Milan DiSFeB
Department of Pharmacological and Biomolecular Sciences

UNIVERSITÀ DEGLI STUDI DI MILANO

Center of Excellence on Neurodegenerative Diseases

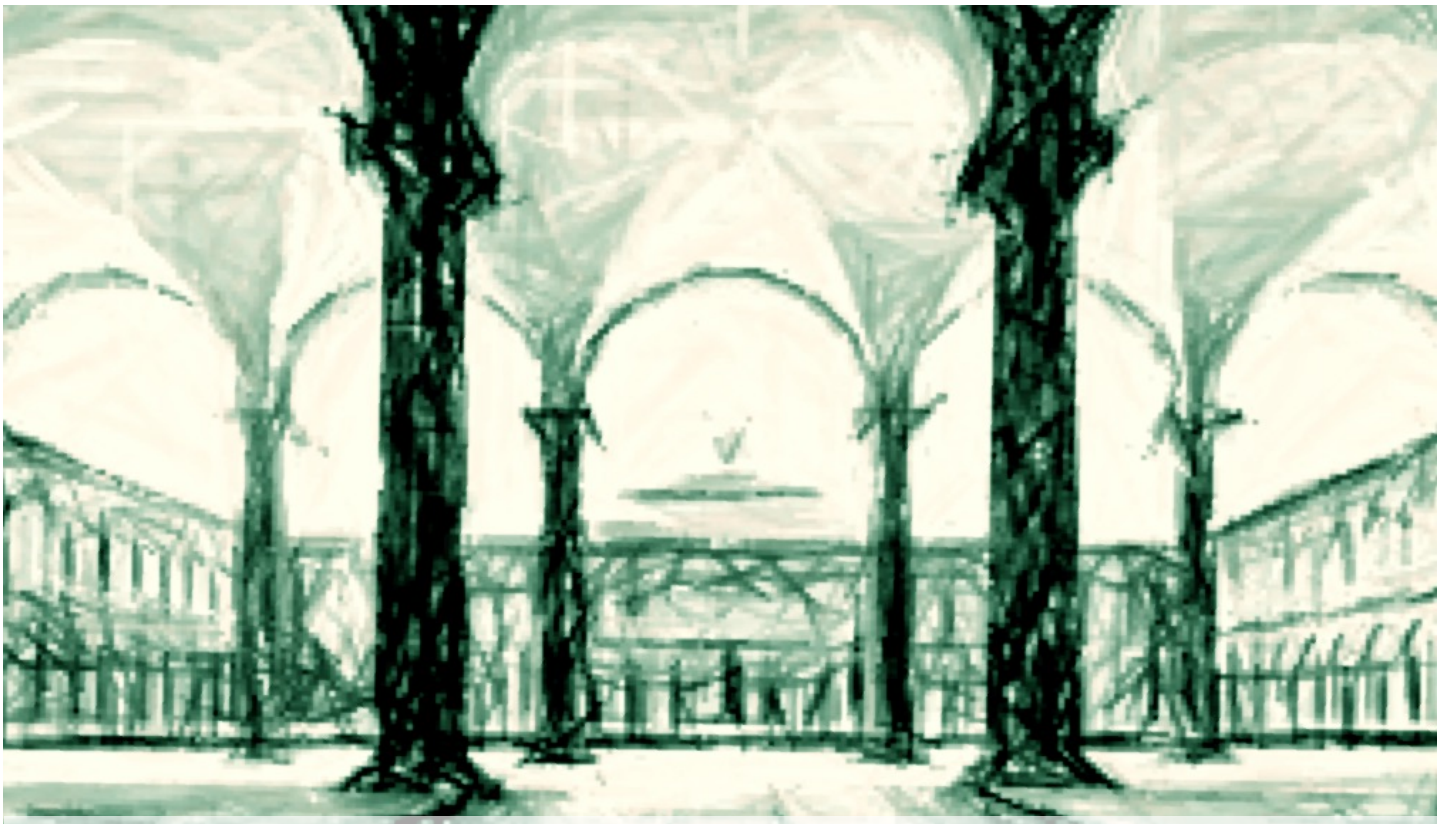
Convegno Monotematico SIF

In collaboration with ***Gruppo di Lavoro SIF di Neuropsicofarmacologia***

The Stressed Brain:

Psychopathologic Implications and Pharmacological Intervention

Scientific Organizers: Marco A. Riva and Fabio Tascedda



March 3 - 4, 2016
Sala Napoleonica – University of Milano
Via S. Antonio 10 - Milano

SOCIETÀ ITALIANA FARMACOLOGIA

FINAL PROGRAM

Thursday, March 3

13:00-14:30	Registration
14:30-14:45	Opening and Congress Presentation *(**Giorgio Cantelli Forti, Monica Di Luca, Marco A. Riva and Fabio Tascedda**)*
14:45-16:00	Oral Communications – Session #1 *(Chairs: **Patrizia Campolongo** and **Cristiano Chiamulera**)*
16:00-16:30	Coffee Break
16:00-17:30	Poster Session *(Chair: **Silvia Alboni**)*
17:30-18:30	Keynote Lecture: **Ronald S. Duman** (Yale Univ. School of Medicine, USA) "Stress, depression, and antidepressants: remodeling synaptic connections" *(Chair: **Giorgio Racagni**)*
19:30	Happy Hour (*www.lebiciclette.com)*

Friday, March 4

9:00-10:15	Oral Communications – Session #2 *(Chairs: **Paola Fadda** and **Mariagrazia Grilli**)*
10:15-11:15	Keynote Lecture**: Elisabeth B. Binder** (Max Planck Inst. of Psychiatry; Germany) "Molecular mechanisms of gene x stress interactions: implications for prevention of psychiatric disorders" *(Chair: **Nicoletta Brunello**)*
11:15-11:45	Coffee Break
11:45-12:45	Oral Communications – Session #3 *(Chairs: **Roberto C. Melcangi** and **Raffaella Molteni**)*
12:45-13:00	Concluding Remarks *(**Marco A. Riva** and **Fabio Tascedda**)*

KEYNOTE SPEAKERS

Ronald S. Duman – New Haven (USA)
Elisabeth B. Binder – Munich (Germany)

SPEAKERS

Alessia Auber – Verona
Valeria Bortolotto – Novara
Annamaria Cattaneo – Brescia
Federica Ferrari – Pavia
Alessia Luoni – Milano
Gian Marco Leggio – Catania
Laura Musazzi – Milano
M.Grazia Morgese – Foggia
Vincenzo Prisco – Napoli
Francesco Papaleo – Genova
Andrea C. Rossetti – Milano
Chiara Ruzza – Ferrara
Stefania Schiavone – Foggia
Paolo Tornese – Milano

CHAIRS

Silvia Alboni – Modena
Nicoletta Brunello – Modena
Patrizia Campolongo – Roma
Cristiano Chiamulera – Verona
Paola Fadda – Cagliari
Mariagrazia Grilli – Novara
Roberto C. Melcangi - Milano
Raffaella Molteni – Milano
Giorgio Racagni – Milano

SCIENTIFIC SECRETARIAT
Silvia Alboni
Raffaella Molteni

SOCIETÀ ITALIANA FARMACOLOGIA

ORAL COMMUNICATIONS

SESSION #1 ***Thursday, March 3, 14:45-16:00***
Chairs: ***Patrizia Campolongo*** *and* ***Cristiano Chiamulera***

- Musazzi Laura (Università degli Studi di Milano) **The stress impact on synaptic function and brain architecture: implications for mood and anxiety disorders.**
- Rossetti Andrea C. (Università degli Studi di Milano) **Effect of the antidepressant agomelatine on the IL-6 pathway in rats exposed to chronic mild stress: role of Suppressor Of Cytokine Signaling 3 (SOCS3).**
- Ruzza Chiara (Università degli Studi di Ferrara) **Effects of nociceptin/orphanin FQ receptor partial agonists in mouse models of anxiety and depression.**
- Auber Alessia (APTUIT, Verona) **Towards pharmacological validation of the novelty-suppressed feeding test in the rat as a model to predict the time-course of anxiolytic drug action.**
- Tornese Paolo (Università degli Studi di Milano) **Acute ketamine treatment modulates brain area-specific deficits induced by chronic mild stress in vulnerable rats.**

SESSION #2 ***Friday, March 4, 9:00-10:15***
Chairs: ***Paola Fadda*** *and* ***Mariagrazia Grilli***

- Schiavone Stefania (Università degli Studi di Foggia) **Brain oxidative stress and suicide: identification of the NADPH oxidase NOX2 as a novel biomarker.**
- Morgese Maria Grazia (Università degli Studi di Foggia) **Lifelong nutritional omega-3 deficiency evokes depressive-like state and hyperactivation of HPA axis: which role for soluble beta amyloid?**
- Ferrari Federica (Università degli Studi di Pavia) **Effect of aging on the energy metabolism of cerebral cortex, hypothalamus and hypophysis: implications for the responsiveness to stress.**
- Luoni Alessia (Università degli Studi di Milano) **Ankyrin-3: a link between early life stress and the vulnerability to mood disorders.**
- Cattaneo Annamaria (IRCCS “San Giovanni di Dio” - Fatebefratelli, Brescia) **Inflammatory related pathways and SGK1 signaling as targets of early life stressful events: role of DNA methylation and miRNAs.**

SESSION #3 ***Friday, March 4, 11:45-12:45***
Chairs: ***Roberto C. Melcangi*** *and* ***Raffaella Molteni***

- Prisco Vincenzo (Università Degli Studi di Napoli) **Influence of temperamental and character traits on antidepressant response in patients affected by major depressive disorder.**
- Papaleo Francesco (IIT, Genova) **Arc/Arg3.1 genetic disruption in mice causes dopamine system alterations and neurobehavioral phenotypes related to schizophrenia.**
- Leggio Gian Marco (Università Degli Studi di Catania) **Genetic-driven reduction of dopamine D3 receptor ameliorates dysbindin-dependent schizophrenia-relevant abnormalities.**
- Bortolotto Valeria (Università del Piemonte Orientale, Novara) **Role of NF-kB p50 in the cross-talk between adult neural progenitor cells and astrocytes.**

POSTER COMMUNICATIONS

Thursday, March 3, 16:00-17:30
Chair: Silvia Alboni

1. **BDNF alterations as consequences of childhood trauma experiences.** Begni V, Tosato S, Tomassi S, Locatelli S, Tarantini L, Lopizzo N, Bollati V, Pariante CM, Cattaneo A, Riva MA (Milano)

2. **Activation of immune signaling related pathways and reduced telomere length in subjects exposed to stressful life events.** Lopizzo N, Begni V, Tosato S, Tomassi S, Riva MA, Pariante CM, Cattaneo A (Brescia)

3. **mRNA-miRNA integration approach in rats and in humans to identify long-term signatures of early life stress.** Malpighi C, Plazzotta G, Luoni A, Mondelli V, Riva MA, Pariante CM, Cattaneo A (Brescia)

4. **Early modulation of microRNAs in rat prefrontal/frontal cortex after acute stress.** Seguini M, Tardito D, Tornese P, Sala N, Merelli I, Milanesi L, Popoli M, Musazzi L (Milano)

5. **Acute foot-shock stress induces time-dependent alterations of glutamatergic synapses in prefrontal cortex of male rats.** Sala N, Musazzi L, Tornese P, Bazzini C, Popoli M (Milano)

6. **Exposure to the chronic mild stress induced cognitive dysfunctions: investigation of molecular mechanisms underlying this deficit.** Brivio P, Calabrese F, Papp M, Riva MA (Milano)

7. **Regulation of clock gene expression in the chronic mild stress model: modulatory activity of the novel drug lurasidone.** Savino E, Calabrese F, Rossetti AC, Papp M, Racagni G, Molteni R, Riva MA (Milano)

8. **Role of central dopamine D3 and serotonin 2C receptors in the control of the mesoaccumbens dopaminergic pathway: implications for the treatment of depression.** Torrisi SA, Leggio GM, Giurdanella G, Caraci F, Platania CBM, Bucolo C, Salomone S, Drago F (Catania)

9. **Role of CRF system in frustration stress-induced binge-like palatable food consumption in female rats.** Giusepponi ME, Micioni Di Bonaventura MV, Ubaldi M, Ciccocioppo R, Rice KC, Shaham Y, Massi M, Cifani C (Camerino)

10. **Epigenetic regulation of adenosine A2A and dopamine D2 receptor gene transcription in frustration stress-induced binge-like palatable food consumption.** Turchetti L, Micioni Di Bonaventura MV, Giusepponi ME, Pucci M, Lambertucci M, Volpini R, D'Addario C, Cifani C (Camerino)

11. **Association of brain amyloidosis with pro-inflammatory gut bacterial strains and peripheral inflammation markers in cognitively impaired elderly.** Provasi S, Cattaneo A, Cattane N, Galluzzi S, Lopizzo N, Plazzotta G, Festari C, Ferraria C, GuerraUP, Paghera B, Muscio C, Bianchetti A, Dalla Volta G, Turla M, Cotelli MS, Gennuson M, Prelle A, Zanetti O, Lussignoli G, Mirabile D, Bellandi D, Gentilep S, Belotti G, Villani D, Padovani A, Boccardi M, Frisoni GB, for the INDIA-FBP group (Brescia)

12. **Pro-BNP as a biomarker of asymptomatic clozapine-related heart dysfunction: possible usefulness for clozapine management.** Prisco V, Petrosino M, Fiore G, Tridente A, La Rocca A, Catapano F, Fabrazzo M (Napoli)

13. **Omega-3 and omega-6 polyunsaturated fatty acid enriched diet in susceptibility to stress response: implication for depression in female rats.** Bove M, Morgese MG, Trabace L (Roma)

14. **Different roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in the modulation of memory retrieval in rats.** Rubino B, Atehourtua Martinez A, Ratano P, Campolongo P (Roma)

15. **Everolimus improves memory and learning while worsening depressive- and anxiety-like behavior in an animal model of depression.** Maida F, Crupi R, Leo A, Citraro R, Cuzzocrea S, De Sarro G, Russo E (Catanzaro)

Abstracts of Oral Communications

The stress impact on synaptic function and brain architecture: implications for mood and anxiety disorders

Musazzi L[1], Tornese P[1], Sala N[1], Milanese M[2], Seguini M[1], Barbon A[3], Nava N[4], Treccani G[1,5], Nyengaard J[4], Racagni G[1], Wegener G[5], Bonanno G[2], Popoli M[1]

[1]Laboratory of Neuropsychopharmacology and Functional Neurogenomics - Dipartimento di Scienze Farmacologiche e Biomolecolari and CEND, Università di Milano, Milano, Italy; [2]Department of Pharmacy - Unit of Pharmacology and Toxicology, Center of Excellence for Biomedical Research, Università di Genova, Genova, Italy; [3]Biology and Genetic Division, Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy; [4]Stereology and Electron Microscopy Laboratory, Centre for Stochastic Geometry and Advanced Bioimaging, Aarhus University Hospital, Aarhus C, Denmark; [5]Translational Neuropsychiatry Unit, Department of Clinical Medicine, Aarhus University, Aarhus, Denmark.

Stressful life events impact on brain and bodily function and represent major risk factors for mood and anxiety disorders. Stressful events represent main risk factors in the pathophysiology of neuropsychiatric disorders. The physiological stress response implies metabolic and adaptive plasticity changes, aimed to promote adaptation and to preserve the memory of the stressor. However, when the stressor is chronic, uncontrollable, or overwhelming, the response could be impaired or overused, in turn leading to impaired function and increased risk to develop stress-related pathologies.

Stressful experiences have been shown to deeply affect structural and functional plasticity, particularly within prefrontal cortex (PFC), a brain area with critical roles in cognitive function. We have shown that acute stress rapidly enhances excitatory (glutamatergic) transmission in PFC, together with an increase in the number of docked vesicles and small excitatory synapses, and that chronic treatment with antidepressants attenuates these effects. We have also shown that the increase of trafficking into the readily releasable pool (RRP) of glutamate vesicles induced by acute stress in PFC is dependent on synaptic, non-genomic action of glucocorticoids.

We are now studying the time-dependent effects of acute stress on brain structural and functional plasticity and related cognitive behavior. We found that acute stress induces early and sustained increase of RRP and activity-dependent glutamate release. Moreover, acute stress dramatically increases the total number of excitatory non-perforated synapses, and induces rapid (24 h) atrophy and remodeling of apical dendrites in prelimbic PFC, sustained for at least 14 days. In behavioral tests for working memory, acute stress improved performance 2 h after stress, while impairing behavioral performance 24 h later. We also measured time-dependent changes in the expression and phosphorylation levels of molecular effectors regulating glutamate release and transmission and involved in the stress response (i.e. corticosterone receptors, mGluR2, synapsin I). Changes in glutamate release, RRP, number of synapses and spines, and behavior are blocked or attenuated by prior chronic treatment with the antidepressant desipramine. Taken together, these results showed that acute stress may result in sustained remodeling of neural architecture and function, suggesting that the stress response within PFC may turn at some point from early excitatory activation into its opposite. The identification of these turning points and the players involved in the switch are crucial for the understanding of the dynamics of stress-related pathology.

Effect of the antidepressant agomelatine on the IL-6 pathway in rats exposed to chronic mild stress: role of Suppressor Of Cytokine Signaling 3 (SOCS3)

Rossetti AC[1], Paladini MS[1], Bruning CA[2], Racagni G[1], Papp M[3], Riva MA[1], Molteni R[1]

[1]Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Milan, Italy; [2]Department of Biochemistry and Molecular Biology, Federal University of Santa Maria, Santa Maria, RS, Brazil; [3]Institute of Pharmacology, Polish Academy of Sciences, Kracow, Poland.

Major depression (MD) is a debilitating disorder whose treatment is being challenged by the high rate of failure and relapse of the pathology. Among the molecular systems thought to be involved in the MD etiology and in the mechanism of action of antidepressant drugs, inflammation has emerged as an important actor (Miller and Raison, 2015). In particular, increased levels of pro-inflammatory cytokines have been observed in the plasma and cerebrospinal fluid of depressed patients and, among these inflammatory mediators, interleukin (IL-) 6 has been recently proposed to play a crucial role (Fonseka et al., 2015). IL-6 triggers a peculiar pathway comprising the JAK/STAT signaling proteins and characterized by a specific negative feedback loop exerted by the cytoplasmic protein, SOCS3 (Suppressor Of Cytokine Signalling-3). We have recently demonstrated that a chronic mild stress (CMS) paradigm able to induce a depressive-like phenotype, up-regulates the expression of different pro-inflammatory cytokines in the rat brain and that pharmacological treatment with the antidepressant agomelatine was able to normalize not only the pathologic phenotype but also the inflammatory state (Rossetti et al., 2015). In this context, the aim of the present work was to further investigate the mechanism underpinning the anti-inflammatory activity of agomelatine by evaluating the impact of the drug on IL-6 pathway in the prefrontal cortex of rats exposed to CMS. As expected, stress was able to activate the IL-6 cascade, including SOCS3 gene and protein expression and JAK1/STAT3 phosphorilation, without any suppressive effect of the feedback-loop inhibition. On the contrary, chronic treatment with agomelatine was able not only to normalize the stress-induced activation of IL-6 signaling, but also to modulate SOCS3 translation and transduction under basal conditions. Given the potentiality of IL-6 signaling as target of antidepressant treatment, we suggest that SOCS3 modulation might be a valuable goal for new drug development.

References:

Miller AH et al., Nat Rev Immunol. (2015); 16(1):22-34.
Fonseka TM et al., Expert Opin Investig Drugs. (2015); 24(4):459-75.
Rossetti AC et al., Pharmacol Res. (2015); 103:1-12.

Effects of nociceptin/orphanin FQ receptor partial agonists in mouse models of anxiety and depression

Ruzza C

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Nociceptin/orphanin FQ (N/OFQ) receptor (NOP) agonists produce anxiolytic-like effects in rodents while antagonists promote antidepressant-like effects. The aim of this study was to investigate the effect on anxiety and depression of NOP receptor partial agonists such as the peptides [F/G]N/OFQ(1-13)NH_2 and UFP-113 and the non-peptide AT-090. In vitro AT-090, UFP-113, and [F/G]N/OFQ(1-13)NH_2 were tested for their ability to promote NOP/G-protein and NOP/β-arrestin 2 interaction, using a bioluminescence resonance energy transfer assay. In vivo, they were tested in mice in the elevated plus maze (EPM) and in the forced swimming (FST) tests. NOP partial agonists effects were systematically compared with to those of full agonists (N/OFQ and Ro 65-6570) and antagonists (UFP-101 and SB-612111). In vitro, AT-090, UFP-113, and [F/G]N/OFQ(1-13)NH_2 promoted NOP/G protein interaction, with maximal effects lower than those evoked by N/OFQ and Ro 65-6570. AT-090 behaved as a NOP partial agonist also in inducing β-arrestin 2 recruitment, while UFP-113 and [F/G]N/OFQ(1-13)NH_2 were inactive in this assay. In vivo, AT-090 induced anxiolytic-like effects in the EPM but was inactive in the FST. Opposite results were obtained with UFP-113 and [F/G]N/OFQ(1-13)NH_2. In conclusion, NOP ligands producing similar effects on NOP/G protein interaction (partial agonism) but showing different effects on β-arrestin 2 recruitment (partial agonism vs antagonism) elicited different actions on anxiety and mood. These results suggest that the action of a NOP ligand on emotional states is better predicted based on its β-arrestin 2 rather than G-protein efficacy.

Towards pharmacological validation of the novelty-suppressed feeding test in the rat as a model to predict the time-course of anxiolytic drug action.

Auber A[1], Guidi A[1], Pedercini M[1]; Corsi M[2], Gerrard P[1]

[1]Dept of Translational Pharmacology; [2]Dept of in vitro Pharmacology, Drug Discovery and Design, Aptuit, Verona, Italy.

Animal models exhibiting predictive validity for the time–course of the response to known pharmacotherapies are greatly needed for studying the mechanisms of the antidepressant/anxiolytic response and to develop novel drugs for the treatment of psychiatric diseases (e.g. Anxiety). Although several models of acute antidepressant effects provide excellent tools for the discovery of novel antidepressant/anxiolytic drugs, they do not permit investigation into their therapeutic effects, which require several weeks of treatment to emerge (e.g. Serotonin Reuptake Inhibitors). The aim of the present study is to pharmacologically validate the Novelty–suppressed Feeding Test (NSF), which has been suggested to exhibit predictive validity for the time–course of the therapeutic effect (Dulawa & Hen, 2005). Rats were food-deprived for 23 hours, placed into a novel environment containing food and the latency to begin eating was recorded. Acute injection of Diazepam (2mg/Kg, i.p., 1 hour before test) reduced latency to eat compared to controls (n=5; one-tailed t-test $p<0.05$). Both acute and chronic treatment with Fluoxetine (10mg/Kg, p.o., 1 hour before test,1 or 28 days respectively) tended to increase latency to eat compared to controls (n=5-6 or n=13-14, one-tailed t-test p=0.05 or 0.06 respectively). In summary, the well-known anxiolytic effect of Diazepam has been observed in the NSFT. Acute Fluoxetine induces an anxiogenic-like effect and this is in line with the exacerbation of anxiety symptoms reported by humans after a single administration of Fluoxetine. Chronic Fluoxetine treatment also induced an anxiogenic–like effect which is in contrast with the anxiolytic-effect observed in humans following chronic administration. Several studies have investigated the effect of chronic Fluoxetine in different animal models of anxiety and contrasting results were observed. It has been demonstrated that chronic Fluoxetine elicits different changes in stressed mice compared to normal animals. This suggests that the anxiolytic effect of Fluoxetine, and therefore the predictive validity of NSFT, may need to be assessed in animals subjected to manipulations inducing a pathological state. Further experiment evaluating the effect of acute vs. chronic Fluoxetine in stressed rats will be needed in order to set-up an animal-model with predictive validity for the time-course of anxiolytic effect.

Acute ketamine treatment modulates brain area-specific deficits induced by chronic mild stress in vulnerable rats.

Tornese P[1], Musazzi La[1], Sala N[1], Bonini D[2], Racagni G[1], Barbon A[2], Popoli M[1]

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Increasing evidence has associated dysfunction of the glutamate system with the pathophysiology of stress-related neuropsychiatric disorders. Clinical studies on depressed patients have shown consistent volumetric and functional changes in brain areas where glutamatergic transmission is predominant, including the hippocampus (HPC) and the prefrontal and frontal cortex (PFC/FC). In parallel, preclinical studies on stress-based animal models of depression demonstrated that chronic stress induces dendrite remodelling and reduction of synaptic contacts in the same brain regions affected in patients. However, while acute stress was shown to rapidly enhance glutamate neurotransmission, an effect blocked by chronic antidepressants, the impact of chronic stress on glutamate release is still largely unknown. Intriguingly, consistent evidence has shown that the NMDA receptor antagonist ketamine (KET) induces rapid and sustained antidepressant effect, both in patients and rodent models of depression. However, although it was proposed that the fast antidepressant effect of KET is linked to a surge of glutamate release, it is still not clear if and how KET modulates glutamate release. Using a chronic mild stress (CMS) rat model of depression, we aimed at studying the effects of chronic stress and KET on glutamate release and on synaptic/intracellular molecular mechanisms involved in the stress response. Rats were subjected for 5 weeks to a variable sequence of mild and unpredictable stressors, including food/water deprivation, crowding, isolation, soiled cage, cage tilting, light-on overnight, light/dark reversal and forced swim. Sucrose Preference Test allowed to distinguish stress resilient (CMS-R) from vulnerable (CMS-V) rats. Weight gain, adrenal glands/body weight and serum corticosterone levels were also measured to evaluate phenotypic changes induced by stress. We found that CMS induced significant phenotypic changes in all rats, while anhedonic behaviour was selectively induced in CMS-V rats. To assess basal and depolarization-dependent glutamate release, the technique of purified synaptic terminals (synaptosomes) in superfusion was used. Selective changes in basal and depolarization-evoked release of glutamate were measured in HPC and PFC/FC of CMS-V rats, while 10 mg/kg KET (administered i.p. 24 h before sacrifice) attenuated these alterations. Changes in the expression and phosphorylation levels of selected proteins were found in total homogenate, synaptosomes and synaptic membranes from HPC and PFC/FC of CMS and KET-treated rats. In particular, we found alterations in proteins known to be involved in stress response (glucocorticoid and mineralocorticoid receptors), in the presynaptic release of glutamate (metabotropic glutamate receptor 2, Synapsin I), and in synaptic plasticity (BDNF). Moreover, in situ hybridization on HPC and PFC/FC slices showed reduced dendritic trafficking of total BNDF mRNA and BDNF splice variant containing exon 6. KET attenuated these changes. In conclusion, these results suggest that chronic exposure to stressful stimuli induces alterations in glutamate release and related molecular mechanisms, which are partly reversed by KET. Further investigation of the mechanisms underlying individual resilience or vulnerability to stress could help to clarify the neurobiological underpinnings of depression and to identify new pharmacological targets for faster, more efficient antidepressant drugs.

Brain oxidative stress and suicide: identification of the NADPH oxidase NOX2 as a novel biomarker

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Recent increasing evidence points towards a role of oxidative stress in suicidality development. However, very few studies have been performed on the possible sources of reactive oxygen species production in subjects with suicidal behaviour. We have previously demonstrated that the NADPH oxidase NOX2-derived oxidative stress plays a major role in the development of neuropathological alterations in an animal model of psychosis.

Here, we investigated the possible increase of NOX2 in post-mortem brain samples of subjects who committed suicide by asphyxia compared to controls and to subjects died by non-suicidal asphyxia. We showed that NOX2 expression was significantly elevated in the cortex of suicidal subjects with respect to the other two groups. NOX2 immuno-staining was mainly detected in GABAergic neurons with a minor presence of NOX2 positive stained cells in glutamatergic and dopaminergic neurons, as well as astrocytes and microglia. A sustained increase in the expression of the 8-hydroxy-2'–deoxyguanosine, an indirect marker of oxidative stress, was also detected in the cortex of subjects who committed suicide compared to controls and to subjects died by non-suicidal asphyxia. A significant increase in cortical interleukin-6 immunoreactivity in suicidal subjects suggested an involvement of cytokine-mediated molecular pathway in NOX2 elevations.

Our results suggest that the increase of NOX2-derived oxidative stress in the brain might be involved in the neuropathological pathways leading to suicidal behaviour. These results will open innovative insights in the identification of new biomarkers to be considered predictive for suicidality and used for suicide prevention.

Lifelong nutritional omega-3 deficiency evokes depressive-like state and hyperactivation of HPA axis: which role for soluble beta amyloid?

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Recent evidence pointed out that the prevalence of depression has reached epidemic proportions in last decades. This increase has been linked to many environmental factors, such as chronic stress and chronic hyperactivation of hypothalamic pituitary adrenal (HPA) axis. In addition, the influence of dietary factors has gained great attention and low n-3 polyunsaturated fatty acid (n-3 PUFA) dietary intake was correlated to the development of depressive and anxiety like symptoms. On the other hand, we have previously found that acute intracerebral injection of the soluble beta amyloid 1-42 (Aβ1-42) peptide induces a depressive like-behavior in rats, associated to altered HPA axis activation and reduced cortical serotonin and neurotrophin levels. Maternal malnutrition is a widely accepted risk factor for developing mental illness in later adulthood, including depression. Thus, aim of the present study was to study the effect of lifelong exposure to diets differently enriched in n-3, n-6 (poor in n-3), as well as n-6/ n-3 PUFA balanced, on immobility time displayed on the forced swimming test (FST), along with neuroendocrine quantification of HPA axis parameters in offspring rats.

Results showed that n-6 PUFA enriched diet increased depressive- and anxiety-like behaviors, as shown by the elevation in the immobility time in the FST test and self-grooming in the open field test. Those pro-depressive effects were accompanied by reduced cortical serotonin and enhanced plasmatic Aβ1-42 levels. Furthermore, plasmatic corticosterone and hypothalamic corticotropin releasing factor levels were significantly increased in animals fed with n-6 rich diet.

In addition, we tested the effects of these diets on Aβ1-42 induced pro-depressive symptoms in rats. We found that the n-3 rich diet prevented the increase in immobility time consequent to the peptide injection in the FST. In conclusion, our data indicate that high n-6 dietary intake sensitizes animals to depressive stimuli, while n-3 rich diet is protective toward depressant-like effect of Aβ1-42 in rats.

Effect of aging on the energy metabolism of cerebral cortex, hypothalamus and hypophysis: implications for the responsiveness to stress.

Ferrari F, Gorini A, Villa RF

Laboratory of Pharmacology and Molecular Medicine of CNS, Department of Biology and Biotechnology, University of Pavia, Pavia, Italy.

Aging is one of the main risk factors for brain disorders and many studies indicate that age-related changes in brain energy metabolism are causative of development/progression of several neurological (Ferrari et al., 2015a; Moretti et al., 2015; Villa et al., 2013a, b) and psychiatric diseases (Moretti et al., 2003). Moreover, according to the neuroendocrine theory, aging modifies the sensitivity of the hypothalamus-pituitary-adrenal axis (HPA) to the homoeostatic signals coming from the cerebral cortex (Dilman, 1971). For these reasons, in this study, the relationships between the energy metabolism of cerebral cortex, hypothalamus and hypophysis have been systematically evaluated respect to aging, being this aspect never considered before.

Brain bioenergetics has been studied through Functional Proteomics, i.e. the catalytic properties of the regulatory enzymes of (i) Krebs' cycle, (ii) Electron Transport Chain, (iii) glutamate and related amino acids' metabolism, and (iv) ammonia detoxification assayed on non-synaptic and intra-synaptic mitochondria, respectively located in vivo in neuronal perikaryon and in synapses. These mitochondria have been purified from the frontal cerebral cortex (FCTX), hypothalamus (HPT) and hypophysis (HYP) of female Sprague-Dawley rats aged 4, 6, 12, 18, 24 and 28 months. The study showed that: (i) the functional proteomics of these mitochondria shows different expression of the enzymatic activities; (ii) FCTX energy metabolism is poorly affected by physiological aging; (iii) at 4-6 months, HPT and HYP possess lower oxidative metabolism respect to the frontal cerebral cortex, while (iv) the opposite situation occurs during aging.

These metabolic modifications try to grant HPA functionality in response to the incoming external stressful stimuli increased during aging. Thus, we propose the following operative hypothesis: (i) in physiological conditions, the brain is characterized by the "eumetabolomic energy state", i.e. the thermodynamic state that balances energy utilisation and energy expenditure; (ii) during aging, a "dismetabolomic energy state" might be created, being the tissue more predisposed to ensuing pathological alterations and possibly evolving into (iii) the "pathometabolomic energy state" with increased entropy (ΔS) (Villa et al., 2012). In fact, before the morphological lesion becomes apparent, subtle changes may accompany the aging process even in unimpaired but aged animals, though gross abnormalities of metabolic and neurotransmitter functions are unlikely to be compatible with survival (Villa and Gorini, 1997). Age-related changes in brain energy metabolism and in mitochondrial functionality should be considered as remarkable factors during stress, physiological aging and related physiopathological events, since they are of main importance as molecular targets for pharmacological treatments (Ferrari et al., 2015b; Moretti et al., 2003, 2015; Villa and Gorini, 1997; Villa et al., 2013b).

References

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Ankyrin-3: a link between early life stress and the vulnerability to mood disorders

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Early life represents a critical period during which the nervous system is highly susceptible to environmental stimuli and adverse events perceived during this critical time may alter the correct trajectory of several systems, thus leading to long-lasting changes in neuronal function.
We used an unbiased genome-wide, cross-species and cross-tissues approach in order to identify genes whose expression may be persistently affected by the exposure to stress early in life through changes in their methylation status. The analysis pointed to the identification of an interesting candidate, Ankyrin-3 (Ank3), which is involved in the cellular trafficking of several molecules, such as receptors and channels, and which has a strong genetic association for psychiatric disorders.
Indeed, we found that the methylation status of Ank3 is affected in all the paradigms of exposure to stress early in life analyzed. In particular, in the prefrontal cortex of adult rats exposed to gestational stress, in the prefrontal cortex and T cells of monkeys exposed to different early-life social and rearing conditions, as well as in the CD34+ cells from human cord blood of newborns from mothers with a perceived stress status during the third trimester of pregnancy.
We deepened the study of the regulation of Ank3 in the rat model of prenatal stress to deeply investigate the possible mechanisms underlying the long-lasting effects observed, and the possible implications for psychopathology. We found that prenatal stress led to a reduction of Ank3 mRNA levels with a specific temporal profile and that this effect was paralleled by changes in its methylation status throughout the postnatal development. Moreover, we found that Ankyrin-G 190kDa, one of the protein encoded by Ank3 gene, was enriched in the postsynaptic compartment where it interacted with GluR1 and with PSD95, thus having a feasible relevant role on synaptic function. Finally, to model in humans a gene by early stress interplay on brain phenotypes during cognitive performance, we demonstrated an interaction between functional variation in Ank3 gene and obstetric complications on prefronto-striatal functional connectivity and behavior during working memory in healthy adult subjects.
Our data, combined with genetic evidence, support a causal role for alterations in Ank3 expression and function in mediating the effects of perinatal stress on the development of psychopathology.

Inflammatory related pathways and SGK1 signaling as targets of early life stressful events: role of DNA methylation and miRNAs.

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Stress and glucocorticoid hormones regulate hippocampal neurogenesis, but the molecular mechanisms mediating these effects are poorly understood. We focused the attention on an important player involved in the regulation of stress response: the Serum Glucocorticoid kinase-1 (SGK-1). Using human neural stem cells, we have previously shown that cortisol increases SGK-1 expression and that SGK-1 mediates the effects of cortisol on neurogenesis and Glucocorticoid Receptor (GR) function. (1).

Here we aimed at characterizing the impact of early life stressful events on SGK-1 signaling pathway, which may be responsible for the increased vulnerability to psychopathologies and at characterizing the role of epigenetic mechanisms in SGK1 modulation by using a cross species approach.

We first analyzed mRNA levels of SGK1 in the hippocampus of male and female adult rats (PND62) exposed or not to a prenatal stress (PS) paradigm, and we compared them to those observed in control animals. We found a significant increase in mRNA levels of SGK1 both in male and female exposed animals (+48%, $p < 0.005$ PS vs CTRL in males; +24%, $p < 0.005$ PS vs CTRL in females). With the aim to assess the potential clinical relevance of changes in SGK1 expression observed in the hippocampus of animals exposed to early life stress, SGK1 mRNA levels were also measured in the blood cells (whole blood mRNA using PaxGene Tubes) of controls and in a group of depressed patients characterized for childhood trauma experiences.

Interestingly we found that SGK1 mRNA levels were significantly increased in the group of subjects with a history of trauma as compared to those who have not experienced trauma (mean of the relative expression ratio: 1.3 ± 0.4 in the subjects with no trauma and 2.6 ± 0.8 in subjects with trauma, +100%, $p = 0.02$; CTRL, $p<0.05$). Depressed patients showed higher SGK1 levels (+30% vs. controls, $p < 0.05$) as compared to controls. Most importantly, the SGK1 increase was higher in those who had both childhood trauma and depression (+45% $p < 0.01$ vs. controls), with significant statistical interactions between groups ($p<0.05$).

We then investigated the role of DNA methylation and miRNAs as possible mechanisms associated with the long lasting changes in SGK1 signaling both in animal and human samples. Interestingly we observed a common hyper methylation within SGK1 gene in both the species and a down-regulation of a panel of miRNAs that target SGK1 including miR-204-3p, rno-miR-151-3p and mir-711 both in the hippocampus of PNS animals and in the blood of subjects exposed to childhood trauma.

Our data indicate that an exposure to early life stressful event cause activation of pathways involved in inflammation and in SGK1 signaling which are associated with enhanced vulnerability for depression development. Moreover the persistence of changes over time is associated with changes in a panel of miRNAs rather than changes in DNA methylation.

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Influence of temperamental and character traits on antidepressant response in patients affected by major depressive disorder

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Several studies have been conducted to evaluate personality characteristics in patients affected by major depressive disorder. Temperamental and character traits may help clinicians to identify responders to antidepressive therapy. The aim of our study was to evaluate these characteristics as possible predictive indices of response to SSRIs or SNRIs. A cohort of forty-one patients was included (30 F and 11 M), whose diagnosis was made according to DSM-IV criteria. Subjects were interviewed using the Hamilton Rating Scale for Depression (HAM-D) and the Hamilton Rating Scale for Anxiety (HAM-A) in basal condition (T0) and after 4 weeks (T1) of antidepressive treatment. Patients with a ≥ 50% reduction of HAM-D and HAM-A score, have been considered as responders, when compared to basal conditions. All subjects were treated with antidepressive monotherapy and evaluated using Temperament and Character Inventory Revised (TCI-R). Data were analysed by SPSS (16.0 version). In patients affected by major depressive disorder, personality assessment was characterised, from a temperamental point of view, by high levels of Persistence and Reward Dependence and, from a character point of view, by high levels of Self Directiveness and Cooperativity. Responders showed an internal locus of control – perceiving themselves much more responsible of their own actions – while non-responders had an external locus of control with a passive attitude towards life events. After 4 weeks of antidepressive treatment, responders to HAM-D had higher levels of Responsibility versus Guilt, which could be considered as a predictor of positive response to SSRIs or SNRIs. Conversely, responders to HAM-A, showed higher values of Responsibility versus Guilt and of Safety versus Fear of uncertainty. These values were predictive of a recovery of anxious sintomatology associated with depressive disorder. Our results are in line with those reported in the literature, indicating that character dimensions “Self Directiveness” and “Cooperativity” could be important predictors of response to antidepressants.

Studies with a larger sample of patients would be useful to evaluate if character and temperamental traits in depressed subjects could represent indices of positive response to treatment. Moreover, additional data on the effects of different types of antidepressants on temperamental and character traits would be useful in treatment response.

Arc/Arg3.1 genetic disruption in mice causes dopamine system alterations and neurobehavioral phenotypes related to schizophrenia.

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Recent human genetic studies highlighted the postsynaptic Activity-regulated cytoskeleton-associated protein (Arc) complex as a convergence signal for several genes implicated in schizophrenia. However, the functional significance of Arc in schizophrenia-related neurobehavioral phenotypes and brain circuits is unknown. Here we demonstrated that genetic disruption of Arc in mice produces deficits in sensorimotor gating, social, and cognitive abilities, as well as altered locomotor/amphetamine responses consistent with schizophrenia-related phenotypes. Furthermore, genetic disruption of Arc led to reduced frontal cortical dopamine release and mesocortical circuit activation concomitant with increased postsynaptic D2 expression in the striatum. These findings identify a previously unexpected role of Arc in the regulation of dopaminergic neurotransmission and show that genetic disruption of Arc can lead to a hypoactive mesocortical and upregulated mesostriatal D2 signaling with schizophrenia-related behavioral phenotypes. These results support the notion that Arc is a point of convergence for the pathophysiology of schizophrenia.

Genetic-driven reduction of dopamine D3 receptor ameliorates dysbindin-dependent schizophrenia-relevant abnormalities

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Dysbindin-1 is encoded by the dystrobrevin-binding protein 1 gene (DTNBP1) and is located in synaptic sites throughout the human and mouse brain (Talbot et al., 2009). Several studies have associated DTNBP1 genetic variations with risk for schizophrenia (Morris et al., 2008) and reduced dysbindin gene function and protein expression have been reported in the hippocampus and prefrontal cortex of schizophrenic patients (Weickert et al., 2004, 2008). Reduced dysbindin levels have also been linked to increased D2-receptor (D2R) abundance on the neuronal surface in vitro (Iizuka et al., 2007; Ji et al., 2009), suggesting a potential pathophysiological link to psychosis, which has long been thought to involve D2 mechanisms (Laruelle et al., 2003). Dysbindin-1 directly interacts with G protein-coupled receptor-associating proteins (GASPs), which modulates lysosomal trafficking of various G protein-coupled receptors, including D2Rs (Whistler et al., 2002). Whether this molecular interaction with dopamine receptors involves others dopamine D2-like receptors is unknown. Indeed, as the D2R, the dopamine D3 receptor (D3R) also binds GASP-1 (Thompson et al., 2011). However, no studies have investigated the possible interaction between D3R and dysbindin-1. Aim of this study was to evaluate the possible in vivo interaction between D3R and dysbindin-1 in the manifestation of schizophrenia-relevant behaviors, by generating a novel double D3R/dysbindin-1 heterozygous mouse (D3R+/- Dys+/-). These double mutants, single D3R+/- and Dys+/- and their wild-type +/+ littermates were then tested in experimental behavioral paradigms such as the startle/prepulse inhibition (PPI) test and the working memory discrete paired-trial variable-delay T-maze task. Dysbindin heterozygous mice (Dys+/-) showed both an increased startle response to acustic stimuli ($p<0.001$) and working memory deficits ($p<0.05$, 4 and 30 seconds of choice-delays). The partial genetic deletion of D3R completely rescued the aberrant acoustic startle response of Dys+/-. Moreover, D3R+/- Dys+/- mice showed a significant increase of PPI response as compared to wild-type mice ($p<0.05$). Finally, in the discrete paired-trial variable-delay T-maze task, D3R+/- Dys+/- showed a better working memory performance as compared to D3R+/+ Dys+/+ ($p<0.05$, 4 and 60 seconds of choice-delays), again rescuing the dysbindin-1-dependent cognitive deficits. These results demonstrate for the first time that the partial reduction of D3R ameliorates dysbindin-dependent schizophrenia-relevant abnormalities. Importantly, these observations suggest a previously unexplored impact of dysbindin on D3R trafficking and signaling.

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Role of NF-κB p50 in the cross-talk between adult neural progenitor cells and astrocytes

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Adult hippocampal neurogenesis (AHN) is a plastic process that occurs in the subgranular zone of the dentate gyrus and relies on a pool of neural stem/progenitor cells that produce new granule neurons throughout life. Several data in literature confirmed that AHN is altered in several neuropsychiatric and neurodegenerative disorders. Additionally, AHN is negatively affected by chronic stress which is considered a contributing risk factor for the development of psychiatric disorders such as depression. Molecular mechanisms which underlie impaired AHN are still largely unknown. Disrupted cross-talk between adult neural progenitors and astrocytes in the adult neurogenic niche represents a potential contributor. Among molecules which regulate AHN and which are also playing a role in response to stress, the NF-κB family of transcription factors has received particular attention. In previous work our group proved that NF-κB members are crucial signalling pathways in the SGZ neurogenic niche and in the response of adult NPC to several clinically relevant drugs, including antidepressants. Within the family, the p50 subunit appears to play a crucial role since p50KO mice display dramatically reduced adult hippocampal neurogenesis in association with short-term memory defects. However, when adult Neural Progenitor Cells (NPC) derived from wt and p50KO mice are cultured in vitro, no significant differences can be observed in their neurogenic potential, suggesting a potential contribution of other cell subpopulations within the niche to defective neurogenesis in mutant mice. Starting from this hypothesis we focused our attention on astrocytes which are known to be implicated in neurodegenerative diseases and inflammatory processes and are integral part of the neurogenic niche. To this purpose we have set up enriched astrocyte cultures from hippocampi of p50KO and wt neonatal mice and studied their influence on wt or p50KO NPC by using astrocyte-conditioned medium (ACM). When wt NPC where exposed to wt ACM, an increased rate of differentiation towards both neuronal and astroglial lineages was observed, in comparison with standard medium. Conversely, p50KO ACM significantly increased the percentage of newly generated astrocytes, but lacked proneurogenic effect on wt NPC. Moreover, wt and p50KO ACM promoted neither neurogenesis nor gliogenesis in p50KO NPC.

Altogether these results suggest that neurogenic defects observed in vivo in p50KO mice may be due to both autonomous and non cell autonomous defects of NPC and that, in absence of p50, astrocytes may not provide adequate proneurogenic support to adult NPC. Ongoing work is currently aimed at identifying proneurogenic or antineurogenic molecules that are differentially secreted by wt and p50KO astroglia. Altogether, these studies will increase our current knowledge on the relevance of astrocyte-NPC communication in the modulation of AHN in physiology and, potentially, in pathophysiological conditions associated with stress.

Abstracts of Poster Communications

#1 BDNF alterations as consequences of childhood trauma experiences

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Several evidences support causal relations between early life stress and psychiatric disorders. Indeed, exposure to childhood trauma experiences is associated with increased vulnerability for several kinds of disorders including mental illnesses (van Winkel et al., 2013). This increased vulnerability is due to the fact that childhood is a critical period for the brain development, where the brain is not completely developed yet and thus high vulnerable to adverse events. The Brain-Derived Neutrotrophic factor (BDNF) is a major neuronal growth factor in the brain, regulating neurogenesis, neuronal maturation and survival, and synaptic plasticity (Tsankova et al., 2007). It has been proposed that BDNF could be the link between early life stress and the vulnerability to psychiatric disorders (Schroeder et al., 2010).

The focus of this work was to investigate BDNF levels in the peripheral blood of control subjects characterized for exposures to stressful experiences during childhood. By using Real Time PCR we measured total BDNF mRNA levels and the results indicated a strong reduction of total BDNF mRNA levels in individuals exposed to stress early in life versus not exposed individuals (-32%, $p<0.01$ vs not exposed subjects). Then, we assessed the contribution of the different BDNF transcripts on BDNF total modulation, and we found that transcripts IV and IX were significantly reduced (-71%, $p<0.05$; -35%, $p<0.05$, respectively). In order to investigate the molecular mechanisms underlying these long lasting changes in BDNF expression, we evaluated the role of DNA methylation and miRNAs as possible epigenetic mechanisms. We assessed the DNA methylation levels of specific CpGs in both the transcripts IV and IX, however, no significant changes in association to stress exposures were observed. Then, we focused on miRNAs targeting BDNF and we identified, by using biostatistical tools, the main predicted and validated miRNAs targeting BDNF 5' UTR region, CDS region and 3' UTR region (total number of miRNAs = 805). Due to the high number of miRNAs targeting BDNF that we identified, we used a whole genome approach and we run a miRNome analysis. We found that 26 of the 805 miRNAs targeting BDNF are significantly up regulated in response to stress early in life; 18 targeting the 5' UTR region, 4 targeting the CDS region and 4 targeting the 3' UTR region (Fold Change>1.1; $p<0,05$ for all the 26 miRNAs). Bioinformatic analysis, using the DIANA miRPath program (v.2.0) identified the signal transduction, the neurotrophin signalling and the long-term potentiation pathways as the most significant pathways modulated by the significant miRNAs.

In conclusion we found that total BDNF mRNA levels are reduced in the peripheral blood of subjects exposed to stressful experiences during childhood and that miRNAs could be in part responsible for these long lasting changes in BDNF expression. In the future, a better characterization of miRNAs modulation may identify novel targets for preventative therapies in subjects with a history of childhood trauma, which are at higher risk to develop psychiatric disorders in the adulthood.

#2 Activation of immune signaling related pathways and reduced telomere length in subjects exposed to stressful life events.

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Exposures to stressful life events are well known to affect mood and behaviors and to increase the vulnerability for several kind of diseases, including stress related mental disorders, in particular depression, post traumatic stress disorder and bipolar disorder (Stroud et al., 2008). Possible mechanisms underlying this association include alterations in the Hypothalamic-Pituitary-Adrenal (HPA) axis system and in immune response and inflammation (Ben-Efraim YJ et al., 2012). Moreover, recent evidences suggest that telomere biology might offer an additional overview to study the mechanisms linking stressful events with disease risk. In particular, several studies reported a link between stressful life events and leukocyte telomere shortening (Verhoeven et al., 2015). Thus, individuals exposed to stressful life events may represent an important tool to identify biological processes and mechanisms modulated by stress across the life span that could also explain the association between stress exposure and enhanced vulnerability for psychiatric illnesses (Schneiderman et al., 2005). In this study, we performed transcriptomic analyses in a group of control subjects characterized for recent stressful life events by using the life events questionnaire (21 subjects with at least one episode of severe stressful event in the previous six months and 51 subjects, age and gender matched, with no severe stressful events). Transcriptomics assays have been performed in blood samples using the Human Gene 1.1st Array Strips on GeneAtlas platform (Affymetrix). CEL files were imported into Partek Genomics Suite 6.6 for data visualization, quality control and statistical analyses. Ingenuity Pathway Analyses Software was then used to identify regulation of molecular signalling pathways and Gene mania was used to build up networks. We found that the exposure to stressful life events caused the modulation of 297 genes (p-value< 0.05), which are involved in several biological systems, including natural killer cell signaling, T-cell receptor signaling and regulation of IL-2 expression in activated T lymphocytes as most significant ones. Using GeneMania, we also investigated the main upstream regulators associated with gene expression changes. We selected the top 20 genes significantly modulated in our dataset (10 up-regulated and 10 down-regulated), and we found that all of them tightly interact each other with co-expression (60.88%) as the main representative interaction type. As it is well known that activation of pathways related to inflammation/immune response and oxidative stress can cause telomere shortening, we investigated in the genomic DNA samples of the same subjects, the relative telomere length (RTL) by Real time PCR, determining the ratio of telomere repeat (T) copy number to single gene (S) copy number (T/S ratio). We found that the telomere length is significantly reduced (-20%) in subjects exposed to stressful life events as compared to subjects not exposed (p-value<0.05). Our results indicate that the exposure to stressful life events in the adulthood can cause the activation of several pathways that are involved in immune system and oxidative stress that in turn may cause a reduction of telomere length. These alterations may render the subjects exposed to stress more vulnerable to develop a wide range of psychopathologies including mood disorders.

#3 mRNA-miRNA integration approach in rats and in humans to identify long-term signatures of early life stress

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Early life exposures to stressful events produce widespread changes on brain function that predispose the individuals to develop a wide range of pathologies later in their life. Although it is still unclear how childhood trauma can induce such vulnerability, it likely involves non-genetic mechanisms that affect complex network of biological pathways [1]. The identification of biological mechanisms relevant to disease vulnerability could lead to the identification of biomarkers as well as new pharmacological targets for preventive therapies. Emerging evidence suggests that microRNAs (miRNAs) may underlie these long lasting changes. MiRNAs are small non coding RNAs (20-22 nucleotides) considered as possible "master regulators" of many cellular processes, including metabolism and energy homeostasis; moreover, evidences suggest that miRNA levels are altered by stress and in depressed patients. On these bases, we used the prenatal stress (PNS) model and the blood of controls with a history of childhood trauma to investigate transcriptomic alterations induced by early life adversities that may contribute to the vulnerability for a large spectrum of disorders in the adulthood. Subsequently we have investigated the impact of miRNAs on transcriptomic changes and we have used a cross species approach to identify peripheral biomarkers with an impact on brain functioning and possibly associated with enhanced vulnerability for psychiatric disorders.

We performed transcriptomic and miRNome analyses in the hippocampus of adult rats, which have been exposed to stress prenatally with the aim to identify coordinated changes in miRNAs and in their target genes. We then validated our gene expression findings in the leukocytes of 40 subjects characterized by childhood trauma exposures. The gene expression analyses conducted in the hippocampus of adult rats identified 873 genes and 68 miRNAs significantly regulated by PNS. Thereafter, a meta-analysis for miRNA binding sites was used to detect possible miRNAs binding sites, and only miRNAs with significant genes interactions, were used to carry out further analyses. We thus filtered out 3 up- and 21 down-regulated miRNAs as the most significantly modulated by PNS and also as highly significantly enriched for their binding sites within deregulated genes. A pathway analyses on these selected miRNAs indicated alterations in several biological processes including metabolic, calcium signalling, MAPK, TGFβ and chemokine signalling pathways. We then selected and measured the expression levels of miR-let7a, miR-322, miR-494 and miR-362 in the leukocytes of healthy subjects which reported a history of childhood trauma to validate their role in the maintenance of stress signature following exposure to early life adversities.

Our data provide support to the notion that early life stress enhances depression vulnerability through changes in gene expression that may be set in motion by coordinated disruption of miRNAs. The characterization of these mechanisms may ultimately lead to the identification of novel targets for pharmacological intervention.

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#4 Early modulation of microRNAs in rat prefrontal/frontal cortex after acute stress

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Stressful events are physical and psychosocial challenges altering the physiological homeostasis, and inducing a stress response aimed at improving survival and adaptation. However, when the stressor is too strong or repeated, especially in genetic vulnerable subjects, the stress response may activate maladaptive mechanisms, leading to increased susceptibility to neuropsychiatric disorders. We have previously shown that acute footshock (FS)-stress rapidly enhances glutamate release/transmission in prefrontal and frontal cortex (PFC/FC). Moreover, acute stress was found to increase remarkably the total number of small excitatory synapses in PFC and to induce rapid and sustained dendrite retraction, suggesting that, unexpectedly, acute stress can induce large-scale changes in brain architecture at a fast pace. However, the molecular mechanisms involved in these rapid effects of stress are still largely unknown. MicroRNAs (miRNAs) have recently emerged as regulators of complex patterns of gene/protein expression changes in the brain, where they have a crucial role in the regulation of neuroplasticity, neurogenesis, and neuronal differentiation. Although much remains to be uncovered regarding their mechanism of action, recent studies showed that miRNAs are involved in the pathophysiology of mood disorders and in the action of psychotropic drugs. The peculiar ability of miRNAs to fine-tune the expression of hundreds of genes makes them potential candidates as fast mediators in the modulation of cellular excitability and morphology induced by stress. Although previous studies showed that chronic stress could modulate the expression of several brain miRNAs, regulating stress susceptibility and leading to changes in behavior, emotion, and cognition, the effect of acute stress on miRNA expression is still mostly unknown. Aim of this study was to verify whether miRNAs could be early mediators of the neuronal and structural changes occurring after acute stress. We studied the expression profile of 21 miRNAs, selected for their involvement in the modulation of synaptic plasticity, and antidepressant effect, in the PFC/FC of adult male rats sacrificed immediately after 40 minutes of acute FS-stress. 5 miRNAs were found to be significantly downregulated after acute stress: miR-135a, miR-206, miR-195, miR-132, miR-134. In order to identify putative target genes of these miRNAs and the molecular pathways involved, bioinformatic analysis was performed by integrating and filtering the results of different miRNA target prediction algorithms, followed by annotation analysis with Gene Ontology subcategories. Bioinformatic analysis results highlighted enrichment of miRNA targets in different pathways, some of them related to neuronal functions, synaptic plasticity and the stress response. Overall, our preliminary data showed for the first time that miRNAs may be early mediators of the stress response and identified new possible target genes that could contribute to better understanding the rapid and sustained effects of acute stress on neuronal functional and structural plasticity.

#5 Acute foot-shock stress induces time-dependent alterations of glutamatergic synapses in prefrontal cortex of male rats

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Maladaptive changes induced by stress in the structure and function of excitatory/inhibitory circuitry have a primary role in the pathogenesis of mood disorders, including anxiety and depression. A large body of neuroimaging data and meta-analyses on depressed patients shows consistent structural abnormalities in brain regions closely associated with the stress response and emotional/cognitive processing, such as hippocampus and cortical regions (including the prefrontal cortex, PFC). Concurrently, rodent studies have assessed that different types of stressors have powerful effects on volume and neuronal morphology in the same brain areas. Interestingly, the vast majority of neurons and synapses that populate those cerebral districts is glutamatergic. Even though previous studies analyzed rapid and early alterations induced by acute stress on the glutamatergic synapses, as well as those induced by chronic stress, little is known about the delayed and sustained changes exerted by acute stress. In order to fill this gap, our study intended to identify functional, molecular/cellular and behavioral short-term and delayed effects of acute stress in PFC of male rats. To this aim, we subjected animals to a single session of acute foot-shock (FS) stress (40 minutes, 20 min total actual shock with random intershock length between 2-8s; 0,8 mA), and sacrificed rats at four different time points after the stress paradigm.
We found that acute stress alters glutamatergic functionality with a rapid but sustained effect (up to 24h): the size of the pool of vesicles ready to release glutamate (RRP) was enlarged and the release of glutamate induced by depolarization was increased. Moreover, after dissection of PFC, we purified three different subcellular fractions (total extract, synaptic terminals, and synaptic membranes) and we measured time-dependent changes in the expression and phosphorylation levels of molecular effectors regulating glutamate release or transmission and involved in the stress response (i.e. corticosterone receptors, type 2 glutamate metabotropic receptor, synapsin I). We also performed t-maze test, a behavioral analysis that evaluates working memory, an executive function mostly regulated by the PFC. Our data showed that acute stress improves working memory 2 hours later, but exerts an opposite effect 24 hours after stress. Taken together, our results show that acute stress can induce early and sustained alterations in the glutamatergic plasticity, and alters cognitive performance in a time-dependent way. Accordingly, a deeper understanding of the mechanism whereby acute stress affects glutamate release and transmission could help in the identification of key determinants in the outcome of stress, paving the way to an easier investigation of new pharmacological targets in the treatment of neuropsychiatric disorders.

#6 Exposure to the chronic mild stress induced cognitive dysfunctions: investigation of molecular mechanisms underlying this deficit

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Serotonin Depression is a complex and heterogeneous disorder that represents a major cause of disability in the world. With this respect, cognitive deterioration is a major problem that can interfere with all areas of a person's life, including work, school and their relationships.
On these bases, it is important to characterize cognitive dysfunctions within the context of a depressive phenotype in order to identify the underlying systems, which may represent an important target for drug intervention. To this aim, adult male Wistar rats were exposed to chronic mild stress (CMS), a well-established model of depression, for 7 weeks before being tested in the novel object recognition (NOR). The animals were then sacrificed immediately after the end of the test session for the molecular analyses. The behavioral analysis shown that CMS rats display, when compared to controls, impaired cognitive functions as indicated by the reduction of the discrimination index in the NOR test. At molecular level, we first investigated the activity-regulated genes (Arc) and the neural PAS domain 4 (Npas4) in the dorsal hippocampus and we found that their expression was markedly increased after the NOR, in control as well as in CMS rats. Among the mechanisms involved in the stress response but also in memory and cognitive functions, we decided to focus on glutamatergic system and on neuroplastic mechanisms. We found that in the crude membrane fraction, the phosphorylation of the NMDA subunit NR2B at the serine 1303 was significantly increased in control rats exposed to NOR, but not in CMS rats. Interestingly, pmTOR (Ser2448), a downstream target of the NMDA receptor, was similarly upregulated after the test in normal animals, but not in those exposed to the chronic stress. Moreover, we observed a significant increase of the mature form of BDNF after the NOR both in control as well as in CMS-rats, whereas the protein levels of its high affinity receptor TrKB were reduced in the synaptosomal fraction after the test only in stressed-animals. Next, we measured one of the element involved in the protein synthesis, namely eukaryotic elongation factor 2 α (eEF2) α and we found an increase in the ratio of the peEF2α/eEF2α after the NOR in control animals but not in those exposed to the CMS paradigm, suggesting a shift from the general translation to the translation of specific mRNA containing the upstream open reading frame (uORF). We believe that the different modulation of these molecular players may contribute to the cognitive impairment observed in CMS rats. On these bases, pharmacological intervention able to correct these alterations might ameliorate functions that are deteriorated in patients with major depression and stress-related disorders.

#7 Regulation of clock gene expression in the chronic mild stress model: modulatory activity of the novel drug lurasidone

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Disruptions in biological rhythms are known to be associated with mood disorders. This has led to hypothesize that abnormalities in the molecular clock may contribute to the development of these disorders and normalization of these changes may be important for therapeutic efficacy. The cellular clock is a transcriptional-translational feedback loop involving a number of different genes that may possess separate functions in circadian rhythms and mood regulation. While this machinery has been extensively characterized in the suprachiasmatic nucleus, little is know on the role exerted by individual clock genes in other brain structures, such as hippocampus and prefrontal cortex, which are important for mood disturbances. In the present study we have employed the chronic mild stress (CMS) model of depression in order to establish if possible alterations in the expression of clock gene machinery in hippocampus and prefrontal cortex. Male Wistar rats were exposed to CMS for 2 weeks and sucrose consumption was used to distinguish between susceptible and non-susceptible animals. Control and CMS-susceptible rats were then randomized to receive chronic vehicle or the novel multi receptor drug lurasidone (3 mg/kg/day) for 5 more weeks, while continuing the stress procedure, in order to evaluate the ability of chronic drug treatment to normalize the phenotype associated with CMS. Our data show that the mRNA levels for Per1 and Per2 are significantly down-regulated in the prefrontal cortex of CMS rats, and this is associated with a slight up-regulation of Bmal1 expression. No changes were found for Clock mRNA levels, whereas a small reduction was found for Cry2 expression. Interestingly, chronic treatment with lurasidone, which per se produced limited changes on clock gene mRNA levels, was able to normalize the molecular changes induced by stress exposure. The modifications of Per1 and Per2 expression after exposure to CMS appear to be anatomically selective, since we did observe similar changes in dorsal or ventral hippocampus. We believe that changes in clock gene expression as a consequence of CMS exposure may contribute to the disturbances associated with mood disorders and may bridge circadian abnormalities with neuronal function in critical brain regions. With this respect, the ability of chronic lurasidone to modulate clock gene expression in association with its ability to normalize the anhedonic phenotype in CMS rats provide further support to its therapeutic properties in ameliorating functions that are deteriorated in patients with major depression and stress-related disorders.

#8 Role of central dopamine D3 and serotonin 2C receptors in the control of the mesoaccumbens dopaminergic pathway: implications for the treatment of depression

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The mesoaccumbens dopamine (DA) pathway, whose activity is functionally deficient in depressed states, is a key structure in mediating the neurochemical and behavioral effects of antidepressant drugs. Specifically, central DA-D3 receptor (D3R), which represents a target for new antidepressant agents, exerts inhibitory controls on mesoaccumbens DA neurons. Also, D3R knock-out mice (D3R−/−), which display increased DA extracellular levels in the nucleus Accumbens (NAc), are more sensitive to antidepressant drugs and exhibit reduced anxiety-like behaviour. The central serotonin2C receptor (5-HT2CR), in keeping with its ability to inhibit the mesoaccumbens DA pathway, represents another major target for improved treatments of affective disorders. Several 5-HT2CRs ligands have shown antidepressant-like activity in various behavioural models, although the mechanisms underlying their effects remain unclear. Thus, the mesoaccumbens DA pathway may serve as a common substrate in mediating the antidepressant effects of D3 and 5-HT2C compounds. The aim of this study was to assess this hypothesis by specifically identifying the relative contribution of D3Rs and 5-HT2CRs in the control of mesoaccumbens DA pathway activity as well as in the depressive-like behaviors. In particular, we used D3R-/- and their wild-type (WT) littermates, treated (7 days) or not with several 5-HT2C ligands (the inverse agonist SB206553, the selective antagonist SB242084 or the combination SB242084 + SB206553) and tested in the tail suspension test (TST) after 8 weeks of exposure to the unpredictable chronic mild stress paradigm (UCMS). Stressed D3R-/- mice, tested in the TST, showed a decreased immobility time as compared to their wild type littermates ($p<0.01$). Wild type mice exposed to the UMCS and treated for the last 7 days of the experimental procedure with the 5-HT2C inverse agonist SB206553 (2.5 mg/kg, i.p.), show a decreased immobility time in the TST as compared with their vehicle control group ($p<0.05$). Conversely, the same treatment with SB206553 increased the immobility time of D3R-/- as compared with their vehicle control group ($p<0.05$). Seven days of treatment with the 5-HT2C antagonist SB242084 (1 mg/kg, i.p.) did not change the immobility time of both stressed D3R-/- and WT mice. Finally, the pre-administration (15 min before) of the 5-HT2C selective antagonist SB242084 blocked the effect of the inverse agonist SB206553 in both stressed D3R-/- and WT mice. These data indicate that the effect of 5-HT2C inverse agonist SB206553 in a depressive-like behavior may be changed by manipulating D3R function.

#9 Role of CRF system in frustration stress-induced binge-like palatable food consumption in female rats

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We recently developed a binge-eating model in which female rats with a history of intermittent food restriction show binge-like palatable food consumption after 15 min exposure to the sight of the food. This "frustration stress" manipulation also activates the HPA stress axis. Here, we determined the role of the stress neurohormones corticosterone and CRF in stress-induced binge eating in our model. We also assessed the role of CRF receptors in the BNST, a brain region implicated in stress responses and stress-induced drug seeking, in this binge-eating model.

We used 4 groups that were first exposed or not exposed to repeated intermittent cycles of regular chow food restriction/re-feeding during which they were also given intermittent access to high caloric palatable food. On test day, we either exposed or did not expose the rats to the sight of the palatable food for 15 min (frustration stress) before assessing food consumption for 2 h.

We found that systemic injections of the CRF1 receptor antagonist R121919 (10-20 mg/kg) and BNST (25-50 ng/side) or ventricular (1000 ng) injections of the non-selective CRF receptor antagonist D-Phe-CRF(12-41) decreased stress-induced binge eating. This manipulation also increased CRF1 receptor mRNA and Fos (a neuronal activity marker) expression in the BNST.

To assess whether corticosterone is involved in the binge eating behavior, rats were treated with metyrapone, a corticosterone synthesis inhibitor at the doses of 50 and 100 mg/kg. It failed to prevent binge eating. Lastly, corticosterone injection (2.5 and 10 mg/kg) did not induce binge eating in restricted and non-stressed rats, in comparison to the control group (non-restricted and non-stressed).

Results demonstrate a critical role of CRF receptors in BNST in stress-induced binge eating in our rat model. CRF1 receptor antagonists may represent a novel pharmacological treatment of stress-induced binge eating.

#10 Epigenetic regulation of adenosine A2A and dopamine D2 receptor gene transcription in frustration stress-induced binge-like palatable food consumption

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Satisfactory treatments for eating disorders, such as binge eating disorder and bulimia nervosa, are not available at present. Using a well-characterized animal model of binge eating, we investigated the epigenetic regulation of the adenosine A2A Receptor (A2AAR) and dopamine D2 (D2R) gene. The animal model included four groups (rats fed normally, and then stressed or not, rats exposed to cycles of restriction/refeeding, and then stressed or not). Gene expression analysis carried out on the amygdala complex of restricted and stressed rats revealed a significant increase of A2AAR and D2R mRNA when compared to non-stressed and non-restricted rats. Administration of the A2AAR agonist (VT 7) induced in restricted and stressed rats a significant increase of A2AAR and D2R mRNA levels when compared to vehicle group, whereas a significant decrease in rats pre-treated with the A2AAR antagonist (ANR 94) was observed. Pyrosequencing analysis revealed a significant reduction of the % of DNA methylation at A2AAR promoter region in restricted and stressed compared to the non-stressed and non-restricted animals. We did not find any difference in D2R DNA methylation among different groups. Significant changes in the DNA methylation status of A2AAR promoter were found in restricted and stressed rats after administration of VT 7 or ANR 94. We observed a decrease of DNA methylation in VT 7 treated rats and a hypermethylation in ANR 94 rats with respect to the vehicle group. The increase in A2AAR mRNA observed in restricted and stressed rats could be due to a compensatory mechanism to counteract the effect of binge eating, suggesting that the A2AAR activation, inducing receptor gene up-regulation, could be relevant to reduce food consumption. We here demonstrated for the first time the epigenetic regulation of A2AAR in an animal model of binge eating.

#11 Association of brain amyloidosis with pro-inflammatory gut bacterial strains and peripheral inflammation markers in cognitively impaired elderly

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[18]http://www.centroalzheimer.org/sito/contenuti/ip_lilly_publications/INDIA-FBP_WORKING_GROUP.pdf

The pathway leading from beta-amyloid (Aβ) deposition to cognitive impairment is believed to be a cornerstone of the pathogenesis of Alzheimer's disease (AD). However, what drives amyloid build-up in sporadic non-genetic cases of AD is still unknown. AD brains feature an inflammatory reaction around amyloid plaques, and a specific asset of the gut microbiota (GMB) may promote brain inflammation. We investigated the possible role of the GMB in AD pathogenesis by studying the association of brain amyloidosis with (i) GMB strains with pro- and anti-inflammatory activity, and (ii) peripheral inflammation in cognitively impaired patients. We measured the stool abundance of selected bacterial GMB strains (Escherichia/Shigella and Pseudomonas Aeruginosa, Eubacterium rectale, Eubacterium Hallii, and Faecalibacterium Prausnitzii) and the blood expression levels of cytokines (IL-6, TNF-α, CXCL2, NLRP3, IL-10, IL-4, IL-13) in cognitively impaired patients with (n=20, Amy+) and with no brain amyloidosis (n=20, Amy-). Amy+ patients had increased levels of IL-6 (+31%, p=0.013), CXCL2 (+29%, p=0.024) and NLRP3 (+24%, p=0.016), and a reduction of IL-10 (-18%, p=0.033) as compared to Amy- patients. Amy+ patients also had lower abundance of Eubacterium rectale (4.4-fold, p<0.001), and higher abundance of Escherichia/Shigella (2.3-fold, p=0.02). Interestingly, the abundance of Escherichia/Shigella correlated positively with the levels of CXCL2, IL-6, NLRP3, and negatively with IL-10. Eubacterium Rectale correlated negatively with CXCL2, IL-6 and NLRP3.
Our data suggest that a pro-inflammatory GMB composition is associated with peripheral inflammatory activation in patients with cognitive impairment and brain amyloidosis. A possible causal relation between GMB-related inflammation and amyloidosis deserves further investigation.

#12 Pro-BNP as a biomarker of asymptomatic clozapine-related heart dysfunction: possible usefulness for clozapine management

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Cardiovascular clozapine-related side effects such as tachycardia and orthostatic hypotension are well recognized, but are rarely clinically important. However, the increasing number of life-threatening drug-related complications are giving rise to concerns about cardiac adverse reactions (myocarditis, cardiomyopathy, pericarditis and heart failure). The diagnosis is usually made considering patient's symptoms, such as tachycardia, slightly increased body temperature, subjective chest pain, dyspnea. However, this symptomatology is not always present in a clozapine-related pericarditis. Some Authors suggest measuring BNP levels to detect early and asymptomatic cardiac dysfunction. We here report the clinical cases of two women, respectively 22 and 28 years old. They both suffered from an early onset resistant schizophrenia. Clozapine was gradually introduced, at a dose of 200 mg/day, in both patients. After about one month in both cases, while the first patient was nearly asymptomatic, apart from the intermittent fever (only PCR and pro-BNP values were elevated, 16.88 mg/dl and 1004 pg/ml, respectively), the second one showed a classic symptomatology suggestive of pericarditis. Clozapine was discontinued in both patients, resulting in progressive resolution of pericarditis. Interestingly, in the patient in which pro-BNP was elevated, after clozapine cessation, the pro-BNP fell down dramatically. Pro-BNP plasma levels appear to be an interesting test in identifying subjects with asymptomatic cardiac impairment. It would be useful to evaluate if early treatment with beta-blockers and ACE-inhibitors may allow the prosecution of clozapine treatment after developing of mild signs of cardiac toxicity in drug resistant schizophrenic patients responsive to clozapine.

#13 Omega-3 and omega-6 polyunsaturated fatty acid enriched diet in susceptibility to stress response: implication for depression in female rats

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Chronic stress is considered a widely accepted risk factor for the development of depressive symptoms. However, the dramatic increase of depression prevalence made mandatory the research of new environmental risk factors. Among others, dietary factors have been importantly taken into consideration. In this regard, unbalanced ratio in polyunsaturated fatty acid (PUFA), particularly omega-6/omega-3 ratio, has been shown to play a crucial role in mental illness development. Indeed, in utero exposure to unbalanced diet can be an important risk factor for mental disorders in later adulthood, especially for depression. Poor omega-3 PUFA diet has been associated to increased depressive- and anxiety-like symptoms in human and in animals subjected to chronic mild stress paradigm. The mechanism that correlates chronic or repeated stressful events to the rise of depression has been identified in the dysregulation of hypothalamic–pituitary-adrenal (HPA) axis. Furthermore, the development of depressive disorders subsequent to chronic stress was considerably exacerbated among women (Beydoun et al., 2015). Thus, the aim of the present study was to evaluate the effect of pre- and post-natal exposure to diets enriched in omega-3 or omega-6 PUFA, as well as omega-3/ omega-6 –balanced diet, on several neurochemical and neuroendocrine factors in female rats. Results showed that, in female offspring rats, omega-6 PUFA enriched diet induced an increase in hypothalamic corticotropin-releasing factor and plasmatic corticosterone content respect to omega-3/ omega-6 –balanced and omega-3 enriched diet. Conversely, omega-3 PUFA enriched diet caused a significant decrease in plasmatic corticosterone measurement. In addition, we have previously demonstrated that intracerebral injection of soluble beta amyloid (sAbeta) in rats, can evoke a depressive-like status, by increasing the immobility time in the forced swimming test (FST), as well as by reducing cortical brain derived neurotrophic factor, nerve growth factor and serotonin levels (Colaianna et al, 2010). In the same animal model, we also found higher cortical and hippocampal concentrations of noradrenaline and lower plasmatic corticosterone, that described an altered HPA axis functioning (Morgese et al., 2014). Thus, we further investigated whether PUFA diet exposure could modify the pro-depressive effects of sAbeta. In particular, we confirmed in female the depressive effect previously shown in male rats. Moreover, we found that omega-3 prevented the depressive effect of sAbeta retrieved in the FST and increased cortical serotonin in treated animals. In addition, omega-3 PUFA enriched diet decreased adrenocorticotropic hormone content in sAbeta-treated rats. In conclusion, our data endorse the protective role of omega-3 PUFA toward either susceptibility to stress and development of depressive-like symptoms sAbeta-related.

References

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#14 Different roles of the endocannabinoids anandamide and 2-arachidonoylglycerol in the modulation of memory retrieval in rats

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To date, our understanding of the relative contribution of the endocannabinoids anandamide and 2-arachidonoylglycerol (2-AG) in the regulation of memory retrieval for emotional experiences is still limited. To address this issue, we investigated the effects induced by pharmacological manipulation of the endocannabinoid signalling in the dorsal hippocampus or in the basolateral complex of the amygdala (BLA) on memory retrieval of stressful experiences. To this aim, adult male Sprague Dawley rats were trained in a Contextual or Auditory Fear Conditioning task (CFC and AFC, respectively). They were bilaterally infused, 60 minutes before memory retrieval, into the BLA or into the hippocampal CA1 field with the FAAH inhibitor, URB597, or the MAGL inhibitor, KML29, respectively increasing endogenous levels of anandamide and 2-arachinoyglycerol (2-AG).
In a first experiment, URB597 (3-30 ng/0.2 µl/side) or KML29 (2-200 ng/0.2 µl/side) were infused into the BLA of rats trained in the CFC task. We found that increasing anandamide or 2-AG signalling in the BLA did not influence the retrieval of fear memories. Conversely, infusion of URB597 (10 ng/0.2 µl) in the BLA of rats trained in the auditory version of the task induced an impairing effect on memory retrieval. KML29 administration in the same brain area did not induce any effect. In a second experiment, URB597 (3-30 ng/0.5 µl/side) or KML29 (2-200 ng/0.5 µl/side), were infused into the CA1 field of the hippocampus. In the CFC task, the infusion of KML29 (2 ng/0.5 µl) impaired retrieval of aversive memory. Intra-CA1 infusions of URB597 in the CFC or of both URB597 and KLM29 in the AFC task did not influence memory retrieval. To investigate whether the impairing effects of intra-BLA infusion of URB597 or intra-CA1 infusion of KML29 were dependent on CB1 receptor activation, we co-infused the effective doses of URB597 (10 ng) or KML29 (2 ng), together with the CB1 receptor antagonist AM251 at a dose not altering behavioral response per se (0.14 ng). AM251 administration blocked the impairing effects induced by URB597 on retrieval of cued fear memory or by KML29 on retrieval of contextual fear memory, thus demonstrating that increasing anandamide levels in the BLA or 2-AG levels in the CA1 region of the hippocampus negatively modulates fear memory retrieval through a CB1 receptor-dependent mechanism. Our results suggest that the endocannabinoid system is crucially involved in the regulation of retrieval of memory for stressful experiences. It might represent a new therapeutic target for the treatment of neuropsychiatric disorders where a previous exposure to traumatic events could alter the response to trauma reminder leading to mental illness.

#15 Everolimus improves memory and learning while worsening depressive- and anxiety-like behavior in an animal model of depression

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Everolimus (EVR) is an orally-administered rapamycin analog that selectively inhibits the mammalian target of rapamycin (mTOR) kinase (mainly mTORC1 and likely mTORC2) and the related signaling pathway. mTOR is a serine/threonine protein kinase regulating multiple important cellular functions; dysfunction of mTOR signaling has also been implicated in the pathophysiology of several neurological, neurodegenerative, developmental and cognitive disorders. EVR is widely used as an anti-neoplastic therapy and more recently in children with tuberous sclerosis complex (TSC). However, no clear correlation exists between EVR use and development of central side effects e.g. depression, anxiety or cognitive impairment.

We studied the effects of a 3 weeks administration of EVR in mice chronically treated with betamethasone 21-phosphate disodium (BTM) as a model of depression and cognitive decline. EVR treatment had detrimental effects on depressive- and anxiety-like behavior while improving cognitive performance in both control (untreated) and BTM-treated mice.

Such effects were accompanied by an increased hippocampal neurogenesis and synaptogenesis. Our results therefore might support the proposed pathological role of mTOR dysregulation in depressive disorders and confirm some previous data on the positive effects of mTOR inhibition in cognitive decline. We also show that EVR, possibly through mTOR inhibition, may be linked to the development of anxiety. The increased hippocampal neurogenesis by EVR might explain its ability to improve cognitive function or protect from cognitive decline. Our findings suggest some caution in the use of EVR, particularly in the developing brain; patients should be carefully monitored for their psychiatric/neurological profiles in any clinical situation where an mTOR inhibitor and in particular EVR is used e.g. cancer treatment, TSC or immunosuppression.

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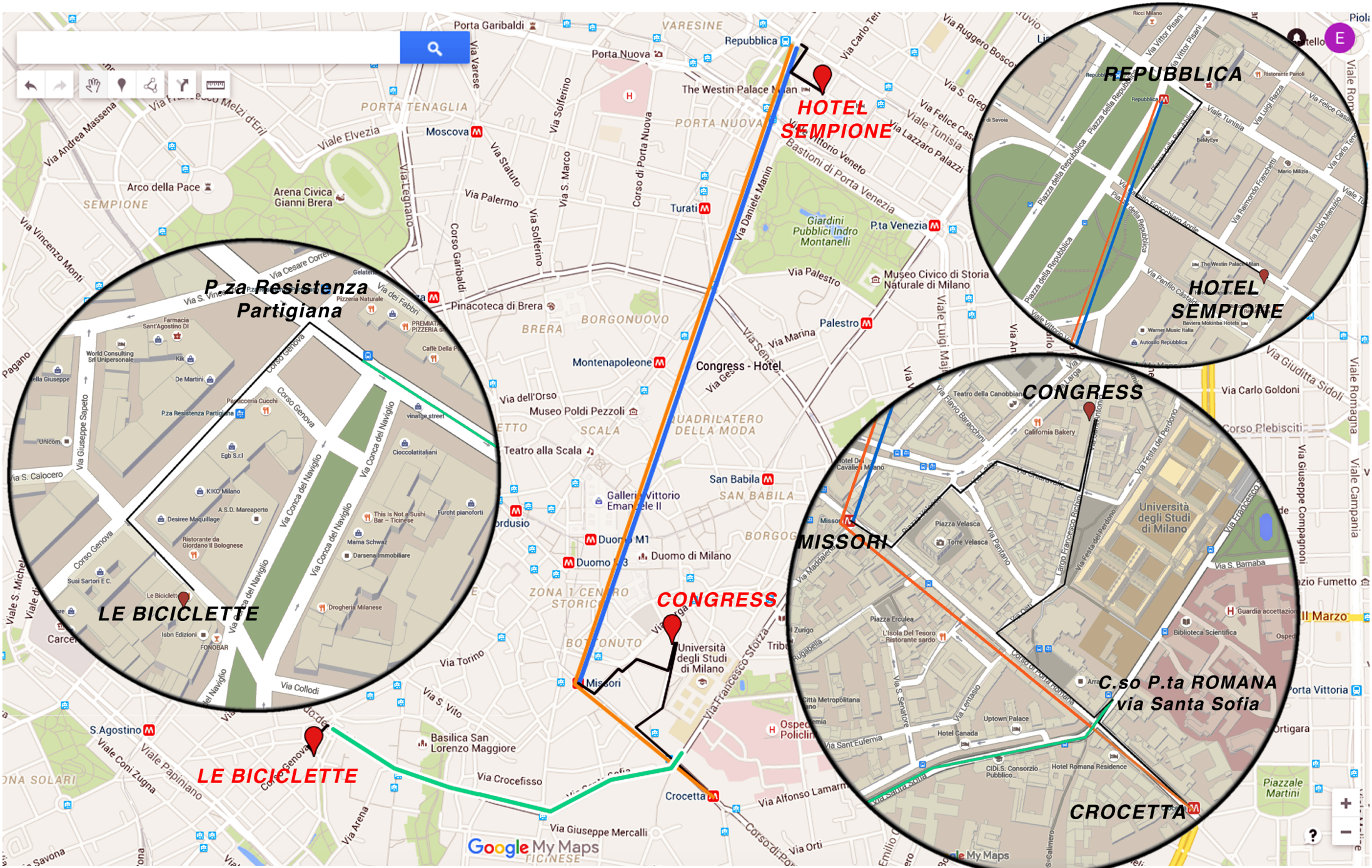
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