

provided the strongest evidence of an association with sexual dysfunction following antidepressant treatment. However, our results are likely due to chance and there was no strong supporting evidence in our replication cohort. The lack of biological plausibility in the identified genes also reduces confidence in our findings. The inclusion of an interaction term in our regression model allowed us to identify genetic variants whose association with sexual dysfunction differed by antidepressant (SSRI or NARI), which are clinically important, but also increased our chances of obtaining spurious associations. Larger, randomised controlled trials are required before pharmacogenetics may be able to guide clinical practice in reducing antidepressant-induced sexual dysfunction.

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#### P.2.a.022 Robust efficacy of 3 $\beta$ -methoxy-pregnenolone (MAP4343) in validated animal models of depression suggests a new antidepressant mechanism

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Depressive disorders (DDs) are common psychiatric disorders worldwide whose pathophysiology is linked to an alteration of central monoaminergic systems. In particular, reduction of serotonergic metabolism was evoked in depressed patients and most current antidepressant drugs are selective serotonin/noradrenaline reuptake inhibitors, such as fluoxetine. However the clinical efficacy of these drugs is restricted to a chronic administration, about half of target patients remain partial or non-responders and these compounds display substantial adverse side effects, responsible for discontinuation of treatment. Accumulating evidences have suggested that such psychiatric disorders are closely associated with abnormalities in brain microtubule function. Since last decade, we have developed various neurosteroids that target the microtubular system. Accordingly, we have proposed that neurosteroids, acting on the cytoskeleton, may constitute a new original therapeutic approach for DDs. Hence, we found that the 3 $\beta$ -Methoxy-pregnenolone (MAP4343), a derivative compound of pregnenolone, displays a very interesting and unique antidepressant activity.

We investigated the antidepressant effects of MAP4343 in two validated animal models of DDs consisting in (i) the Wistar Kyoto (WKY) rats, a spontaneous model which is known to be resistant to classic antidepressants, and (ii) the tree-shrews subjected to psychosocial stress which is considered as a non-human primate model. These animals received either acute (4 days in WKY rats) or chronic (4 wks in three shrews) administration of MAP4343. We assessed in WKY rats the ‘depressive-like’ behavior using the forced-swimming (FST) test and the ‘anxious-like’ behavior using the open-field. In tree-shrew, locomotor activity (LMA) and

avoidance behavior were videotaped, while various physiological parameters known to be disturbed in DDs (sleep activity, core body temperature and hormone secretion) were measured. Finally, expressions of  $\alpha$ -tubulin isoforms (tyrosinated versus detyrosinated) were measured in hippocampus from these two models, thereby the infrared Western-blot technique, and the ratio tyrosinated Tubulin/detyrosinated Tubulin (Tyr/Glu-Tub ratio) was taken as index of microtubular dynamics.

When compared to vehicle-treated WKY rats, we found that acute administration of MAP4343 (10 mg/kg/day, s.c.) significantly reduced the immobility in FST (–39%,  $p < 0.01$ ,  $n = 8$ ) whereas fluoxetine displayed an inverse effect. MAP4343 was able to increase the LMA in open-field arena (+56%,  $p < 0.001$ ,  $n = 8$ ), while fluoxetine had no effect. In tree-shrews, chronic oral administration of MAP4343 (50 mg/Kg/day, *per os*) significantly reversed stress-induced LMA reduction and stress-elicited avoidance behavior. In addition, MAP4343 can prevent stress-induced hypothermia, sleep disturbances and noradrenaline hypersecretion. Finally, a reduced Tyr/Glu-Tub ratio was found in these two models showing an alteration of microtubular dynamics in DDs. However, this alteration was not prevented by MAP43, suggesting that MAP43 does not directly act at the level of microtubular assembly.

Taken together, these data display a robust and potent antidepressant effect of MAP4343 compound, evidenced under both acute and chronic administration, in two validated models of depression from two species. Relevant antidepressant effects were demonstrated here on translational (both behavioral and physiological) parameters, known to be disturbed in DDs. Further investigations, using more accurate techniques, are in progress to elucidate the cellular mechanism of action of MAP4343, in particular on microtubular dynamics.

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#### P.2.a.023 Common and specific blood microRNA alterations in unipolar and bipolar depression

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MicroRNAs (miRNAs) are small non-coding RNAs (20–22 nucleotides) which regulate protein synthesis post-transcriptionally by base-pairing to target mRNAs. Generally, miRNAs inhibit protein synthesis either by repressing translation or by inducing deadenylation and degradation of target mRNAs, and they are predicted to regulate more than 50% of all the protein-coding genes. It has been reported that almost 50% of all the so far identified miRNAs are expressed in the human brain, with putative target genes regulating synaptogenesis and other basic neuronal processes. A role for miRNAs in neurogenesis, neuronal differentiation and survival, as well as in neuroplasticity, is now well established. Moreover, recent studies suggest that miRNAs may be involved in the pathophysiology of neuropsychiatric disorders and in the action of psychotropic drugs [1]. Alterations in

miRNA expression have been observed, among others, in major depression (MD) and bipolar disorder (BD), and their modulation has been described after treatments with antidepressant [3] and mood stabilizer drugs. In particular, intriguing findings concern the identification of disease- and treatment-associated miRNA signatures in patients' peripheral tissues, such as blood; this may provide further insights into the etiopathogenesis of MD and BD and contribute to identify new biomarkers for diagnostic assessment improvement and treatment personalization.

Thus, the aim of this study was to investigate miRNA expression levels in the blood of 20 MD and 20 BD patients, compared to 20 healthy controls. All the patients were in a depressive state [Hamilton Depression Rating Scale (HAMD) score  $\geq 15$ ] and drug-free from antidepressants; BD patients were in treatment with mood stabilizers. Total RNA was extracted from 2.5 mL of peripheral blood with the PAXGene Blood miRNA Kit (Qiagen) and analyzed with the GeneChip miRNA 3.0 Arrays (Affymetrix), which cover all the 1733 mature miRNAs and 1658 pre-miRNAs annotated in miRBase (online miRNA database, <http://www.mirbase.org>) version 17 (April 2011). Data were then analyzed with Partek Genomics Suite software, and the most significant results were confirmed by real-time PCR using the TaqMan MicroRNA Assays (Applied Biosystems). Five miRNAs were detected as differentially expressed only in MD patients, 5 only in BD patients, while 3 miRNAs were commonly modulated in MD and BD patients. A pathway analysis, which works by identifying the target genes of selected miRNAs, was performed with the online tool DIANA-miRPath (<http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=mirpath/index>) and revealed the presence of common altered pathways in MD and BD, such as long-term potentiation and depression, axon guidance, ErbB, TGF-beta, mTOR, Wnt, PI3K-Akt and neurotrophin signaling pathways, and the specific involvement of dopaminergic and glutamatergic synapse pathways for BD.

These results highlight a dysregulation in the expression of specific blood miRNAs in patients suffering from mood disorders and suggest putative specific alterations in the regulation of genes related to the dopaminergic and glutamatergic systems in BD patients.

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## P.2.a.024 Regulation of HDAC5 and Sirt2 by chronic stress and imipramine treatment in the prefrontal cortex

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Epigenetic mechanisms mediate stable functional changes in brain plasticity in response to environmental stimuli. Among them, stress-mediated changes in histone acetylation, regulated by the superfamily of histone deacetylases (HDAC's), could contribute to the pathogenesis of depression. Likewise, antidepressant therapy might counteract these effects [1,2].

Emerging clinical interest in the mechanisms that link exposure to stressful situations to the perpetuation of depressive disorders and/or increased vulnerability to relapse has stimulated the study of mouse specific models from a longitudinal perspective, aiming to identify stable neuroadaptations induced by stress. The chronic social defeat stress (CSDS) model has its theoretical rationale in the induction of 'social subordination' caused by short periods of struggle with a dominant animal followed by fellowship in an adjacent cage. CSDS induces long-lasting anhedonia, a core symptom of clinical depression, but also anxiety and social avoidance. Interestingly, some of these behaviours are effectively reversed or prevented by different monoaminergic antidepressants [3].

We hypothesise that the long-term behavioural deficits induced by CSDS could be related to neuroadaptive changes in areas such as the prefrontal cortex, which, is compromised in depressed patients. Using the chronic social defeat stress (CSDS) model of depression we aimed to study the HDAC's regulation by stress and imipramine treatment in the prefrontal cortex (PFC), their functional implications and relevance to depression.

**Methods:** Mice were exposed to CSDS (10 days) followed by saline or imipramine (10 mg/kg/day, 4 weeks). The PFC was obtained 24 h after the last drug administration and *hdac5*'s mRNA was studied and compared to human studies using the TaqMan Low Density Arrays (TLDA) microfluidic card technology. The protein expression of selected HDAC's, histone acetylation and other synaptic plasticity markers was explored by western-blot. Further, the effect of repeated treatment with specific HDAC's inhibitors in histone acetylation and the expression of synaptic plasticity markers were also explored.

**Results:** While CSDS increased *hdac5* and *sirt2* mRNA, repeated imipramine downregulated mRNA of these enzymes. However, HDAC5 protein and its phosphorylated cytoplasmic form (p-HDAC5) were strikingly upregulated by imipramine. Sirt2 protein was oppositely regulated by stress and imipramine. Moreover, imipramine-induced Sirt2 inhibition correlated with increased acetylated  $\alpha$ -tubulin, the Sirt2 cytoplasmic substrate. Subsequently, while CSDS decreased acetylated histone 3 and CREB levels, imipramine induced an increase of both acetylated histone 3 and 4 as well as pro-BDNF and CREB.

Further, both the class II HDAC inhibitor MC1568 (25 mg/kg, 3 weeks) and the sirt2 inhibitor 33i (1 mg/kg, three weeks) elevated acetylation levels of histone 4 and increased expression of pro-BDNF and CREB.

**Conclusion:** Our results suggest a role for HDAC5 and Sirt2 in stress-induced neuronal adaptations and in the mechanism of action of imipramine treatment, by which, it might stimulate the expression of specific genes involved in synaptic plasticity.