDY OF CRMPS FUNCTIONS IN MAP6 DEPENDENT AXONAL OUTGROWTH INDUCED BY SEMAPHORIN 3E

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Structural microtubule associated proteins (MAPs) stabilize microtubules, a property that is thought to be essential for development, maintenance and function of neuronal circuits. Our group is working on the microtubule associated protein 6 (MAP6) family; we demonstrate a role for MAP6 in brain wiring that is independent of microtubule binding. We find that MAP6 deletion disrupts brain connectivity and is associated with a lack of post-commissural fornix fibres. MAP6 contributes to fornix development by regulating axonal elongation induced by Semaphorin 3E. MAP6-KO mice display a large spectrum of social and cognitive impairments that positively respond to chronic neuroleptic treatment. These features are reminiscent of human psychiatric conditions and MAP6-KO mice have been a useful animal model for schizophrenia's pathophysiology study for the last ten years.

Moreover, three members of the Collapsin Response Mediator Proteins (CRMP) family have been recently identified as protein partners of MAP6 in our group. These CRMPs were originally identified for their function in semaphorin signalling. In the present study we focus on i) the involvement of these partners in the sema3E-dependent signalling ii) the function of CRMPs-MAP6 interactions in this pathway and iii) the need of these partners for the fornix formation. To do so, we use or develop various techniques such as primary cells cultures and electroporation to test semaphorin signalling, proteins purification and pulldown experiments to challenge interaction domains, histological slices and tissue clearing for fornix analysis.

Bearing in mind that disorders in the fornix formation are associated with schizophrenia, a better understanding of the fornix formation would provide new evidence in favour of the neurodevelopmental origin of psychiatric diseases.

Key words: Axonal guidance, semaphorine, CRMP, MAP6, psychiatric diseases

ROLE OF HUNTINGTIN IN SKELETAL MUSCLE CALCIUM RELEASE

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Huntington's disease (HD) is a rare multisystemic neurodegenerative disorder combining psychiatric, cognitive and motor impairments. Muscle manifestations are recently proved to be independent from neurodegeneration. Therefore, specific mechanisms may be involved in muscle. HD is caused by an increase in CAG repeats in the huntingtin gene, resulting in an expansion of polyglutamine stretch in the protein. This induced a loss of the huntingtin protein (HTT) normal function associated with production of a mutant protein. HTT is a ubiquitous microtubules associated protein, with numerous functions among which vesicles and organelles traffic along microtubules. One of its functions could be the traffic of reticulum vesicles to form contact point with the plasma membrane in neurons. In skeletal muscle, the contact points between sarcoplasmic reticulum and plasma membrane (T-Tubule), called the triads, are of major importance. These contact points are the basis of calcium release and excitation-contraction coupling. This study explores the potential role of HTT in skeletal muscle calcium release.

Key words: Huntingtin, intracellular traffic, muscle, triad, excitation, contraction coupling, Huntington's disease

Hyaluronic acid hydrogel combined to mesenchymal stem cell therapy after acute ischemic stroke.

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Cerebrovascular disease is the leading cause of disability in adults and there is no effective treatment to avoid stroke damages after the first hours. Various types of engineered biomaterial matrices, which in combination with stem cells, have shown promises for brain tissue replacement and reduce post-stroke disability. Hydrogels are tree-dimensional cross-linked networks of water-soluble polymers. Hydrogel polymers can be engineered in a variety of physical forms. They have excellent nutrient and oxygen permeability, allowing cell survival in the scaffold. The advantage of this kind of biomaterial is injectable. The aim of this work is to optimize the cell therapy and to improve the stem cell engraftment by co-administration with hyaluronic acid hydrogel. For this purpose rats Sprague Dawley male were submitted to a model of ischemic stroke by occlusion of middle cerebral artery and treated by stereotaxic intracerebral injection of mesenchymal stem cells co-administrated with a hydrogel, seven day after experimental stroke. During one month, rats were evaluated by a sensorymotor tests and Magnetic Resonance Imaging (MRI) follow up. The survival of stem cells and the biocompatibility of hydrogel were investigated by histological analysis.

Key words Stroke; MRI; MSC; hydrogel; MCAO; rat

P10

REPROGRAMMING REACTIVE GLIAL CELLS INTO FUNCTIONAL GABAERGIC NEURONS

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Direct cellular reprogramming of non-neuronal cells into neurons emerges as an innovative strategy to regenerate lost neurons for brain repair. It has been shown that glial cells can be reprogrammed into neurons, both in vitro and in vivo, by forced expression of neurogenic transcription factors. The next challenge in this field is to reprogram somatic cells, residing within the injured brain, into fully functional neurons that acquire a specific phenotype, functionally integrate into the existing neuronal networks, and modulate their activity with beneficial effects. Epilepsy is a major clinical problem and about 30% of epileptic patients suffer from seizures that cannot be controlled with currently available medications. In particular, Mesio-Temporal Lobe Epilepsy (MTLE), the most common form of intractable epilepsies, is characterized by recurrent seizures occurring in the hippocampus. MTLE is associated with a loss of GABAergic neurons in the epileptic hippocampus, which has been suggested to participate in the increased neuronal excitability responsible for seizures. There is also an increasing body of evidence suggesting that astrocytes exert proepileptic effects. In the present study we investigated in this pathological context whether glial cells can be reprogrammed to generate induced GABAergic neurons that functionally integrate into hippocampal epileptic networks and modulate their activity. To answer this guestion, we used a MTLE mouse model and two complementary approaches: (i) in vivo direct reprogramming of endogenous glial cells and (ii) transplantation of glia-derived neurons generated in vitro. We forced expression of different combinations of genes in glial cells and showed that retrovirus-mediated expression of Ascl1 alone or combined with Sox2 induced the conversion of reactive glial cells into neurons. In addition, glia derived neurons generated in vitro by expression of Ascl1 survived and differentiated into mature neurons once transplanted within the epileptic hippocampus. These results show that glia-to-neuron conversion can be achieved in the epileptic hippocampus.

Key words: reprogramming, glial cells, GABAergic neurons, integration, epilpsy

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P11 DIVIDE AND DECIDE: CORTICAL DISSOCIATION DURING PAST EXPERIENCES PROCESSING

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We make decisions every waking day of our life. Facing our options, we tend to pick the most likely to get our expected outcome. Taking into account our past experiences and their outcome is mandatory to identify the best option. This cognitive process is called reinforcement learning.

Past animal studies revealed the major role of dopaminergic neurons of the substantia nigra in reward-based learning thanks to a specific modulation of their firing rate. However, there is no evidence of a role of these neurons in punishment-avoidance learning. Recent work in human functional neuroimaging suggests two distinct cortical systems could co-exist in the brain to ensure both reward-based and punishment-avoidance learning. Despite this promising theory, electrophysiological mechanisms involved in this double cortical system remain unclear.

Here, we describe these electrophysiological mechanisms during reinforcement learning using stereo-EEG recordings in drug-resistant epileptic patients. To date, 20 patients were included. They performed a probabilistic learning task (75/25%) where they had to identify, via trial and error, the best stimulus among pairs so as to maximize their monetary payoff. At all times, a computational model estimates (1) the subjective value of the chosen stimulus, necessary to identify the best stimulus, and (2) the prediction error (difference between actual and expected reinforcements), allowing a behavioral adaptation.

Interestingly, broadband gamma activity (50-150Hz) amplitude increases in the anterior insula and in the lateral orbitofrontal cortex after the loss of money. On the contrary, a similar increase following a gain of money has been highlighted within the ventromedial prefrontal cortex. The model brings out these activations relate to a functional encoding of the absolute value of the obtained reinforcement rather than of the expected one. Finally, the larger the magnitude of this gamma insular response, the better the patient performance at avoiding punishments, linking electrophysiological response and behavior.

Results presented here are consistent with a cortical dissociation in appetitive and aversive reinforcement processing during economic decision making. Insights from the sEEG are patent with the exceptional spatiotemporal resolution reached to address on-hold issues about the cortical dynamics of reinforcement learning in humans.

Key words: decision making, reinforcement learning, stereoEEG, broadband gamma activity, anterior insula, orbitofrontal cortex

NOTES :

EXPERIMENTAL APPROACHES IN RODENT TO DECIPHER THE PATHOPHYSIOLOGICAL MECHANISMS OF NEUROPSYCHIATRIC SYMPTOMS IN PARKINSON'S DISEASE

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Parkinson's disease (PD) is associated with motor dysfunctions involving the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SNc). However, in addition to these cardinal features of PD, a plethora of non-motor manifestations may occur, ranging from anxiety, depression and apathy (defined as a reduction of motivated behaviors) to a complex group of behavioral addictions, termed as impulse control disorders (ICDs).

Besides their beneficial effects on the motor function, classical treatments of the disease may aggravate or even induce these neuropsychiatric symptoms. Indeed, deep brain stimulation of the subthalamic nucleus (STN-DBS) is suspected to promote apathy by itself, whereas dopamine replacement therapies, by increasing impulsivity favor the development of ICDs in PD patients.

We therefore wonder how apathy and impulsivity can fluctuate, after dopaminergic denervation, STN-DBS and under pharmacological treatment. We hypothesized that 1) STN-DBS induces apathetic-like behavior through the modulation of the activity of the dopaminergic system and 2) denervation of the dopaminergic nigrostriatal system would promote the development of ICDs when combined with dopaminergic agonist treatments.

To answer this question, we used a rodent model of non-motor symptoms of PD recently developed in our team. Briefly, rats were injected bilaterally with the neurotoxin 6-OHDA into the SNc in order to induce a selective, and partial denervation of dorsal striatum, inducing motivational deficit. Then, rats were treated either with STN-DBS and/or with pramipexole, a D2/D3 receptors (D2R/D3R) agonist used to reverse motivational impairment but known to favor the development of ICDs in PD patients. 1) Apathetic like behavior was evaluated in a sucrose self-administration task in which rats have to press on a lever to

obtain sucrose solution (2,5%). 2) Impulsivity was assessed in a delay discounting task in which rats have to press a lever and choose between a small, but immediate, reward (5% sucrose) or a larger, but delayed, reward (10% sucrose).

We observed that 1) STN-DBS induces motivational impairment in sham animals or exacerbated this deficit in 6-OHDA lesioned rats and it was alleviated by pramipexole. In addition, 2) chronic pramipexole treatment increases impulsivity in our rodent model, both in sham and 6-OHDA lesioned rats.

These results show that 1) STN-DBS seems to aggravate the hypodopaminergic state related to the neurodegenerative process (i.e., loss of dopaminergic neurons) by acting on D2R/D3R function. Studies to evaluate the impact of STN-DBS on D2R/D3R are under process. 2) Pramipexole treatment increases impulsivity in our rodent model. We are currently investigating the cellular pathways (e.g mTORC1) potentially responsible for the synaptic dysfunctions that underlie these impulsive behaviors.

Finally, we would like to aim the influence of STN-DBS on the transition from hypo to hyperdopaminergia and the subsequent development of ICDs

Key words: Parkinson's disease, apathy, DBS, STN, pramipexole, Impulsivity

NOTES :

P13 MOLECULAR MECHANISMS OF MICROTUBULE BUNDLING BY TAU: DIFFERENTIAL ROLES OF TAU PROJECTION DOMAIN AND REPEAT MOTIFS.

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Microtubules (MTs) are key components of the eukaryotic cytoskeleton and are involved in major cellular events including cell division, motility and morphogenesis. MTs are organized into stable bundles within axons and dendrites to maintain the polarized shape of neurons and to insure cargo transport. Tau is one of the neuronal MAPs (Microtubule-Associated Proteins) that promote MT assembly and bundling. Although the MT-stabilizing properties of tau have been widely studied, the mechanisms by which this protein spatially organizes MTs remain elusive. By reconstituting in vitro MT bundling, we could identify molecular features involved in MT organization by tau. We used various tau isoforms and truncated fragments either in tau's N-terminal projection domain or C-terminal MT-binding repeats to decipher the role of each subdomain in MT bundling. We found that the projection domain of tau has an inhibitory effect on MT bundling whereas the two hexapeptides responsible for the formation of Alzheimer paired helical filaments (PHFs) are fundamental in this process. We also showed that tau phosphorylations on specific sites, with some of them being abnormally phosphorylated in Alzheimer's disease, differentially regulates MT bundling and stabilization. Overall, our study reveals that tau modulates MT bundling and dynamics via distinct domains, and that these two activities can be dissociated by tau phosphorylation.

Key words: Tau, Microtubules, Actin, Phosphorylation

P14

Dynamic regulation of BDNF axonal transport by neuronal activity

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Dynamic remodeling of axonal connections is a *sine qua none* condition to adapt proper response to environmental stimuli. BDNF, the most abundant neurotrophin in the adult brain, is a key regulator of axonal remodeling, dendritic sprouting and synaptic plasticity, and its release at the synapse depends on neuronal activity. But how does neuronal activity direct axonal vesicles containing BDNF toward activated synapses to promote branching? Here developed microfluidics systems compatible with high-resolution we videomicroscopy connected to multielectrode arrays (MEA) to isolate corticostriatal connections and to monitor BDNF axonal transport within active axonal networks. Using this multicomplex system with segregated cortical and striatal primary cultures, we analyzed the evolution of BDNF trafficking during the maturation of the corticostriatal pathway. The formation and maturation of the network was assessed using a variety of fluorescent markers and sensors: MAP-2 (dendritic marker) and Tau-1 (axonal marker) to analyze the growth of dendritic and axonal branches; synaptophysin (presynaptic marker) and PSD-95 (postsynaptic marker) to analyze the formation of synaptic contacts; iGluSnFr (glutamate sensor) to visualize active glutamatergic corticostriatal synapses; GCamp5 (Calcium sensor) to analyze global and individual neuronal activity. We found that corticostriatal formation and maturation correlate with changes in BDNF trafficking properties. We are now studying the molecular and cellular events responsible for this regulation and its effect on the connection probability of the corticostriatal pathway.

Key words: BDNF, Cleavage, Maturation



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