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IL PROMETEO

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La luce della scienza cerco e 'l beneficio

LUX SCIENTIAE

DOTTORATO
INTERNAZIONALE
DI
NEUROFARMACOLOGIA

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Meccanismi Neuro anatomici alla Base della Predisposizione alla Farmacodipendenza

Se dovessi definire il mio anno appena trascorso in Francia, direi, senza pensarci neanche un momento che è stata un esperienza tanto formativa, quanto intensa ed esaltante.

Quando ho saputo di essere stata ammessa al XXV ciclo di Dottorato di Ricerca Internazionale in Neurofarmacologia, coordinato dal Professore Filippo Drago, sapevo che ci sarebbe stata l'opportunità di andare all'estero per qualche tempo…si trattava di Francia e mi sembrava già un buon inizio! Carica, quindi, della giusta grinta, di qualche paura e di un'italianissima moka lo scorso settembre mi sono trasferita a Bordeaux. Una città stupenda in un paese, la Francia, che si distingue da sempre per civiltà e savoir faire; Ma in particolare un paese che dona grandi attenzioni e supporti alla ricerca scientifica.

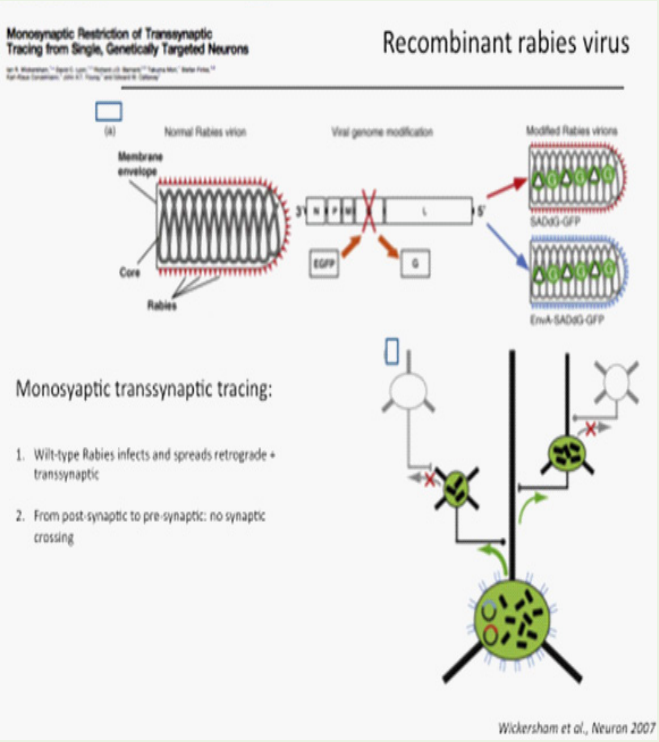
Per chi, come me, viene da una realtà come l'Italia in cui la genialità di alcuni validi individui è continuamente soffocata da uno stato totalmente disinteressato al progresso scientifico e che traduce la sua noncuranza sia in una mancanza di fondi economici che di una mancanza organizzativa, non può che non rimanere affascinata da uno dei più grossi centri europei di Neurobiologia in cui ho avuto l'onore di essere ospitata e formata per più di un anno: le Neurocentre Magendie.

L'INSERM (Institut National De la Santé et de la Recherche Medicale)U862, ospita il Neurocentro Magendie fondato

e diretto dal 2007 da PierVincenzo Piazza. L'istituto è un centro multidisciplinare dedicato a uno studio integrato delle neuroscienze che va dalle patologie neurologiche e comportamentali ai meccanismi cellulari e molecolari dell'attività neurale. In particolare, l'obbiettivo specifico del centro è la comprensione della fisiopatologia della plasticità neuronale; Si studiano quindi i meccanismi alla base delle malattie del cervello sviluppando una ricerca che è al limite tra la ricerca di base e quella applicata, quindi la base che porta poi allo sviluppo di nuovi farmaci. Il centro è composto da 150 persone altamente qualificate e specializzate, divise in 9 gruppi di ricerca e 5 strutture comuni. I 9 gruppi lavorano separatamente, ma in una continuità di scambi e di rapporti: settimanalmente si partecipava ai journal club in cui si discuteva in maniera critica un buon articolo scientifico oppure gli hot topic in cui uno studente o un team leader, indipendentemente dalla loro gerarchia, discuteva il suo progetto con punti di forza e di debolezza in modo da ricercare aiuto e collaborazioni all'interno dell'istituto stesso.

Questa generosità nell'offrire e nel rimettere al bene comune il proprio sapere, così come la semplicità nel tendere la mano per chiedere aiuto è quello che rende questo istituto uno dei poli di attrazione Europei per i giovani scienziati.

Io ho lavorato con il gruppo di Andreas Frick, che proveniva dall'istituto Max Planck di Monaco in Germania, che si distingueva per multiculturalità: eravamo 8 persone provenienti ognuno da diverse parti del mondo, cosa che ha particolarmente arricchito la mia esperienza. Andreas che da molti anni ormai si occupa dei meccanismi della plasticità corticale, mi ha aiutato a sviluppare un progetto in collaborazione con il gruppo di Piervincenzo Piazza sui Meccanismi Neuro anatomici alla Base della Predisposizione alla Farmacodipendenza.
In particolare abbiamo riprodotto un modello animale di farmacodipendenza su ratti maschi wistar di circa 300 grammi in cui sono stati iniettati mediante iniezione stereotassica, due tipi diversi di virus: AAV e un Virus Ricombinante della Rabbia. Questo ultimo entra a far parte di una raffinata tecnica : il Monosynaptic Transsynaptic tracing.

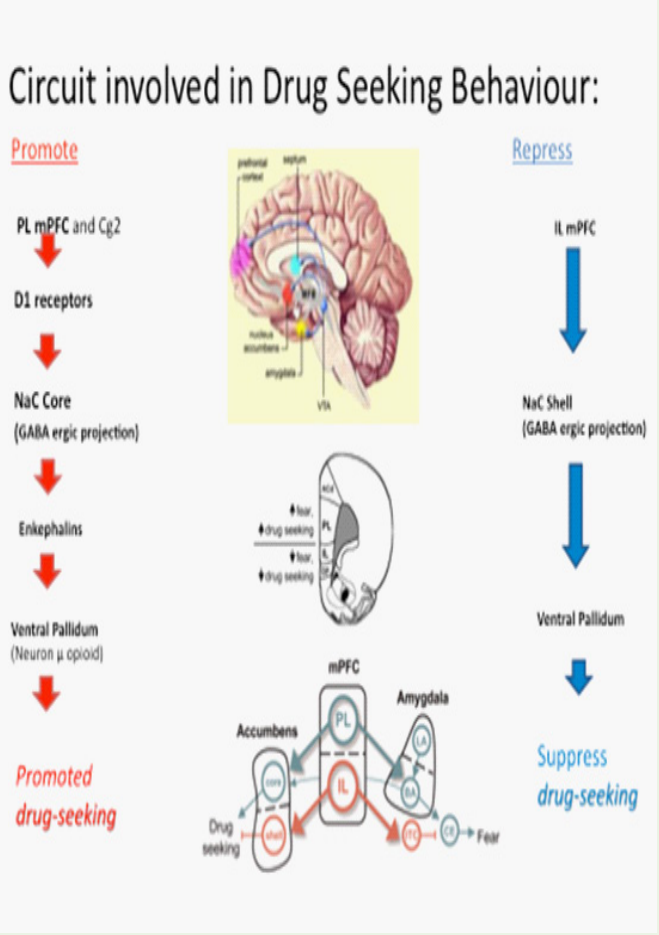


Il virus è un virus geneticamente modificato della Rabbia in cui è stata sostituita la proteina G con il marcatore eGFP. Una volta iniettato il virus in una precisa area del cervello (es: corteccia prefrontale- layer 2), questo penetrava all'interno dei terminali assonici rimontando a ritroso la fibra nervosa riuscendo a risalire la giunzione sinaptica per marcare il neurone a monte. In tal modo si poteva evidenziare un circuito dineuronico ed identificare in tal modo la rete neurale che controlla il sito iniettato. A questo punto l'osservazione al microscopio mi permetteva di ricostruire la topografia dei siti marcati permettendomi di compare i diversi siti iniettati.

Questo metodo è stato eseguito sia su animali trattati per 1,2,3 mesi con cocaina, sostanza che come è noto induce dipendenza, e in animali wild type, usati come controllo. Le osservazioni sperimentali hanno evidenziato l'esistenza di circuiti che si realizzano de novo negli animali trattati. Infatti, in questi animali sono state evidenziate proiezioni ai siti iniettati che erano assenti negli animali wt. La costituzione di tali connessioni anatomiche negli animali trattati con la cocaina suggerisce l'esistenza di una possibile correlazione di essi con le stimmate comportamentali proprie degli animali tossicodipendenti.

Future ricerche dovranno verificare questa ipotesi mediante test funzionali e comportamentali.

Volendo concludere questa mia breve descrizione scientifica ed umana a Bordeaux, devo riconoscere di essere orgogliosa di aver portato un sia pur modesto contributo alla conoscenza dei meccanismi neurofisiologici che sottostanno a quel processo ancora oscuro che si definisce come tossicodipendenza e che tanto rilievo assume oggi nel contesto sociale dei paesi ad economia sviluppata.



Neuropathic pain – translational research and impact for patients care

Prof. Dr. Ralf Baron

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Neuropathic pain syndromes, i.e., pain after a lesion or disease of the peripheral or central nervous system, are clinically characterized by spontaneous pain (ongoing, paroxysms) and evoked pains (hyperalgesia, allodynia). Basic science has revealed a variety of distinct pathophysiological mechanisms in the peripheral and central nervous system which operate in concert: In some patients the nerve lesion triggers molecular changes in nocicepetive neurons that become abnormally sensitive and develop pathological spontaneous activity (up-regulation of sodium channels and receptors, e.g., vanilloid TRPV1-, menthol-sensitive TRPM8-receptors). These phenomena may lead to spontaneous pain, shooting pain sensations as well as heat hyperalgesia and cold hyperalgesia. Spontaneous activity in damaged non-nociceptive A-fibers may lead to paresthesias. All these changes may also occur in uninjured neurons driven by substances released by adjacent dying cells. The hyperactivity in nociceptors in turn induces secondary changes (hyperexcitability) in processing neurons in the spinal cord and brain. This central sensitization causes input from mechanoreceptive A-fibers to be perceived as pain (mechanical allodynia). Neuroplastic changes in the central descending pain modulatory systems (inhibitory or faciliatory) may lead to further hyperexcitability. Other patients suffer from severe spontaneous pain in combination with a profound sensory loss but no hyperalgesia or allodynia. The pain is due to spontaneous activity in deafferented central neurons.

A new hypothetical concept was proposed in which pain is analyzed on the basis of underlying mechanisms rather than on the basis of the etiology. If a precise phenotypic characterization is combined with a selection of drugs acting at those particular mechanisms, it should ultimately be possible to design optimal treatments for the individual patient. Such research can only be performed in large cohorts of patients, ideally on a Research Network level. The German Research Network on Neuropathic Pain established a large data-base that includes epidemiological, clinical and history data as well as a standardized quantitative sensory testing (QST). Up to now more than 2000 patients with different neuropathic pain states have been examined. Furthermore, epidemiological and clinical data on the symptomatology of 4000 patients with painful diabetic neuropathy (DPN), postherpetic neuralgia (PHN) and radiculopathy from a cross sectional survey (painDETECT) are available.

Based on these data different sensory profiles could be analyzed in several entities which occur in different frequencies. The second step to be solved is the question whether an individual somatosensory phenotype really mirrors distinct pain mechanisms? For this purpose the technique of somatosensory pattern recognition was used and first results have been achieved. This approach uses the somatosensory patterns that are specific for human surrogate models of pain in which the underlying mechanisms are relatively well understood. The last and decisive question is whether these different phenotypes (which are presumably related to different mechanisms) are really associated with different treatment outcomes. The results of such multi-center network trials will ultimately substantiate the mechanism based treatment concept in neuropathic pain.

Mechanisms of chronic visceral pain: from epidemiology to evidence-based management

L. Ashley Blackshaw

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Chronic abdominal pain (CAP) has no effective treatment, but results in more primary care consultations than headache, chest pain or high blood pressure, and has a major impact on quality of life. Irritable Bowel Syndrome (IBS) is the most common manifestation of abdominal pain, which is diagnosed as a recurrent sense of abdominal discomfort or pain accompanied by altered bowel movements. There are no physiological markers, so all approaches to the condition are based on bowel habit, with patients mainly categorized as diarrhoea predominant (D-IBS), or constipation predominant (C-IBS). While these classifications provide options for the management of bowel habit, it is important to note that pain crosses these subgroups and beyond into gastrointestinal inflammatory and neuromuscular diseases. Pain is the most debilitating symptom and the most intractable to treatment. Currently the best insight into the aetiology of CAP is the increased risk of development following gastrointestinal infection, clearly implicating the immune system in the initiation of disease. Termed post-infectious (PI-IBS), the clinical characteristics of these patients correlate robustly with D-IBS. Thorough investigation of biopsies from PI-IBS patients consistently reveal a chronic but low grade immune activation, with increased numbers of resident immune cells including mast cells and T-cells. More recent investigations have extended to the circulating peripheral blood mononuclear cell (PBMC) population. These cells migrate to target tissues, and in IBS are more likely to target the gastrointestinal tract. Evidence is presented that in accurately categorized cohorts of IBS patients there are marked differences in their cytokine status and the way in which these interact with sensory nerves from the colon. Events in the intestine are signaled by the lumbar splanchnic and sacral pelvic sensory afferent nerves. They possess a rich diversity of afferent subtypes that signal the full range of painful and physiological stimuli including distension, urge and contractile events. Colonic afferent function is actively modulated by inflammation, and increased peripheral levels of cytokines correlate with symptoms of altered motility in D-IBS patients, such as diarrhea and urgency. Immune mediators from PI/D-IBS patients sensitize colonic afferents to mechanical stimuli. We have characterized the immune profile in sub-classes of IBS patients and how altered immune function interacts with sensory pathways to give rise to symptoms of pain, thereby identifying potential targets for the treatment of this elusive disease. There are also clear molecular mechanisms underlying visceral sensory neuroimmune interactions involving subtypes of cytokine receptors and ion channels.

Molecular Mechanism of Migraine

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The molecular and cellular origins of migraine headache are among the most complex problems in contemporary neurology. To meet these challenges, researchers have successfully applied the tools of neuroimaging, neurogenetics, neuropharmacology and neurophysiology. With recent advances, we now have a clearer description of cellular events that characterize the migraine visual aura such as cortical spreading depression.

This presentation will review translational advances with special emphasis on the evidence implicating genes regulating ion channels and pumps, sex hormones and migraine prophylactic drugs as modulators of cortical spreading depression (CSD). Cortical spreading depression, a slowly propagating wave of neuronal and glial depolarization, was first linked to visual aura in the 1940's based on the close correspondence between CSDs known neurophysiological characteristics and the evolving visual per-

cept of migraine. High field strength, near-continuous BOLD imaging recordings during visual aura substantiate this association and implicate CSD as a noxious event capable of triggering headache. In knock-in mice expressing the Familial Hemiplegic Migraine -1 missense mutation in a gene encoding a subunit of Cav2.1 (P/Q calcium channel), susceptibility to evoked CSDs is enhanced, but especially in female mice. In addition to sex hormones and genes regulating ion translocation, CSD is modulated in normal rats by chronic administration of migraine prophylactic drugs. These and other experimental data are consistent with the notion that headaches developing after migraine aura are caused by CSD-induced release and extracellular build-up of noxious molecules normally sequestered in neuronal and non-neuronal cellular compartments. The translational relevance and congruence of this body of work to the phenotype of common forms of migraine will be discussed

Pain as a channelopathy

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Mendelian heritable pain disorders have provided insights into human pain mechanisms and suggested new analgesic drug targets. Interestingly, many of the heritable monogenic pain disorders have been mapped to mutations in genes encoding ion channels. Studies in transgenic mice have also implicated many ion channels in damage sensing and pain modulation. It seems likely that aberrant peripheral or central ion channel activity underlies or initiates many pathological pain conditions. Understanding the mechanistic basis of ion channel malfunction in terms of trafficking, localization, biophysics, and consequences for neurotransmission is a potential route to new pain therapies. Pain serves to protect the body from harm and promote healing of damaged tissues. Chronic pain, however, remains a major clinical challenge, which, if unmet, significantly diminishes quality of life in the affected individuals. The genetics of human pain has been the subject of intense study in the past decade. Substantial evidence indicates that a large component of the pain experience, such as acute pain thresholds or efficacy of analgesics, is inherited. The involvement of channelopathies in human pain conditions has been highlighted by evidence from analysis of pain phenotypes in transgenic animal models. Aberrant channel expression has also been linked to chronic pain evoked by physical insults. Hence understanding the ion channels involved in the pathophysiology of human pain conditions could provide opportunities for the development of novel therapeutic agents as well as furthering our insights into the functioning of the nervous system

Pain in Cancer Patients: Pathophysiology and treatment

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Cancer pain is a complex issue regarding the majority of patients with advanced cancer.
Anticancer therapy, including surgery and chemotherapy, may provide some analgesia, which is difficult to quantify. Radiotherapy provides an effective symptomatic treatment for local bone pain causing transitory events. However, the pharmacological treatment will be necessary in most cases.
Analgesic drug therapy is the cornerstone of cancer pain therapy. Current treatment is based on the analgesic ladder recommended by WHO, which involves a stepwise approach to the use of analgesic drugs and is essentially a framework of principles rather than a rigid protocol. Many drugs are available and provide efficient analgesia in most cases. However, a minority of patients require more complex strategies, including opioid switching and route of administration. Interventional procedures may occasionally support the pharmacological treatment in a selected population of cancer patients with pain.

Peripheral mechanisms of pain and analgesia

Christoph Stein
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We aim at the discovery of novel molecules and therapeutic approaches devoid of central side effects such as addiction, tolerance, respiratory depression or sedation. Opioid receptors are present and upregulated on peripheral sensory neurons, and opioid peptides are expressed in immune cells within injured tissue. Environmental stress and releasing agents can lead to secretion of these peptides and to local analgesia by inhibiting the excitability of peripheral sensory neurons. We have examined G-protein coupling and signaling of opioid receptors in sensory neurons, opioid peptide processing, release and extracellular degradation by immune cells, and adhesion molecules, chemokines and growth factors governing the migration of opioid containing cells to inflamed tissue. As a result of the interaction between immune cell-derived opioid peptides and opioid receptors on peripheral sensory neurons, tolerance does not develop to the analgesic effects of locally applied exogenous opioids in inflammatory pain. Clinical data will be presented demonstrating that peripherally active or locally administered opioids can potently inhibit acute and chronic inflammatory pain associated with surgery or arthritis.

How to integrate pain treatment in psychiatric patient's care

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Although the association between psychiatric disorders and chronic pain has been established, the more in depth research concerning the pathogenesis and treatment modalities is needed.
While analysing the various paradigms of pain, the more detailed association of pain and mental disorders will be covered in order to detect overlaps in the various phenomenological states and epidemiology. On examples of posttraumatic stress disorders (PTSD) and major depressive disorder (MDD) most recent findings from applied neuroscience and genetics will be covered in order to introduce the specific hallmarks of the chronic pain states comorbid with these mental disorders.
The third issue will be the implementation of person-centred medicine and personalized medicine in the management of chronic pain and psychiatric disorders, covering the basic similarities and differences in pain diagnosis and management, varying from person-centred diagnosis to personalized approach using biomarkers to improve outcome in the area of pain management. The current problems of pain management in patients with psychiatric disorders will be addressed.
Possible research issues arising in these paradigms and concerning the evidence-based approach in holistic surrounding and the need for interdisciplinary work in both clinical management and pain research will be addressed with the several recommendations for the future research.