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Ulrike Wiechern
Elsevier
PO Box 993
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The Netherlands

Tel: +31 20 485 2934
Fax: +31 20 485 2940
E-mail: u.wiechern@elsevier.com

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S.01.01 Induced pluripotent stem cells and in vitro models of neurodevelopmental disorders

J. Price[1]*. [1]*King's College London, Institute of Psychiatry, London, United Kingdom*

There is increasing interest in developing cellular models of psychiatric disorders [1]. On the one hand, the idea of a cellular model of a complex disorder such as schizophrenia or autism would seem absurd. These are profoundly human conditions, which surely cannot be reduced to events in a tissue culture dish. Moreover, one is very aware of the checkered history of animal models in psychiatry. Can cellular models hope for more success, particularly as the pivotal features of these disorders are likely to involve features only emergent at the system level?

The prospects for cellular models rest on a series of technical and scientific advances of recent years. First, the genetics of autism, schizophrenia, and bipolar disorder has produced latterly some strong candidates, either from genome wide association studies (GWAS), or from copy number variations (CNVs). This means that we can look for the causative features of disease among the phenotypes associated with specific genetic variants. Second, there have been a number of pivotal advances in stem cell biology. Several sources of human stem cells are now available to researchers including somatic and fetal stem cells, such as neural stem cell populations, and pluripotent cells, such as embryonic stem (ES) cells and more recently induced pluripotent (iPS) cells. These cells allow two significant advances. First, neural development can be recapitulated in culture. Not completely of course, but stem cells in vitro can enact in vitro many of the seminal features of neural development, such as neurogenesis, gliogenesis, axon outgrowth, and synapse formation. This includes many of the features that are putatively disorganised in neurodevelopmental disorders. Second, by combining our understanding of the genetics with our current ability to manipulate genomes, we can generate human cells in vitro that carry precisely the genetic variants that are found associated with disease. So, we can assay precisely how these variants impact development, and thereby entertain precise hypotheses about the developmental bases of psychiatric disorders. For example, our laboratory has used neural progenitor cells taken from the human hippocampus to study the effects of stress factors, such as corticosterone, and antidepressant drugs on hippocampal neurogenesis [2]. We have been able reproduce many features of depression observed in both patients and animal models, but the tractability of the culture system has allowed us to study the molecular basis of disease aetiology that would have been impossible in whole organism approaches

iPSC technology represents the pinnacle of this approach. This allows researchers to take somatic cells from a patient – typically from skin, hair, or blood – and 'reprogram' them to give pluripotent cells, essentially indistinguishable in their potential from the pluripotent cells that otherwise exist only at the earliest stages of embryonic development. These iPSCs can be used to derive neural tissue (or indeed any tissue type) in a manner that largely mimics normal development, and can be used to study the deviation from normality that accompanies neurodevelopmental disorders. For example, we have grown hair keratinocytes from autistic patients carrying microdeletions of SHANK3, a structural protein of the synapse. We are now actively engaged in studying synaptogenesis in neurons derived from these iPSCs and comparing this to what we see in control cells.

Among the phenotypes that can be studied in these cellular models are cellular phenotypes (the differentiation of neurons, the growth of axons, etc) but also molecular phenotypes, such as gene expression and epigenetic variation. For example, we have discovered recently that neural stem cells in vitro show random monoallelic gene expression [3]. This is a phenomenon whereby some cells express just a single allele at a particular genetic locus. It appears to be random, because some clones of neural stem cells express one allele, other sister clones from the same donor express the alternate allele, while a third set of clones are biallelic expressing both alleles. The choice seems to be made randomly at some point during development, then maintained robustly right through the differentiation of the stem cells into neurons and glia. We estimate that probably 5 to 10% of expressed genes have this property. This is of interest in the aetiology of psychiatric disorders for two reasons. First, if it occurs during normal development (which has yet to be demonstrated), then it would mean that the brain would be a mosaic of clones of cells, each with a unique combination

of mono-allelically expressed genes. Thus each clone would be subtly different. It is easy to imagine how this could drive familiar features such as discordance between twins. Second, there is an over representation among these mono-allelic loci of genes associated with both autism and schizophrenia. We do not yet know how to interpret this finding, but it suggests a particular involvement of these loci in neural development, and a sensitivity of these loci to gene dosage.

These multiple avenues mean that increasingly disease aetiology can be approached in tissue culture systems. How far this endeavour can go remains to be seen, and the iPS technology in particular has much promise, but limited delivery so far. Surely, much disease pathophysiology will be beyond the scope of these unsophisticated cellular models. Nonetheless, much of the proximal impact of genetic variants is likely to appear at the cellular level, and these approaches should aid our understanding of these early steps. Finally, the potential of cellular systems as drug discovery assays and for target identification and validation has not escaped the attention of the pharmaceutical industry. This could be an important development, given the paucity of new therapies in the field of psychiatry.

Reference(s)

[1] Bray, N.J., Kapur, S., and Price, J. 2012. Investigating schizophrenia in a "dish": possibilities, potential and limitations. World Psychiatry 11, 153–155.

[2] Anacker, C., Zunszain, P.A., Cattaneo, A., Carvalho, L.A., Garabedian, M.J., Thuret, S., Price, J., and Pariante, C.M. 2011. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry 16, 738–750.

[3] Jeffries, A.R., Perfect, L.W., Ledderose, J., Schalkwyk, L.C., Bray, N.J., Mill, J., and Price, J. 2012. Stochastic Choice of Allelic Expression in Human Neural Stem Cells. Stem Cells 30, 1938–1947.

Disclosure statement: I act as consultant to ReNeuron Ltd., a UK biotech company developing stem cells for therapeutic and drug discovery applications.

S.01.02 Novel strategies for probing 3D structures of GPCRs

F. Marshall[1]*. [1]*Heptares Therapeutics, CSO, Hertfordshire, United Kingdom*

G protein coupled receptors are a major class of cell surface proteins responding to hormones and neurotransmitters. Around 40% of drugs on the market – particularly in the CNS area – mediate their activity through GPCRs including drugs such as olanzapine for schizophrenia, opioids for pain and dopamine agonists for Parkinson's disease, however there have been few recent successes with only 10 new GPCRs being drugged in the last decade. New GPCRs of interest including the receptors for neuropeptides and lipids have proved more difficult to find modulators with good in vivo activity, including selectivity, safety and pharmacokinetics. High throughput screening is frequently used to discover starting points for GPCR drug discovery, however oftentimes the leads derived from this approach have a high molecular weight and lipophilicity. Such compounds often fail during clinical development due to poor pharmacokinetics and off target toxicity.

The use of modern biophysical and structure based techniques in drug discovery have been very effectively applied to soluble targets such as enzymes. Structural biology of membrane proteins including GPCRs has been hampered by low levels of expression, heterogeneity of the protein, flexibility, the existence of different conformational states, instability when removed from the membrane and lack of domains for making contacts in crystal lattices. A range of protein engineering strategies have now been developed which address many of these issues and have resulted in the structures of over 15 GPCRs being solved in the last few years [1].

Heptares Therapeutics uses the technique of conformational thermostabilisation to generate GPCRs with an increased stability in a chosen conformational state [2]. These stabilised receptors are known as StaRs. StaRs have been produced for over 30 different GPCRs in different sub families including family A, B and C. An increase in thermostability is measured by solubilising the protein in detergent and heating in the presence of a ligand of defined pharmacology. The melting point or Tm is defined as the temperature at which 50% of the protein is unfolded. The addition of a small number of point mutations results in a pronounced increase in the Tm of as much as 30ºC. As well as having an increased thermostability StaRs can be purified in harsh short chain detergents that are more useful for crystallisation.

Fluorescence size exclusion chromatography (FSEC) is a technique whereby GFP is fused to the C-terminus of the receptor. The solubilised protein is analysed by FSEC and the shape of the peak eluting from the column is indicative of the quality of the protein. A sharp single peak shows a monodisperse protein suitable for crystallisation. Native GPCRs generally show a broad profile with several subpeaks. This is improved by the addition of ligand, thermostabilising mutations as well as modifications to the protein such as truncations. Prior to crystallisation the protein construct is optimised by making truncations and removing sites for post-translational modification. GPCRs are usually expressed in the baculovirus insect cell system

and purified on an IMAC Nickel affinity column using a His-tag at the C terminus. Other expression systems that have been used for structural studies with GPCRs include yeast, E. coli and mammalian cells. Crystallisation screens are set up in a wide range of different detergent conditions. Two approaches are routinely used: vapour diffusion in short chain detergents and lipidic cubic phase (LCP) crystallisation. The lipidic cubic phase is a lipid matrix which produces a more native, membrane-like environment. Extensive screening and optimisation of crystallisation conditions is then required to obtain the best diffracting crystals. Crystallisation in LCP is frequently facilitated by making fusion proteins with the GPCR which increase crystallisation contacts. For example T4 lysozyme may be fused into the third intracellular loop of the receptor. Antibodies may also be included as crystallisation chaperones. The resulting GPCR crystals are small and must be taken to specialised microfocussed X-ray beams at the synchrotron. Selection of the ligand for co-crystallisation studies is also important. In the absence of thermostabilising mutations a high affinity stabilising ligand is required to obtain crystals. The presence of thermostabilising mutations reduces this requirement and enables multiple co-crystal structures of weaker ligands to be obtained. This is important when using co-structures during the lead optimisation stage of drug discovery.

Once the structure is solved a model of the receptor structure is obtained and this is used for virtual screening and structure based drug discovery. X-ray structures are highly enabling for GPCR drug discovery as they allow drug candidates to be designed which fit efficiently into the ligand binding pocket [3]. Compounds can be optimised for affinity, kinetics and selectivity using structure based approaches. Heptares have applied this approach to a range of CNS discovery projects including an adenosine A_{2A} antagonist for the treatment of Parkinson's disease, an orexin receptor antagonist for the treatment of sleep disorders and a highly selective muscarinic M1 agonist for the treatment of Alzheimer's disease.

Reference(s)

[1] Katritch V, Cherezov V, Stevens RC. (2012). Diversity and modularity of G protein-coupled receptor structures. Trends Pharmacol Sci. 33(1):17–27.
[2] Tate CG. (2012). A crystal clear solution for determining G-protein-coupled receptor structures. Trends Biochem Sci. 37(9):343–52.
[3] Congreve M, Langmead C, Marshall FH. (2011). The use of GPCR structures in drug design. Adv Pharmacol. 62:1–36.

Disclosure statement: I am an employee of Heptares Therapeutics a company working on GPCR structure based drug discovery

Posters

P.1.001 3-Nitropropionic acid induces autophagy by forming mitochondrial permeability transition pore, not by activating mitochondrial fission

M.E. Solesio[1]*, S. Saez-Atienzar[2], J. Jordan[2], M.F. Galindo[1]. [1]*Complejo Hospitalario Universitario Albacete, Unidad de Neuropsicofarmacología Traslacional, Albacete, Spain;* [2]*Universidad de Castilla-La Mancha Facultad de Medicina, Grupo de Neurofarmacología, Albacete, Spain*

Purpose of study: Huntington's disease is a neurodegenerative process associated with mitochondrial alterations. Inhibitors of the electron-transport channel complex II, such as 3-nitropropionic acid (3NP), are used to study the molecular and cellular pathways involved in this disease [1]. We studied the effect of 3NP on mitochondrial morphology and dynamics, as well as its involvement in macrophagy and apoptosis activation.

Methods used: Pharmacological and biochemical methods were used to characterise the effects of 3NP on autophagy and mitochondrial dynamics and apoptosis, in a dopaminergic cell culture model (SH-SY5Y). 24 h before the transfections, SH-SY5Y cells were plated. The day after, there were transfected with GFP-LC3, GFP-Drp1 or GFP-Bax to ascertain their role and intracellular localisation after 3NP treatment, using confocal microscopy. Mitochondrial morphology was studied by transfecting our cell cultures with the chimeric protein pDs-Red2Mito and then visualising them using confocal microscopy. In some experiments, data obtained by confocal microscopy were corroborated by WB analysis.

Summary of results: Untreated SH-SY5Y cells presented long, tubular and filamentous net of mitochondria. After 3NP (5mM) treatment, mitochondria became shorter and rounder (filamentous mitochondria: 74±5 in control vs 16±2 in 3NP-treated cells). 3NP induces formation of mitochondrial permeability transition pore, both in cell cultures and in isolated liver mitochondria, and this process was inhibited by cyclosporine A. After 3 h of treatment, 3NP induces autophagy activation (4±2 dotted cells in control vs 17±1.2 in 3NP-treated cells). Participation of the mitochondrial fission pathway was excluded because 3NP did not induce translocation of the dynamin-related protein 1 (Drp1) to the mitochondria (8±2 cells with Drp1 translocated in control vs 11±5 in 3NP treated-cells). The Drp1 inhibitor Mdivi-1 did not affect the observed changes in mitochondrial morphology, (47±2 cells with fragmented mitochondria in control vs 52±3 in

3NP treated cells). After 12 h of 3NP addition, we observed Bax mitochondrial translocation, (26±5 Bax traslocation under control conditions vs 41±2 after 3NP treatment). Finally, scavengers of reactive oxygen species failed to prevent mitochondrial alterations, while cyclosporine A, but not Mdivi-1, prevented the generation of ROS. Data shown are mean±SEM, unless otherwise stated. Statistical significance of differences between groups was determined by ANOVA followed by a Newman–Keuls post hoc analysis. The level of statistical significance was set at $P < 0.05$.

Conclusions: There was a direct correlation between formation of mitochondrial permeability transition pore and autophagy induced by 3NP treatment. Activation of autophagy preceded the apoptotic process and was mediated, at least partly, by formation of reactive oxygen species and mitochondrial permeability transition pore. 3NP induces mitochondrial swelling at short time, thus Drp1 is not able to migrate to the mitochondria. Moreover, cell apoptosis is activated through the intrinsic pathway.

Reference(s)

[1] Beal, M.F., Brouillet, E., Jenkins, B.G., Ferrante, R.J., Kowall, N.W., Miller, J.M., Storey, E., Srivastava, R., Rosen, B.R., Hyman, B.T., 1993. Neurochemical and histological characterisation of striatial excitotoxic lesions produced by mitochondrial toxin 3-nitropropionic acid. J Neurosci 13, 4181–4192.

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P.1.002 Pharmacological characterisation of positive allosteric modulators acting on the metabotropic glutamate receptor 2

K.A. Bennett[1]*, A. Weaver[1], F. Marshall[1], C.J. Langmead[1]. [1]*Heptares Therapeutics, Department of pharmacology, Hertfordshire, United Kingdom*

There are eight subtypes of metabotropic glutamate (mGlu) receptors which bind the neurotransmitter glutamate. The mGlu2 and mGlu3 receptors are expressed pre-synaptically in the cortex, thalamus, striatum, amygdala and hippocampus. Hyperactivity in glutamatergic transmission in these regions is associated with anxiety disorders and psychosis and may be ameliorated by pharmacological activation of mGlu2. The selectivity and tolerance issues faced by orthosteric agonists of mGlu receptors are circumvented by allosteric modulators, which modulate the affinity (α) and/or efficacy (β) of orthosteric ligands. Here we show how reported positive allosteric modulators (PAMs) of mGlu2 (BINA, LY487379 and JNJ-40068782) affect the affinity and efficacy of glutamate.

Binding studies were established using the orthosteric antagonist [^{3}H]LY341495 in HEK293 cell membranes transiently expressing mGlu2. A three-way competition binding assay was used to study the interaction between [^{3}H]LY341495, glutamate and the PAMs (BINA, 2 μM – 2 nM; LY487379 and JNJ-40068782, 30 μM – 10 nM) allowing estimation of affinity co-operativity between PAM and glutamate (α′). Data was analysed globally using the simple allosteric ternary complex model [1,2]. An inducible mGlu2 expressing Jump-In HEK cell line (Life Technologies) was used to measure $G_{\alpha i/o}$ activity using a commercially available cAMP kit (HTRF; CisBio). Receptor expression was induced with 1 μg/ml doxycycline (16 h) and, prior to challenge, 3 U/ml glutamate pyruvate transaminase and 5 mM sodium pyruvate was applied to eliminate endogenous glutamate. When necessary, cells were pre-treated with PAM (15 min) before the addition of glutamate. Data was analysed globally using the operational model of allosterism [3] to elucidate net affinity/efficacy co-operativity parameters (αβ) and efficacy co-operativity factors (β).

[^{3}H]LY341495 bound with high affinity (K_d ± S.E.M = 0.61±0.03 nM) in mGlu2 expressing cell membranes and was completely displaced by glutamate (pK_i 5.69±0.13) and the orthosteric agonists LY367385 and LY354740 (pK_i values 4.73±0.08 and 7.68±0.15, respectively). Although they did not modulate equilibrium [^{3}H]LY341495 binding (α = 1), BINA, LY487379 and JNJ-40068782 all increased glutamate affinity (α′; Table 1). Functional analysis revealed, in this system, all three PAMs behaved as partial allosteric agonists and were able to increase the potency of glutamate (glutamate pEC_{50} 4.26±0.09). The combined co-operativity values (αβ; Table 1) obtained from the functional data were consistent with positive allosteric modulation (αβ > 1). Derivation of β (i.e ability of PAM to modulate efficacy; Table 1) revealed that BINA and LY487379 were weak positive modulators of glutamate efficacy whilst JNJ-40068782 behaved as a 'neutral' modulator (i.e JNJ-40068782 co-binding did not alter the efficacy of glutamate).

Activation of mGlu2/3 receptors negatively feeds back to reduces glutamate release at glutamatergic synapses. PAMs of mGlu2 have the ability to increase the response to endogenous glutamate – reducing glutamate release. The results from this study highlight subtle differences in the ability of mGlu2 PAMs BINA, LY487379 and JNJ-40068782 to modulate the affinity and efficacy of

glutamate. LY487379 equally modulated glutamate affinity and efficacy, BINA modulated affinity to a greater extent than efficacy whilst JNJ-40068782 acted solely to increase affinity of glutamate.

Table 1. Properties of mGlu2 PAMs

Compound	Co-operativity in binding (α'; mean±S.E.M. n=3)	Net co-operativity effect ($\alpha\beta$; mean±S.E.M. n=3)	Co-operativity in function (β; mean±S.E.M. n=3)
BINA	4.81±0.10	23.44±1.48	2.96±0.37
LY487379	2.25±0.25	5.50±1.39	2.11±0.41
JNJ-40068782	6.65±0.44	8.95±1.27	1.16±0.13

Reference(s)

[1] Lazareno S, Birdsall NJ., 1995. Detection, quantitation, and verification of allosteric interactions of agents with labeled and unlabeled ligands at G protein-coupled receptors: interactions of strychnine and acetylcholine at muscarinic receptors. Mol Pharmacol. Aug;48(2):362–78.

[2] Bradley SJ, Langmead CJ, Watson JM, Challiss RA., 2011. Quantitative analysis reveals multiple mechanisms of allosteric modulation of the mGlu5 receptor in rat astroglia. Mol Pharmacol May;79(5):874–85. Feb 14.

[3] Leach K, Sexton PM, Christopoulos A., 2007. Allosteric GPCR modulators: taking advantage of permissive receptor pharmacology. Trends Pharmacol Sci. Aug;28(8):382–9.

P.1.003 Inflammatory response in the brain of rats exposed to chronic mild stress

C. Zecchillo[1]*, F. Macchi[1], R. Molteni[1], G. Racagni[1], M. Papp[2], M.A. Riva[1]. *[1]University of Milan, Scienze Farmacologiche e Biomolecolari, Milan, Italy; [2]Institute of Pharmacology, Polish Academy of Sciences, Krakow, Poland*

Major depressive disorder (MDD) is a common disorder that represents a leading cause of disability in the world. It is thought to originate from the interaction between susceptibility genes and environmental events, such as stress, to which an individual can be exposed in different moments of life [1]. One of the major problems of depression is the relevant percentage of patients who do not show an adequate response to antidepressant therapy, as well as the high rate of relapse. Growing evidence suggests that the activation of the inflammatory/immune system contributes to the pathogenesis of depression. In particular, depression shows comorbidity with cancer, arthritis rheumatoid, cardiovascular and neurodegenerative diseases, characterised by inflammatory alterations [2]. In addition, elevated blood levels of the pro-inflammatory cytokines including interleukin (IL)-1b, IL-6 and tumour necrosis factor (TNF)-α are commonly found in depressed patients [3]. A role for inflammation in depression is also supported by the findings that cytokine administration induces depressive symptoms, as occurs in the 30% of hepatitis C patients who are treated with the immune activator interferon-alpha.

On these bases, it is important to characterise the changes of immune/inflammatory response in animal models of depression in order to establish their relationship with the depressive phenotype as well as the involvement in antidepressant response.

In order to do this we investigated the inflammatory response of rats exposed to a chronic mild stress (CMS) paradigm, which represents a well-established animal model of depression, for 8 weeks. Moreover, a group of animals (sham or CMS) were chronically treated with the antidepressant imipramine (10 mg/kg/day starting from week 2), in order to evaluate the ability of the antidepressant treatment to interfere with inflammatory alterations.

As expected, chronic mild stress caused a gradual decrease in the consumption of 1% sucrose solution over the 8-week period. When investigating the molecular changes of inflammatory markers, we found that the gene expression of two cytokines, IL-1b and IL-6, was significantly increased in the hippocampus of stressed animals (+49%, $p < 0.05$; +49%, $p < 0.01$). Also the mRNA levels for CD11b, a marker of microglia activation, were increased after CMS (+83%, $p < 0.01$). Moreover, we found that chronic imipramine treatment was able to normalise the depressive phenotype caused by CMS paradigm, although it did not alter the changes of the inflammatory response (IL-1b (+47%), IL-6 (+61%), and CD11b (+119%)).

In summary, these data provide support for a link between inflammation and depression, suggesting that a depressive state may be associated with significant alterations of the inflammatory response in selected brain regions. Interestingly, the failure of antidepressant treatment to normalise the inflammatory changes, while correcting the 'anhedonic' phenotype, suggests that classical pharmacological intervention may not work on the full spectrum of changes that characterise the depressive phenotype. This concept, when translated to humans, may be relevant for the presence of residual symptoms that are associated with enhanced the risk of relapse.

Reference(s)

[1] Caspi, A., Sugden, K., Moffitt, TE., Taylor, A., Craig, IW., Harrington, H., McClay, J., Mill, J., Martin, J.,

Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 301(5631):386–9.
[2] Anisman, H., Merali, Z., Hayley, S. 2008. Neurotransmitter, peptide and cytokine processes in relation to depressive disorder: comorbidity between depression and neurodegenerative disorders. Prog Neurobiol. 85(1):1–74.
[3] Connor, TJ., Leonard, BE. 1998. Depression, stress and immunological activation: the role of cytokines in depressive disorders. Life Sci. 1998;62(7):583–606.

P.1.004 RNAi-mediated serotonin transporter suppression rapidly increases serotonergic neurotransmission and hippocampal neurogenesis

A. Ferrés-Coy[1]*, F. Artigas[1], A. Bortolozzi[1]. [1]*IIBB-CSIC (IDIBAPS), Neurochemistry and Neuropharmacology, Barcelona, Spain*

Despite extensive research, the neurobiology of major depressive disorder (MDD) remains poorly understood due to lack of biomarkers, relatively low rates of heritability, and heterogeneity of precipitating factors, including stress. Serotonin (5-HT) participates in the aetiology and treatment of MDD. In fact, selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressant drugs. However, SSRIs take weeks to months to produce a therapeutic response and are only moderately effective, leaving more than one-third of depressed individuals resistant to drug treatments [1]. The serotonin transporter (SERT) is a key regulator of serotonergic neurotransmission, as it controls the active fraction of 5-HT. RNA interference (RNAi) plays a critical role in regulating gene expression and normal cell development. It also provides new alternatives to pharmacological treatments to modulate brain neurotransmission through the use of exogenous small interference RNA (siRNA). Previously, we showed the feasibility to silence the expression of 5-HT_{1A}-autoreceptor in 5-HT neurons [2,3]. Here we used a multifaceted approach to assess the ability of RNAi to reduce SERT expression in dorsal raphe of adult mice. We examined downstream changes on brain variables linked to antidepressant efficacy and compared SERT-siRNA effects with those of a standard SSRI (fluoxetine) treatment. Local SERT-siRNA infusion for 4-days (0.7 nmol/dose) suppressed SERT expression in DR (40%). This was accompanied by a selective and widespread reduction of SERT-binding sites and protein levels throughout the brain. Moreover, a 4-day regimen with intra-DR SERT-siRNA modified brain variables considered to be key markers of antidepressant action, such as: **(a)** reduced expression and sensitivity of 5-HT_{1A}-autoreceptors; **(b)** augmented extracellular 5-HT in DR-projecting areas such as striatum and hippocampus; **(c)** increased hippocampal neurogenesis (BdrU-labelled cells to 144±8% of vehicle-treated mice) and **(d)** increased plasticity-associated gene expression (BDNF, VEGF and Arc) in hippocampus. In contrast, a 4-day regimen with fluoxetine (20 mg/kg/day) did not alter any of these variables and only started to modify them after 15-day treatments. Furthermore, the intranasal administration of conjugated SERT-siRNA for 7-days, but not fluoxetine, reversed the behavioural dysfunction induced by three weeks of corticosterone treatment (reduced immobility time in tail suspension test, reduced latency to feed in response to stress in novelty suppressed feeding test and increased the preference and intake of sucrose solution compared with water). These findings highlight the critical role of SERT in the control of serotonergic function, including serotonin-mediated neural plasticity. They also support the use of siRNA targeting serotonergic genes (SERT, 5-HT_{1A}-autoreceptor) as a new generation of antidepressant therapies with a potential greater efficacy faster onset of action than current treatments.

Reference(s)

[1] Wong, M.L., Licinio, J., 2001. Research and treatment approaches to depression. Nature Rev Neurosci 2, 343–351.
[2] Ferrés-Coy, A., Santana, N., Castañé, A., Cortés, R., Carmona, M.C., Toth, M., Montefeltro, A., Artigas, F., Bortolozzi, A., 2013. Acute 5-HT1A autoreceptor knockdown increases antidepressant responses and serotonin release in stressful conditions. Psychopharmacology (Berl) 225, 61–76.
[3] Bortolozzi, A., Castañé, A., Semakova, J., Santana, N., Alvarado, G., Cortés, R., Ferrés-Coy, A., Fernández, G., Carmona, M.C., Toth, M., Perales, J.C., Montefeltro, A., Artigas, F., 2012. Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong anti-depressant-like effects. Mol Psychiatry 17, 612–623.

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P.1.005 Role of cannabinoid CB1 receptors in modulation of dopamine output in the prefrontal cortex associated with food restriction in rats

V. Licheri[1]*, G. Talani[2], L. Dazzi[1], F. Biggio[1], C. Utzeri[1], V. Lallai[1], S. Lutzu[1], G. Biggio[1,2], E. Sanna[1,2].
[1]University of Cagliari, Life and Environmental Sciences, Section of Neuroscience, Monserrato (CA), Italy; [2]Institute of Neuroscience National Research Council, Life and Environmental Sciences, Monserrato (CA), Italy

An extensive body of literature has documented the importance of endocannabinoid system in the regulation of appetite and feeding behaviour in mammals. The cannabinoid CB1 receptor is abundant in the prefrontal cortex (PFC), which plays key roles in working memory and reward. Principal dopaminergic afferents arising from the ventral tegmental area (VTA) are crucial for PFC function and dopamine type 2 (D2) as well as CB1 receptors are co-expressed on γ-aminobutyric acid (GABA)-containing terminals in the PFC [2].

To elucidate the role of CB1 receptors in the regulation of dopamine release in the PFC associated with feeding behaviour in rats, we exposed Sprague-Dawley rats to a restricted food regimen, with availability of food limited to a 2-h period daily for 3 weeks. Control rats were given ad libitum access to food. Microdialysis, Western-blot as well as patch clamp experiments were performed in order to test our hypothesis.

Food-restricted (FR) rats showed a marked increase in the extracellular dopamine concentration in the PFC 80 min before food presentation, with the concentration peaking during food consumption and returning to baseline after food removal. These changes were attenuated by the CB1 antagonist SR141716A but were unaffected by the agonist WIN 55212-2. Patch clamp experiments performed in principal medial PFC (mPFC) neurons revealed that during the anticipatory phase before food presentation there is a significant decrease of the inhibitory effect of WIN 55212-2 on GABAergic spontaneous inhibitory post-synaptic currents (sIPSC) frequency in FR rats compared with control animals (one-way ANOVA: $p = 0.017$ vs controls). Moreover, endocannabinoid-dependent DSI of GABAergic inhibition was abolished in FR rats during this phase (one-way ANOVA: $p = 0.001$ vs controls). The basal sIPSC frequency was also reduced in mPFC neurons of FR rats compared with control animals, suggestive of an altered control of presynaptic GABA release (one-way ANOVA: $p = 0.0001$ vs controls). A complex innervations between mPFC and VTA has been previously described by other authors. Briefly, evidence suggests that endocannabinoids may modulate dopamine release in the PFC indirectly by activating CB1 receptors located on presynaptic GABAergic terminals, thereby inhibiting GABA release and resulting in disinhibition of glutamatergic neurons that project to the VTA. An increase in glutamate release in the VTA would stimulate dopaminergic neurons that project back to the mPFC and thereby increase the release of dopamine in this region [3]. Current-clamp recordings revealed an increased excitability in both mPFC and VTA neurons of FR animals, relative to control animals, an effect that correlates well with the decrease of GABA release observed in mPFC inhibitory synapses (t-test: $p < 0.05$ vs controls). Finally, CB1 expression in the PFC was reduced in FR rats before food presentation compared with controls accordingly with other previews reports [1].

Together, these data support a role for the endocannabinoid system in regulation of dopamine release in the PFC, and they suggest that the feeding-associated increase in dopamine output in the PFC of FR rats might be due to down-regulation of CB1 in this brain region that in turn regulates the function of the whole meso-cortical neuronal circuitry.

Reference(s)

[1] Bello, N.T., Coughlin, J.W., Redgrave, G.W., Ladenheim, E.E., Moran, T.H., Guarda, A.S, 2012. Dietary conditions and highly palatable food access alter rat cannabinoid receptor expression and binding density. Physiol Behav 105: 720–726.
[2] Chiu, C.Q., Puente, N., Grandes, P., Castillo, P.E., 2010. Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex. J Neurosci 30: 7236–7248.
[3] Egerton, A., Allison, C., Brett, R.R., Pratt, J.A., 2006. Cannabinoids and prefrontal cortical function: insights from preclinical studies. Neurosci Biobehav Rev 30: 680–695.

P.1.006 Oxytocin controls CRF gene expression through TORC3: implications for stress-related disorders

B. Jurek[1]*, D.A. Slattery[1], Y. Liu[2], I.D. Neumann[1], G. Aguilera[2], E.H. van den Burg[1]. *[1]University of Regensburg, Dept of Neurobiology and Animal Physiology, Regensburg, Germany; [2]Eunice Kennedy Shriver National Institute of Child Health and Human Development, Section on Endocrine Physiology, Bethesda MD, USA*

Increased fear and stress responses following a threatening stimulus are considered normal emotional and physiological reactions. However, they become pathological when

exaggerated or prolonged, and can then lead to severe psychological and somatic disorders including depression and anxiety. The neuropeptide corticotropin-releasing factor (CRF), which is produced in the hypothalamus, is a key regulator of both fear/anxiety and stress responses/reactions. It controls the hypothalamo–pituitary–adrenal (HPA)-axis to stimulate the release of the stress hormone cortisol or corticosterone via ACTH. Its expression in turn is regulated by the transcription factor CREB and its cofactor TORC (transducer of regulated CREB activity, a.k.a. CRTC) that, upon an appropriate stimulus, translocates from the cytoplasm to the nucleus to form a complex with CREB and activate gene transcription by binding to a promoter region [1].

We have shown that another neuropeptide, namely oxytocin, is anxiolytic within the hypothalamus, and has anti-stress properties. The underlying molecular mechanisms of these effects are unknown, yet are important for the quest for pharmaca that may dampen (exaggerated) stress responses. As CRF cells express the oxytocin receptor, oxytocin might inhibit HPA-axis activity directly through altering CRF gene expression or CRF release. Therefore, in the present study, we hypothesised that oxytocin inhibits CRF gene transcription through prevention of CREB phosphorylation and/or TORC translocation and assessed this using a combination of in vivo and in vitro assays.

Icv administration of oxytocin in male rats delayed and prolonged restraint stress-induced CRF transcription, as measured by hnRNA levels using quantitative real-time PCR. CRF hnRNA levels were significantly lower in oxytocin-infused rats when compared with vehicle-infused rats 10 min after the onset of the stressor ($p < 0.05$, $n = 4$). In contrast, while CRF hnRNA levels returned to basal values in the vehicle-treated group after 30 min, they remained elevated in the OXT-treated group ($p < 0.05$, $n = 8$). In line with these in vivo findings, Western blot analyses using primary hypothalamic neurons showed that the oxytocin receptor agonist TGOT lowered the maximum CRF hnRNA response to forskolin by 20% ($p < 0.05$, $n = 7$) after 30 min of stimulation, but prolonged forskolin-induced CRF expression (up to 90 min of stimulation; $p < 0.05$, $n = 7$). In a hypothalamic cell line (H32), TGOT did not influence forskolin-induced CREB phosphorylation nor TORC2 nuclear levels at any timepoint tested, but delayed the forskolin-induced increase of nuclear TORC3 concentrations from 10 to 60 min of incubation (10 min: $p < 0.05$; 60 min: $p < 0.001$; $n = 4$–8). TGOT/oxytocin without forskolin/stress showed no effect on any parameter tested.

These results indicate that oxytocin controls CRF gene expression by modulating nuclear TORC3 levels, thus decelerating CRF synthesis during the initial phase of an acute stress response. Indeed, this was mirrored via a delay in peak plasma ACTH levels from 10 min in the vehicle-treated group to 15 min in the oxytocin-treated group (interaction factor time × treatment; $F_{(3,28)} = 3.372$, $p < 0.05$). Taken together, our findings indicate that oxytocin acts on the CRF system to modulate the acute HPA-axis response to a stressor. Therefore, drugs, such as oxytocin, that target TORC3 activity may represent novel candidates for the treatment of stress-related disorders.

Reference(s)

[1] Liu, Y., Knobloch, S., Grinevich, V., Aguilera, G., 2011. Stress induces parallel changes in corticotrophin-releasing hormone (CRH) transcription and nuclear translocation of transducer of regulated cAMP response element-binding activity 2 in hypothalamic CRH neurones. J Neuroendocrinol, 216–223.

P.1.007 A proteomic and functional analysis reveals that 5-HT$_6$ receptors modulate neuronal differentiation by recruitment of Cdk5

F. Duhr[1]*, M. Séveno[2], C. Mannoury la Cour[3], D. Dupuis[3], M.J. Millan[3], J. Bockaert[1], P. Marin[1], S. Chaumont-Dubel[1]. *[1]Institut de Génomique Fonctionnelle (IGF), Neurobiologie, Montpellier, France; [2]Institut de Génomique Fonctionnelle (IGF), Plateforme de Protéomique Fonctionnelle, Montpellier, France; [3]Institut de Recherche Servier, Psychopharmacologie, Croissy sur Seine, France*

Purpose of the Study: The serotonin 5-HT$_6$ receptor is expressed in CNS regions involved in the pathogenesis of disorders like Alzheimer's disease and schizophrenia. 5-HT$_6$ receptors are a promising target for treatment of the accompanying cognitive deficits, since their blockade consistently enhances mnemonic performance in rodents [1]. Paradoxically, still little is known about 5-HT$_6$ receptor-associated signalling pathways, an issue we have addressed by a proteomic approach. Previously, we showed physical association of the 5-HT$_6$ receptor with several members of the mTOR pathway and demonstrated that mTOR recruitment by prefrontal 5-HT$_6$ receptors perturbs cognition in schizophrenia [2]. Here we show that 5-HT$_6$ receptors interact with Cyclin-dependent kinase 5 (Cdk5), a protein which controls actin cytoskeleton dynamics and modulates neurodevelopmental processes such as neuron migration, neurite outgrowth, synaptogenesis and dendritic spine morphogenesis.

Methods and Results: The present study explored the role of Cdk5, under the control of 5-HT$_6$ receptors, in

the differentiation of NG108–15 neuroblastoma cells and rat striatal neurons in primary culture, assessed by neurite outgrowth. Expression of voltage-gated Ca^{2+} channels was also analysed in NG108–15 cells using Fura2 imaging. The unpaired Student's t-test and one-way ANOVA followed by Dunnett's test were performed (using the Prism software) for two-sample and multiple comparisons, respectively. Expressing 5-HT_6 receptors in NG108–15 neuroblastoma triggered neurite outgrowth and induced expression of functional voltage-gated Ca^{2+} channels. These effects were not further enhanced by an agonist (WAY181,187, 1 μM) but were prevented by SB258,585 (10 μM), a selective 5-HT_6 antagonist. Thus, upon SB258,585 treatment, cells showed a decrease in neurite length of 48.4% compared to untreated cells (22.5±1.5 μm vs. 43.5±1 μm, $n = 4$, $p < 0.0001$). Expression of a dominant-negative (DN) Cdk5 or treating cells with roscovitine (pharmacological inhibitor of Cdk5) likewise inhibited NG108–15 cells differentiation induced by 5-HT_6 receptor expression (control: 69.8±3.6 μm; DN Cdk5: 42.8±3 μm, $p < 0.0001$; roscovitine: 28.1±1.5 μm, $n = 3$, $p < 0.0001$). SB258,585 also impaired the association of 5-HT_6 receptor with Cdk5 in NG108–15 cells, as determined by co-immunoprecipitation, suggesting that this interaction was necessary for induction of differentiation. Treating striatal neurons with either SB258,585 or roscovitine immediately after seeding decreased neurite length, as determined 24 hrs later (untreated: 29.9±0.7 μm; SB258,585: 23.7±0.6 μm, $p < 0.0001$; roscovitine: 20.2±1.0 μm, $n = 4$, $p < 0.0001$). Conversely, exposure of neurons to WAY181,187 did not significantly affect neurite outgrowth ($p = 0.43$). Further supporting a role of endogenously expressed receptors in differentiation of striatal neurons, silencing 5-HT_6 receptor expression in cultured neurons significantly reduced neurite length (control siRNA: 29.6±0.8 μm, 5-HT_6 receptor siRNA: 17.2±0.9 μm, $n = 3$, $p < 0.0001$).

Conclusions: Complementing work indicating that 5-HT_6 receptors modulate neuronal migration [3], the present data show that 5-HT_6 receptors developmentally promote neuronal differentiation and reveal a critical role for Cdk5 in this process. These novel insights into molecular substrates underlying neurodevelopmental effects of 5-HT_6 receptors are of potential importance to the pathophysiology and treatment of early-onset CNS conditions like autism-spectrum disorder and schizophrenia.

Reference(s)

[1] Mitchell, E.S., and Neumaier, J.F. 2005 5-HT6 receptors: a novel target for cognitive enhancement. Pharmacol Ther 108, 320–333.

[2] Meffre, J., Chaumont-Dubel, S., Mannoury la Cour, C., Loiseau, F., Watson, D.J., Dekeyne, A., Seveno, M., Rivet, J.M., Gaven, F., Deleris, P., Herve, D., Fone, K.C., Bockaert, J., Millan, M.J., and Marin, P. 2012 5-HT(6) receptor recruitment of mTOR as a mechanism for perturbed cognition in schizophrenia. EMBO Mol Med 4, 1043–1056.

[3] Riccio, O., Potter, G., Walzer, C., Vallet, P., Szabo, G., Vutskits, L., Kiss, J.Z., and Dayer, A.G. 2009 Excess of serotonin affects embryonic interneuron migration through activation of the serotonin receptor 6. Mol Psychiatry 14, 280–290.

P.1.008 V_{1B}/CRF_1 receptor heterodimerisation as a key mechanism of vasopressin and corticotropin-releasing factor synergism

J. Mion[1]*, V. Boulay[1], M.J. Millan[2], G. Guillon[1], M. Corbani[1]. *[1]Institut de Génomique Fonctionnelle CNRS UMR 5203 – INSERM U.661 Universités de Montpellier I et II, Endocrinologie, Montpellier, France; [2]Institut de Recherches SERVIER, Unité de Recherche et Découverte, Croissy-sur-Seine, France*

Purpose of the study: Vasopressin (AVP) and Corticotropin-Releasing Factor (CRF) are involved in the stress response, mainly by regulating ACTH secretion from the pituitary and by increasing catecholamine and corticosteroid secretion from the adrenal medulla. In these structures, AVP and CRF act in synergism via V_{1B} and CRF_1 receptors, respectively. Recently, we suggested that synergism operates via both second messenger crosstalk and putative mechanism involving receptors heterodimerisation [1]. To further validate this last original mechanism, we monitored the influence of receptor heterodimerisation selectivity and of receptor heterodimerisation disruption on functional synergism.

Methods: We co-expressed in HEK293 cells either CRF_1 or CRF_2 receptors on one hand, and either V_{1B} or V_{1A} receptors on the other hand. For each pair of transfected receptors, the degree of physical interaction was evaluated by BRET experiments. AVP receptors were tagged with the Renilla Luciferase and CRF receptors were tagged with Yellow Fluorescent Protein at their C-terminal domain. In contrast to a previous study by Young et al. [2], tagging the receptors at their C terminus preserved their functionality and was a key element for the success of this approach. For each pair of transfected receptors, functional synergism was measured by respective second messenger accumulation: Inositol Phosphates (IPs) for V_{1B} and V_{1A} and cAMP for CRF_1 and CRF_2). We also generated a mutant of CRF_1 receptor unable to heterodimerise with

V_{1B} receptor and looked for its ability to induce functional synergism.

Results: Only for V_{1B}/CRF_1 receptors, there was a clear association between receptor heterodimerisation (BRETmax reach from x1 = Fluorescence/Luminescence = 0.005 to x2 = 0.02) and crosstalk-independent potentiation in both IPs (27% under 10 nM AVP and 10 nM CRF) and cAMP (29% under 1 nM CRF and 10 nM AVP) signalling pathways. By contrast, for co-transfection of V_{1B} and CRF_2 receptors, no molecular association could be observed (no saturation curve) and hormone-stimulated second messenger potentiation only reflected signalling crosstalk. Evidence for the existence of V_{1A}/CRF_1 heterodimers was found (x1 = 0.015; x2 = 0.03) but they conformationally differed to V_{1B}/CRF_1 heterodimers, and potentiation of signalling independent of second messenger crosstalk could only be observed for formation of IPs (13% under 10 nM AVP and 10 nM CRF).

To further correlate heterodimerisation and crosstalk-independent potentiation, we generated a chimerical CRF_1-CRF_2 receptor. BRET experiments show that this mutant has lost capacity to heterodimerise with V_{1B} receptors. Moreover, despite it still activated cAMP pathway in a dose-dependent manner, this CRF_1 mutant was no longer able to potentiate vasopressin-stimulated IPs accumulation via a mechanism independent of second messengers crosstalk.

Conclusion: These results suggest that AVP-CRF synergism at least partially reflects the existence of V_{1B}/CRF_1 heterodimers, and that their formation modifies signalling at these sites. Studies underway are in line with the existence of molecular physical association of V_{1B}/CRF_1 heterodimers in native tissues. Further, the present work is consistent with a role for V_{1B}/CRF_1 heterodimers controlling ACTH secretion – and the activity of corticolimbic structures controlling mood – in the pathogenesis of stress-related psychiatric conditions like depression, anxiety disorders and schizophrenia.

Reference(s)

[1] Murat, B., Devost, D., Andrés, M., Mion, J., Boulay, V., Corbani, M., Zingg, H.H., Guillon, G., 2012 V1b and CRHR1 receptor heterodimerization mediates synergistic biological actions of vasopressin and CRH. Mol Endocrinology 26(3), 502–20.

[2] Young, S.F., Griffante, C., Aguilera, G., 2007 Dimerization between vasopressin V1b and corticotropin releasing hormone type 1 receptors. Cell Mol Neurobiol 27, 439–461.

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P.1.009 Cell synchronisation as a tool to optimise expression of metabotropic glutamate receptors in inducible mammalian expression system

B. Chruscicka[1]*, P. Branski[1], G. Burnat[1], A. Pilc[1].
[1]*Polish Academy of Sciences, Neurobiology, Krakow, Poland*

Background: The metabotropic glutamate receptors (mGluRs) are class C G protein-coupled receptors that play an important neuromodulatory role throughout the brain, as such they are involved in a number of psychiatric and neurological disorders like anxiety, depression and schizophrenia [1]. Expression of metabotropic glutamate receptors in heterologous mammalian cells is a method for their functional characterisation, and tool to study the agonist, antagonist and allosteric effects which are very attractive targets for therapeutic intervention. However, the constitutive expression of mGluRs seems to have a toxic effect on the HEK293 cells. Thus, an inducible expression system (in which the mGluRs expression is activated in the presence of tetracycline) is used [2]. Unfortunately an expression of receptors was confirmed in approximately 30% of cells after tetracycline induction.

Aim: Our goal was to increase the number of HEK293 cells with an induced expression of mGlu2 receptor using synchronisation of cell cycle.

Methods: cDNA fragment encoding mGlu2 receptor was cloned into a pcDNA5/FRT/TO vector under the control of a tetracycline regulated promoter. HEK293 cells containing inducible mammalian expression system (Flp-In T-REx host cells line from Invitrogen) were treated with GeneJuice transfection reagent, and then selected by hygromycin. Synchrony of stably transfected cells was performed by means of three different synchronisation protocols. To obtain G_2/M phase block, cells were supplemented with nocodazole for 18 h. To arrest cells at the beginning of S phase a double thymidine block was used (16 h and 18 h). G_0/G_1 population of cells was obtained by serum starvation for 48 h [3]. The efficacy of cell synchronisation was verified by flow cytometric analysis of DNA content after propidium iodide-staining. For evaluation a surface expression of mGlu2 receptor flow cytometer immunofluorescence staining was performed.

Results: We received 45.2±0.9% cells at the beginning of S phase after double thymidine block, 60.6±0.9% cells in M phase after nocodazole treatment and 77.9±8.0% cells in G_0/G_1 phase after serum starvation. Untreated cells are characterised by 52.7±1.8% cells in G_0/G_1, 31.3±0.9% in S and 15.0±1.0% in G_2/M phase of cycle. Cell synchronisation seems to have statistically

significant effect on mGlu2 receptor expression level [One-way ANOVA, $F(3.41) = 22.8$, $p < 0.0001$]. Moreover Dunnett's Multiple Comparison Test analysis shows that level of mGlu2 receptor surface expression in cells after double thymidine block was significantly higher compared to receptor expression level in asynchronously growing cells ($p < 0.01$). In contrast, the expression level of mGlu2 receptor was significantly decreased in M phase blocked cells ($p < 0.01$) and cells after serum starvation ($p < 0.01$).

Conclusions: The results indicate that modification of the inducible expression system by cell synchronisation seems to have significant effect on surface mGluR2 expression level.

Reference(s)

[1] Palucha, A., Pilc, A., 2007. Metabotropic glutamate receptor ligands as possible anxiolytic and antidepressant drugs. Pharmacology & Therapeutics 115, 116–147.

[2] Van Craenenbroeck, K., Vanhoenacker, P., Leysen, J.E., Haegeman, G., 2001. Evaluation of the tetracycline- and ecdysone-inducible systems for expression of neurotransmitter receptors in mammalian cells. European Journal of Neuroscience 14, 968–976.

[3] Yu, J.N., Ma, S.F., Miao, D.Q., Tan, X.W., Liu, X.Y., Lu, J.H., Tan, J.H., 2006. Effects of cell cycle status on the efficiency of liposome-mediated gene transfection in mouse fetal fibroblasts. Journal of Reproduction and Development 52, 373–382.

P.1.010 The identification of protein tyrosine phosphatase, non-receptor type 1 in hippocampal modulation of food anticipatory behaviour

E. Kostrzewa[1]*, L.A.W. Verhagen[1], C. Gelegen[1], H.A. van Lith[2], D.A. Collier[3], M. Mitsogiannis[1], E. de Vries[1], M. van Gestel[1], R.A. Adan[1], M.J.H. Kas[1].

[1]Rudolf Magnus Institute of Neuroscience, Neuroscience and Pharmacology, Utrecht, The Netherlands; [2]Utrecht University, Faculty of Veterinary Medicine, Utrecht, The Netherlands; [3]King's College, Institute of Psychiatry, London, United Kingdom

Background: Activity based anorexia (ABA) is a rodent model of hyperactivity evoked by food restriction. The paradoxical hyperactivity during food restriction is reminiscent of that observed in patients suffering from eating disorders. One of the parameters of this model, food anticipatory activity (FAA) enables us to study neurobiological mechanisms that synchronise the timing of food availability and changes in arousal in order to be prepared for upcoming relevant environmental events [1].

Methods: To identify chromosomes contributing to FAA, 21 mouse strains from a chromosome substitution panel (host strain expressing FAA (C57BL/6J) and a donor strain lacking FAA (A/J)) were tested using the ABA model ($n = 8–10$ mice per strain). Subsequently, we generated an F2 population from chromosome substitution strains 2 that lacks FAA. F2 mice ($n = 125$) were genotyped and phenotyped to fine map a locus on mouse chromosome 2 associated with FAA. In addition, genome-wide micro-array gene expression analysis was performed to identify differentially expressed genes in the hippocampus of F2 mice selected for their high or low FAA-levels. To functionally validate the candidate gene we generated shRNA viral vector (pAAV-shbase containing GFP cassette, titer 7.8×10^{12} genomic copies/ml) and injected C57BL/6J mice in the dentate gyrus (DG) three weeks prior to the ABA model. We verified virus spread using ISH for GFP and assessed the potential neuronal damage by performing IHC for NeuN and ISH for miRNA124. Level of candidate gene knockdown was quantified by radioactive ISH. Animals' body weight changes, food intake and running wheel activity (RWA) during ABA were recorded.

Results: We genetically mapped a Quantitative Trait Locus on mouse chromosome 2 associated with FAA. The combined genetic fine mapping and genome-wide gene expression data revealed Ptpn1 (protein tyrosine phosphatase, non-receptor type 1) as a candidate gene for FAA. To validate this candidate gene, we performed stereotactic injections of a virus containing anti-Ptpn1 shRNA. The virus infected neurons in DG and partly in CA fields without detectable neuronal damage. The expression of Ptpn1 in DG was significantly reduced in the anti-Ptpn1 group in comparison to the control virus injected group (Student t-test: $t(8) = 2.50$, $p < 0.05$). There were no differences in body weight, food intake nor baseline RWA (Student t-test: $t(8) = –0.493$, $p = 0.635$) between anti-Ptpn1 and control virus treated mice. Similarly, total RWA during the restriction phase did not differ significantly between the groups (Student t-test: $t(8) = 0.126$, $p = 0.903$ respectively). Normalised FAA, (total FAA as a percentage of total RWA), was found to be significantly decreased in the Ptpn1 knockdown group compared to the control group (Student t-test: $t(8) = 2.459$, $p < 0.05$). A positive association was found between the level of Ptpn1 mRNA expression in the DG and the magnitude of FAA ($r = 0.642$, $p = 0.045$).

Conclusions: This data confirm that FAA levels are associated with Ptpn1 gene expression in the DG. As Ptpn1 is a key regulator of insulin and leptin receptor sensitivity, these findings provide a possible mechanism through

which metabolic hormones mediate the timing of arousal necessary to be prepared for upcoming environmental events.

Reference(s)

[1] Kas, M.J., Adan, R.A., 2011. Animal models of eating disorder traits. Curr Top Behav Neurosci 6:209–27.

P.1.011 Identification of a significant role for the ventral hippocampus in neuropeptide S-elicited anxiolysis

I.A. Ionescu[1]*, J. Dine[2], J. Stepan[2], Y.C. Yen[3], L. Herrmann[1], F. Holsboer[4], C.T. Wotjak[3], R. Landgraf[5], M. Eder[2], U. Schmidt[1]. *[1]Max-Planck-Institut für Psychiatrie, RG Molecular Psychotraumatology, München, Germany; [2]Max-Planck-Institut für Psychiatrie, RG Neuronal Networks Dynamics, München, Germany; [3]Max-Planck-Institut für Psychiatrie, RG Neuronal Plasticity, München, Germany; [4]Max-Planck-Institut für Psychiatrie, MPI of Psychiatry, München, Germany; [5]Max-Planck-Institut für Psychiatrie, RG Behavioral Neuroendocrinology, München, Germany*

Purpose of the study: Past research increasingly points towards a dual role of the hippocampus, where the dorsal hippocampus is involved in memory-related tasks and the ventral hippocampus (VH) modulates emotional processes, including fear and anxiety. Whereas pharmacological targeting of the VH has been associated with anxiogenic effects, reports on VH-mediated anxiolysis are still scarce. Here, we describe for the first time a role for the VH in the anxiolytic effects of neuropeptide S (NPS), a neuropeptide with a high potential as a novel therapy for anxiety disorders such as posttraumatic stress disorder (PTSD).

Methods: NPS uptake in the hippocampus was investigated by intranasal administration of fluorophore-labelled NPS. Effects of NPS treatment on hippocampal expression of candidate proteins involved in the glutamatergic system and in synaptic plasticity were examined by real-time PCR and immunoblotting in C57BL6/N mice as well as in two mouse models of anxiety disorders, namely the high anxiety behaviour (HAB) mice and a mouse model of PTSD. To elucidate the effects of NPS treatment on VH physiology, field potential recordings were conducted in VH slices. The impact of NPS treatment on neuronal activity flow in the VH was measured using voltage-sensitive dye imaging (VSDI). Finally, the role of the VH in behavioural effects of NPS was examined by intra-VH injection of NPS or vehicle and monitoring of locomotion in the open field and of anxiety-like behaviour in the light-dark test and in the elevated plus maze (EPM).

Results: Our experiments showed uptake of fluorescently labelled NPS in the CA1, CA2 and CA3 regions and in the dentate gyrus (DG) of the murine hippocampus, indicating hippocampal expression of the functional NPS receptor (NPSR). On a molecular level, intranasal NPS treatment modulates hippocampal expression of the GluR1 subunit of AMPA receptors, of the glutamate transporter Glt-1 and of synapsin isoforms differentially in C57BL6/N mice and in HAB mice [1]. Additionally, NPS increases hippocampal synapsin expression in the mouse model of PTSD, where synapsin expression had previously been found decreased [2]. On a physiological level, intranasal administration of NPS led to decreased paired-pulse facilitation and long-term potentiation (LTP) at CA3-CA1 synapses in C57BL6/N mice as well as in HAB mice. Moreover, in the VSDI assay, bath application of NPS to VH slices resulted in weakened stimulus-evoked activity flow from DG to CA1. Finally, intra-VH injection of NPS significantly decreased anxiety-like behaviour on the EPM.

Conclusions: Here, we identified the VH as a novel target region of NPS that had not been previously associated with NPS-mediated anxiolysis. In the hippocampus, NPS treatment may contribute to modulation of hippocampal synaptic plasticity by regulating expression of proteins involved in the glutamatergic circuit and in synaptic plasticity. Furthermore, we prove that targeting the VH is also sufficient to mediate NPS-elicited anxiolysis in vivo, possibly by modulating amygdala activity via bidirectional connections to and from the ventral CA1 region. In conclusion, our data point towards an important role of the hippocampus in mediating anxiolytic effects of NPS.

Reference(s)

[1] Ionescu, I.A., Dine, J., Yen, Y.-C., Buell, D.R., Herrmann, L., Holsboer, F., Eder, M., Landgraf, R., Schmidt, U., 2012. Intranasally administered Neuropeptide S (NPS) exerts anxiolytic effects following internalization into NPS receptor-expressing neurons. Neuropsychopharmacology 37, 1323–37.

[2] Herrmann, L., Ionescu, I.A., Henes, K., Golub, Y., Wang, N.X.R., Buell, D.R., Holsboer, F., Wotjak, C.T., Schmidt, U., 2012. Long-lasting hippocampal synaptic protein loss in a mouse model of posttraumatic stress disorder. PLoS One 7, e42603.

P.1.012 Stress-induced vulnerability of presynaptic glutamatergic terminals and effect of desipramine

N. Nava[1]*, M. Popoli[2], L. Musazzi[2], G. Wegener[1], J.R. Nyengaard[3]. [1]*Center for Psychiatric Research, Aarhus University, Aarhus, Denmark;* [2]*Laboratory of Neuropsychopharmacology and Functional Neurogenomics, University of Milano, Milano, Italy;* [3]*Stereology & Electron Microscopy Laboratory, Aarhus University, Aarhus, Denmark*

Background: Growing evidence has highlighted a deep impairment in stress-related disorders of limbic structures such as amygdala, hippocampus and prefrontal cortex, where glutamatergic transmission is considered to be prevalent [1,2].

A number of studies have shown the cumulative effects of stress and its major mediators, glucocorticoids, on brain volume and dendritic remodelling, in both humans and rodents. Nevertheless, very little is known on the ultrastructural changes exerted by behavioural stress on glutamatergic synapses responsible for neuronal communication. Excitatory synapses provide synaptic communication by neurotransmitters which are stored within the presynaptic terminal in morphologically distinct pools of vesicles, namely the readily-releasable pool of vesicles (RRP), docked to the active zone and ready for release, and the reserve pool of vesicles. When a neurotransmitter is released, exchange of information takes place through interaction of glutamate with receptors sitting on the post-synaptic density.

Alterations of such synaptic ultrastructure might result in impairment of glutamatergic release and transmission.

Aim: The main goal of the present study was therefore to shade light on the consequences of acute behavioural stress and chronic antidepressant treatment on the ultrastuctural features of asymmetric terminals, commonly regarded as excitatory, within the medial prefrontal cortex (mPFC).

Methods: Rats were treated chronically with either desipramine (DMI) or vehicle (2 weeks). At the end of the treatment, animals were subjected to acute Foot-Shock (FS) stress and soon after they were deeply anaesthetised and perfused. mPFC subareas (prelimbic area, dorsal anterior cingulate and medial precentral area) were identified, based on their noticeable cytoarchitectural characteristics, and overall volume quantified with the Cavalieri estimator [3]. Through serial section electron microscopy, asymmetric synapses were identified and the number of vesicles in the readily-releasable pool (RRP) and the reserve-pool estimated. Extension of the post-synaptic density as well as of the active zone area was measured; presynaptic terminal volume was also assessed.

Results: Acute behavioural stress induced a strong increase in the number of vesicles docked to the presynaptic membrane and ready for release, which was prevented by chronic treatment with DMI. Post-synaptic density area was strongly reduced after acute FS-stress; previous treatment with DMI only partially prevented this change. Moreover, following acute stress, the volume of presynaptic glutamatergic terminals was reduced. No effect was observed on the volume of medial prefrontal cortex.

Conclusions: In the present study we showed that acute stress was able to significantly change the ultrastructure of glutamatergic terminals, by inducing a large increase in the number of the RRP vesicles, as well as decreasing the area of the post-synaptic density and the volume of the whole presynaptic terminal. Chronic treatment with DMI seemed to only partially prevent such stress-induced changes. Identifying the effects of stress on excitatory transmission may provide further knowledge for developing drugs directly targeting the glutamatergic system.

Reference(s)

[1] Musazzi, L., Milanese, M., Farisello, P., Zappettini, S., Tardito D., Barbiero, V.S., Bonifacino T., Mallei A., Baldelli P., Racagni G., Raitieri M., Benfenati F., Bonanno G., Popoli M., 2010. Acute stress increases depolarization-evoked glutamate release in the rat prefrontal/frontal cortex: the dampening action of antidepressants. PloS One 5(1):e8566.

[2] Ansell E.B., Rando K., Tuit K., Guarnaccia J., Sinha R., 2012. Cumulative adversity and smaller gray matter volume in medial prefrontal, anterior cingulate, and insula regions. Biol Psychiatry 72:57–64.

[3] Van Eden C.G., Uylings H.B. 1985. Cytoarchitectonic development of the prefrontal cortex in the rat. J Comp Neurol 241:253–67.

P.1.013 Chronic (−)cannabidiol produces antidepressant-like effects in bulbectomised mice, acting on 5-HT_{1A} and CB_1 receptor functionality

R. Linge[1]*, A. Pazos[1], A. Diaz[1]. [1]*University Cantabria/ CIBERSAM, Instituto de Biomedicina y Biotecnologia de Cantabria, Santander, Spain*

Depression is one of the most prevalent psychiatric disorders in our society. The classic antidepressant drugs, such as the selective serotonin reuptake inhibitors (SSRIs), act raising the monoamine levels in different regions. In

spite of the fact that >90% these antidepressants enhance monoaminergic transmission by blocking neurotransmitter reuptake, the fast neurotransmitter increase in presynaptic areas activates autoreceptors such as the 5-HT_{1A}, inhibiting neuronal firing. Thus, the therapeutic effect is only obtained after some weeks of sustained treatment.

As the classic monoaminergic hypothesis fails to explain all the features of the pathology, other hypotheses have been proposed involving other neurotransmitter receptors as the cannabinoid receptors.

In this sense, the major non-psychoactive constituent of marihuana, (−)cannabidiol (CBD), has been recently proposed as a potential therapy for mood disorders [1]. The acute administration of this drug has been proven to have an anxiolytic effect. However, the consequences of chronic treatment, particularly in an animal model of depression, remain unknown.

In this study we have tested the effect of chronic treatment with CBD in the bilateral olfactory bulbectomy (OBX) mice model of comorbid depression and anxiety, both in behavioural paradigms and in the functionality of CB_1 and 5-HT_{1A} receptors in different brain areas.

Materials and Methods: Mice were bulbectomised or sham operated and after a recovery period of one month, they received CBD or vehicle resulting into four experimental groups. CBD 50 mg/kg/day was administered intraperitoneally for 3 days followed by CBD 10 mg/kg/day for 11 days. Aversive open field and sucrose intake tests were conducted for behavioural characterisation at the end of the treatment. The animals were sacrificed and the brains were removed, frozen and cut into 14 μm slices. G-protein coupling to CB_1 and 5-HT_{1A} receptors was analysed by [^{35}S]GTPγS binding in brain areas relevant for depressive pathology, using WIN55,212–2 and 8-OH-DPAT agonists, respectively.

Results: CBD treatment prompted a decrease in peripheral hyperactivity of OBX mice in the open field, but no modification was reported in the total locomotor activity of sham counterparts. As for the central activity, no significant changes were noticed, either in OBX or in sham animals. In the sucrose intake test, OBX mice exhibited a decrease in sucrose consumption, resembling an anhedonic state that was reversed by two weeks CBD treatment. Regarding receptor functionality, 8-OH-DPAT-stimulated [^{35}S]GTPγS autoradiography revealed that 5-HT_{1A} functionality was decreased in OBX limbic areas, such as amygdala, hippocampus and entorhinal cortex, and it was reverted by chronic administration of CBD. On the other hand, WIN55,212–2-stimulated [^{35}S]GTPγS autoradiography showed an increased CB_1 functionality in hypothalamus of the OBX group compared to their sham counterparts, and CBD restored the normal activity.

Conclusions: Taken together, these results indicate that CBD improves the depression-like features of OBX mice, acting like classic antidepressant drugs. The modulation of 5-HT_{1A} and CB_1 receptors functionality in limbic areas could underlie these effects.

Reference(s)

[1] El-Alfy AT, Ivey K, Robinson K, Ahmed S, Radwan M, Slade D, Khan I, ElSohly M, Ross S, 2010. Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from Cannabis sativa L. Pharmacol Biochem Behav. 95(4):434–42.

P.1.014 Intracerebroventricular administration of interleukin-1β elevates brain kynurenic acid and disrupts prepulse inhibition in C57BL/6 mice

M. Larsson[1]*, L. Schwieler[1], G. Engberg[1], S.B. Powell[2], S. Erhardt[1]. *[1]Karolinska Institute, Physiology and Pharmacology, Stockholm, Sweden; [2]University of California, Department of Psychiatry, San Diego, USA*

Purpose: Patients with schizophrenia and bipolar disorder display elevated central levels of the pro-inflammatory cytokine interleukin-1β (IL-1β) [1] and of the endogenous NMDA receptor antagonist kynurenic acid (KYNA) [2]. Both KYNA and IL-1β are predominantly elevated in those bipolar patients that have had a psychotic episode. Moreover, in similarity with the psychotomimetic compounds ketamine and PCP, pharmacologically elevated levels of KYNA in rodents have been shown to disrupt prepulse inhibition (PPI) [3]. The aim of the present study was to investigate if IL-1β influences the synthesis of brain KYNA in mice and if administration of IL-1β affects PPI.

Methods: Adult C57BL/6 mice were injected intracerebroventricularly (i.c.v.) with 0.5, 1, 5, or 10 ng of IL-1β in PBS under isoflurane anaesthesia. Control animals recieved vehicle only. Six hours post-injection the animals were swiftly sedated with isoflurane and then euthanised by cervical dislocation and their brains were harvested. KYNA was then quantified by means of reversed phase high performance liquid chromatography. Another cohort of mice received i.c.v. injections of 0.5, 5, or 50 ng IL-1β and were tested for PPI deficits at several time points post-injection.

Results: Administration of 0.5 ng IL-1β, but not 1, 5, or 10 ng, significantly elevated brain KYNA levels compared to vehicle (9.49±1.88 nM vs. 3.40±0.51 nM, one-way ANOVA with Bonferroni's post hoc test, $F(4,30) = 4.737$,

$p < 0.05$). I.c.v. administration of IL-1β significantly disrupted PPI at the lowest dose only (0.5 ng).

Conclusion: Present results support the hypothesis that IL-1β and KYNA are important players in the pathophysiology of psychotic diseases, such as schizophrenia and bipolar disorder. Notably, only administration of the lowest dose IL-1β had a PPI disruptive effect, indicating that this effect may be mediated by the increased brain KYNA concentrations observed at this dose. Present data are also in line with recent in-vitro data from our laboratory showing that IL-1β, by induction of tryptophan 2,3-dioxygenase, increase KYNA production in human cortical astrocytes.

Reference(s)

[1] Söderlund, J., Schröder, J., Nordin, C., Samuelsson, M., Walther-Jallow, L., Karlsson, H., Erhardt, S., Engberg, G., 2009. Activation of brain interleukin-1beta in schizophrenia. Mol Psychiatry 14, 1069–71.

[2] Erhardt, S., Blennow, K., Nordin, C., Skogh, E., Lindström, LH., Engberg, G., 2001. Kynurenic acid levels are elevated in the cerebrospinal fluid of patients with schizophrenia. Neurosci. Lett. 313, 96–98.

[3] Erhardt, S., Schwieler, L., Emanuelsson, C., Geyer, MA., 2004. Endogenous kynurenic acid disrupts prepulse inhibition. Biological Psychiatry. 56, 255–260.

P.1.015 Time-dependent adaptations at the level of dopamine D2 receptor in stress-resilient rats

D. Zurawek[1]*, A. Faron-Gorecka[1], M. Kusmider[1], M. Kolasa[1], P. Gruca[2], M. Papp[2], M. Dziedzicka-Wasylewska[1]. *[1]Polish Academy of Sciences, Department of Biochemical Pharmacology, Krakow, Poland; [2]Polish Academy of Sciences, Department of Behavioural Pharmacology, Krakow, Poland*

Recent research has focussed on the mechanisms that underlie the susceptibility to stress. Long-lasting mild stress, in contrast to single short-term traumatic event, may be a more important factor leading to depression. Few studies have examined the molecular differences characteristic for stress-resilient individuals [1] therefore, factors that contribute to stress-resilience may be worth of investigation. Stress exerts strong influence on mesolimbic dopamine system and elicits specific responses depending on duration and type of aversive stimuli [2]. Therefore, the main purpose of this study was to examine stress-induced changes in mesoaccumbal dopamine D2 mRNA expression and D2 receptor protein density in two groups of animals with different behavioural responses to CMS paradigm.

We used Chronic Mild Stress [3], a well-established behavioural animal model of depression, in which male rats were subjected to unpredictable, variable stress conditions for 2 and 5 consecutive weeks. After 2 weeks of the CMS we identified three different groups of animals: 1. control, 2. stress-reactive which significantly decreased drinking of a palatable sucrose solution ($F_{2,29} = 14.74$, $p < 0.005$ vs. control) and 3. stress-resilient animals with unaltered drinking profile ($F_{2,29} = 14.74$, $p > 0.05$ vs. control). Similar groups were identified after 5 weeks of the CMS: 1. control, 2. stress-reactive ($F_{2,29} = 10.79$, $p < 0.05$ vs. control) and 3. stress-resilient ($F_{2,29} = 10.79$, $p > 0.05$ vs. control). [^{3}H]Domperidone was used as a ligand to label dopamine D2 receptors in nucleus accumbens (Nacc) shell and core, and ventral tegmental area (VTA). In situ hybridisation method was used to examine dopamine D2 receptor mRNA changes in all tested brain regions. Data were analysed using appropriate ANOVA analysis of variances followed by Bonferroni's post hoc test.

Table 1. The effect of 2 and 5 weeks of CMS on specific [^{3}H]domperidone binding in various regions of the rat brain

	2 weeks CMS			5 weeks CMS		
	Control	Stress-reactive	Stress-resilient	Control	Stress-reactive	Stress-resilient
Nacc shell	45.69±3.937	34.14±4.236	19.95±2.829***	36.44±4.690	19.88±2.827**	23.45±2.802*
Nacc core	46.70±4.649	32.49±3.979	21.44±3.097***	45.59±7.104	23.42±3.506*	32.08±4.228
Medial VTA	46.45±4.732	43.55±7.477	30.72±4.740	34.99±4.522	17.49±1.653*	35.02±5.901
Lateral VTA	44.18±4.474	40.69±6.720	20.59±3.019**	24.35±2.361	13.10±1.822*	28.11±5.271

Data represent the mean±SEM; n = 10 (fmol/mg tissue).
*p < 0.05, **p < 0.01, ***p < 0.001 vs. control.

CMS affected the mesolimbic dopamine circuit in stress-resilient animals after 2 weeks, and in stress-reactive group of rats after 5 weeks, as observed by a decrease in the level of dopamine D2 receptor protein without alterations in D2 mRNA expression. The increase in D2 mRNA expression returned the dopamine D2 receptor density to control levels in stress-resilient rats after 5 weeks of CMS, while that effect was not observed in stress-reactive animals.

Conclusions: Despite earlier blunting, stress-resilient animals, but not stress-reactive animals, effectively adapted to the extended stress and coped with it by increasing dopamine D2 receptor mRNA expression and protein density in mesoaccumbens system after 5 weeks of stress. Stress-reactive rats did not show such adaptations or it may be delayed, leading to anhedonia, a core symptom of depression.

Reference(s)

[1] Blugeot A, Rivat C, Bouvier E, Molet J, Mouchard A, Zeau B, Bernard C, Benoliel JJ, Becker C (2011) Vulnerability to depression: from brain neuroplas-

ticity to identification of biomarkers. J Neurosci 31(36):12889–99.
[2] Cabib S, Giardino L, Calzá L, Zanni M, Mele A, Puglisi-Allegra S (1998) Stress promotes major changes in dopamine receptor densities within the mesoaccumbens and nigrostriatal systems. Neuroscience 84(1):193–200.
[3] Willner P, Towell A, Sampson D, Sophokleous S, Muscat R (1987) Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 93(3):358–64.

P.1.016 Modulation of dopamine D_{2L} and D_3 receptor signalling and cell surface-cytosol trafficking by dysbindin in CHO cells

N. Schmieg[1]*, C. Rocchi[2], R. Maggio[2], M.J. Millan[1], C. Mannoury la Cour[1]. [1]*Institut de Recherche Servier, Unité Recherche et Découverte Neuroscience, Croissy-sur-Seine, France;* [2]*University of L'Aquila, Biotechnological and Applied Clinical Sciences Department, L'Aquila, Italy*

Purpose of the studies: Schizophrenia is a severe neuropsychiatric disorder with many risk factors, both environmental and genetic. As regards genetic factors, dystobrevin binding protein 1 (dysbindin) is a susceptibility gene and its levels are reduced in schizophrenia [1]. Further, genetically reducing dysbindin expression enhanced cell surface expression of D_2 receptors in frontal cortex [2]. In view of these observations suggesting altered control of dopaminergic transmission in schizophrenia by dysbindin, we investigated the influence of dysbindin overexpression on signalling and cell surface localisation of human (h) dopamine D_{2L} versus D_3 receptors stably expressed in CHO cells.

Methods and Results: CHO cells stably expressing D_{2L} or D_3 receptors (B_{max} (in fmol/mg protein) 8283±438 and 9766±347, respectively) were transiently transfected with dysbindin (3 μg) with no significant effect on B_{max} values. Employing the non-membrane penetrant radioligand, [^{3}H]-methoxysulpride, it was shown that in dysbindin-treated compared to control cells (100%) there was a reduction in the density of hD_{2L} and hD_3 receptors to 61±5 and 59±4%, respectively, without any change in radioligand affinity (K_D): 0.22±0.05 and 0.23±0.05, for hD_{2L} and hD_3 receptors, respectively. In line with these data, using Alphascreen Surefire™ assays, dysbindin overexpression was found to markedly decrease the inhibitory effect of DA stimulation on forskolin-activated adenylyl cyclase activity. As compared to control values (defined as 100%), values in the presence of dysbindin were 62±7% and 41±5% for hD_{2L} and hD_3 receptors, respectively – as compared to 98±6% for D1 receptors, which were not affected in a control study. As shown by Western Immunoblotting, in the presence of dysbindin, and as compared to control (100%) values, the maximal effect of DA on Akt (Ser473 and Thr308), GSK-3β (Ser9) and ERK1/2 phosphorylation at hD_{2L} receptors was 42±3, 58±5 and 48±3% respectively and, at hD_3 receptors, it was 41±4, 53±3 and 22±4%, respectively. Despite these alterations in maximal effect there was, by contrast, little or no change in the potency of DA for all measures of hD_{2L} and hD_3 receptor activation. Finally, employing the inhibitor of clathrin/caveolar-mediated receptor internalisation, methyl-beta-cyclodextrin (MβCD), we acquired evidence that this mechanism may be involved in the influence of dysbindin upon signalling and membrane-cytosol shuttling at both hD_{2L} and hD_3 receptors.

Conclusions: These data show that overexpression of dysbindin markedly reduces the efficacy (but not potency) of DA-mediated signalling at hD_{2L} and hD_3 receptors as concerns both inhibition of adenylyl cyclase and recruitment of Akt/GSK-3β and ERK1/2 pathways. These observations were parallelled by decreases in cell surface expression of hD_{2L} and hD_3 receptors and may, at least partially, reflect a decrease of receptor density involving clathrin/caveolar-mediated internalisation. These data support studies of dysbindin silencing in suggesting that abnormalities in dysbindin control of D_3 and D_{2L} receptor signalling and localisation may be related to the pathogenesis and symptoms of psychosis.

Reference(s)

[1] Ghiani C.A. and Dell'Angelica E.C., 2011. Dysbindin-containing complexes and their proposed functions in the brain: From zero to (too) many in a decade. ASN neuro 3. pii: e00058.
[2] Ji, Y., Yang, F., Papaleo, F., Wang, H.X., Gao, W.J., Weinberger, D.R., Lu, B., 2009. Role of dysbindin in dopamine receptor trafficking and cortical GABA function. Proc Natl Acad. Sci. 106, 19593–19598.

P.1.017 Histaminergic regulation of presumed serotonin neurons in the dorsal raphe nucleus

K. Panetta[1]*, D. Belelli[1], J. Lambert[1]. [1]*University of Dundee, Division of Neuroscience, Dundee Scotland, United Kingdom*

Introduction: Sleep is essential for our cognitive and physical wellbeing, yet 25% of the UK population

suffer from sleep disorders which can have major consequences on their daily lives. Neural control of the sleep-wake cycle results from the complex interaction of neurotransmitters systems, which arise from anatomically and chemically-defined brain structures. Serotonergic and histaminergic neurons of the dorsal raphe nucleus (DRN) and tuberomammillary nucleus (TMN) respectively are proposed to be wake-promoting neurons, although how they act in concert to influence behavioural state is not well understood [1]. H_1 receptor antagonists and certain selective serotonin (5-HT) receptor antagonists have been shown promote sleep in humans [2,3] highlighting the possible importance of these compounds as sleep therapeutics. The purpose of this study was to utilise electrophysiological techniques to better understand how 5-HT neurons within the DRN are regulated by histamine.

Methods and Results: Firstly extracellular single-unit recordings were performed from putative 5-HT neurons within mouse brain slices containing the DRN. The bath application of 10 μM histamine caused an increase in the firing frequency of all cells tested. Secondly whole-cell voltage-clamp recordings ($V_H = -60$ mV) were performed from putative 5-HT neurons within mouse brain slices containing the DRN. Under these recording conditions, the bath application of 10 μM or local pressure delivery of 300μM, (5–10 ms, 10psi) histamine caused an inward current associated with increased membrane noise, indicative of the opening of associated ion channels. To identify the receptors underlying the histamine-evoked current, the effects of selective ligands of the histamine receptor subtypes (H_1, H_2 and H_3 receptors (Rs) known to be present in the DRN were investigated. The bath application of the H_1R antagonist, histabudifen (20–60μM) completely abolished the histamine-induced inward current. In contrast, both the H_2R antagonist, ranitidine (10μM), and the H_3R antagonist thioperamide (1μM) had no effect on the histamine-induced current. Interestingly, the bath application of either of two structurally distinct H_1 inverse agonists, mepyramine (300nM) and dimethendene (100nM) also inhibited the histamine-induced inward current, but additionally caused an outward shift in the holding current thus, suggesting the presence of an endogenous tonic histaminergic conductance. However, the competitive antagonist histabudifen while blocking the histamine-evoked response did not produce an outward current, thus suggesting H_1Rs to be constitutively (i.e. spontaneously) active rather than being gated by ambient levels of histamine.

Conclusions: Collectively, these results demonstrate that histamine is able to modulate presumed 5-HT neurons and moreover that a histamine tone is present in the DRN. Importantly, the findings reported here provide the first example of a spontaneous H_1 receptor activity in the DRN. Future studies will assess the physiological relevance of this constitutive activity **in vivo** to explore whether H_1 inverse agonists may be used as therapeutic agents to treat sleep disorders.

Reference(s)

[1] Saper, C.B., Scammell, T.E., Lu, J., (2005). Hypothalamic regulation of sleep and circadian rhythms. Nature 437, 1257–1263.

[2] Landolt, H.P., Meier, V., Burgess, H.J., et al. (1999). Serotonin-2 receptors and human sleep: effect of a selective antagonist on EEG power spectra. Neuropsychopharmacology 21, 455–466.

[3] Morairty, S.R., Hedley, L., Flores, J., Martin, R., Kilduff, T.S., (2008). Selective 5HT2A and 5HT6 receptor antagonists promote sleep in rats. Sleep 31, 34–44.

P.1.018 Dissecting the neural mechanisms underlying impaired threat detection in the Ahi1 knockout mouse

A. Lotan[1]*, T. Lifschytz[1], A. Slonimsky[1], S. Abedat[2], Y. Fellig[3], H. Cohen[4], O. Lory[5], G. Goelman[5], B. Lerer[1]. *[1]Biological Psychiatry Laboratory, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [2]Cardiovascular Research Center, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [3]Dept of Pathology, Hadassah-Hebrew University Medical Center, Jerusalem, Israel; [4]Anxiety and Stress Research Unit, Faculty of Health Sciences Ben-Gurion University of the Negev, Beer-Sheva, Israel; [5]MRI Laboratory, Hadassah-Hebrew University Medical Center, Jerusalem, Israel*

Purpose: The Abelson helper integration site (AHI1) gene plays a pivotal role in brain development. Studies by our group, replicated by several others, have demonstrated association of single nucleotide polymorphisms in AHI1 with susceptibility to schizophrenia and autism [1,2]. To elucidate the mechanism whereby alteration in AHI1 expression may be implicated in the pathogenesis of endophenotypes related to severe neuropsychiatric disorders, we studied Ahi1 heterozygous knockout mice ($Ahi1^{+/-}$). We employed behavioural paradigms that model different aspects of schizophrenia, pharmacological challenges, histological studies and resting state functional magnetic resonance imaging (rsfMRI).

Methods: We evaluated the behavioural effects of reduced Ahi1 expression by comparing performance of $Ahi1^{+/-}$ and control wildtype mice ($Ahi1^{+/+}$) on tests

that model positive (prepulse inhibition, MK-801 induced hyperlocomotion), negative (sociability) and cognitive (Morris water maze) aspects of schizophrenia, as well as tests measuring anxiety such as elevated plus maze (EPM), light dark box (LDB) and open field (OF) tests. Serum cortisol and core body temperature were measured following behavioural and pharmacological experiments. We sought structural abnormalities in the brains of $Ahi1^{+/-}$ mice by light microscopy and functional disconnectivity by rsfMRI.

Results: Western blots showed lower Ahi1 protein levels in $Ahi1^{+/-}$ mice, although the difference was significant only in newborns. We did not detect abnormalities on behavioural measures reflecting positive, negative and cognitive domains of schizophrenia. On the other, we observed consistently lower levels of anxiety in $Ahi1^{+/-}$ mice. Compared to $Ahi1^{+/+}$, $Ahi1^{+/-}$ mice spent more time in the OF arena centre ($p < 0.01$), the EPM open arms ($p < 0.01$) and the LDB lit zone ($p < 0.001$). Serum cortisol levels and core body temperature, two highly consistent endophenotypes relevant to the mammalian stress response, were significantly lower in $Ahi1^{+/-}$ than $Ahi1^{+/+}$ mice after exposure to environmental stress in the EPM ($p = 0.02$ and $p = 0.03$, respectively). However, following a direct anxiogenic challenge with caffeine 80 mg/kg i.p., cortisol levels were similar across genotypes. Detailed examination of brain structure using H&E staining revealed normal morphology and cytoarchitecture in $Ahi1^{+/-}$ mice. Surface areas of both the cerebellum, which is grossly distorted in Ahi1-null mice, and the amygdala, which is implicated in threat detection, were not reduced. However, rsfMRI data indicated that a dense network of symmetrical corticolimbic connections and highly significant functional connections between the cerebellum and amygdala, both evident in $Ahi1^{+/+}$ mice, were markedly diminished in $Ahi1^{+/-}$ mice ($p < 0.01$).

Conclusions: Our results indicate that decreased Ahi1 expression is associated with a defect in threat detection and/or in generation of an adequate stress response. Although we did not observe structural brain abnormalities in $Ahi1^{+/-}$ mice, rsfMRI suggested corticolimbic disconnectivity. The present findings imply that neurodevelopmental abnormalities associated with reduced expression of Ahi1 during the fetal and neonatal period may have significant long-term behavioural and functional consequences in the adult. Such a mechanism is consistent with currently postulated neurodevelopmental theories of schizophrenia and autism. The present data provide a translational evidence for the role of AHI1, a gene replicably associated with severe neuropsychiatric disorders, in the pathogenesis of endophenotypes related to threat detection and anxiety.

Reference(s)

[1] Lerer, B., Segman, R.H., Hamdan, A., Kanyas, K.O., Kohn, Y., Korner, M., Lanktree, M., Kaadan, M., Turetsky, N., Yakir, A., Kerem, B., Macciardi, F., 2003. Genome scan of Arab Israeli families maps a schizophrenia susceptibility gene to chromosome 6q23 and supports a locus at chromosome 10q24. Mol Psychiatry 8, 488–98.

[2] Amann-Zalcenstein, D., Avidan, N., Kanyas, K., Ebstein, R.P., Kohn, Y., Hamdan, A., Ben-Asher, E., Karni, O., Mujaheed, M., Segman, R.H., Maier, W., Macciardi, F., Beckmann, J.S., Lancet, D., Lerer, B., 2006. AHI1, a pivotal neurodevelopmental gene, and C6orf217 are associated with susceptibility to schizophrenia. Eur J Hum Genet 14, 1111–9.

P.1.019 Role of the 5-HT_{2A} receptor in the mechanism of action of antidepressant drugs: a translational human–mouse study

G. Quesseveur[1]*, A.C. Petit[2], F. Gressier[2], R. Colle[2], D.J. David[1], A.M. Gardier[1], C. Verstuyft[3], E. Corruble[2], B.P. Guiard[1]. *[1]University of Paris Sud 11, EA 3544 Laboratoire de Neuropharmacologie, Chatenay Malabry Cedex, France; [2]University of Paris Sud 11, INSERM U 669 Department of Psychiatry Bicêtre University Hospital, Le Kremlin Bicêtre, France; [3]Hôpital Bicêtre, Service de Génétique Moléculaire Pharmacogénétique et Hormonologie, Le Kremlin Bicêtre, France*

Selective serotonin reuptake inhibitors (SSRIs) are commonly prescribed for the treatment of major depression. However, 50% of depressive patients do not respond adequately to these medications. Although evidence incriminates the overactivation of the 5-HT_{1A} autoreceptor in this poor response [1], other serotonergic receptors could be recruited to modulate the therapeutic activity of SSRIs [2]. In agreement with this hypothesis, growing arguments suggest that variants at gene encoding the 5-HT_{2A} receptor are associated with antidepressant responses [3] but the results of pharmacogenetic studies in human are still matter of debate.

The purpose of this translational study was to determine the effects of 5-HT_{2A} receptor inactivation on the electrophysiological, neurochemical and behavioural activity of SSRIs in mice. On the other hand, this work evaluated the impact of two putatively functional single nucleotide polymorphisms of the 5-HT_{2A} receptor gene (rs6313 and rs6314) [3] on SSRIs responses in depressed patients from the French cohort METADAP. In particular, we studied the percentage of responders and

the improvement of their depression scores from various relevant scales.

In wild-type 5-$HT_{1A}^{+/+}$ mice, the acute administration of the SSRIs escitalopram or fluoxetine decreased the firing rate of dorsal raphe (DR) 5-HT neurons, while the administration of the selective 5-HT_{1A} receptor antagonist WAY100635 reversed this effect. Remarkably, the electrophysiological response induced by both SSRIs persisted in 5-$HT_{1A}^{+/+}$ mice pretreated with WAY100635 or in 5-$HT_{1A}^{-/-}$ mice thereby demonstrating the involvement of another serotonergic receptor type in the inhibitory activity. The observation that the 5-HT_{2A} receptor antagonist MDL100907 also reversed escitalopram-induced decrease in DR 5-HT neuronal activity indicates that the simultaneous blockade of 5-HT_{1A} and 5-HT_{2A} receptors is required to prevent the acute inhibitory effects of SSRIs upon the serotonergic system. It also suggests that the activity of SSRIs might be enhanced in 5-$HT_{2A}^{-/-}$ mice after chronic treatment. However, the genetic inactivation of the 5-HT_{2A} receptor significantly attenuated the ability of repeated administration of escitalopram or fluoxetine to increase the firing rate of DR 5-HT neurons and reduced their antidepressant-like effects in the tail suspension test or the novelty suppressed feeding paradigm. Finally, the enhancement of adult hippocampal neurogenesis induced by prolonged administration of SSRIs was blunted in 5-$HT_{2A}^{-/-}$ mice.

In depressed patients, rs6313 and rs6314 genetic variants were not associated with SSRIs response. We extended this observation to the fact that separately analysed neither escitalopram nor fluoxetine responses were altered for the rs6313. In marked contrast, a trend toward a lower percentage of improvement of depression score was detected after escitalopram treatment in homozygous individuals for the C allele of the rs6314 ($p = 0.06$).

Altogether, these preclinical and clinical data indicate that a functional variant of the 5-HT_{2A} receptor gene may be associated with a poor SSRIs response resulting, at least in part, from an impairment of serotonergic neurotransmission. Although this study has to be completed by determining the consequence of the C allele on the function/expression of the 5-HT_{2A} receptor in the human brain, these results could be of particular importance to select appropriate antidepressant treatment according to the patient's genotype.

Reference(s)

[1] Gardier AM, Malagié I, Trillat AC, Jacquot C, Artigas F. Role of 5-HT1A autoreceptors in the mechanism of action of serotoninergic antidepressant drugs: recent findings from in vivo microdialysis studies. Fundam Clin Pharmacol. 1996;10(1):16–27.

[2] Quesseveur G, Nguyen HT, Gardier AM, Guiard BP. 5-HT2 ligands in the treatment of anxiety and depression. Expert Opin Investig Drugs. 2012 Nov;21(11):1701–25.

[3] Serretti A, Drago A, De Ronchi D. HTR2A gene variants and psychiatric disorders: a review of current literature and selection of SNPs for future studies. Curr Med Chem. 2007;14(19):2053–69. Review.

P.1.020 The role of CREB/BDNF/TrkB signalling in the zinc deficiency model of depression

U. Doboszewska[1]*, B. Szewczyk[1], M. Sowa-Kucma[1], K. Mlyniec[2], G. Nowak[1]. *[1]Polish Academy of Sciences Institute of Pharmacology, Department of Neurobiology, Krakow, Poland; [2]Jagiellonian University Medical College, Department of Biochemical Toxicology, Krakow, Poland*

Background: Induced zinc deficiency in adult rats significantly decreases the number of progenitor cells and immature neurons in the dentate gyrus of the rat hippocampus. Zinc deficiency, which occurs during both lactation and adulthood, increases hippocampal apoptosis. This suggests that the trace element zinc may be involved in the regulation of hippocampal neurogenesis during the early stages of life through to adulthood [1]. Neurotrophins are an important class of signalling molecules essential for the development of the central nervous system (CNS). Brain-derived neurotrophic factor (BDNF) is the best characterised of these neurotrophins in terms of its role in synaptic plasticity as well as its potential role in the pathology and treatment of a variety of psychiatric disorders. BDNF gene expression is increased by the transcription factor cyclic AMP response-element binding protein (CREB). Mature BDNF signals via the high-affinity tropomyosine-related kinase B receptor (TrkB). BDNF signalling via TrkB receptors divides into three pathways, all of which converge on the transcription factor CREB, which in turn up-regulates gene expression [2].

Aim: To examine whether zinc deprivation induces changes in the protein levels of the phosphorylated forms of CREB (pCREB), BDNF and TrkB in the prefrontal cortex and hippocampus of rats.

Methods: Male Sprague-Dawley rats (5-week-old) were fed a 50 mg Zn/kg (control group) or a 3 mg Zn/kg (zinc-deficient group) diet for four weeks. The protein levels of pCREB, BDNF and TrkB were measured using western blotting.

Results: Four weeks of dietary zinc deprivation resulted in a significant decrease in the protein levels of pCREB,

BDNF and TrkB in the hippocampus but not in the prefrontal cortex. The level of pCREB protein was decreased by 91% whereas the level of BDNF protein was decreased by 73% in the hippocampus of the zinc-deficient rats when compared with that of the control animals. At the same time point a decrease (by 40%) in TrkB protein was observed in the hippocampus of the zinc-deficient group compared to the control group.

Conclusions: The previous studies indicated that dietary-induced zinc deficiency leads to the development of depressive like behaviour (e.g. increase in the immobility time in the forced swim test) and that experimentally induced zinc deficiency might be a model of depression. The present data suggests that pCREB, BDNF and TrkB expression is modulated by zinc deficiency and that the decreased expression of these proteins after dietary zinc deprivation is linked to the depressive like behaviour in the zinc deficiency paradigm. It seems that the hippocampus, the region of the brain that plays a critical role in neurogenesis, is more susceptible to the biochemical changes caused by zinc deficiency than the prefrontal cortex. This is consistent with data obtained during experiments where acute and chronic stress paradigms were found to decrease the expression of BDNF in the rodent hippocampus [1].

Reference(s)

[1] Levenson, C.W., Morris, D., 2011. Zinc and neurogenesis: making new neurons from development to adulthood. Adc Nutr 2, 96–100.

[2] Autry, A.E., Monteggia, L.M., 2012. Brain-derived neurotrophic factor and neuropsychiatric disorders. Pharmacol Rev 64, 238–258.

P.1.021 Chronic administration of haloperidol in rats and its effect on microglial cell density and whole brain weight and volume

P.S. Bloomfield[1]*, O.D. Howes[1], V. de Paola[2]. *[1]MRC Clinical Sciences Centre, Psychiatric Imaging, London, United Kingdom; [2]MRC Clinical Sciences Centre, Neuroplasticity and Disease, London, United Kingdom*

Introduction: Patients with schizophrenia are routinely prescribed antipsychotic medication. Magnetic resonance imaging (MRI) in newly medicated patients has demonstrated how, over a 5 year period, there is an average whole brain structural deficit of 8%, with regional losses of up to 20%, when compared to baseline [1]. Another study investigating schizophrenic patients demonstrate an increase in binding with the [C_{11}]PK11195 positron emission tomography (PET) radiotracer, which acts as a marker of neuroinflammation [2]. While these are changes since the start of medication, it is not possible to differentiate between changes arising from medication and those occurring with the progression of the disease. Here we dose naïve rats with haloperidol (Hal) to test the hypotheses that anitpsychotic drugs induce brain volume loss and microglial activation.

Methods: We used subcutaneous drug pellets to slowly release Hal over a two-week period in randomised mixed cages of control (n = 9) and medicated (n = 9) male Sprague Dawley rats. The drug pellets released a 0.05 mg/kg/day dose, control animals were implanted with a placebo pellet. The dose used is a comparatively low dose in terms of preclinical literature, with a similarly low D_2 receptor occupancy when compared with clinical data (calculated from Samha et al., 2008 [3]).

Following perfusion, brains were dissected for post mortem analysis. The weights of brains were measured and whole brain volumes were calculated using water displacement. The brain tissue was then processed for immunohistochemistry, where changes in microglial cell number were determined to assess neuroinflammatory associated changes.

Results: Here we show that there is a significant reduction in both brain weight and volume in Hal treated animals (2.57 g (±0.088 SEM), 1583.3 mm^3 (±105.4 SEM)) when compared with placebo controls (2.78 g (±0.111 SEM), 2166.7 mm^3 (±153.7 SEM)) ($p < 0.05$, independent samples t-test). Immunohistochemical quantification of colocalised Iba-1 and DAPI stains was used to determine the number of microglial cells in the following 800 μm^2 regions of interest (ROIs); the prefrontal cortex, hippocampus and ventral striatum. There was a consistent increase in microglial number across the 3 regions in drug treated animals (see table), which trended toward significance ($p = 0.07$).

Mean Iba-1 positive cells counts in cortical ROIs

	PFC (±SEM)	Hippocampus (±SEM)	Striatum (±SEM)
Placebo	59.0 (±11.1)	25.3 (±5.1)	69.2 (±16.1)
Haloperidol	62.0 (±12.4)	51.3 (±13.2)	85.3 (±19.4)

Conclusions: The results we have seen here show how a low dose of a typical antipsychotic medication is able to produce significant changes in the brains of naïve rats. Further investigation is required to determine the full effects of these changes and the mechanism involved. An experiment investigating the effects of a higher dose regime is in progress and will be used to investigate inflammatory changes further alongside

cortical morphology. While evaluating the results of this preliminary investigation, the impact of function requires consideration, as the changes we see here may not be detrimental to cognition. We propose further investigation into the impact of such cortical changes on function.

Reference(s)

[1] Thompson, P.M., et al., Mapping adolescent brain change reveals dynamic wave of accelerated gray matter loss in very early-onset schizophrenia. Proceedings of the National Academy of Sciences, 2001. 98(20): p. 11650–11655.
[2] Doorduin, J., et al., Neuroinflammation in schizophrenia-related psychosis: A PET Study. Journal of Nuclear Medicine, 2009. 50(11): p. 1801–1807.
[3] Samaha, A.-N.L., et al., Less Is More: Antipsychotic drug effects are greater with transient rather than continuous delivery. Biological Psychiatry, 2008. 64(2): p. 145–152.

P.1.022 Role of trace amine-associated receptor 1 (TAAR1) in the modulation of the dopaminergic system and cortico-striatal signalling

S. Espinoza[1]*, I. Sukhanov[1], G. Lignani[1], L. Medrihan[1], S. Maggi[1], G. Giannotti[2], F. Fumagalli[2], F. Benfenati[1], V. Tucci[1], R. Gainetdinov[1]. *[1]Italian Institute of Technology, NBT, Genova, Italy; [2]University of Milan, Pharmacology, Milan, Italy*

Purpose of the study: Mammalian Trace Amine Associated Receptor 1 (TAAR1) is a G protein-coupled receptor (GPCR) that is mainly expressed in limbic regions and monoaminergic nuclei, such as ventral tegmental area, dorsal raphe and nucleus coeruleus [1]. There is evidence indicating that TAAR1 is involved in the modulation of dopaminergic system [2]. In mice lacking TAAR1 (TAAR1-KO mice), amphetamine induces more pronounced locomotor stimulation and dopamine release. Moreover, it has been reported that D2 receptor function is altered in TAAR1-KO mice. Dopamine system is involved in many physiological functions and has been implicated in various pathological states such as schizophrenia and Parkinson's disease. Previously, we showed that TAAR1 and D2 could heterodimerise and this interaction could influence TAAR1 signalling in vitro and D2-induced behaviour in vivo [3]. In this study, our purpose was to describe how TAAR1 could influence dopamine system in vivo, in particular we evaluated TAAR1 role in D2-related signalling in the striatum.

Methods used: Binding: the total number of D1 and D2 receptors was evaluated by binding experiments. Western blot: total form and phosphorylated form of various proteins was investigated with the analysis of different brain regions extracts. BRET: β-arrestin2 recruitment to D2L receptor was monitored by using a BRET approach. Electrophysiology: NMDA and AMPA functionality was measured by patch clamp recordings in slices. Behaviour: deficits of cortico-striatal circuitry were assessed using a timing task, namely, fix interval and peak interval.

Results: With binding experiments, we found that D2 but not D1 number was decreased in striatum of TAAR1-KO mice. Moreover, we analysed D2 receptor-related signalling, both G protein-dependent as well as G protein-independent pathway. In striatum of TAAR1-KO mice, a significant reduction in AKT and GSK-3β phosphorylation was found as well as a reduction of β-catenin, with no difference in phosphorylation of DARPP32, ERK and CREB, demonstrating that the AKT/GSK3 pathway was activated. In order to understand the mechanism of this activation, we also studied β-arrestin2 recruitment to D2R using BRET and its role in vivo in striatum of TAAR1-KO mice. While in vitro it seems that TAAR1 does not modulate β-arrestin2 recruitment to D2R, in vivo we found an alteration of the complex AKT/PP2A. Since TAAR1 modulates dopaminergic neurons located in VTA we made investigations in the prefrontal cortex, particularly NMDA receptor functionality. By western blot we noted that NMDA receptor was less phosphorylated at the serine 896 suggesting a reduction in NMDA functionality. By using patch clamp recordings we found that NMDA receptor located in pyramidal neurons in layer V were less active. Finally, we performed a timing task, namely a fix interval and peak interval. While it seems that the timing it is not altered in TAAR1-KO mice, it is evident a deficit in the general performance, suggesting a deficit in cognition and an alteration in cortico-striatal circuits.

Conclusions: These data indicates that TAAR1 is able to modulate dopamine system, in particular D2 receptor functions in striatum, and also prefrontal cortex NMDA functions, neurons excitability and some behaviours related to cortico-striatal pathways.

Reference(s)

[1] Lindemann L and Hoener MC (2005) A renaissance in trace amines inspired by a novel GPCR family. Trends Pharmacol Sci 26(5):274–281.
[2] Sotnikova TD, Caron MG, and Gainetdinov RR, Trace amine-associated receptors as emerging therapeutic targets. Mol Pharmacol 76 (2009) 229–35.
[3] Espinoza S, Salahpour A, Masri B, Sotnikova TD, Messa M, Barak LS, Caron MG, and Gainetdinov RR,

Functional interaction between trace amine-associated receptor 1 and dopamine D2 receptor. Mol Pharmacol 80 (2011) 416–25.

P.1.023 Survival role of embryonal proteins in Alzheimer's disease linked dementia via regulation of oxidative stress and level of catecholamines

K. Yenkoyan[1]*, M. Aghajanov[1]. [1]*Yerevan State Medical University, Department of Biochemistry, Yerevan, Armenia, Republic of*

Purpose of the study: Main event in the pathogenesis of such a neurodegenerative disorder as Alzheimer's disease (AD) is generation and deposition of amyloid beta (Ab). Oxidative stress and generation of free radical species have implications in the formation of Ab and its subsequent neurotoxicity. Degeneration of aminergic brainstem nuclei such as the locus coeruleus which is very vulnerable to the free radicals and the selective exhaustion of catecholamines near the locus coeruleus of brainstem can appear as a potential pathogenesis of AD. That's why our study was focussed to find some parallels between oxidative stress and changes in catecholamines concentration experimental model of AD, and find new biological agents which will be able to regulate them. As a bioregulator we chose the complex of proteoglycans of embryonal genesis (PEG). PEG contains the pool of proteoglycans of embryonal genesis which are associated with alpha-fetoprotein, chorionic gonadotrophin, beta1-glycoprotein, carcinoembryonic antigen, and the carbohydrate antigens Ca-19–9 and Ca-125.

Methods used: The experimental model of AD was made in rats by intracerebroventricular injection of aggregated Ab (fragment 25–35). The animals were divided into five groups: the control group consisted of vehicle-treated animals; the 1st experimental group (PEG-control) was subcutaneously injected with PEG (0.5 mg/100 g) only; the 2nd experimental group was i.c.v. injected with aggregated Ab; the 3rd experimental group was subcutaneously injected with PEG (0.5 mg/100 g) 7 days before Ab injection (group PEG-1); the 4th experimental group was subcutaneously administered PEG (0.5 mg/100 g) 7 days before Ab injection and on the 31st day after it (group PEG-2). Oxidative stress and antioxidant capacity were measured by use of chemoluminescence method (spontaneous, induced by UV and Fe^{2+} ions) in cerebral cortex, hippocampus and brainstem. Adrenergic structures of brainstem were studied by glyoxylic acid condensation using luminescence microscope and accompanied by the HPLC study of norepinephrine (NE) and metabolites of adrenergics.

Summary of results and Conclusion: After injection of Ab received data testify the increase in the level of chemoluminescence (both spontaneous and inducible) in the cerebral cortex, hippocampus and brainstem. On the other hand the increase in intensity of luminescence of catecholamine granules near the locus coeruleus was also detected. At the same time HPLC results show high elevation in concentration of NE in the brainstem. In PEG treated animals all types of chemoluminescence in mentioned brain structures decreased and the antioxidant capacity increased without any significant difference between PEG-1 and PEG-2 groups. Almost no changes of adrenergic structures were shown compared with the control rats in all PEG treated groups. At the same time in PEG-1 group concentration of NE was less than in control, whereas in PEG-2 group it was about control level. It should be certainly pointed that the results of biochemical determination of NE and metabolites of monoamines in brainstem were in unison with the luminescent study. Summarising, it seems that regulation of oxidative stress and disturbance in adrenergic structures can be of importance for neuronal rescue in Alzheimer's pathology and regulation of these both processes can lead to neuroprotection.

P.1.024 Endocannabinoid-mediated plasticity at inhibitory synapses on dopamine cells as a marker of vulnerability to addiction

C. Sagheddu[1]*, M. Melis[1], M. Pistis[1]. [1]*University of Cagliari, Biomedical Sciences, Cagliari, Italy*

Addiction is a psychiatric disorder, whose aetiology involves interaction of inherited predispositions and environmental factors. Both clinical and preclinical findings indicate that there are important genetic variations in vulnerability to drug addiction, and that such differences may be mediated by the same biological mechanisms. Addictive drugs share the properties of being self-administered by laboratory animals, and of activating the brain reward circuitry, which stems from the ventral tegmental area (VTA) where dopamine (DA) cells are located. Endocannabinoids serve as retrograde signalling molecules at many synapses in the brain, including the VTA, and regulate reward seeking by modulating DA signalling. Among synaptic inputs that regulate DA neuron impulse activity, those arising from the newly identified rostromedial tegmental nucleus (RMTg) play a major role [1,2].

In this study we took advantage of significant differences between pairs of lines of rats selectively bred for their voluntary alcohol preference or aversion, that is the Sardinian alcohol-preferring (sP) or nonpreferring (sNP) rat line. We investigated their electrophysiological properties and synaptic plasticity both in vivo and in vitro. Extracellular single unit recordings in anaesthetised rats revealed a difference in baseline firing activity of DA neurons between sP and sNP rats consistent with our previous study [3]. More particularly, sP rats showed an increased spontaneous neuronal activity. Remarkably, this increase was parallelled by a reduced strength of RMTg inputs. This enhanced impulse activity of DA cells in sP rats negatively correlated with the duration of inhibition elicited by electrical stimulation of the RMTg.

Since DA neurons can escape afferent inputs by releasing endocannabinoids, we examined one form of short-term synaptic plasticity mediated by endocannabinoids at inhibitory synapses in the brain that is depolarisation-induced suppression of inhibition (DSI). VTA DA neurons make no exception to this rule and express this form of plasticity. We have found that DSI is differently expressed by two discrete sets of inhibitory synapses arising from rostral and caudal afferents onto VTA DA neurons. This phenomenon is selectively mediated by the endocannabinoid 2-arachidonoylglycerol (2-AG), which activates presynaptic type 1-cannabinoid (CB1) receptors. However, the two discrete DSI do not seem to depend upon differences in CB1 number and/or function, since the dose-response relationship curves showed no differences. On the other hand, we found that the difference in DSI can be ascribed to the rate 2-AG is degraded. Thus, 2-AG by differently depressing inhibitory synapses arising from either rostral or caudal afferents might indirectly alter DA neuron functional state, and enhance the responsiveness of the reward pathway to phasic DA.

Given that sP rats are vulnerable phenotype, and that they possess this endocannabinoid-mediated form of short term plasticity, our results suggest that differences in the equipment of the endocannabinoid system machinery might control specific sources of vulnerability.

Reference(s)

[1] Lecca S, Melis M, Luchicchi A, Muntoni AL, Pistis M., 2012. Inhibitory inputs from rostromedial tegmental neurons regulate spontaneous activity of midbrain dopamine cells and their responses to drugs of abuse. Neuropsychopharmacology. Apr;37(5):1164–76.

[2] Lecca S, Melis M, Luchicchi A, Ennas MG, Castelli MP, Muntoni AL, Pistis M., 2011. Effects of drugs of abuse on putative rostromedial tegmental neurons, inhibitory afferents to midbrain dopamine cells. Neuropsychopharmacology 36(3):589–602.

[3] Melis M, Pillolla G, Perra S, Colombo G, Muntoni AL, Pistis M., 2009. Electrophysiological properties of dopamine neurons in the ventral tegmental area of Sardinian alcohol-preferring rats. Psychopharmacology 201(4):471–81.

Behavioural pharmacology

Lectures

S.02.01 Genetic models of psychiatric disorders: phenotyping mutant mice

J. Waddington[1]*. [1]*Royal College of Surgeons in Ireland, Molecular and Cellular Therapeutics, Dublin, Ireland*

This presentation reviews the application of mutant mouse phenotypes to the study of neuropsychiatric disorders in general and psychotic illness in particular, with schizophrenia as the primary exemplar. It considers these issues at the levels of behavioural, psychopharmacological and cellular phenotypes of putative import for understanding disease processes and drug development. Mutant models appear to be heuristic at two main levels; firstly, by indicating the functional roles of neuronal components thought to be of relevance to the putative pathobiology of psychotic illness, they help resolve overt behavioural and underlying cellular processes regulated by those neuronal components; secondly, by indicating the functional roles of genes associated with risk for psychotic illness, they help resolve overt behavioural and underlying cellular processes regulated by those risk genes. This presentation focusses initially on models of dopaminergic and glutamatergic dysfunction. Then, it considers advances in the genetics of schizophrenia and mutant models relating to replicable risk genes. There is continuing need to address not only numerous technical challenges but also to develop more 'real world' paradigms that reflect the milieu of gene × environment and gene × gene interactions that characterise psychotic illness and its response to antipsychotic drugs. Thus, the presentation extends this discussion by exemplifying two new, variant approaches in mutant mice that may serve as prototypes for advancing understanding of disease processes and drug development. An enduring challenge is the translational relationship between mouse phenotype, clinical psychopathology and, to the extent known, disease pathobiology.

S.02.02 Developmental models of schizophrenia and their behavioural characterisation

K.C.F. Fone[1]*, D.J.G. Watson[1], A. McIntosh[1], M.V. King[1]. [1]*The University of Nottingham, School of Biomedical Sciences, Queen's Medical Centre, Nottingham, United Kingdom*

Schizophrenia is a chronic debilitating neuropsychiatric disorder which affects approximately 1% of the population. Symptoms fall into three categories; positive (such as auditory and visual hallucinations and thought disorder), negative (e.g. emotional blunting, social withdrawal and anhedonia) and cognitive dysfunction (including impaired executive function, working memory and attention). The cause of schizophrenia remains unclear but the risk of developing this disorder is influenced by both genetic and environmental factors. Reliable, predictive animal models of complex psychiatric disorders, such as schizophrenia, are therefore essential both to increase our understanding of its neurobiological basis and to develop drugs with improved efficacy. Animal models offer a more rapid platform to monitor disease progression than in man, the ability to perform invasive monitoring of anatomical and molecular changes that underlie the disease, and to test novel medicine impossible in patients. Numerous animal models of schizophrenia have been produced either by drug-administration, neuronal lesioning, genetic manipulation or exposure to early-life adversity [1]. These include: (i) repeated administration of amphetamine or the NMDA receptor antagonist, phencyclidine, to produce chronic changes in dopaminergic and/or glutamatergic function, (ii) gestational exposure to the antimitotic agent, methylazoxymethanol, to alter neurogenesis, (iii) neonatal ibotenic acid lesion of the ventral hippocampus and (iv) alteration of the early-life environment, whose long-lasting impact on anatomical and behavioural deficits in the resultant rats will be compared. Most rodent models have behavioural phenotype changes which resemble 'positive-like' symptoms of schizophrenia, probably reflecting altered mesolimbic dopamine function, but fewer models also show altered social interaction and learning and memory impairment analogous to the negative and cognitive symptoms, respectively. As the negative and cognitive impairments in schizophrenia are resistant to treatment with current antipsychotics, even after remission

of the psychosis, it is clearly vital that animal models should replicate these changes. All useful animal models should have the appropriate triad of face (symptom homology), construct (replicate the theoretical neurobiological rationale and pathology) and predictive (show the expected pharmacological response to treatment by known antipsychotics and potential new compounds) validity to the clinical disorder being modelled. For schizophrenia a suitable constellation of behavioural and neurochemical abnormalities would include postpubertal onset, loss of hippocampal and cortical connectivity and function, limbic dopamine dysregulation, cortical glutamatergic hypofunction, vulnerability to stress, abnormal response to reward, social withdrawal and cognitive impairment. These features will be discussed using the neurodevelopmental rat model of schizophrenia produced by rearing rats in social isolation from weaning [2]. A perplexing problem is how to evaluate core symptoms of mood disorders like feelings and thoughts which are uniquely human traits. A further problem is that there is no current 'gold standard' medication available to treat symptoms which can be used as a definitive positive control in preclinical studies. Furthermore, many of the current antipsychotics may have a small therapeutic window of effect before sedation and other non-specific motor suppressant actions confound interpretation in tasks designed to assess negative and cognitive function. Within a colony rats display a defined social structure and develop a hierarchy which plays a critical impact on their development. Thus social deprivation of rat pups from the age of weaning (by placing them in separate cages from littermates) alters brain development and causes behavioural deficits at adulthood which are unaltered by social re-integration in later life. Post-weaning social isolation of rats induces locomotor hyperactivity, enhanced responses to novelty (neophobia), sensorimotor gating deficits and learning and memory deficits. Collectively, these behavioural changes have been termed the "isolation syndrome" and several of these resemble core symptoms of schizophrenia. The hyperactivity may relate to changes in mesolimbic dopamine activity which reflect changes in positive symptoms seen in schizophrenia and are accompanied by an increased proportion of striatal D_2^{High} receptors which may contribute to dopamine supersensitivity demonstration the utility of animal models to determine underlying molecular mechnaisms of the behavioural impact observed. Furthermore when isolation is combined with neonatal treatment with phencyclidine in the form of a 'dual adverse hit' resultant offspring show reduced social interaction with an age- and weight-matched conspecific which may serve as a very useful index of the negative symptoms of schizophrenia that has proved very difficult to model in rodents. Several of the neurobiological changes in the brain of isolation-reared rats resemble some of those seen in patients with schizophrenia, suggesting the model has good construct validity. For example, isolation reared rats show a selective reduction in prefrontal cortex volume measured by MRI, accompanied by decreased dendritic spine density and altered spine morphology and a reduction in parvalbumin positive GABAergic chandelier cartridges on interneurones in the prefrontal cortex. Cytoskeletal alterations and a selective loss of parvalbumin and calbindin-containing interneurones have also been identified in the hippocampus of isolation reared rats, similar to changes seen in schizophrenic patients. This presentation will describe the behavioural and neurochemical abnormalities and findings from treatment with current antipsychotics (such as risperidone) or novel drugs acting on dopaminergic [3] and non-dopaminergic targets, such as 5-HT_6 receptor antagonists, mGluR2/3 agonists, memantine and nicotinic agonists, to reverse these changes and illustrate the predictive validity of the paradigm.

Reference(s)

[1] Jones, C.A., Watson, D.J.G. & Fone K.C.F. 2011. Animal models of schizophrenia. Brit J Pharmacol 164, 1162–1194.

[2] Fone, K.C.F. & Porkess, M.V. 2008. Behavioural and neurochemical effects of post-weaning social isolation in rodents – relevance to developmental neuropsychiatric disorders. Neurosci & Biobehav Rev 32, 1087–1102.

[3] Watson, D.J.G., Loiseau, F., Ingaleseni, M., Millan, M.J. & Fone, K.C.F. 2012. Selective blockade of dopamine D3 receptors enhance while D2 receptor antagonism impairs social novelty discrimination and novel object recognition in rats: a key role for the prefrontal cortex. Neuropsychopharmacol 37, 770–786.

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Posters

P.2.001 Citalopram given to stressed and control pregnant rats causes sex-dependent changes in behaviour and CRH mRNA expression in their offspring

I. Zohar[1]*, M. Weinstock[1]. [1]*Hebrew University of Jerusalem, Pharmacology, Jersualem, Israel*

Rationale: Exposure to stressful life events during pregnancy increases the likelihood of anxiety and depression in the young and adult offspring of humans and experimental animals. An increasing number of pregnant women exposed to stress and/or suffering from depression are being treated with antidepressant drugs that are selective serotonin reuptake inhibitors (SSRIs) but it is not clear whether any benefits of treatment outweigh the potential adverse effects on the offspring.

Objectives: To compare the effect of maternal treatment with citalopram, a frequently prescribed SSRI, on behaviour in prenatally-stressed (PS) and control rats of both sexes. Since we had previously found that the increased anxiety induced by prenatal stress was associated with sex-dependent alterations in the gene expression of members of the corticotropin-releasing hormone (CRH) family in the amygdala and the paraventricular nucleus in the hypothalamus (PVN) [1] we also determined how these were affected by maternal citalopram treatment.

Methods: Citalopram (10 mg/kg/day) was administered in the drinking fluid to pregnant individually housed rats from day 7 of gestation until after their pups were weaned when aged 21 days. Half of the pregnant rats were subjected to once-daily varied stress from day 14–21 of gestation. Each stressor was shown to elevate maternal plasma COR more than threefold and there was no sign of adaptation to them. Offspring behaviour and gene expression of the CRH family, CRHR1, CRHR2 and CRH binding protein (CRH-BP) was evaluated in the amygdala and the PVN in adulthood.

Results: Stressed females were more anxious than controls three weeks after parturition and citalopram treatment prevented the increased anxiety. Adult PS males and females were also more anxious than controls in the elevated plus maze and showed depressive-like behaviour in the forced swim test. Maternal citalopram treatment reduced anxiety in PS females but not in males and did not affect depressive-like behaviour in either sex. Moreover, citalopram treatment of control mothers induced depressive-like behaviour in the offspring of both sexes. In both the amygdala and the PVN, PS females had a higher expression of CRH mRNA and lower expression of CRHR2 mRNA than controls and males had a lower expression of CRH-BP and of CRHR2 which could explain their increased anxiety. Citalopram treatment had little effect on CRH signalling in PS males in both brain areas but reduced the expression of CRH, its binding protein and receptors in PS females in the amygdala and increased CRHBP in the PVN.

Conclusion: Although citalopram can prevent anxiety in stressed rat mothers it does not normalise behaviour of their offspring and even induces anxiety in those of control rats. It is possible that blockade of the 5HT transporter by citalopram at a critical time during development could lead to reduced activation of postsynaptic 5HT1A receptors which changes CRH signalling in the amygdala and the PVN and induces depressive-like behaviour in adulthood.

Reference(s)

[1] Zohar I, Weinstock M., 2011. Differential effect of prenatal stress on the expression of corticotrophin-releasing hormone and its receptors in the hypothalamus and amygdala in male and female rats. J Neuroendocrinol 23(4):320–328.

P.2.002 Behavioural, molecular and glutamatergic changes in a developmental model of schizophrenia, and reversal by a 5-HT_6 receptor antagonist

M.V. King[1]*, O. Negm[2], P. Tighe[2], S. Knapp[1], P. Wigmore[1], K.C.F. Fone[1]. [1]*The University of Nottingham, School of Biomedical Sciences, Nottingham, United Kingdom;* [2]*The University of Nottingham, Molecular Medical Sciences, Nottingham, United Kingdom*

Post-weaning social isolation in the rat, a neurodevelopmental model of schizophrenia, impairs memory in several tasks which map to distinct cognitive domains deficient in this disorder, including hippocampal-dependent novel object discrimination (NOD) and conditioned emotional responses (CER). These impairments are accompanied by structural alterations within the hippocampus, including reduced cell proliferation and survival, dendritic length and spine density. Reduced parvalbumin and calbindin-containing GABA interneurones and decreased expression of Vesicular Glutamate Transporter 1 (VGLUT1) indicate disruption of inhibitory and excitatory neurotransmission and also resemble changes seen in schizophrenia [1]. In contrast 5-HT_6 receptor antagonists reverse drug-induced deficits in NOD and CER, and elevate hippocampal cell proliferation and glutamate efflux in group-housed rats [2],

so may potentially reverse isolation-induced cognitive impairments. The aims of this study were to utilise protein microarray, immunohistochemical and glutamate microsensor techniques to further characterise isolation-induced hippocampal changes, and examine the ability of a 5-HT_6 receptor antagonist, SB-399885, to reverse the neurochemical and behavioural alterations in this model.

Male Lister hooded rats (University of Nottingham or Charles River UK) were weaned on post-natal day 21–24 and housed individually or in groups (3–4). Rats received minimal handling until assessment (starting 5–6 weeks later) of locomotor activity, NOD, and contextual and cue-mediated CER. Rats received i.p. vehicle (1% Tween 80, 1 ml/kg) or 10 mg/kg SB-399885 on six occasions (n = 11/housing-treatment combination) either 30 min prior to testing or immediately after CER acquisition to preclude potential nociceptive/affective confounds. Rats were killed 24 h after the final injection and hippocampi collected for quantification of VGLUT1–3 expression using Western blots, cell proliferation using Ki67 immunohistochemistry and expression of intracellular signalling molecules using protein microarray. Glutamate signalling in hippocampal slices from separate groups of drug-free rats (n = 7/housing condition) was measured using enzyme-coated microsensors.

Isolation-rearing prevented discrimination of the novel object during NOD ($P > 0.05$ versus familiar, whereas group-housed $P < 0.001$), such that the discrimination ratio (DR) was significantly reduced from 0.68 ± 0.02 to 0.56 ± 0.03 ($P < 0.01$). Isolation-rearing also reduced cue-mediated freezing during CER retention from 245 ± 12s to 162 ± 24s ($P < 0.01$), without affecting contextual freezing. SB-399885 restored NOD ($P < 0.001$ versus familiar) such that the DR increased to 0.71 ± 0.03 ($P < 0.01$), and partially reversed the CER deficit (freezing duration 193 ± 17s) and the increase in VGLUT2 and decrease in Ki67 expression ($P > 0.05$ versus isolate and group vehicle controls in each case). Isolation also elevated Rac/CDC42 (Rho GTPases which regulate microtubule stabilisation and dendrite morphogenesis, and have been implicated in schizophrenia), while SB-399885 decreased TAK1 and pSTAT3 (members of the SAPK/JNK signalling cascade, whose stimulation via Rac/CDC42 reduces pSTAT3) in isolates only. Isolation tended to lower basal extracellular glutamate in hippocampal slices, from $4.32 \pm 1.40\mu$M to $1.99 \pm 0.41\mu$M ($P = 0.0708$), but the combination of 3μM SB-399885 and 120mM KCl stimulated glutamate release irrespective of housing.

The 5-HT_6 receptor antagonist reversed isolation-induced cognitive impairments and partially reversed deficits in hippocampal cell proliferation in this neurodevelopmental model of schizophrenia. These findings further support the use of 5-HT_6 antagonists to treat cognitive dysfunction, and the value of isolation-reared rats to investigate the underlying neurobiology of schizophrenia and evaluate novel treatments for the cognitive symptoms.

Reference(s)

[1] Jones, C.A., Watson, D.J., Fone, K.C., 2011. Animal models of schizophrenia. Br J Pharmacol 164:1162–1194.

[2] King, M.V., Marsden, C.A., Fone, K.C., 2008. A role for the 5-HT1A, 5-HT4 and 5-HT6 receptors in learning and memory. Trends Pharmacol Sci 29:482–492.

P.2.003 Sub-anaesthetic ketamine modulates intrinsic blood oxygen level-dependent (BOLD) connectivity between the hippocampus and the prefrontal cortex in the rat

N. Gass[1]*, A. Sartorius[1], A.J. Schwarz[2], E. Schenker[3], C. Risterucci[4], M. Spedding[3], L. Zheng[1], A. Meyer-Lindenberg[5], W. Weber-Fahr[1]. *[1]Central Institute of Mental Health Medical Faculty Mannheim University of Heidelberg, Neuroimaging, Mannheim, Germany; [2]Eli Lilly and Company, Translational Medicine, Indianapolis, USA; [3]Institut de Recherches Servier, Neuroscience Drug Discovery Unit, Croissy s/Seine, France; [4]F. Hoffmann-La Roche, CNS Biomarker Pharmaceuticals Division, Basel, Switzerland; [5]Central Institute of Mental Health Medical Faculty Mannheim University of Heidelberg, Department of Psychiatry and Psychotherapy, Mannheim, Germany*

Purpose: Resting state fMRI (rsfMRI) is well-established in humans and has been shown to be sensitive to pharmacological modulation. More recently, consistent intrinsic connectivity networks have been demonstrated in the rat, but drug effects on rsfMRI are only beginning to be characterised in the rodent. The utility of rsfMRI as a translational biomarker depends on (1) its sensitivity to pharmacological modulation in preclinical species and (2) the degree of convergence with effects using the same compound in humans. Ketamine, a potent N-methyl-D-aspartate (NMDA) receptor antagonist, is of substantial current interest both (a) as a pharmacological model of glutamatergic dysfunction in psychiatric disease [1], and (b) as a rapidly acting antidepressant, effective in treatment-resistant depressive patients [2]. The aim of this work was to systematically characterise the effects of ketamine on rsfMRI in the rat.

Methods: Male Sprague-Dawley rats (368–447 g) in 4 parallel groups (N = 10/group) received either vehicle

(saline) or one of three sub-anaesthetic doses of S-ketamine (5, 10 and 25 mg/kg; s.c.). Rats were scanned under 0.14 mg/kg/h medetomidine infusion. Three 8.5-min rsfMRI datasets were acquired from each rat at 9.4T scanner: pre-injection and 15 and 30 min post-injection. Blood samples were taken from each rat after scanning to determine exposure to ketamine. Seed-based connectivity mapping was used to test the hypothesis that ketamine modulates functional connectivity within the hippocampal-prefrontal system. An ROI–ROI correlation analysis, based on a parcellation of the brain into 44 atlas-derived regions, was also performed to profile the ketamine effects throughout the brain.

Results: Pharmacokinetic/pharmacodynamic (PK/PD) image analysis revealed an increased functional connectivity between the hippocampus (except its ventral part) and regions in the prefrontal cortex that positively correlated with ketamine plasma levels. The effects were strongest 30 min post-injection. ROI–ROI correlation analysis revealed dose-dependent increases in connectivity within prefrontal cortex (PFC) structures and between the PFC and cortical structures, in particular the temporal and parietal association cortices, 15 min post-injection.

Conclusions: The observed increases in functional connectivity reveal possible neural mechanisms underlying established behavioural effects of ketamine, including increased wakefulness and locomotor activity, and are consistent with ketamine-induced increases in cortical EEG gamma band coherence. This pattern might result from increased glutamate levels which could then bind other type of glutamate receptors (e.g. AMPA receptors) and also induce synaptogenesis similar to long-term potentiation. Additionally, PFC increased connectivity could also reflect a psychotomimetic aspect observed after ketamine intake in humans. This study provides further evidence that rsfMRI is a sensitive probe of central pharmacological effects in preclinical species, and characterizes the effects of ketamine – a tool compound of considerable current interest in psychiatry research – on rsfMRI in the rat. These results provide an important comparator to (a) other preclinical modalities and (b) analogous rsfMRI experiments emerging in humans.

Reference(s)

[1] Large, C., 2007. Do NMDA receptor antagonist models of schizophrenia predict the clinical efficacy of antipsychotic drugs? J Psychopharmacol 21(3): 283.

[2] Zarate, C.A. Jr., Singh, J.B., Carlson, P.J., Brutsche, N.E., Ameli, R., Luckenbaugh, D.A., Charney, D.S., Manji, H.K., 2006. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. Arch Gen Psychiatry 63(8):856–64.

P.2.004 The dopamine β-hydroxylase inhibitor nepicastat suppresses different chocolate-motivated behaviours in rats

A. Zaru[1]*. [1]*Neuroscience Institute – National Research Council of Italy, Department of Medicine, Monserrato (CA), Italy*

Background and Aims: Chocolate is the most craved food and its excessive consumption may lead to overweight and addiction-like behaviours, including stress-induced relapse to chocolate-seeking and -taking. Animal models indicate that the reinforcing and motivational effects of abusive drugs and palatable foods are mediated by similar neuronal circuitries and molecular mechanisms [1]; accordingly, compounds affecting the reinforcing and motivational properties of abusive drugs may also affect the reinforcing and motivational properties of palatable foods. Recent data demonstrated that the selective dopamine β-hydroxylase inhibitor, nepicastat, suppressed the reinstatement of cocaine-seeking behaviour in rats [2]. The present study was therefore designed to investigate the effect of nepicastat on different chocolate-motivated behaviours in rats.

Methods: Male Wistar rats were trained to self-administer a chocolate-flavoured beverage (CFB; 5% powdered Nesquik® in water) under an operant (lever-responding) procedure in daily 30-min sessions [3]. After training, rats were allocated to 3 separate experiments, assessing the effect of nepicastat (0, 25, 50, and 100 mg/kg; i.p.) on: (a) reinforcing properties of CFB [to this end, rats were exposed to a fixed ratio 10 schedule of reinforcement, in which the response requirement (RR; i.e., the 'cost' – in terms of number of lever-responses – of each CFB presentation) was kept fixed throughout the session] (Experiment 1); (b) motivational properties of CFB [rats were exposed to a progressive ratio schedule of reinforcement, in which RR was progressively increased after the delivery of each reinforcer; the lowest ratio not completed (breakpoint) was taken as measure of the motivational properties of CFB] (Experiment 2); (c) reinstatement of CFB-seeking [after extinction of CFB self-administration, non-reinforced lever-responding for CFB was triggered by the non-contingent presentation ('priming') of CFB] (Experiment 3). Experiment 4 assessed the effect of nepicastat on spontaneous locomotor activity.

Results: All rats easily acquired and steadily maintained CFB self-administration, with baseline values of ~1200 lever-responses and ~50 ml/kg CFB consumed per session. In Experiment 1, treatment with 25, 50, and 100 mg/kg nepicastat reduced by ~15%, 35%, and 45%, respectively, the number of lever-responses for CFB [$F(3,33) = 14.85$,

$P < 0.0001$]. In Experiment 2, treatment with 25, 50, and 100 mg/kg nepicastat reduced by ~15%, 35%, and 65%, respectively, the breakpoint for CFB [$F(3,24) = 10.60$, $P < 0.0005$]. In Experiment 3, limited and non-contingent presentation of CFB robustly reinstated lever-responding; treatment with 25, 50, and 100 mg/kg nepicastat reduced by ~50%, 60%, and 95%, respectively, the number of lever-responses (CFB-seeking) [$F(3,28) = 10.45$, $P < 0.0001$]. In Experiment 4, no dose of nepicastat affected locomotor activity [$F(3,31) = 1.57$, $P > 0.05$].

Conclusions: Treatment with non-sedative doses of nepicastat reduced the reinforcing and motivational properties of CFB and totally suppressed the reinstatement of CFB-seeking in rats. The suppressing effect of nepicastat on reinstatement of CFB-seeking replicated the drug effect on reinstatement of cocaine-seeking [2], suggesting that common neuronal circuitries underlie relapse-like behaviours for different rewards. Based on the mechanism of action of nepicastat, these results imply a role for brain noradrenaline and dopamine in CFB-seeking and -taking behaviours. These results suggest that nepicastat is potentially effective in the treatment of 'chocoholism' and addictive-like, food-related behaviours.

Reference(s)

[1] Volkow, N.D., Wise, R.A., 2005. How can drug addiction help us understand obesity? Nat Neurosci 8, 555–560.

[2] Schroeder, J.P., Cooper, D.A., Schank, J.R., Lyle, M.A., Gaval-Cruz, M., Ogbonmwan, Y.E., Pozdeyev, N., Freeman, K.G., Iuvone, P.M., Edwards, G.L., Holmes, P.V., Weinshenker, D., 2010. Disulfiram attenuates drug-primed reinstatement of cocaine seeking via inhibition of dopamine β-hydroxylase. Neuropsychopharmacology 35, 2440–2449.

[3] Maccioni, P., Pes, D., Carai, M.A.M., Gessa, G.L., Colombo, G., 2008. Suppression by the cannabinoid CB1 receptor antagonist, rimonabant, of the reinforcing and motivational properties of a chocolate-flavoured beverage in rats. Behav Pharmacol 19, 197–209.

P.2.005 The trans-isomer of resveratrol acutely improves motivation in rat

J. Samardzic[1]*, L. Gojkovic-Bukarica[1], D.I. Obradovic[1].
[1]*Medical Faculty University of Belgrade, Pharmacology Clinical Pharmacology and Toxicology, Belgrade, Serbia*

Purpose of the study: Resveratrol (3,5,4′-trihydroxy-trans-stilbene), a natural non-flavonoid polyphenol antioxidant, exists as two geometric isomers: cis- and trans-. It has been shown that more stable trans-resveratrol demonstrates a variety of pharmacological activities including antioxidant, anti-inflammatory, neuroprotective properties and amelioration of learning and memory impairment [1–3]. However, its behavioural profile still remains controversial. The goal of the present study was to examine the influence of trans-resveratrol and compare its dose-response effects on memory and depression-like behaviour.

Methods used: We independently studied the effects of trans-resveratrol (5–20 mg/kg) on retention versus acquisition of active avoidance (AA) and depression-like behaviour in the forced swim test (FST). AA test was performed in automated two-way shuttle boxes and programming recording units (Campden Instruments, Sileby, UK). In the first part of the study, the active avoidance task was elaborated by 100-trial 2-day sessions, and in the second part the influence of trans-resveratrol on the acquisition rate was checked in a procedure lasting five consecutive days, with 50 trials per day. FST was performed in a glass cylinder, 45 cm high, 20 cm diameter filled with water up to a height of 30 cm, with a temperature of 21–23ºC. Male Wistar rats were exposed to two swimming sessions (an initial 15-min pretest session, followed 24 h later by a 5-min test session). A rat was considered immobile whenever it floated passively in the water and only made movements necessary to keep its head above the water line. We also tested the locomotion to exclude the excitatory or inhibitory effects. The measurement of locomotor activity was performed in a clear Plexiglas box ($40 \times 25 \times 35$ cm), for 30 min, without any habituation period. For all the experiments, the behaviour of the animals was recorded by a digital camera, and the data were analysed by one-way ANOVA, followed by Dunnett's t test.

Summary of results: Treatment with trans-resveratrol significantly affected retrieval of avoidance responses on the second day of shuttle box testing ($F(4,45) = 2.88$, $p < 0.05$). Dunnett's test indicated that the trans-resveratrol avoidance-facilitatory dose was 20 mg/kg. However, it did not induce significant differences in acquisition rate during 5 days training. In FST, during the test session, the average immobility time of animals, in seconds, for the vehicle and resveratrol (5, 10, 20 mg/kg) was 145.3, 147.3, 94.7 and 66.0, respectively. ANOVA indicated statistically significant effects of resveratrol ($F(3,20) = 50.95$, $p < 0.001$). Dunnett's analysis showed that resveratrol significantly decreased immobility time at the doses of 10 and 20 mg/kg, exerted acute antidepressant-like effects. ANOVA did not show a significant effect of treatment on the total immobility time of the animals during 30 min of monitoring of spontaneous locomotor activity ($F(3,20) = 1.68$, $p > 0.05$).

Conclusions: Our results experimentally support the findings that under certain circumstances, trans-resveratrol, produces acute memory-enhancing and antidepressant-like effects. Furthermore, these effects were not confounded by locomotor influences. This study will be extended in the further characterising the behavioural profile of resveratrol, as well as clarifying the probable central mechanism of its action.

Reference(s)

[1] Kumar, A., Naidu, P.S., Seghal, N., Padi, S.S., 2007. Neuroprotective effects of resveratrol against intracerebroventricular colchicine-induced cognitive impairment and oxidative stress in rats. Pharmacology 79, 17–26.

[2] Ranney, A., Petro, M.S., 2009. Resveratrol protects spatial learning in middle-aged C57BL/6 mice from effects of ethanol. Behav Pharmacol 20, 330–336.

[3] Xu, Y., Wang, Z., You, W., Zhang, X., Li, S., Barish, P.A., Vernon, M.M., Du, X., Li, G., Pan, J., Ogle, W.O., 2010. Antidepressant-like effect of trans-resveratrol: Involvement of serotonin and noradrenaline system. Eur Neuropsychopharmacol 20, 405–413.

P.2.006 Effects of serotonin (5-HT)$_{1B}$ receptor ligands on amphetamine-seeking behaviour in rats

J. Miszkiel[1]*, E. Przegalinski[1]. [1]*Polish Academy of Sciences, Department of Pharmacology, Krakow, Poland*

Background: Amphetamine and cocaine addiction is a serious medical and social problem; however, despite many years of research, there is still no efficient therapy [1]. Both drugs belong to the psychostimulants and among other behavioural effects they show rewarding properties. These effects – evoked by enhanced activation of the brain dopamine system, especially by elevated extracellular dopamine level in the nucleus accumbens – determine their addictive properties [2]. Interestingly, it was established that manipulation of the serotonin (5-HT)$_{1B}$ receptor can modify the behavioural effects of these psychostimulants including their reinforcing activity [3]. However, the role of the 5-HT$_{1B}$ receptor in amphetamine self-administration and extinction/reinstatement model is still unclear. In order to substantiate a role of those receptors in incentive motivation for amphetamine we used the extinction/reinstatement model to examine the effects of the 5-HT$_{1B}$ receptor ligand on reinforcement of extinguished amphetamine-seeking behaviour.

Methods: Male Wistar rats (250–300 g) were trained to self-administer amphetamine in a standard operant chambers, during 2-hour daily sessions, 6 days per week (maintenance). The FR schedule was gradually elevated from FR1 to FR5 once animals showed the stable responding. Every each 5 completed active lever presses resulted in a 5-s infusion of amphetamine and a 5-s presentation of a stimulus complex. Rats were maintained on the 0.06 mg/kg/infusion amphetamine dose until they met the criteria. When rats met the self-administration criteria, the extinction procedure was begun on the following day. During the extinction animals received intravenous saline infusions (instead of amphetamine) without presentation of the conditioned stimulus (light and sound). Once they reached the extinction criteria rats were divided into separate groups to run reinstatement experiment. Reinstatement was triggered by amphetamine injection (i.p.) or the presentation of the stimulus complex paired with threshold dose of amphetamine (i.p.). Separate groups of rats were pretreated with either SB 214461 (a 5-HT$_{1B}$ receptor antagonist; 2.5–7.5 mg/kg; i.p.), CP 94253 (a 5-HT$_{1B}$ receptor agonist; 0.03–5 mg/kg; i.p.) or both before the appropriate test sessions. Data were analysed by t-Student test or by one- or two-way analysis of variance followed by Dunnett or Newman–Keuls tests.

Results: The 5-HT$_{1B}$ receptor antagonists SB 216641 (5–7.5 mg/kg) attenuated the amphetamine (1.5 mg/kg)-[$F(3,24) = 9.351$, $p < 0.001$] and amphetamine-associated cue combined with the threshold dose of amphetamine-induced [$F(3,25) = 4.808$, $p < 0.01$] reinstatement of amphetamine-seeking behaviour. The 5-HT$_{1B}$ receptor agonist CP 94253 (1.25–5 mg/kg) also inhibited amphetamine-seeking behaviour induced by amphetamine (1.5 mg/kg) [$F(5,38) = 7.963$, $p < 0.0001$] but not by a cue combined with threshold dose of amphetamine-induced [$F(4,31) = 0.507$] reinstatement. The inhibitory effect of CP 94253 on amphetamine-seeking behaviour remained unaffected [$F(1,21) = 0.16$] by this 5-HT$_{1B}$ receptor antagonist.

Conclusion: Our results indicate that tonic activation of 5-HT$_{1B}$ receptors is involved in amphetamine- and amphetamine associated cue combined with the threshold dose of amphetamine-induced reinstatement of amphetamine-seeking behaviour and that the inhibitory effects of 5-HT$_{1B}$ receptor antagonists on these phenomena are directly related to motivational aspects of amphetamine abuse. The inhibitory effect of CP 94253 on amphetamine-seeking behaviour seems to be unrelated to the 5-HT$_{1B}$ receptor activation and may result from a general reduction of motivation.

Reference(s)

[1] Jupp, B., Lawrence, A.J. 2010. New horizons for therapeutics in drug and alcohol abuse. Pharmacol Ther. 125, 138–168.

[2] Pontieri, F.E., Tanda, G., Di Chiara, G. 1995. Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the 'shell' as compared with the 'core' of the rat nucleus accumbens. Proc Natl Acad Sci U S A. 19, 12304–12308.
[3] Miszkiel, J., Filip, M., Przegalinski, E. 2011. Role of serotonin (5-HT)1B receptors in psychostimulant addiction. Pharmacol Rep. 63, 1310–1315.

P.2.007 Tryptophan depletion impairs emotion recognition in healthy women

M. Defrancesco[1]*, W. Parson[2], J. Marksteiner[3], E.A. Deisenhammer[1], J. Kemmler[1], H. Niederstätter[2], H. Hinterhuber[1]. *[1]Medical University Innsbruck, Department of General Psychiatry, Innsbruck, Austria; [2]Medical University Innsbruck, Institute of Legal Medicine, Innsbruck, Austria; [3]LKH Hall, Department of Psychiatry, Innsbruck, Austria*

Purpose of the study: The brain serotonin system modulates neural circuits that regulate emotion and mood in humans [1]. Tryptophan depletion (TD) is an established method to decrease cerebral serotonin levels [2]. The serotonin transporter promoter polymorphism (5-HTTLPR) is suggested to influence emotion recognition [3]. This study examined the effect of TD on the recognition of emotional faces in healthy women carrying a s-allele of the 5-HTTLPR polymorphism.

Methods: A prospective clinical study was conducted for 38 healthy women carrying an s-allele of the 5-HTTLPR polymorphism. Participants completed computerised tests (Penn Emotion Recognition Test, and Penn Emotion Acuity Task) and a visual analogue scale (VAS) before, five and seven hours after the administration of acute TD. Blood samples were taken at these three time points to analyse biological markers (plasma serotonin, total plasma tryptophan (Trp), 5-hydroxyindolacetic acid (5-HIAA), long neutral amino acids (LNAAs), Trp /LNAA ratio). Comparability of the two genetic subtypes with respect to socio-demographic variables was checked by means of the adequate statistical tests (t-test, Mann–Whitney U-test, chi-square test), depending on the type of variable. Repeated-measures analysis of variance (ANOVA) was performed to analyse the biochemical parameters in the course of time (baseline, 5 hours and 7 hours after TD). Genotype (5-HTTLPR) was included in the model as a between-subjects factor allowing statistical testing for both a main effect of genotype and time-by-genotype interaction.

Results: Thirty-eight female participants with a mean age of 23.18±1.96 (mean±SD), mean years of education of 16.65±1.69 and a mean BMI of 21.14±2.31 completed the study. All participants had euthymic depression scores in Beck Depression Inventory at baseline testing 0.97±1.97. After TD, mood (VAS) decreased significantly within 5 hours ($p < 0.001$) and then increased again after 7 hours ($p = 0.020$) compared to 5 hours after TD. Further, total plasma Trp, 5-HIAA, and Trp/LNAA ratio were significantly decreased by TD over time. Concerning genotype, repeated measures ANOVA showed a significant time-by-genotype interaction regarding the time course of Trp plasma levels ($p = 0.038$). Homozygous carriers of the 5-HTTLPR s-allele (−10.6 μg/ml ±2.5) showed a significantly stronger reduction of plasma Trp than heterozygous individuals (−8.2 μg/ml ±3.0) between baseline and 5 hours after TD ($p = 0.026$). Compared to baseline, subjects recognised angry faces significantly better 7 hours after TD ($p < 0.001$). TD increased the specificity for the emotions sad and happy but decreased the sensitivity for the emotion sad over the course of the test day.

Conclusions: In summary, we found that TD impairs the sensitivity for negative emotions in faces in healthy females carrying the s-allele of the 5-HTTLPR polymorphism. In addition, our data suggest that changes in plasma levels of tryptophan, 5-HIAA and the Trp/LNAA ratio are directly associated with the mood lowering effect of TD in genetically vulnerable healthy females. These results substantiate the putative role of the serotonergic system in the processing of emotional faces.

Reference(s)

[1] Heninger G.R., Charney D.S., and Sternberg D.E. 1984. Serotonergic function in depression. Prolactin response to intravenous tryptophan in depressed patients and healthy subjects. Archives of General Psychiatry 41: 398–402.
[2] Marsh A.A., Finger E.C., Buzas B., Soliman N., Richell R.A., Vythilingham M., Pine D.S., Goldman D., and Blair R.J.R. 2006. Impaired recognition of fear facial expressions in 5-HTTLPR S-polymorphism carriers following tryptophan depletion. Psychopharmacology 189: 387–394.
[3] Roiser J.P., Muller U., Clark L., and Sahakian B.J. 2007. The effects of acute tryptophan depletion and serotonin transporter polymorphism on emotional processing in memory and attention. International Journal of Neuropsychopharmacology 10: 449–461.

P.2.008 5XFAD mouse model of Alzheimer's disease: a dissociation between brain pathology and behavioural phenotype

K. Sonn[1]*, K. Jaako[1], R.K. Jain[1], A. Zharkovsky[1]. [1]*University of Tartu, Dept. of Pharmacology, Tartu, Estonia*

Alzheimer's disease (AD) is a neurodegenerative disease clinically characterised by progressive cognitive impairment and pathologically by extracellular β-amyloid (Aβ)-containing senile plaques, intraneuronal aggregates of hyperphosphorylated τ-protein and neurofilaments as well as synaptic loss. The amyloid cascade hypothesis postulates that deposition of toxic Aβ species is the primary cause of AD. 5XFAD mice is a transgenic model of AD known for rapid brain accumulation of Aβ [1].

Our aim was to characterise behaviour and brain findings of 5XFAD mice dynamically in order to gain insight into pathophysiology of AD and to identify possible new drug targets. Male and female 5XFAD mice and wild-type (WT) littermates of 2 and 6 months of age were used; n = 5–15.

Morris water maze test was performed on 6 consecutive days. Training lasted 4 days, platform was made visible on day 5 and removed on day 6. Two months old 5XFAD mice displayed impaired learning (no decrease in time to find hidden platform during training days), but not memory (% time spent in quadrant of the pool where platform had been) compared to WT mice. Swimming velocity and vision did not differ between groups at 2 months. At 6 months of age, there was no difference in learning, memory or vision between 5XFAD and wild-type mice. However, swimming velocity of 5XFAD mice was significantly lower at 6 months compared to WT mice.

Elevated plus maze task was performed on 3 consecutive days. 5XFAD mice demonstrated a decrease in % of open arm entries on days 2 and 3 at 2, but not 6 months of age. At 2 but not 6 months, 5XFAD mice showed a trend towards increased % of open arm entries compared to WT mice.

At 2 months, 5XFAD mice had a significant increase in microglial activation (demonstrated immunohistochemically by a greater Iba-1 positive area fraction in hippocampus and amygdala) compared to WT mice. The difference increased further by 6 months. Diameter of Congo-red positive Aβ plaques in hippocampus and amygdala significantly increased in 5XFAD mice between 2 and 6 months of age.

The number of polysialylated neural cell adhesion molecule (PSA-NCAM)-positive cells in the hippocampus was significantly higher in 2, but not in 6 months old 5XFAD mice. By 6 months, the number of PSA-NCAM-positive cells decreased significantly in 5XFAD and WT mice with no difference between the groups. Immunoblotting showed significantly higher levels of PSA-NCAM in 6 months old and a trend for increase in 2 months old 5XFAD mice.

Levels of synaptic proteins synaptophysin and PSD-95 and neurofilament H as well as phosphorylated neurofilament H were measured by Western blot in hippocampus and cortex of 5XFAD and WT mice at 2 and 6 months. No difference in the levels of either synaptic or axonal markers was found in either age group between experimental groups.

Our data suggest existence of compensatory mechanisms in 5XFAD mice such as enhanced neurogenesis or neurite outgrowth which need identification. Additionally, our findings raise the question of suitability of 5XFAD mice as an AD model.

Reference(s)

[1] Oakley H, Cole SL, Logan S et al., 2006. Intraneuronal beta-amyloid aggregates, neurodegeneration, and neuron loss in transgenic mice with five familial Alzheimer's disease mutations: potential factors in amyloid plaque formation. The Journal of Neuroscience 26, 10129–10140.

P.2.009 Effect of early life experiences on brain structure and function: neurogenesis and decision making

M. Loi[1]*, S. Koricka[1], L. de Visser[1], M.J.H. Kas[1], P.J. Lucassen[2], M. Joels[1]. [1]*Rudolf Magnus Institute of Neuroscience, Neuroscience and Pharmacology, Utrecht, The Netherlands;* [2]*Swammerdam Institute of Life Sciences, Centre for Neuroscience, Amsterdam, The Netherlands*

Exposure to stress during early postnatal life can alter adult brain structure and function and can e.g. enhance stress responsiveness later in life and increase vulnerability for psychiatric disorders. Previous studies suggest that 4 days of treatment with mifepristone prevents a reduction in hippocampal neurogenesis induced by chronic stress through a blockage of the glucocorticoid receptors [1].

To investigate effects of early life stress on neurogenesis and decision making, we used the maternal deprivation (MD) paradigm in rats that leads to increased levels of circulating corticosterone (CORT). First we wish to study the effects of MD on neurogenesis at postnatal day (PND) 29. Second, we shall assess the effects of MD on decision-making processes during young-adulthood. Finally, the effects of glucocorticoid receptor blockade mifepristone (RU38486) during early adolescence will be examined both on structural and behavioural alterations induced by MD.

Wistar rats were exposed to 24 h MD at PND3. On PND21, pups were group housed. At PND 26–28 half of the maternal deprived and control groups received either mifepristone or vehicle (VEH) treatment through oral gavage administration. At PND29, females were perfused. Proliferation and neurogenesis were quantified using immunohistochemistry for Ki-67 and doublecortin, respectively. To assess funcional effects of MD on executive functioning, 12 week old male rats performed the Rodent Iowa Gambling Task (r-IGT) that measures the ability to choose a long-term advantageous option with immediate moderate rewards, over a long-term disadvantageous option with immediate high rewards. After the last trial, brains were processed for c-fos immunochemistry.

Good performing rats (no MD, and MD treated with mifepristone groups), showed a higher fraction of visits into the advantageous arm: NoMD+VEH (mean±SEM: 0.6±0.2 n = 8); NoMD+RU38486 (mean±SEM: 0.5±0.1 n = 11); MD+RU38486 (mean±SEM: 0.5±0.1 n = 12) compared to saline-treated maternal deprived animals (mean±SEM: 0.2±0.1 n = 12). Good performing rats, during the training, improved in choosing the long-term advantageous arm while MD+VEH rats did not improve. Post-hoc comparison revealed significant difference in performance between NOMD+VEH vs MD+VEH ($p = 0.015$) as well as between MD+MIF compared to MD+VEH ($p = 0.025$). Good performing rats showed a lower fraction of visits into the empty arms (mean±SEM: 0.3±0.08) than poor performing rats (mean±SEM: 0.5±0.08), a measure of non-reward related exploration.

Our data show MD impairs adult decision-making performance, as deprived rats did not learn to choose for the long-term advantageous option. Treatment with RU38486 during early adolescence rescued the negative effects of MD and improved task performance. As r-IGT performance relies on the integrity of the prefrontal cortex, we hypothesize the poor performance in the r-IGT may be caused by MD effects on prefrontal cortex development.

Reference(s)

[1] Oomen CA, Mayer JL, de Kloet ER, Joëls M, Lucassen PJ., 2007. Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. Eur J Neurosci 26: 3395–3401.

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P.2.010 Effects of L-DOPA and sarizotan treatments in a parkinsonian rat model of depression

N. Schintu[1]*, X. Zhang[1], A.A. Mathé[1], P. Svenningsson[1].
[1]*Karolinska Institute, Clinical Neuroscience, Stockholm, Sweden*

Parkinson's disease (PD) is the second most common neurodegenerative disorder. The diagnostic symptoms of PD are based on the motor dysfunctions, bradykinesia, tremor, rigidity and postural imbalance. The standard drug for PD is L-DOPA, but its therapeutic efficacy is gradually lost and dyskinesias appear after 5–10 years of treatment. One possible explanation for the development of dyskinesia is that, when most dopaminergic neurons are degenerated, L-DOPA is converted to dopamine in serotoninergic neurons and thus released in a non-controlled manner [1]. In this context, serotonin 5-HT_{1A} agonists, such as sarizotan, have been found to have antidyskinetic properties. Beside motor symptoms, PDs patients have non-motor symptoms of which depression is the most frequent. Because of the increased awareness of the negative impact of depression for the quality of life of PD patients, there is a growing interest in developing optimised treatments that alleviate both motor and non-motor symptoms of PD.

In this study, unilateral 6-OHDA lesioning was performed in the Flinders Sensitive Line (FSL), a rat strain associated with distinct behavioural and neurochemical features of major depression, and their littermates, the Flinders Resistant Line (FRL). Two weeks later, rats were injected with apomorphine (1 mg/kg) and their controlateral rotations were measured over 30 minutes to determine the success of nigrostriatal denervation. The effectiveness of the lesion was further confirmed postmortem by dopamine transporter autoradiography in the striatum. Four weeks after surgery, rats were administered with saline, L-DOPA/benserazide (10/7.5 mg/kg), sarizotan (2.5 mg/kg) alone or in combination with L-DOPA once daily for 23 days. Once per week, rotational behaviour and abnormal involuntary movements (AIMs) [2] were measured. 30 minutes after the last injection, rats were sacrificed and brains rapidly removed for in situ hybridisation study of immediate early genes including c-fos in the striatum.

The results showed that the first administration of L-DOPA and L-DOPA/sarizotan induced similar contralateral turning behaviour in both FRL and FSL rats. After chronic treatment with L-DOPA, a significant increase in the number of rotations was observed in the FRL group. In contrast, FRL rats receiving chronic co-administration of sarizotan/L-DOPA did not develop behavioural sensitisation. In the FSL group, chronic

treatment with neither L-DOPA nor L-DOPA/sarizotan induced any significant behavioural sensitisation. Chronic treatment with L-DOPA and L-DOPA/sarizotan induced AIMs in both lines of rats. However, FRL rats treated with L-DOPA spent more time in AIMs compared to FSL rats. Moreover, co-administration with sarizotan reduced significantly L-DOPA-induced AIMs in FRL, but not in FSL rats. In situ hybridisation analysis showed that treatment with L-DOPA and L-DOPA/sarizotan significantly increased c-fos mRNA levels in the lesioned side as compared to the nonlesioned side of saline treated FRL and FSL rats. Interestingly, c-fos mRNA level in FSL group after chronic L-DOPA treatment was significantly lower than in FRL group receiving the same treatment. Moreover, co-administration with sarizotan reduced significantly L-DOPA-induced c-fos in FRL, but not in FSL, rats. The results suggest that parkinsonian animals with distinct depressive-like phenotypes react differently to PD treatments in terms of dyskinetic behaviour and striatal neuronal-like activity.

Reference(s)

[1] Carta M, Carlsson T, Kirik D, Björklund A. Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain. 2007 Jul;130(Pt 7):1819–33. Epub 2007 Apr 23.

[2] Pinna A, Tronci E, Schintu N, Simola N, Volpini R, Pontis S, Cristalli G, Morelli M. A new ethyladenine antagonist of adenosine A(2A) receptors: behavioural and biochemical characterization as an antiparkinsonian drug. Neuropharmacology. 2010 Mar;58(3):613–23.

P.2.011 GluA1 and its Postsynaptic density protein 95 (PSD-95)/Discs large/Zonula occludens-1 (PDZ)-interaction: a role in experience-dependent behavioural plasticity in the forced swim test

F. Freudenberg[1]*, V. Marx[2], V. Mack[3], L.E. Layer[3], M. Klugmann[4], P.H. Seeburg[3], R. Sprengel[3], T. Celikel[2].
[1]University of Würzburg, Department of Psychiatry Psychosomatics and Psychotherapy, Würzburg, Germany; [2]Radboud University Nijmegen, Donders Institute for Brain Cognition and Behavior Centre for Neuroscience, Nijmegen, The Netherlands; [3]Max Planck Institute for Medical Research, Department of Molecular Neurobiology, Heidelberg, Germany; [4]University of New South Wales, Department of Physiology, Sidney, Australia

Although current pharmacological treatment for depression mainly targets the monoaminergic system, recent studies indicate that glutamatergic neurotransmission is more principally involved in the neuropathology of depression [1]. Importantly, pharmacological blockade of NMDA receptors (e.g. with ketamine) has relatively long-lasting antidepressant effects with rapid onset. The antidepressant properties of ketamine require activation and synaptic incorporation of GluA1-containing AMPA receptors [2]. Moreover, hippocampal samples from clinically depressed patients display reduced mRNA levels for GluA1. These findings argue that GluA1-dependent synaptic plasticity might be critically involved in the development of depression [1,2].

Here we tested this hypothesis by exposing wild type (WT) and GluA1 transgenic mice to two sessions of forced swimming, 24-hours apart (FST1 and FST2 respectively), and assessed experience-dependent expression of behavioural despair by quantification of latency to immobility and cumulative immobility (Table 1).

Table 1. Latency to immobility and cumulative immobility during the first (FST1) and second day (FST2) of testing for all genotypes.

Genotype	Latency to immobility (s)		Cumulative immobility (%)	
	FST1	FST2	FST1	FST2
WT (N = 10)	96.1±9.5	35.7±8.5**	46.3±5.3	60.5±2.6**
GluA1$^{-/-}$ (N = 7)	284.0±66.8	244.7±75.4	25.9±3.2	31.4±6.4
TG8.1 (N = 9)	221.3±61.3	210.8±59.0	26.7±1.1	26.2±2.0
A1.1 (N = 7)	327.3±41.7	102±16.2***	27.7±2.4	35.5±3.6*
WT-Cre (N = 7)	201.7±37.2	65.1±9.9***	35.6±2.3	47.4±2.2**
ΔdHpc (N = 6)	120.5±15.3	132.2±39.6	40.0±1.8	39.3±3.0
ΔvHpc (N = 13)	142.9±10.3	148.5±36.0	38.9±1.7	36.9±1.8

Statistical significance after post-hoc Tukey's t-test (FST1 vs FST2): *p <0.05, **p < 0.01, ***p < 0.001.

In WT mice latency was significantly reduced and immobility was significantly increased during FST2 (compared to FST1), suggesting successful experience-dependent induction of behavioural despair. Interestingly, mice globally lacking GluA1-containing AMPA receptors (GluA1$^{-/-}$ mice) were impaired in the experience-dependent expression of behavioural despair in the FST, as suggested by comparable latency and immobility on both days of testing. By transgenically expressing a GFP-tagged GluA1-subunit (^{GFP}GluA1) in the GluA1$^{-/-}$ background (A1.1 mice) we were able to rescue this deficit. However, when the ^{GFP}GluA1-subunit lacked the final C-terminal amino acid (leucine) (TG8.1 mice), responsible for PDZ-interaction of GluA1 [3] the deficit seen in GluA1$^{-/-}$ mice remained (i.e. unchanged latency and immobility), arguing that GluA1–PDZ interaction is critical for expression of learned behavioural despair.

To investigate the neural circuits involved, we selectively removed GluA1 in hippocampus of mice with 'floxed' GluA1 alleles by stereotaxic delivery of a Cre-expressing recombinant adeno-associated virus (rAAV). While control mice (WT mice injected with the Cre-

expressing rAAV; WT-Cre) showed normal reduction in latency and increased immobility, mice preferentially lacking GluA1 in dorsal (ΔdHpc) or ventral (ΔvHpc) hippocampus did not display any changes in latency and immobility across sessions and were indistinguishable from GluA1$^{-/-}$ mice, suggesting that hippocampal GluA1-containing AMPA receptors are critical for the experience-dependent expression of behavioural despair in the FST.

Taken together, this study provides evidence that hippocampal GluA1-containing AMPA receptors and their interaction with PDZ-domain proteins are critical for the induction of learned behavioural despair, thereby linking mechanisms of hippocampal GluA1-dependent synaptic plasticity with the pathophysiology of depression.

Reference(s)

[1] Skolnick, P., Popik, P., Trullas, R., 2009. Glutamate-based antidepressants: 20 years on. Trends in pharmacological sciences 30, 563–569.

[2] Duman R.S., Voleti B., 2012. Signaling pathways underlying the pathophysiology and treatment of depression: novel mechanisms for rapid-acting agents. Trends in neurosciences 35, 47–56.

[3] Cai C., Coleman S.K., Niemi K., Keinänen K., 2002. Selective binding of synapse-associated protein 97 to GluR-A alpha-amino-5-hydroxy-3-methyl-4-isoxazole propionate receptor subunit is determined by a novel sequence motif. The Journal of biological chemistry 277:31484–31490.

P.2.012 Chronic modulation of 5-HT$_4$ and 5-HT$_6$ serotonergic receptors: a new hope in the treatment of cognitive deficits?

A. Quiedeville[1]*, T. Freret[1], V. Bouet[1], M. Boulouard[1].
[1]*Université de Caen Basse-Normandie, Groupe Mémoire et Plasticité comportementale – EA4259, Caen, France*

Drugs designed for patients suffering from neurodegenerative diseases like Alzheimer's act on a single molecular target, and have most of the time only symptomatic effects. As hitting a single target seems more and more inadequate, Multiple Medication Therapies (MMT) are gaining interest. With the aim to use a low dose of each component and therefore to diminish possible adverse effects, MMT could represent a real improvement in the treatment of neurodegenerative diseases.

Serotonin is a central neurotransmitter known to be involved in a great variety of physiological and cognitive functions, including learning and memory processes. Among the fourteen subtypes of serotonin receptors, the 5-HT$_4$ and 5-HT$_6$ receptors (5-HT$_4$R and 5-HT$_6$R) are interesting therapeutic targets for the treatment of memory disorders and their co-modulation could represent an innovative MMT strategy. Neurochemical and behavioural studies have shown that activation of 5-HT$_4$R or blockade of 5-HT$_6$R both improve memory performances. Accordingly, a therapeutic approach combining a simultaneous modulation of these two receptors could be an interesting and innovative strategy in the treatment of memory disorders.

In order to find the best therapeutic strategy, we first selected sub-efficient doses of a 5-HT$_4$R partial agonist (RS 67333) and of a 5-HT$_6$R antagonist (SB-271046), administered chronically (14 days; i.p.) to adult male NMRI mice. We chose to assess their effects in the object recognition test, given that it assesses episodic memory, which is the first memory affected by normal or pathological aging and Alzheimer's disease. The test was performed in a Y-maze [1], with an inter-sessions interval of 72 h, a duration that does not allow significant recognition in normal mice and therefore is appropriate to observe promnesiant effects. This test sets up a criterion of 20s of exploration for both objects, thus the exploration time of the novel object will be compared to the chance level of 10s with a Student t test.

Results showed that chronic activation of 5-HT$_4$R by RS 67333 improved episodic-like memory performances, at the dose of 5 μg/kg [time spent exploring the novel object, mean±SEM, Student t test versus 10 s, 11.17±0.37, $p = 0.004$], but not at 1 μg/kg [11.00±0.67, $p = 0.073$]. Chronic blockade of 5-HT$_6$R by SB-271046 improved memory performances at very low doses such as 0.001 μg/kg [11.42±0.34, $p = 0.0007$], but not below.

We demonstrated that chronic activation of 5-HT$_4$R or blockade of 5-HT$_6$R, both at very low doses, improved episodic-like memory performances. Besides, a preliminary study assessing the effects of a chronic co-modulation of these two receptors with sub-efficient doses of RS 67333 and SB-271046 showed the high potential synergistic effect of this strategy. These results bring new hopes in finding an effective strategy to restore memory performances both in physiological and pathological situations, along with potential suppression of adverse effects.

Reference(s)

[1] Leger, M., Quiedeville, A., Paizanis, E., Natkunarajah, S., Freret, T., Boulouard, M., Schumann-Bard, P., 2012. Environmental enrichment enhances episodic-like memory in association with a modified neuronal activation profile in adult mice. PLoS ONE 7(10): e48043. doi:10.1371/journal.pone.0048043.

P.2.013 Modelling serotonergic neuromodulation of behavioural performance in spatial working memory

M. Cano-Colino[1]*, R. Almeida[2], A. Compte[1].
[1]*IDIBAPS, Theoretical Neurobiology, Barcelona, Spain;* [2]*Karolinska Institute, Department of Neuroscience, Stockholm, Sweden*

The executive functions of prefrontal cortex (PFC) are regulated by ascending monoaminergic systems. In particular, the dorsal PFC is associated with spatial working memory (SWM), linked to persistent neuronal activity, and it receives anatomical projections from the brainstem monoamine centres. Catecholamine regulation of SWM has been studied at mechanistic and computational levels. However, the role of serotonin (5-HT) in SWM is unclear. Thus, while 5-HT is clearly associated with orbitofrontal cortex functions, its association with SWM is controversial, with positive and negative reports. An indirect association between PFC 5-HT and SWM is suggested by the pro-cognitive effects of antipsychotic drugs targeting 5-HT receptors. Several neurocognitive problems are core deficits of schizophrenia and SWM deficits mark a genetic predisposition for the disease.

These weak associations between 5-HT and SWM are in contrast with neurobiological observations predicting a stronger link. Indeed, 5-HT receptors are densely expressed in various PFC subfields, and serotonergic projections from the raphé nuclei target specifically PFC and modulate neuronal activity. Moreover, 5-HT_{2A} receptor activation/blockade in dorsal PFC reduces persistent neuronal activity during SWM tasks.

Here, we sought to develop a computational model of serotonergic modulation of the PFC network in order to address the possible reasons for this discrepancy. We used a computational network model of spiking neurons [1] which is consistent with PFC neurophysiological data from behaving monkeys in SWM. This model falls within the general class of attractor models proposed to account for behavioural deficits of schizophrenia, and provides a rich set of behavioural output predictions that can be experimentally tested. We incorporated 5-HT receptor mechanisms in the model following electrophysiological evidence, and we tested the effects of 5-HT receptor ligands on network simulation performance during SWM.

We found that 5-HT modulated network's SWM performance non-monotonously via 5-HT_{2A} and especially via 5-HT_{1A} receptors, following an inverted-U shape. This may partly explain the weak behavioural effects of serotonergic agents in previous SWM studies. Our simulations showed that errors committed at low and high 5-HT concentrations are due to different network dynamics instabilities, suggesting that these two conditions can be distinguished experimentally based on their dependency with delay length, on the response confidence declared in error trials and on PFC activation contrasts in neuroimaging studies. We derived specific predictions regarding the expected behavioural effects of serotonin agents in two classic working-memory tasks: an oculomotor delayed-response task and a delayed match-to-sample task. In brief, the probability of memory storage in the oculomotor task and the rate of misses in the match-to-sample task will be most sensitive to serotonin modulation, depending on delay duration for increases but not for decreases in baseline 5-HT. Our study underscores the relevance of identifying different error types in SWM tasks in order to reveal the association between neuromodulatory systems and SWM. Testing these model-derived predictions in electrophysiological and psychophysical studies of SWM will help advance our understanding of the neural basis of SWM and its neuromodulation by 5-HT receptors.

Reference(s)

[1] Compte A., Brunel N., Goldman-Rakic P.S., Wang X.J., 2000. Synaptic mechanisms and network dynamics underlying spatial working memory in a cortical network model. Cereb Cortex 10:910–923.

P.2.014 Serotonin receptor 2B knockout mice present schizophrenic-like behaviour

P.M. Pitychoutis[1]*, L. Maroteaux[1]. [1]*INSERM UMR-S 839 Institut du Fer à Moulin, Université Pierre et Marie Curie F75005, Paris, France*

Purpose: Impulsivity, very broadly defined as action without foresight, novelty-seeking and hyperlocomotion, shares common playground with numerous mental disorders, among them chronic substance abuse, attention deficit hyperactivity disorder, and schizophrenia [1]. In a recent study conducted in a Finnish cohort, we reported that a population-specific serotonin 2B receptor (5-HT_{2B}R) stop-codon (i.e. HTR2B Q20*) predisposes to severe impulsivity [2]. Moreover, the genetic ablation of the 5-HT_{2B}R gene in mice (5-$HT_{2B}^{-/-}$) yielded a hyperlocomotor and novelty-seeker phenotype, characterised by enhanced impulsivity, as assessed in the delay discounting task [2,3]. In the same cohort, psychosis was numerically more prevalent in HTR2B Q*20 carriers, with early-onset schizophrenia being observed in this group [2]. However, the extent to which 5-HT_{2B}R is implicated in the neurobiology of schizophrenia has never been investigated

per se. Therefore, in the present study we investigated the effects of the genetic ablation of the 5-HT_{2B} receptor, across a battery of schizophrenia-relevant behavioural paradigms and demonstrate herein that loss of function of 5-HT_{2B}R confers a wide spectrum of schizophrenic-like behavioural and psychopharmacological phenotypes in mice.

Methods: Adult male 5-$HT_{2B}^{-/-}$ mice and their respective wild-type (WT) control mice were used in the present study. All mice were tested in the prepulse inhibition of acoustic startle (PPI) and the three-compartmental sociability and preference for social novelty tests. Putative attentional and cognitive deficits were assessed in the novel object recognition (NOR), the contextual and cued fear conditioning and the latent inhibition (LI) paradigms. Locomotor response to the systemic administration of the psychostimulants dizocilpine (i.e. MK-801) and amphetamine was also assessed. Dopamine (DA) and glutamate content, as well as the mRNA expression of dopaminergic and glutamatergic receptors was assessed in the dorsal striatum (dSTR) and the nucleus accumbens (NAC) of 5-$HT_{2B}^{-/-}$ and WT mice.

Results: Domains related to the positive, negative and cognitive symptom-clusters of schizophrenia appear to be affected in 5-$HT_{2B}^{-/-}$ mice. In particular, genetic ablation of the 5-HT_{2B}R induced deficits in sensorimotor gating (i.e. impaired PPI performance) and selective attention (i.e. lack of LI establishment). Moreover, 5-$HT_{2B}^{-/-}$ mice exhibited increased locomotor response to the psychostimulant properties of dizocilpine and amphetamine, as well as deficits in social interaction, NOR and fear memory that closely resemble the behavioural alterations observed in schizophrenic patients. At the neurochemical level, ablation of the 5-HT_{2B}R induced a region-selective decrease of DA concentrations in the dSTR and glutamate content in the NAC accompanied by respective alterations in dopaminergic and glutamatergic receptors' expression.

Conclusions: Herein we report that 5-HT_{2B}R deficiency may induce a wide spectrum of schizophrenic-like behavioural phenotypes accompanied by robust region-distinctive dopaminergic and glutamatergic neurochemical alterations. The latter implies an important role for 5-HT_{2B}R in the regulation of dopaminergic and glutamatergic activity. This genetic mouse model holds heuristic value in elucidating the relevant neurodevelopmental, epigenetic, and physiological mechanisms that may be sensitive to 5-HT_{2B}R genetic variations. Given that many currently marketed atypical antipsychotic drugs present high affinity for the 5-HT_{2B}R our findings bear broader significance for the use of these drugs in psychotic disorders.

Reference(s)

[1] Humby, T., Wilkinson, L.S., 2011. Assaying dissociable elements of behavioural inhibition and impulsivity: translational utility of animal models. Curr Opin Pharmacol 11, 534–539.

[2] Bevilacqua, L., Doly, S., Kaprio, J., Yuan, Q., Tikkanen, R., Paunio, T., Zhou, Z., Wedenoja, J., Maroteaux, L., Diaz, S., Belmer, A., Hodgkinson, C.A., Dell'osso, L., Suvisaari, J., Coccaro, E., Rose, R.J., Peltonen, L., Virkkunen, M., Goldman, D., 2011. A population-specific HTR2B stop codon predisposes to severe impulsivity. Nature 468, 1061–1066.

[3] Doly, S., Valjent, E., Setola, V., Callebert, J., Herve, D., Launay, J.M., Maroteaux, L., 2008. Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. J Neurosci 28, 2933–2940.

P.2.015 Low performing rats model the inattentive subtype of adult ADHD in the 5-choice continuous performance task (5C-CPT)

A. Tomlinson[1]*, K.M. Marshall[2], J.C. Neill[1]. *[1]University of Bradford, School of Pharmacy, Bradford West Yorkshire, United Kingdom; [2]University of Manchester, School of Pharmacy, Manchester, United Kingdom*

The 5-choice continuous performance task (5C-CPT) is an enhanced version of the 5-choice serial reaction time task (5-CSRTT). The 5C-CPT assesses vigilance in a way that is similar to the human CPT, in comparison to the 5-CSRTT that assesses sustained attention. The 5C-CPT allows for the measurement of response inhibition in terms of false-alarm responding to non-signal stimuli [1,2]. Disturbances in attention and inhibitory control play a central role in the symptomatology of ADHD. Measuring response inhibition is important when validating animal models of ADHD because increased false-alarm responding is often mediated by impairments in performance of the CPT in humans. Selection of rats within a normal 'population' that display reduced sustained attention in 5-CSRTT training sessions may provide a more translational model of ADHD [3]. The present study extends these findings by selecting rats that display reduced sustained attention and vigilance in both the 5-CSRTT and the 5C-CPT.

The aim of the current study was to investigate the effects of psychostimulant and non-stimulant drugs

on attention, impulsivity and performance in adult rats separated into high and low-performers in the 5C-CPT.

The effects of acute methylphenidate (MPH) 0.5, 1.0, and 2.0 mg/kg i.p, and atomoxetine (ATMX) 0.2, 1.0, 2.0 mg/kg i.p were assessed in the 5C-CPT in female Lister-hooded rats (n = 40). Animals were trained for 60 sessions and then divided into two groups (high and low performers) based on set criteria. Animals were challenged on test days by increasing the inter-trial interval (ITI) from 5s to 10s.

ATMX significantly reduced premature responding in low performers at 1.0 and 2.0 mg/kg ($p < 0.05$). ATMX (2.0 mg/kg) significantly increased % accuracy and reduced false alarm rate ($p < 0.05$) in poor performers. ATMX (1.0 mg/kg, 2.0 mg/kg) significantly increased sensitivity index in low performers ($p < 0.05$; $p < 0.01$), and significantly decreased responsivity index ($p < 0.05$) MPH at 2.0 mg/kg significantly improved accuracy ($p < 0.05$), and reduced premature responding ($p < 0.05$) in low performers and increased premature responding in high performers ($p < 0.05$). MPH significantly increased correct rejections in high performers at 2.0 mg/kg ($p < 0.05$), and decreased correct rejections at all doses in low performers ($p < 0.01$, $p < 0.05$, $p < 0.05$ respectively). MPH significantly increased sensitivity index in low performers at 0.5 and 1.0 mg/kg ($p < 0.01$).

In summary, ATMX (noradrenaline reuptake inhibitor, non-stimulant) reduced impulsivity in low performers in both the 5-CSRTT (premature responses) and in the 5C-CPT (false alarm rate). ATMX enhanced sustained attention in the 5CSRTT and vigilance in 5C-CPT in low performers. Methylphenidate (dopamine/noradrenaline re-uptake inhibitor, psychostimulant) enhanced sustained attention in the 5-CSRTT, vigilance in the 5C-CPT (% correct rejections) and marginally reduced impulsivity in low performers in the 5C-CPT. This would suggest that low performers are more sensitive to the effects of both stimulant and non-stimulant drugs. These data provide the validation of a rat model for the inattentive subtype of adult ADHD. The model utilises the 5C-CPT to select for those with deficits in sustained attention and vigilance, which can then be enhanced by ADHD medication.

Reference(s)

[1] Young, J.W., Light, G.A., Marston, H.M., Sharp, R. & Geyer, M.A. 2009. The 5-choice continuous performance test: evidence for a translational test of vigalence for mice PLoS One, 4, e4227.

[2] Young, J.W., Powell, S.B., Scott, C.N., Zhou, X. & Geyer, M.A. 2011. The effect of reduced dopamine D4 receptor expression in the 5-choice continuous performance task: Separting response inhibition from premature responding Behaviour and Brain Reseach, 222, 183–192.

[3] Puumala, T., Ruotsalainen, S., Jakala, P., Rickkinen, P. & Sirvio, J. 1996. Behavioural and pharmacological studies on the validation of a new animal model for attention deficit hyperactivity disorder. Neurobiology Learning and Memory 66, 198–211.

P.2.016 Exposure to an alternative reward does not reduce cocaine-seeking behaviour

C. Nicolas[1]*, C. Lafay-Chabassier[2], M. Solinas[1].
[1]Laboratoire de Neurosciences Expérimentales et Cliniques-LNEC, INSERM U-1084 Université de Poitiers, Poitiers Cedex, France; [2]Laboratoire de Neurosciences Expérimentales et Cliniques-LNEC INSERM U-1084 Université de Poitiers, Department of Clinical Pharmacology, Poitiers University Hospital, Poitiers Cedex, France

Exposure to positive environmental conditions, such as environmental enrichment, decreases vulnerability to drugs and reduces the risks of relapse to drug-seeking behaviour [1]. Environmental enrichment is comprised of several elements such as social interactions, novelty and sensory and physical activity, which have been described to be rewarding in rodents. Because access to alternative rewards decreases addiction-related behaviours [2], it could be speculated that environmental enrichment produces its positive effects on addiction by acting as an alternative reward [3]. On the other hand, whereas in most studies alternative rewards are presented together with drugs, environmental enrichment is provided chronically as a living environment that is temporally and physically distinct to drug-related conditions. Here we investigated whereas chronic access to a natural reinforcer such as sucrose, during period of withdrawal under conditions similar to those used for environmental enrichment, could reduce drug craving.

For this, we let rats to self-administer cocaine during 10 experimental sessions (0.15 mg/injection, 6 hours per day) whereas yoked-saline rats received only saline injections. At the end of the self-administration training period, rats were subjected to a withdrawal period of 30 days during which they were separated into three groups: a) the first group had a constant access to one bottle of water and one bottle containing sucrose (10% w/v, sucrose continuous SC); b) the second group had access to water and sucrose intermittently (10% w/v, sucrose intermittent, SI) and c) the third group was a control group and had access to two bottles of water (water, WAT). At the end of the

withdrawal period, rats were tested for cocaine seeking behaviour during a single session that lasts 6 hours.

We found that, during abstinence, SC and SI rats avidly consumed sucrose: they drank 3 times more than WAT rats and they completely ignored water bottles. Sucrose consumption and preference were constant over the entire period of withdrawal and did not differ between cocaine and yoked-saline rats. After the withdrawal period of 30 days, rats that were exposed to cocaine show high levels of drug-seeking behaviour but no difference was found among SC, SI and WAT rats.

Altogether, these results show that exposure to a natural reward such as sucrose during withdrawal period does not affect cocaine seeking behaviour. These results suggest that exposure to an alternative reward that is temporally and physically distinct from drug-related environments is not sufficient to reduce the risks of relapse. In addition, they suggest that elements of enrichment other than alternative reinforcement play a more important role in reducing drug seeking or, at least, that alternative reinforcement must be combined with other elements of enrichment to be effective in decreasing drug seeking.

Reference(s)

[1] Chauvet, C., Lardeux, V., Goldberg, SR., Jaber, M., Solinas, M., 2009. Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine. Neuropsychopharmacology 34(13):2767–78.

[2] Ahmed, S.H., 2005. Imbalance between drug and non-drug reward availability: A major risk factor for addiction. Review, Eur J Pharmacol 5;526(1–3):9–20.

[3] Solinas, M., Thiriet, N., Chauvet, C., Jaber, M., 2010. Prevention and treatment of drug addiction by environmental enrichment. Prog Neurobiol 92(14):572–92.

P.2.017 The hallucinogen 5-methoxy-N,N-dimethyltryptamine (5-MeO-DMT) disrupts cortical function: reversal by antipsychotic drugs

M.S. Riga[1]*, F. Artigas[1], P. Celada[1]. [1]*IIBB-CSIC (IDIBAPS) CIBERSAM, Neuropharmacology and Neurochemistry, Barcelona, Spain*

5-MeO-DMT, a natural hallucinogen component of Ayahuasca (Amazonian beverage), is a non-selective serotonin 5-HT_{1A}/5-HT_{2A} receptor agonist. Its potential interest in schizophrenia lies in its ability to mimic psychotic symptoms such as hallucinations. We previously reported that other hallucinogens (the non-competitive NMDA-R antagonist phencyclidine and the 5-$HT_{2A/2C}$ agonist DOI) markedly disrupt cortical synchrony in the low frequency range (<4 Hz) in rodent prefrontal cortex (PFC), an effect reversed by antipsychotic drugs [1–3].

The aims of the present study are: (1) to examine whether 5-MeO-DMT disrupts cortical synchrony in PFC, (2) to examine the ability of antipsychotic drugs to reverse its effects, and (3) to identify other brain areas sensitive to the action of the hallucinogen. We used electrophysiological techniques – single unit extracellular recording of mPFC pyramidal neurons, local field potential (LFP) and epidural electrocorticogram (ECoG) recordings – and blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) in anaesthetised animals. Drugs were administrated intravenously (i.v.) in rat and subcutaneously (s.c.) in mice. Statistical analyses were conducted using Student's t-test, one- or two-way ANOVA followed by multiple comparison test. Statistical significance was set at $p < 0.05$.

5-MeO-DMT (0.1 mg/kg i.v.), in combination with clorgyline (MAO-A inhibitor, to prevent peripheral degradation of 5-MeO-DMT) altered pyramidal discharge (Student's t-test; $p < 0.001$; $n = 42$) and concurrently reduced low frequency cortical oscillations (LFCO; <4 Hz) to 64±2% of basal values (one-way ANOVA; $p < 0.000001$; $n = 58$) in rat PFC. Likewise, 5-MeO-DMT (1 mg/kg s.c.) differentially reduced LFCO in the PFC of wild-type (WT) mice (to ~50% of basal values) and 5-HT_{2A} knockout mice (KO2A) (transiently to ~75% of basal values) (two-way ANOVA; $p < 0.03$). The 5-MeO-DMT-induced reduction in LFCO was significantly reversed by M100907 (5-HT_{2A} receptor antagonist) and WAY-100635 (5-HT_{1A} receptor antagonist) in the rat. Likewise, the effect produced by 5-MeO-DMT in KO2A mice was fully prevented by WAY-100635. Overall, these data indicate that 5-MeO-DMT reduces cortical synchrony via activation of 5-HT_{2A}, and to a lesser extent, 5-HT_{1A} receptors. The antipsychotic drugs haloperidol, clozapine and risperidone and the mGluR2/3 agonist LY379268 reversed 5-MeO-DMT effects on LFCO.

Moreover, fMRI studies showed significant alterations in BOLD response in several cortical areas (PFC, primary visual (V1), somatosensory (S1) and auditory (Au1) cortices). Further electrophysiological experiments indicated that 5-MeO-DMT reduced LFCO in V1, S1 and Au1 areas in rats and WT mice. Interestingly, 5-MeO-DMT altered LFCO in V1 of KO2A mice.

Together with previous findings [1–3], the present results indicate that reductions in LFCO are a common neurophysiological signature of hallucinogens. The reversal of these effects by antipsychotic drugs with different mechanisms of action suggests a clear association with their therapeutic activity, regardless of their initial target.

This supports the usefulness of the LFCO model in PFC to examine the neurobiological basis of hallucinations and in target identification during antipsychotic drug development.

Moreover the present results point to the prefrontal and sensorial cortical areas as sites of action of this hallucinogen and suggest the involvement of 5-HT_{1A} receptors in the action of indoleamine hallucinogens, in addition to their well-known action on 5-HT_{2A} receptors.

Reference(s)

[1] Kargieman L, Santana N, Mengod G, Celada P, Artigas F (2007). Antipsychotic drugs reverse the disruption in prefrontal cortex function produced by NMDA receptor blockade with phencyclidine. Proc Natl Acad Sci U S A 104: 14843–14848.

[2] Celada P, Puig MV, Diaz-Mataix L, Artigas F (2008). The hallucinogen DOI reduces low-frequency oscillations in rat prefrontal cortex: reversal by antipsychotic drugs. Biol Psychiatry 64: 392–400.

[3] Kargieman L, Riga MS, Artigas F, Celada P (2012). Clozapine reverses phencyclidine-induced desynchronization of prefrontal cortex through a 5-HT(1A) receptor-dependent mechanism. Neuropsychopharmacology 37: 723–733.

P.2.018 Characterisation of the effects of partial agonist of a4β2* nACh receptor cytisine in the two-choice serial reaction time task

G. Makshakov[1]*, O. Dravolina[1], A. Bespalov[1], E. Kayukova[1], M. Dorofeikova[1], E. Zvartau[1].
[1]Pavlov State Medical University, Valdman Institute of Pharmacology, St. Petersburg, Russia

It has been suggested that nicotine has attention-enhancing properties and attenuates response inhibition. Nicotine was found to improve target detection and to decrease reaction time, but also to impair the ability to withhold premature responses (i.e. to increase impulsivity). These effects of nicotine are thought to be mediated by α4β2*, α4β4* and α3β2* nicotinic acetylcholine receptor (nAChR) subtypes [1]. The purpose of this study was to investigate effects of cytisine, a partial agonist of α4β2* nAChR, on the performance in the two-choice serial reaction time task (2-CSRTT).

The study was conducted in male Wistar rats. At the beginning of the training, all animals (n = 10) were placed on food restriction maintaining 85% of their free-feeding weight. Experiment sessions lasted for 30 min or until completion of 100 trials, whichever occurred first. Rats were trained to detect a brief visual stimulus (three light bulbs) presented randomly in one of two locations and to press the lever beneath highlighted bulbs during the stimulus presentation (1 s) or the limited hold period (2 s). Such a response ('correct') was followed by a food pellet presentation. 'Incorrect' responses or failure to respond within the limited hold period ('omissions') were recorded and punished by the extinction of the house light for 5 s ('time-out' period). Just after a 'correct' response and collection of the reward or an 'incorrect' response/an omission and time-out, the hole on the opposite wall was illuminated and a nose-poke was required to initiate the next trial. Lever responses before trial initiation ('pre-initiation' responses) were recorded but had no programmed consequence. After trial initiation, animals had to avoid lever pressing for 5 s before visual stimulus presentation, otherwise they were punished by time-out and premature responses were recorded. Criterion of 70% correct responding and less than 10% omissions during two consecutive sessions required to proceed the drug treatment phase. Various doses of cytisine (0, 0.3, 1.0 and 3.0 mg/kg, s.c.) and nicotine (0, 0.075, 0.15, 0.3 and 0.6 mg/kg, s.c.) were administered in a pseudo-random order.

Both compounds had no reliable effects on percent of 'correct' responses and number of premature, perseverative, and time-out responses, although nicotine tended to increase premature responses. Latencies to make a 'correct' response or to collect a food reward were also unchanged from baseline. However, administration of the highest dose of cytisine (3.0 mg/kg) attenuated the number of completed trials due to significantly increased number of 'pre-initiation' responses. Further analysis found that only number of 'pre-initiation' responses (on both levers) performed after a 'correct' response became persistently elevated following cytisine administration.

In summary, the results of the present study demonstrate that cytisine induced performance disruption indicative of increased 'pre-initiation', but not premature responding. Such deficits might reflect an aspect of response disinhibition, other than loss of impulse control, that represents an additional measure of compulsivity related to cognitive inflexibility.

Reference(s)

[1] Blondel A., Sanger D., Moser P., 2000 Characterisation of the effects of nicotine in five-choice serial reaction time task in rats: antagonists studies. Psychopharmacology, 149: 293–305.

P.2.019 The mismatch hypothesis: a new way of linking early experiences and adult environment to vulnerability to stress

S. Santarelli[1]*, K.V. Wagner[1], J. Hartmann[1], X.D. Wang[1], M.V. Schmidt[1]. [1]*Max Planck Institute of Psychiatry, Neurobiology of Stress, Munich, Germany*

Purpose of the study: Chronic stress is considered one of the main risk factors for depression. Interestingly, not all individuals develop psychopathology after chronic stress. In contrast to the prevailing view that stress effects are cumulative and increase stress vulnerability later in life, the recently formulated mismatch hypothesis proposes that individuals experiencing high levels of psychosocial stress early in life are programmed for dealing with high psychosocial stress and are therefore resilient to high stress levels in later life [1]. We here test this hypothesis by comparing the developmental effects of two different early life conditions, when tested under two opposite adult environments.

Methods used: We used female BALB/c mice that underwent either adverse early life conditions (limited nesting material) or a supportive environment (early handling) from PND2 to PND9. At weaning, the animals of each group were assigned to either group housing (supportive environment) or single housing (social deprivation, adverse environment). At adulthood, we compared the molecular and the behavioural effects of the interaction between early and adult environment, in particular on anxiety like and depressive like behaviour.

Table 1. Experimental groups

	Group Housed (GH)	Single Housed (SH)
Early Handling (EH)	EH GH (matched)	EH SH (mismatched)
Limited Nesting Material (LM)	LM GH (mismatched)	LM SH (matched)

Summary of results: Animals that underwent two adverse environments (mismatched) and those that underwent two supportive experiences (matched) have a similar behavioural profile. They are less vulnerable to stress when compared to the ones that underwent only one stressful experience, either during early life or during adulthood. Parameters like weight of organs, spontaneous behaviour in an open field or floating in the forced swim stress were significantly reduced in animals with mismatched environments compared to animals with matched environments. Also corticosterone levels, a measure of hypothalamic–pituitary–adrenal axis responsiveness, have been significantly affected by the interaction of the early and adult environment. In contrast, anxiety like behaviour in the elevated plus maze was mainly affected by early life experience, whereas sociability behaviour was mainly affected by the adult environment. We also looked for new molecular markers for stress vulnerability, and we found that the expression levels of SLC6A15, a novel candidate for depression vulnerability, are increased in the hippocampus of mismatched animals.

Conclusions: We conclude that animals placed at adulthood in a mismatched environmental condition compared to their early life environment, are more vulnerable to stress in specific behavioural and neuroendocrine parameters. Still further analyses are needed to better understand the effects of this paradigm on other behaviours and to clarify the molecular mechanisms underlying those behavioural modifications. Based on these findings, we hypothesize that adult vulnerability to stress could be modulated by a mismatch between early-life and adult environment, in combination with a more resilient or vulnerable genetic make-up.

Reference(s)

[1] Nederhof, E., Schmidt, M.V., 2012. Mismatch or cumulative stress: Toward an integrated hypothesis of programming effects. Physiology & Behavior, 106 (5), 691–700.

P.2.020 Disruption of 5-HT$_7$ receptors accelerates age-related episodic-like memory decline

G. Beaudet[1]*, M. Brehin[1], T. Freret[1], G. Nee[1], V. Delaunay[1], M. Boulouard[1], E. Paizanis[1]. [1]*Université de Caen Basse-Normandie, GMPc EA4259, Caen, France*

Increasing incidence of cognitive impairments including learning and memory deficits, with early alterations of episodic memory, related to population ageing, is a major public health challenge. Thus it is crucial to identify new therapeutic targets, not only focussing on symptomatic strategies, but also aiming at the prevention and health recovering. In this way, modulation of serotonergic 5-HT$_7$ receptors (5-HT$_7$R) could be an innovative approach since (1) gene expression of 5-HT$_7$R early decreases during senescence in rats (between 3 and 12 months-old), (2) synaptic plasticity of the hippocampus is altered in 5-HT$_7$R knocked-out KO) mice, and (3) as we recently have shown in preliminary experiments, pharmacological modulation of 5-HT$_7$ improves episodic-like memory performances in mice. However, considering that episodic-like memory assessed in an object recognition task [1] seems not to be altered in 5-HT$_7$ KO mice at 3 months of age, we hypothesized that a deficit could appear later.

Therefore, in order to identify the implication of 5-HT_7R on the onset of the age-related memory decline, memory performances of 7–8 months-old and 10–12 months-old KO C57BL/6J mice with homozygous disruption of the 5-HT_7R gene were compared to paired wild type mice (WT). Recognition memory performances were assessed in a Y-maze with a 1-hour delay between presentation and test [2]. Spatial working memory performances were assessed by recording spontaneous alternation behaviour in a Y-maze during a 5-minutes session. In addition locomotor activity was assessed in an activity cage and anxiety-related behaviour was examined using the elevated plus-maze (EPM).

Our data showed that 10–12 months-old WT mice did not discriminate the novel object contrary to 7–8 months-old WT mice, which spent more time exploring the novel object compared to the chance level (univariate t-test: $p < 0.05$). In addition, disruption of 5-HT_7R led to an impaired object discrimination in 7–8 and 10–12 months-old mice. By contrast, ANOVA analysis revealed that mutant mice exhibited equivalent levels of spontaneous alternation compared to WT mice (around 75%). In addition, statistical analysis of locomotor activity did not revealed any significant effect of genotype or genotype × age interaction, indicating equivalent basal level of locomotion. Finally, no differences between mice of both genotypes were detected in the global activity or the anxiety-related parameters assessed in the EPM.

Our results therefore suggest that in our experimental conditions, the onset of episodic-like memory decline is situated between 7–8 and 10–12 months-old of age in C57BL/6J mice, and that the lack of 5-HT_7R could accelerate the onset of this decline, with no relation to any inhibition of locomotor activity or increased anxiety-like behaviour. By contrast, working memory performances seem not to be affected between 7–8 and 10–12 months-old in KO and WT mice. Further pharmacological (using a selective 5-HT_7 antagonist) and molecular (5-HT_7R expression) studies in WT mice would confirm this statement.

Reference(s)

[1] Sarkisyan, G., Hedlund, P.B., 2009. The 5-HT7 receptor is involved in allocentric spatial memory information processing. Behavioural Brain Research 2002, 26–31.

[2] Leger, M., Quiedeville, A., Paizanis, E., Natkunarajah, S., Freret, T., Boulouard, M., Schumann-Bard, P., 2012. Environmental enrichment enhances episodic-like memory in association with a modified neuronal activation profile in adult mice. PLoS One 7, 10.

P.2.021 Role of adenosine $(A)_{2A}$ receptor in nicotine addiction − pharmacological and genetic aspects

J. Czyzyk[1]*, E. Nowak[1], M. Bader[2], K. Fuxe[3], M. Filip[1]. *[1]Polish Academy of Sciences, Department of Pharmacology, Krakow, Poland; [2]Max-Delbrück-Centrum, Molecular Medicine, Berlin, Germany; [3]Karolinska Institutet, Department of Neuroscience, Stockholm, Sweden*

Nicotine is one of the very strong addictive psychoactive substance which is commonly available and accepted by society. The abuse of nicotine is linked to the enhancement of dopaminergic (DA) neurotransmission and indirect activation of DA D_2 receptors in the brain mesolimbic system. A number of data indicate antagonistic interaction at the molecular, neurochemical and behavioural levels between D_2 and adenosine $(A)_{2A}$ receptors that occurs in the striatum [1]. This interaction may have a significance to control behavioural effects induced by nicotine [2].

We studied the effects of the A_{2A} receptor agonist CGS 21680 and antagonist KW 6002 on the development and expression of nicotine sensitisation in wild-type (WT) rats. Moreover, we addressed the role of A_{2A} receptors in the locomotor effects to repeated treatment with nicotine in transgenic (TG) rats overexpressing A_{2A} receptors.

Sensitisation was evoked by 5 daily injections of nicotine (0.4 mg/kg, sc) and a challenge dose of the drug (0.4 mg/kg, sc) on day 10. During the first 5 days of development phase WT animals received the following injections: CGS 21680 (0.2–0.4 mg/kg, ip) or KW 6002 (0.25–0.5 mg/kg) in combination with nicotine, while TGR were treated with vehicle or nicotine (0.4 mg/kg, sc), then on 6–9 days the animals remained drug-free in home cages. On day 10, all rats received nicotine challenge dose. In studies into expression of sensitisation, the WT animals received repeatedly (5 days) vehicle or nicotine (0.4 mg/kg, sc), while on day 10 they were pretreated by CGS 21680 (0.05–0.2 mg/kg, ip) or KW 6002 (0.25–0.5 mg/kg, ip) before a challenge dose of nicotine. Conditioned locomotion was evoked by 5-daily injections of nicotine (0.4 mg/kg, sc) in experimental chambers followed by a 4-day drug-free period and a vehicle challenge in experimental chambers on day 10. Locomotor activity measurements started immediately after the injection of nicotine and were recorded for 60 min.

During development of sensitisation CGS 21680 in dose of 0.4 mg/kg (but not 0.2 mg/kg) given repeatedly with nicotine significantly ($P < 0.05$) decreased the locomotor

activity to the nicotine challenge dose, while KW 6002 was inactive in this procedure. Expression of nicotine sensitisation was reduced by pretreatment with CGS 21680 (by 60% in a dose of 0.2 mg/kg, and by 55% in a dose of 0.1 mg/kg), whereas KW 6002 did not change the locomotor activity induce by nicotine. TG animals, like the WT rats, showed 2-fold enhancement in locomotor activity to repeated nicotine treatment (days 1–5, and day 10). There was no evidence of conditioned locomotion in TG rats; only WT rats treated repeatedly (1–5 days) with nicotine and challenged with vehicle on day 10 showed environment-triggered locomotion.

The present results show that stimulation of adenosine A_{2A} receptors reduces the development and expression of nicotine sensitisation as well as drug-associated environmental cues in WT animals. The latter effect was confirmed in TG rats overexpressing A_{2A} receptors. These findings extent our recent data on cocaine [3] and may rise the possibility of using A_{2A} receptor agonists in drug addiction therapy.

Reference(s)

[1] Filip, M., Zaniewska, M., Frankowska, M., Wydra, K., Fuxe, K., 2012. The Importance of the Adenosine A2A Receptor-Dopamine D2 Receptor Interaction in Drug Addiction. Curr Med Chem. 19(3), 317–55.

[2] Castañé, A., Soria, G., Ledent, C., Maldonado, R., Valverde, O., 2006. Attenuation of nicotine-induced rewarding effects in A2A knockout mice. Neuropharmacology. 51, 631–40.

[3] Filip, M., Frankowska, M., Zaniewska, M., Przegalinski, E., Muller, C.E., Agnati, L., Franco, R., Roberts, D.C., Fuxe, K., 2006. Involvement of adenosine A2A and dopamine receptors in the locomotor and sensitizing effects of cocaine. Brain Res. 1077, 67–80.

P.2.022 Oxytocin in the central nucleus of the amygdala mediates social buffering of fear

E. Rickenbacher[1]*, M. Moita[1]. [1]*Champalimaud Foundation, Champalimaud Neuroscience Programme: Behavioral Neuroscience, Lisbon, Portugal*

Fear responses are innate defensive behaviours exhibited by animals in response to aversive stimuli. During fear conditioning an animal can learn to fear a neutral stimulus (e.g. tone) when it is paired with an aversive one (e.g. footshock), such that this previously neutral stimulus comes to elicit fear responses. This form of learning may be crucial as it allows animals to use cues associated with aversive events to avoid future threats. Interestingly, there is evidence that when animals are in the presence of a conspecific they show decreased fear responses to a stimulus previously paired with an aversive event; demonstrating that social interactions can buffer fear responses [1].

As we are interested in the neural mechanisms of social buffering, we have developed a paradigm to study social buffering in fear conditioned rats. In our experiment rats were conditioned to fear a tone by pairing it with footshocks. After conditioning, rats were tested for their fear of the tone through the presentation of the tone in the absence of shock. Rats quickly learned to fear the tone, displaying a robust fear response, freezing. However, we found that rats re-exposed to the tone in the presence of their naïve cage-mate ($n = 8$) showed less freezing than if tested alone ($n = 11$) (average freezing response across tone presentations ($p < 0.01$)). Moreover, it is known that exposure to the tone in the absence of footshocks leads to a subsequent decrease in tone-evoked fear responses. This decrement in freezing results either from animals learning that the stimulus is now safe, while still remembering that it was previously predictive of shock, or because they update their memory of the association between tone and shock, losing the initial memory.

Thus, we asked whether being re-exposed to the tone in the presence of the cage-mate affected how rats responded to the tone in future exposures. When tested subsequently, now alone, rats that were previously re-exposed in the presence of their cage-mate ($n = 17$) continued to freeze at a decreased level compared to animals that had been re-exposed alone ($n = 16$), showing that social buffering has both short term ($p < 0.001$) and long term effects on fear expression ($p < 0.01$).

Although oxytocin has been implicated in this process, its underlying neural mechanisms remain poorly understood. We have therefore assessed the role of oxytocin in the central nucleus of the amygdala (CeA), a major output station that controls several defence responses [2,3]. Animals received bilateral CeA cannula implants one week prior to fear conditioning and bilateral oxytocin antagonist or vehicle infusions immediately prior to the first tone re-exposure session. Our data show that the infusion of an oxytocin antagonist compared to vehicle into the CeA, prior to re-exposure to the tone, blocks the immediate and long lasting effect of social buffering on the elicited fear memory and response ($p < 0.01$).

Our study shows long lasting effects of social buffering on fear and elucidates the neural mechanisms underlying the phenomenon. These data contribute important knowledge pertaining to underlying causes of anxiety disorders and possible treatment applications.

Reference(s)

[1] Kiyokawa, Y., Takeuchi, Y., Mori, Y., 2007. Two types of social buffering differentially mitigate conditioned fear responses. Eur J Neurosci. 26(12): p. 3606–13.

[2] Wilensky, A.E., Schafe G.E., Kristensen M.P., LeDoux J.E., 2006. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. J Neurosci. 26(48): p. 12387–96.

[3] Ciocchi, S., Herry C., Grenier F., Wolff S.B., Letzkus J.J., Vlachos I., Ehrlich I., Sprengel R., Deisseroth K., Stadler M.B., Müller C., Lüthi A., 2010, Encoding of conditioned fear in central amygdala inhibitory circuits. Nature. 468(7321): p. 277–82.

P.2.023 The antagonist of 5-HT$_7$ receptors, SB-269970, and amisulpride both reverse ketamine-induced cognitive inflexibility in rats

D. Rafa[1]*, A. Nikiforuk[1], P. Popik[1]. [1]*Polish Academy of Sciences, Department of Behavioural Neuroscience and Drug Development, Krakow, Poland*

The prefrontal cortex mediates higher-order executive functions, including among other, the cognitive flexibility, i.e., the ability to modify behaviour in response to changing task demands. This aspect of executive function is impaired in schizophrenia. Cognitive flexibility may also be assessed in rodents in the attentional set-shifting task (ASST) [1]. In this paradigm, rats must select a bowl containing a food reward based on the ability to discriminate the odours and the media covering the bait. The ASST requires rats to initially learn a rule and form an attentional 'set' within the same stimulus dimensions. At the extradimensional (ED) shift, animals must switch their attention to a new, previously irrelevant stimulus dimension and, for example, discriminate between the odours and no longer between the media covering the bait. The animals' performance at the ED phase is impaired in N-methyl-D-aspartate receptor antagonist (e.g., ketamine)-treated animals [2], regarded as pharmacological model of schizophrenia-like symptoms.

The 5-HT$_7$ (5-hydroxytryptamine 7, serotonin 7) receptor, one of the most recently identified members of the serotonin receptor family, may play an important role in the pathophysiology and treatment of many psychiatric disorders. Recent data suggest that the blockade of 5-HT$_7$ receptors may exert procognitive effects in animal models of schizophrenia [3]. This issue might be of special interest, since several atypical antipsychotics, e.g., amisulpride, are characterised by a high affinity for 5-HT$_7$ receptors. Nevertheless, little is known about the efficacy of 5-HT$_7$ antagonists the in models of schizophrenia-like cognitive inflexibility.

The aim of the present study was to investigate the role of a potent and selective 5-HT$_7$ receptor antagonist (SB-269970), and amisulpride (an atypical antipsychotic with high affinity to 5-HT$_7$ receptors) on ketamine-induced deficits in the ASST task in rats.

Ketamine (10 mg/kg) was administered to Sprague-Dawley rats subcutaneously 75 min prior to the test. SB-269970 or amisulpride were given intraperitoneally 15 min before ketamine injection. The number of trials required to achieve the criterion of 6 consecutive correct responses was recorded for each rat and for each discrimination phase. Data were calculated using two-way mixed-design ANOVAs followed by the Newman–Keuls post-hoc test.

Ketamine administration significantly and specifically impaired rats' performance at the ED stage of ASST as indicated by an increased number of trials to criterion during this phase. SB-269970 (0.3 and 1 mg/kg) and amisulpride (3 mg/kg) reversed ketamine-induced cognitive inflexibility. Additionally, SB-269970 at a dose of 1 mg/kg also improved ED performance as compared to control rats. There were no significant drug effects during any other discrimination stage. Analyses of variance revealed significant interactions of discrimination phase and treatment: $F[18,204] = 26.93$, $P < 0.001$ (SB-269970) and $F[12,156] = 16.84$, $P < 0.001$ (amisulpride).

Present study demonstrated the efficacy of the 5-HT$_7$ antagonist, SB-269970, and amisulpride in ameliorating frontal-like deficits relevant to the psychopathology of schizophrenia. It thus seems likely that the antagonism of 5-HT$_7$ receptors may represent a useful pharmacological approach for cognitive enhancement in schizophrenia.

Reference(s)

[1] Birrell, J.M., Brown, V.J., 2000. Medial frontal cortex mediates perceptual attentional set shifting in the rat. J Neurosci 20, 4320–4324.

[2] Nikiforuk, A., Golembiowska, K. and Popik, P., 2010. Mazindol attenuates ketamine-induced cognitive deficit in the attentional set shifting task in rats. Eur Neuropsychopharmacol 20, 37–48.

[3] Horiguchi, M., Huang, M., Meltzer, H.Y., 2011. The role of 5-hydroxytryptamine 7 receptors in the phencyclidine-induced novel object recognition deficit in rats. J Pharmacol Exp Ther 338, 605–14.

P.2.024 The effects of positive allosteric modulators of α7 nicotinic receptors on rats' performance in the odour span test

A. Potasiewicz[1]*, A. Nikiforuk[1], P. Popik[1]. [1]*Polish Academy of Sciences, Department of Behavioral Neuroscience and Drug Development, Krakow, Poland*

It is widely accepted that cognitive deficits are a core feature of schizophrenia. Schizophrenic patients develop a wide range of neuropsychological dysfunctions including deficits in working memory (WM). Specifically, they suffer from shortages in WM span capacity, displaying reduced number of information kept concurrently in the WM.

Nicotinic acetylcholine receptors (nAChRs) play an important role in the regulation of cognitive processes. Particularly, selective activation of α7-nAChRs represents a promising target for the development of pharmacological treatments for cognitive dysfunctions in schizophrenia. It appears however, that positive allosteric modulators (PAMs) of α7-nAChRs may demonstrate a more favorable profile than orthosteric agonists [1]. Current data has demonstrated a positive influence of nicotine on WM span capacity assessed using the odour span task (OST) in rats [2,3]. However, while the role α7-nAChRs in the nicotine-induced enhancement in span capacity has been suggested, the effects of α7-nAChRs PAMs have not been assessed on that task.

The aim of the present study was to investigate the effects of α7-nAChRs PAMs: CCMI (type I) and PNU-120596 (type II) in comparison to nicotine on the WM capacity in the OST in rats. Type I PAMs have little or no effect on desensitisation processes, while the action of type II PAMs is accompanied by a retardation of the kinetics of desensitisation.

Male Sprague-Dawley rats were trained to remember an increasing number of odours according to a nonmatching to sample rule. Rats had to dig for food rewards in scented cups containing woodchips. Once they retrieved the reward, a new differently scented cup was added. Rats had to ignore previous cups and choose a new one in order to gain a reward. After each correct response a new cup was introduced. The number of consecutive correct responses served as a measure of the span length. Also the number of errors was counted during the session. The effect of nicotine (0.05 and 0.1 mg/kg), PNU 120596 (1 and 3 mg/kg), CCMI (0.3 and 1 mg/kg) and vehicle (saline) were evaluated after asymptotic performances were obtained. Drugs doses were administered in a Latin square design order. Data were calculated using main effect-design ANOVAs followed by the Newman–Keuls post-hoc test.

Nicotine (0.05 and 0.1 mg/kg), administered subcutaneously (SC) 10 minutes before the test, caused a statistically significant improvement of the span length ($p < 0.05$). It also significantly decreased a number of errors ($p < 0.05$ and $p < 0.001$ for a dose of 0.05 and 0.1 mg/kg, respectively). Neither CCMI, nor PNU120596 administered SC 30 minutes before the test significantly affected the span length. However, a number of errors was significantly decreased by CCMI at both doses tested ($p < 0.05$).

Although the α7-nAChRs PAMs represent a promising alternative to orthosteric ligands, their behavioural activity is still poorly understood especially with regard to the differences between type I and type II compounds. Hence, despite the limited efficacy of CCMI and PNU120596 on the span capacity in cognitively unimpaired animals, it seems interesting to further investigate α7-nAChRs PAMs in the models of schizophrenia-like OST deficits.

Reference(s)

[1] Hurst, R.S., Hajos, M., Raggenbass, M., Wall, T.M., Higdon, N.R., Lawson, J.A. et al., 2005. A novel positive allosteric modulator of the alpha7 neuronal nicotinic acetylcholine receptor: in vitro and in vivo characterization. J Neurosci. 25:4396–4405.

[2] Rushforth, S.L., Allison, C., Wonnacott, S., Shoaib, M., 2010. Subtype-selective nicotinic agonists enhance olfactory working memory in normal rats: a novel use of the odour span task. Neurosci Lett 471:114–118.

[3] Rushforth, S.L., Steckler, T., Shoaib, M., 2011. Nicotine improves working memory span capacity in rats following sub-chronic ketamine exposure. Neuropsychopharmacology 36:2774–2781.

P.2.025 Ventral medial prefrontal cortex inactivation reduces context-induced reinstatement of nicotine seeking

R.F. Struik[1]*, J. Peters[1], S. Jonkman-Tielemans[1], T.J. De Vries[2]. [1]*Free University Medical Centre (VUmc), Anatomy and Neuroscience, Amsterdam, The Netherlands;* [2]*Free University (VU), Molecular & Cellular Neurobiology Neuroscience Campus Amsterdam CNCR, Amsterdam, The Netherlands*

Environments and circumstances strongly contribute to relapse to smoking in humans. In rodents as well, when returned to a drug-paired context, rats will reinstate drug seeking (or relapse), termed context-induced reinstatement [1]. Preclinical data suggests prefrontal cortex involvement in inhibitory control over drug seeking

behaviour [2]. Inactivation of neurons in the ventral mPFC reduced context-induced reinstatement of heroin seeking [3]. Data on the role of the ventral mPFC in nicotine seeking are lacking. Here, in a pharmacological inactivation study, we determined the involvement of the ventral mPFC in context-induced reinstatement of nicotine seeking.

Thirty-two male Sprague Dawley rats were implanted with intravenous catheters and guide cannulas in the ventral mPFC. Animals were trained to self-administer nicotine and saline in separate contexts during one-hour sessions per day over 19 days. Contexts consisted of an operant chamber containing a nose-poke hole or lever, a discrete light/tone cue as well as tactile, odour, circadian and sound cues. Responding was extinguished in a third context. One hour prior to being returned to the nicotine context, the saline context and the extinction context, animals were infused in the ventral mPFC with 0.3 microlitre of either ACSF or a mixture of the GABA-A/GABA-B agonist baclofen (1 mM)/ muscimol (0.1 mM). Two extinction sessions separated each test session.

Responding at the end of self-administration is higher for nicotine (48.1±3.90, mean±SEM) than saline (8.33±1.26), indicating rats selectively respond for nicotine (one-way ANOVA, $p < 0.001$). The vehicle group reinstated when returned to the nicotine context (17.9±2.8) compared to the extinction context (5.6±2.26; one-way ANOVA, $p < 0.01$). The baclofen/muscimol group exhibited blunted relapse when returned to the nicotine context (8.0±2.4) compared to the extinction context (4.0±1.3). Lower responding in the baclofen/muscimol group in the nicotine context compared to the vehicle group (one-way ANOVA, $p < 0.05$), indicates that inactivation of the ventral mPFC reduces context-induced reinstatement of nicotine seeking. In the saline context, no difference was seen between responding in vehicle (13.2±6.4) versus baclofen/muscimol (6.1±3.6) groups. This seems to indicate ventral mPFC inactivation had no effect on responding in the saline context.

These results suggest that, similar to studies in context-induced reinstatement of heroin seeking, activity in the ventral mPFC is critical for context-induced reinstatement of nicotine seeking. However, ventral mPFC activity is not essential for responding in a saline context and rats are able to distinguish a nicotine-associated context from a saline-associated context. Since responding in an extinction context is unaffected by ventral mPFC inactivation, this area is likely not involved in context-associated inhibitory control over nicotine seeking. Future studies are designed to determine the ventral mPFC neuronal subtypes and projections involved in context-induced reinstatement of nicotine seeking.

Reference(s)

[1] Crombag, H.S., Bossert, J.M., Koya, E., Shaham, Y., 2008, Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond B Biol Sci. 363(1507), 3233–43.

[2] Kalivas, P.W., The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci. 10(8):561–72.

[3] Bossert, J.M., Stern, A.L., Theberge, F.R., Cifani, C., Koya, E., Hope, B.T., Shaham, Y., 2011. Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. Nat Neurosci. 14(4):420–2.

P.2.026 Pro-inflammatory cytokines induce anhedonia in mice and increase monoamine transporter activity in the nucleus accumbens

F. van Heesch[1]*, J. Prins[1], K.G.C. Westphal[1], G.A.H. Korte-Bouws[1], B. Olivier[1], A.D. Kraneveld[1], S.M. Korte[1]. [1]*Utrecht University, Pharmacology, Utrecht, The Netherlands*

A growing body of evidence suggests that pro-inflammatory cytokines contribute to the pathogenesis of depression due to a general medical condition [1]. Lipopolysaccharide (LPS), a component of the outer membrane of gram-negative bacteria, binds to toll-like receptor 4 (TLR4) leading to the rapid release of pro-inflammatory cytokines. In rats these LPS-induced pro-inflammatory cytokines induce anhedonia, i.e. the inability to experience pleasure, a core symptom of major depressive disorder. This was shown by increased thresholds in an intracranial self-stimulation (ICSS) paradigm [2]. The underlying mechanisms are largely unknown. We hypothesize that peripheral cytokines reach the brain and affect central monoamine signalling via the enhancement of monoamine reuptake which leads to anhedonia.

To demonstrate that peripheral cytokines induce anhedonia in mice, C57BL/6 mice were trained in the ICSS paradigm. After establishment of stable ICSS thresholds, mice were treated with saline or LPS (133 μg/kg, i.p.). Animals were tested in the ICSS paradigm 1 h, 4 h, 24 h, 48 h, 72 h and 96 h after administration. In another group of mice we measured extracellular monoamine (metabolite) concentrations in the nucleus accumbens (NAc). In the first microdialysis study, mice were treated with saline followed by a saline or LPS injection (133 μg/kg, i.p.) 30 min later. To test the involvement of monoamine transporters, in the second study, animals were injected with the triple reuptake inhibitor DOV 216,303 (5 mg/kg, i.p.) 30

minutes before the saline or LPS injection (133 μg/kg, i.p.). During the microdialysis studies 30-minute samples were collected during a period of 6.5 h.

Results: In the ICSS study, repeated measures ANOVA with time as within factor and treatment as between-subjects factor revealed a significant time × treatment interaction and a significant effect of treatment ($F(1.7,24.0) = 6.263$, $p = 0.009$ and $F(1,14) = 9.2$, $p = 0.009$ respectively). One-way ANOVA per time point revealed significant elevations of ICSS thresholds in the LPS group at time points 1 h and 4 h after injection. Peripheral LPS injection did not affect serotonin and dopamine levels in the NAc. However, repeated measures ANOVA revealed significant time × treatment interactions and effects of treatment for the metabolites (5-HIAA: $F(3.6,35.9) = 16.8$, $p < 0.001$ and $F(1,10) = 12.6$, $p < 0.01$ respectively; DOPAC: $F(2.9,28.9) = 9.4$, $p < 0.001$ and $F(1,10) = 10.2$, $p < 0.01$ respectively and HVA: ($F(3.0,30.0) = 34.5$, $p < 0.001$ and $F(1,10) = 16.0$, $p < 0.01$ respectively). Pre-exposure to DOV 216,303 reduced or even prevented the LPS-induced increase in monoamine metabolite levels (time × treatment interaction and effect of treatment: 5-HIAA: $F(2.7,32.0) = 15.0$, $P < 0.001$ and $F(1,12) = 8.0$, $p < 0.05$ respectively; DOPAC: $p > 0.05$ and $p > 0.05$ respectively and HVA: $F(1.9,22.0) = 4.1$ and $p > 0.05$ respectively).

Altogether, these results indicate that pro-inflammatory cytokines play a role in the communication between the immune system and central nervous system. The increased elevations in ICSS thresholds after exposure to LPS suggest that pro-inflammatory cytokines induce anhedonia. The microdialysis data suggest that monoamine transporters in the nucleus accumbens are involved in this process, since pre-exposure to the triple reuptake inhibitor DOV 216,303 reduced or even prevented the increased reuptake of monoamines by LPS, as reflected by the decreased extracellular levels of monoamine metabolites.

Reference(s)

[1] Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 1, 46–56.

[2] Borowski, T., Kokkindis, L., Merali, Z., Anisman, H., 1998. Lipopolysaccharide, central in vivo biogenic amine variations, and anhedonia. Neuroreports 17, 3797–802.

P.2.027 α7 nicotinic acetylcholine receptor (α7 nAChR) agonists or antagonists as potential cognition enhancers?

N.P. Van Goethem[1]*, J. Prickaerts[1]. [1]*Maastricht University, School for Mental Health and Neuroscience, Maastricht, The Netherlands*

α7 nicotinic acetylcholine receptors (α7 nAChRs) are ligand-gated ion channels expressed primarily in the brain. These receptors have been implicated in modulating many cognitive functions like attention, episodic memory and learning. α7 nAChRs are located both pre- and postsynaptically and modulate glutamate, GABA and dopamine. Furthermore, α7 nAChRs are directly involved in hippocampal long-term potentiation (LTP), the putative cellular mechanism underlying learning and memory. Activation of α7 nAChRs through selective, partial, or full agonists and/or modulators, has been shown to improve cognitive function in both animal and human studies. The main cognitive improvement with these compounds relate to memory, in accordance with the high level of expression of α7 nAChRs in the frontal-cortex and hippocampus. Hence, α7 nAChR agonists/modulators may be attractive drug candidates to improve cognition in Alzheimer's disease (AD) and schizophrenia patients [1]. The objective of the current study was to investigate the cognition enhancing properties of low dose administration of an α7 nAChR antagonist in rats. Memory performance was assessed with the object recognition task (ORT). The compound used for these studies was methyllycaconitine (MLA). MLA is a norditerpenoid alkaloid produced by many species of Delphinium (larkspurs) and is often used because of its ability to potently antagonise the α7 nAChRs. As a potent selective antagonist of the α7 nAChR, MLA is often used in animal models to show that putative α7 nAChR agonists indeed work through the α7 nAChR mechanism by blocking the procognitive effects of α7 nAChR agonists by means of MLA administration [1]. In contrast, low doses of MLA have sporadically been reported to improve cognition in animals. Furthermore, MLA has been shown to facilitate LTP induction in hippocampal region CA1 in rats. In the current study, it was found that MLA significantly improved memory of rats in the ORT paradigm (24 h interval ORT, repeated measures ANOVA; MLA ($F7,119 = 4.37$; $P < 0.001$) optimal dose range 0.003–0.1 mg/kg i.p.). Moreover, it was found that a dose that was too high (1.0 mg/kg, i.p.) to improve memory in the natural forgetting paradigm (24 h retention interval), was also sufficient to induce a memory deficit in the 1 h retention interval ORT, an interval that normally

leads to good memory performance of rats (1 h interval ORT, repeated measures ANOVA; MLA ($F3,51 = 7.33$; $P < 0.001$). This indicates that MLA could act both as a cognition enhancer and as a deficit model in this paradigm. Among other possibilities, one explanation for these findings could be that α7 nAChR antagonists promote α7 nAChR resensitisation. Since α7 nAChRs desensitise fast, by occupying a subset of α7 nAChRs with selective antagonists without intrinsic value, these receptors could have an opportunity to recover or re-sensitise. This hypothesis is currently investigated. In summary, while the main focus of the α7nAChR as a target for cognition enhancement lies on agonists and positive modulators, the antagonists of these receptors might prove to be a valuable tool for cognition enhancement in AD or schizophrenia.

Reference(s)

[1] van Goethem, N.P., Prickaerts, J., Chesworth, R., Shapiro, G., Boess, F.G., Methfessel, C., Reneerkens, O.A.H., Flood, D.G., Hilt, D., Gawryl, M., Bertrand, S., Bertrand, D., König, G., 2012. EVP-6124, a novel and selective a7 nicotinic acetylcholine receptor agonist, improves memory performance by potentiating the acetylcholine response of a7 nicotinic acetylcholine receptors. NeuroPharmacology, 62(2), 1099–1110.

P.2.028 Enhanced vulnerability to cocaine reinforcing effects in female H/Rouen mice selectively bred for depressive-like behaviour

V. Rappeneau[1]*, A.L. Morel[1], P.H. Luppi[1], J.M. Vaugeois[1], M. El Yacoubi[1], A. Bérod[1]. [1]*Centre de Recherche en Neurosciences, CNRS UMR 5292 INSERM U1028, Lyon, France*

Cocaine addiction is strongly associated with depression, however the mechanisms underlying this co-occurrence are not well understood. We hypothesize that depression strongly enhances vulnerability for cocaine abuse. Females are particularly more vulnerable to depression, and to key phases of the addiction process. Consequently, we employed a genetically-induced mouse model of depression: the Helpless mice (H/Rouen) versus the Non Helpless mice (NH/Rouen). These lines have been selectively bred for respectively high versus low spontaneous immobility in the Tail Suspension Test (TST), a stress paradigm aimed at screening potential antidepressants [1]. The aim of our study was to determine the sensitivity to cocaine psychomotor stimulant and reinforcing effects of female H/Rouen and NH/Rouen mice, and to shed light on the neurobiological mechanisms by which depression-like states enhance addictive behaviours.

To determine the sensitivity to acute cocaine psychomotor stimulant effects in H/Rouen and NH/Rouen mice, we compared the locomotor activity to acute cocaine administration (0–20 mg/kg i.p.). As expected, acute cocaine induced a dose-dependent increase in locomotor activity in both lines. Interestingly, a right shift of the dose-response curve was observed in H/Rouen mice (interaction treatment × line [$F(10,187) = 4.90$); $p < 0.0001$]). We then evaluated the behavioural sensitisation induced by chronic cocaine treatment (10 mg/kg for 5 days). The sensitisation test was assessed by a cocaine challenge (1 mg/kg), after a drug free period of 7 days [2]. We observed a similar motor activation in H/Rouen and NH/Rouen mice ($p < 0.0001$) pre-treated with cocaine. These results indicate similar sensitivity to chronic cocaine psychomotor stimulant effects in both lines, despite differences observed after acute cocaine treatment.

We also investigated the sensitivity to cocaine rewarding effects in the conditioned place preference paradigm, the predominant model of drug-seeking behaviour in mice, where animals were trained for 4 days to associate a specific environment to reinforcing properties of cocaine (10 mg/kg i.p.). The time spent in the drug-paired compartment was measured during the preference test as an indicator of cocaine rewarding effects [3]. Results showed a stronger conditioned place preference in H/Rouen compared to NH/Rouen mice (interaction treatment × line [$F(1,57) = 3.91$; $p = 0.05$]). The cocaine preference score were interestingly correlated to the immobility time in the TST ($r = 0.42$; $p = 0.003$).

The main conclusion that may be drawn from our experiments is that, compared to NH/Rouen mice, H/Rouen mice were more sensitive to cocaine rewarding effects. Neuroanatomical studies were consequently undertaken to explore the neural substrates that mediate the increased sensitivity to cocaine rewarding effects observed in H/Rouen females. Cocaine conditioned place preference was associated with an increase in Fos in the nucleus accumbens (Acb), subregions of the prefrontal cortex (PFC), basolateral amygdala (BLA) and dorsal hippocampus (Hc) in H/Rouen and NH/Rouen females compared to controls. Moreover, preliminary results showed that in H/Rouen mice, infralimbic and cingulate cortices and the Acb core were less activated whereas BLA and Hc were more activated during expression of the cocaine conditioned place preference.

Reference(s)

[1] El Yacoubi M. et al., 2003. Behavioral, neurochemical, and electrophysiological characterization of a genetic

mouse model of depression. Proc Natl Acad Sci USA 100(10):6227–32.
[2] Post R.M. et al., 1992. Conditioned sensitization to the psychomotor stimulant cocaine. Ann N Y Acad Sci 654:386–99.
[3] Tzschentke T.M. et al., 2007. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol 12(3–4):227–462.

P.2.029 Serotonin regulates hippocampal synaptic plasticity and object memory in mice

S.P. Fernandez[1]*, A. Gruart i Massó[2], P. Gaspar[1].
[1]*Institut du Fer a Moulin, UMR-S 839 INSERM/UPMC, Paris, France;* [2]*Pablo de Olavide University, Division of Neurosciences, Seville, Spain*

Low levels of serotonin (5-HT) have been associated with the learning and memory deficits seen in Alzheimer's disease, autism and major depression [1]. Studies performed in healthy volunteers have shown that 5-HT depletion disrupts consolidation of new information, specifically in tasks involving delayed recall and/or recognition of visually-presented words, spoken words, pictures or abstract figures [1]. Despite this evidence, the mechanism by which 5-HT regulates learning and memory function is not clear.

We used a genetic model of 5-HT depletion, Pet1 knock-out mice (Pet1 KO), to understand the role that 5-HT serves in modulating learning and memory function. In Pet1 KO mice, the levels of 5-HT are highly decreased, specifically associative cortical areas and the hippocampal formation are completely depleted of 5-HT axons [2]. Our results showed that Pet1 KO mice are capable of acquiring associative fear conditioning, reward reinforced associative learning and motor learning; however, they do not recall familiarisation with objects. The novel object recognition test was used to measure hippocampal-dependent declarative memory. The animals are trained to familiarise with two identical objects, and then subjected to a test session where one object is replaced by a novel one.

Pet1 KO mice consistently failed to spend more time exploring the novel object, suggesting impairment of object recognition memory (67±3% wild-type vs 51±4% KO, $P < 0.01$). This phenotype was reversed if 5-HT levels were previously incremented in the brain by administration of 5-HTP (5-HT precursor), which is without effect in control mice (69±3% wild-type vs 70±5% KO, $P > 0.05$). This observation strongly linked object recognition deficit with 5-HT depletion; however does not specify the underlying mechanism. Previously it was shown that the consolidation of object memory involves the enhancement of synaptic strength across the CA3-CA1 hippocampal connexion; a phenomenon called as long-term potentiation (LTP). To test the hypothesis that LTP deficit is responsible for the memory impairment observed in Pet1KO mice, we performed in vivo recordings of field potentials in the CA1 region of the hippocampus of mice while performing the object memory task. We found that in Pet1 KO, experience-dependent LTP-like synaptic mechanisms are exaggerated compared to wild-type mice (126±10% wild-type vs 181±12% KO, $P < 0.001$), possibly explaining the aberration seen in forming correct maps of the object presented.

Our results from in situ hybridisation studies showed the inhibitory 5-HT_{1A} receptors are highly expressed in the CA1 region of the hippocampus. We performed pharmacological studies to determine whether the activation of this receptor subtype could correct object memory impairment. Pet1 KO mice injected with 8-OHDPAT (0.5 mg/Kg), a 5-HT_{1A} agonist, spent significantly more time exploring the novel object, suggesting that memory function was re-established in these animals.

These results established for the first time a direct link between central 5-HT depletion, memory impairment and synaptic plasticity deficits. We hypothesize that 5-HT neurons located in the median raphe contact pyramidal neurons in the CA1 region of the hippocampus, and through the activation of post-synaptic 5-HT_{1A} regulate synaptic plasticity and memory function.

Reference(s)

[1] Schmitt JA, Wingen M, Ramaekers JG, Evers EA, Riedel WJ, 2009. 5-HT and human cognitive performance. Curr Pharm Des 12, 2473–86.
[2] Kiyasova V, Fernandez SP, Laine J, Stankovski L, Muzerelle A, Doly S, Gaspar P, 2011. A Genetically Defined Morphologically and Functionally Unique Subset of 5-HT Neurons in the Mouse Raphe Nuclei. J Neurosci 31, 2756–68.

P.2.030 Are nutritional variables associated with cognition in stimulant-dependence?

C.F. Whitelock[1]*, K.D. Ersche[1]. [1]*University of Cambridge, Department of Psychiatry and Behavioural and Clinical Neuroscience Institute, Cambridge, United Kingdom*

Introduction: Recently, there has been increasing interest in the role of nutrition in the development of cognitive functions in both normal and disordered populations.

For example, diet has been linked to the progression of cognitive impairments in ageing and to the hyperactive and attentional symptoms characteristic of Attention Deficit Hyperactivity Disorder [1]. Alcohol- and drug-dependent individuals show a variety of cognitive deficits relative to non-drug using individuals. There is a well-documented link between these deficits and nutritional factors in alcoholism: some of the deficits seen in alcoholics may be due to their reduced ingestion of the vitamin thiamine for example [2]. However, there has been very little investigation into the relationship between nutrition and cognition in drug dependence. Anecdotally, drug-dependent individuals seem to differ in their diets from non-drug using individuals. If diet can affect cognitive function, a change in diet may alleviate some of the symptoms associated with stimulant addiction.

Objectives: We hypothesised that dietary differences between stimulant (cocaine and amphetamine)-dependent individuals and healthy volunteers would be associated with differences in cognition.

Methods: Stimulant-dependent individuals (N = 58) and age-matched non-drug using healthy volunteers (N = 63) completed a Food Frequency Questionnaire to assess their usual food intake and a battery of neuropsychological tests on the Cambridge Automated Neuropsychological Testing Battery (CANTAB, www.camcog.com). Participants also completed a selection of clinical questionnaires.

Results: Stimulant-dependent individuals consumed significantly higher calories and alcohol than healthy volunteers. Controlling for these differences, the dietary intake of drug users was also found to be significantly higher in fats, vitamin B6, fibre, magnesium, and monounsaturated fatty acids, and lower in the consumption of fruit and fructose. Stimulant-dependent individuals performed significantly worse on the CANTAB test of sustained attention than controls. In correlational analyses, nutrients such as fruit ($r = 0.400$, $p < 0.01$) and fibre ($r = 0.425$, $p < 0.01$) were positively related with reaction times on this task. However, this was only true in the healthy volunteers.

Conclusions: Some of the nutritional differences between stimulant-dependent individuals and healthy volunteers were associated with performance on a test of sustained attention in healthy volunteers only. Therefore, although a healthy diet may bestow health benefits, the cognitive impairments of stimulant-dependent individuals may be such that they are not amenable to dietary intervention. Alternatively, stimulant use may inhibit the actions of nutrients. Nutrition as having a role in the treatment of drug-dependence may be an area worth further research and consideration. Changing one's diet may be a relatively simple and inexpensive, yet important, first step in recovery from stimulant-dependence. For example, alleviating lack of attention is likely to aid individuals in achieving optimal treatment outcomes.

Reference(s)

[1] Millichap, J.G., Yee, M.M., 2012. The diet factor in attention-deficit/hyperactivity disorder. Pediatrics 129, 330–337.

[2] Kiela, P.R., 2010. Unraveling the pathophysiology of alcohol-induced thiamin deficiency. Am J Physiol Renal Physiol 299, F26-F27.

P.2.031 The CBA/J mouse as a genetic model of visceral hypersensitivity with co-morbid anxiety and depression: role of glutamate transport

R.D. Moloney[1]*, T.G. Dinan[1], J.F. Cryan[2]. *[1]University College Cork, Psychiatry, Cork, Ireland; [2]University College Cork, Anatomy and Neuroscience, Cork, Ireland*

Visceral pain is a pronounced and, at times, dominant feature of a variety of gastrointestinal disorders. To date there are no effective pharmacotherapeutic approaches to selectively treat this visceral hypersensitivity as the underlying molecular mechanisms remain largely unknown. Responses to painful stimuli differ between populations, ethnic groups, genders and even among individuals of a family. Strain surveys of common inbred and outbred mice are a useful way to gain insight into the genetic contribution to complex disorders. To date, there has been a lack of detailed information on the response of various strains in the context of visceral nociception. This study aimed to redress this issue and investigate strain differences in visceral nociception and its relationship with glutamate transporter (EAAT 1; excitatory amino acid transporter 1) expression in the spinal cord. Anxiety and depressive-like behaviours were also investigated as patients suffering from chronic pain disorders commonly display co-morbid anxiety and depression. Furthermore, we investigated the underlying mechanisms (5HT1a receptor and brain derived neurotrophic factor, BDNF) of these comorbidities in the hippocampus, a key brain region involved in such processes.

Adult male mice of the following strains were used: CBA/J, C3H/HeN, BALB/cOla, C57BL/6JOla, DBA/2JRcc, CAST/EiJ, SM/J, A/JOla, 129P2/Ola, FVB/NHan, Swiss Webster, CD-1. Colorectal distension (CRD) was performed as an indicator of visceral pain [1]. The open field and forced swim test were performed to assess anxiety and depressive-like behaviours respectively. mRNA expression in the lumbar spinal cord and hippocampus was quantified using real time PCR (qRT-PCR).

There was a differential response to CRD in the strains with the CBA/J and C3H/HeN strains displaying a significantly greatest response to CRD ($F_{(11,117)} = 5.358$, $p < 0.001$, post hoc analysis, $p < 0.05$). Furthermore, these two strains travelled significantly less in the inner zone of the open field ($F_{(11,116)} = 24.300$, $p < 0.001$, post hoc analysis, $p < 0.05$) and display increased immobility in the forced swim test compared with C57BL/6J mice ($F_{(11,113)} = 34.565$, $p < 0.001$, post hoc analysis, $p < 0.05$). At a molecular level the viscerally hypersensitive CBA/J mice have reduced EAAT 1 mRNA expression in the spinal cord compared with the normosensitive C57BL/6J mice ($t_{(18)} = 8.242$, $p < 0.001$). Furthermore CBA/J mice display reduced 5HT1a receptor expression ($t_{(18)} = 2.615$, $p < 0.05$) and BDNF expression ($t_{(18)} = 7.322$, $p < 0.001$) in the hippocampus compared with C57BL/6J mice.

This data demonstrates that strain differences occur in visceral nociception with both CBA/JHsd and C3H/HeNHsd strains exhibiting visceral hypersensitivity. Moreover, these two strains display anxiety and depressive-like behaviours, which are commonly co-morbid with chronic pain disorders. These findings may pave the way for future epigenetic studies utilising techniques such as QTL mapping to identify discrete regions of DNA associated with a specific phenotypic trait, in particular, regions specific to visceral pain processes. Moreover, it is well known that inter-individual differences occur in response to analgesic treatment both clinically and in animal models. These variations in efficacy could also be explored in inbred mouse strains. Taken together this data may aid future work aimed at elucidating the mechanisms underlying disorders where visceral pain and psychopathology are co-morbid such as irritable bowel syndrome.

Reference(s)

[1] Moloney, R.D., O'Leary, O.F., Felice, D., Bettler, B., Dinan, T.G., Cryan, J.F., 2012 Early-life stress induces visceral hypersensitivity in mice. Neurosci Lett, 512, 99–102.

P.2.032 Nanoparticles as disease-modifying mediators for brain therapy: focus on Huntington's disease

B.M.D.C. Godinho[1]*, J.R. Ogier[2], R. Darcy[2], C.M. O'Driscoll[3], J.F. Cryan[1]. *[1]University College Cork, Department of Anatomy and Neuroscience, Cork City, Ireland; [2]University College Dublin, Centre for synthesis and Chemical Biology, Dublin, Ireland; [3]University College Cork, Pharmacodelivery Group School of Pharmacy, Cork City, Ireland*

Neuropsychiatric disorders have been associated with altered gene expression profiles or mutations within specific genes which can dramatically influence and determine disease progression. Silencing the expression of such disease-linked genes through short interfering RNA (siRNA) has recently been suggested as a promising disease-modifying approach for treating neurodegenerative diseases, such as Huntington's Disease (HD), but also to treat depression [1,2]. However, neurons are notoriously difficult to transfect and the lack of appropriate and safe siRNA delivery systems has halted progression of RNA interference (RNAi) technologies. Modified cyclodextrins (CDs) are oligosaccharide-based non-viral siRNA vectors that have recently shown to be able to deliver siRNA to neurons in vitro without causing marked toxicity [3]. Thus, the aim of this study was to investigate behavioural improvements in the transgenic mouse model of HD (R6/2 mouse model), after knockdown of the expression of the mutant Huntingtin (HTT) gene using a CD-based siRNA delivery system.

Modified CDs (SC12 CD Click Propylamine) and HTT siRNAs were prepared and complexed as previously described in O'Mahony et al. 2012 [3]. R6/2 mice were subjected to stereotaxic surgery at 4–5 weeks of age for bilateral implantation of cannulas or to perform direct injections into the striatum. Reduction in HTT gene expression was assessed by RT-qPCR. CD.siRNA nanoparticles were injected over 5 weeks (2.5 uL in each side of the striatum) and behaviour assessment carried out. Rotarod task, open field task and clasping behaviour were monitored for up 14 weeks of age. All values are represented in average ±SEM and one-way repeated measures ANOVA with Bonferroni's Post-hoc test was carried out for statistical analysis.

Delivery of CD.siRNA nanoparticles to the R6/2 mouse brain enabled reduction of HTT gene expression. Gene knockdown was found to be sustained for up to 7 days post-injection at the site of injection. Nevertheless, no significant HTT gene knockdown was observed in regions of the brain away from the site of injection. Behaviour assessment revealed that CD.siRNA nanoparticles significantly delayed rotarod deficits when compared to untreated R6/2 mice or naked siRNA treated R6/2 mice ($F(2,26) = 3.906$, $P = 0.033$). Moreover, deterioration of rotarod deficits was observed when treatment was ceased. No significant improvements were observed in the open field task or in clasping behaviour.

Our data showed that local delivery of CD.siRNA nanoparticles to the striatum reduces expression of the HTT gene and leads to improvements in the rotarod task of R6/2 mice. Accumulation of the mutant HTT protein in other regions of the R6/2 mouse brain might have contributed to the selective improvement in motor behaviour. Using HD as a disease-model, here we demonstrate that CD-based RNAi delivery can be used

effectively to regulate gene expression in the brain. Finally, the use of this technology is not restricted to HD but applicable to other neuropsychiatric disorders.

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Reference(s)

[1] Thakker, D.R., Hoyer, D., Cryan, J.F., 2006. Interfering with the brain: use of RNA interference for understanding the pathophysiology of psychiatric and neurological disorders. Pharmacology & therapeutics, 109(3), 413–438.

[2] Bortolozzi, A., Castañe, A., Semakova, J., Santana, N., Alvarado, G., Cortés, R., Ferrés-Coy, A., Fernández, G., Carmoná, M.C., Toth, M., Perales, J.C., Montefeltro, A., Artigas, F., 2012. Selective siRNA-mediated suppression of 5-HT1A autoreceptors evokes strong anti-depressant-like effects. Molecular psychiatry 17, 612–623.

[3] O'Mahony, A.M., Godinho, B.M.D.C., Ogier, J., Devocelle, M., Darcy, R., Cryan, J.F., O'Driscoll, C.M., 2012. Click-Modified Cyclodextrins as Nonviral Vectors for Neuronal siRNA Delivery. ACS Chemical Neuroscience 3, (10), 744–752.

P.2.033 Does serotonin depletion augment or counteract the aggression-provoking effect of testosterone in mice?

E. Studer[1]*, J. Näslund[1], L. Westberg[1], E. Eriksson[1].
[1]*Institute of Neuroscience and Physiology, Department of Pharmacology, Göteborg, Sweden*

While sex hormones increase aggression in most mammals [1], the neurotransmitter serotonin has been reported to exert the opposite effect [2]. Since testosterone influences various indices of serotonergic transmission, one possibility would be that it exerts its pro-aggressive effects by reducing a tonic anti-aggressive serotonergic influence, either by actions at the level of the raphe nuclei and the serotonergic cellbodies, e.g. affecting serotonin synthesis or altering autoreceptor function. The hormone could also directly affect serotonin signalling in terminal areas for serotonergic pathways by alterations in serotonin receptor function or density. Alternatively, the hormone and the transmitter may regulate aggression by parallel, independent paths in several brain loci where they have opposing effects on one or several points of convergence.

This study aimed at investigating the validity of the interaction models proposed above by assessing if testosterone is capable of enhancing aggression also in the absence of serotonin. On the assumption that testosterone enhances aggression by reducing serotonergic output, serotonin depletion would be at least as effective as testosterone in enhancing aggression, and no additional pro-aggressive effect of testosterone above that induced by serotonin depletion would be found.

Male C57Bl/6 mice were gonadectomised, implanted with slow release testosterone (T), releasing 250 μg of hormone per day, or blank (B) pellets and housed individually after 3 weeks of recovery. Following 9 days of isolation, to allow for establishment of territorial behaviour, baseline aggression was assayed using the standard resident intruder paradigm. Briefly, an unfamiliar group-housed intruder mouse was introduced and behaviour was recorded for 15 minutes under IR illumination. Starting the next day mice were treated with the serotonin synthesis inhibitor para-chlorophenylalanine (pCPA) or saline for 3 days and re-tested 24 hours after the final dose of pCPA. While both groups of testosterone-treated animals displayed enhanced aggression as compared to hormone-depleted animals serotonin depletion did not enhance aggression in mice lacking testosterone, and did hence also not diminish the difference between testosterone-treated and hormone-depleted animals (Duration of aggressive behaviour (s); T-pCPA = 75.4 SD = 39.9; B-pCPA = 0.0 SD = 0.0; $t(14) = 5.7$, $p < 0.001$ and T-Saline = 26.4 SD = 31.0; B-Saline = 0.1 SD = 0.1; $t(15) = 2.5$ $p < 0.05$). On the other hand, serotonin depletion did enhance aggression further in testosterone-treated mice ($t(16) = -2.9$, $p = 0.011$).

In conclusion, our data suggest that androgen-induced aggression is not dependent on intact serotonergic activity, but, on the contrary, is enhanced by serotonin depletion, suggesting that testosterone does not exert its pro-aggressive effect by inhibiting serotonergic output – instead serotonin seems to dampen the influence of testosterone on aggression via a parallel inhibitory pathway. In addition, our results suggest that presence of androgens is an indispensable prerequisite for serotonin depletion to enhance aggressive behaviour, i.e. that the form of aggression dampened by serotonin is an androgen-dependent one.

Reference(s)

[1] Barfield RJ, Busch DE, Wallen K. 1972. Gonadal influence on agonistic behavior in the male domestic rat. Hormones and behavior 3, 247–59.

[2] Sánchez C, Arnt J, Hyttel J, Moltzen EK. 1993. The role of serotonergic mechanisms in inhibition of isolation-induced aggression in male mice. Psychopharmacology 110, 53–59.

P.2.034 Swim-stress exposure effects on nociceptive behaviour and the endocannabinoid system in two rat strains differing in stress responsivity

E.M. Jennings[1]*, B. Okine[1], W.M. Olango[1], M. Roche[2], D.P. Finn[1]. [1]*National University Ireland Galway, Pharmacology & Therapeutics College of Medicine NCBES Centre for Pain Research and Neuroscience Cluster, Galway, Ireland;* [2]*National University Ireland Galway, Physiology College of Medicine, Galway, Ireland*

Forced swim-stress produces hyperalgesia in Sprague-Dawley (SD) rats [1], but its effects in strains that are stress hyper-responsive (e.g. Wistar-Kyoto rat [WKY]) have not been assessed. The endocannabinoid system in the spinal cord and brain plays a key role in both stress and pain [2]. The aim of this study was to compare the effects of forced swim-stress exposure on nociceptive responding to noxious thermal and inflammatory stimuli in two rat strains (SD and WKY) that differ in their stress responsivity, and to determine associated alterations in the endocannabinoid system.

Adult male SD and WKY rats ($n = 8$ per group) were exposed to repeated forced swim sessions over ten days; naive rats served as controls ($n = 8$). The hot-plate and formalin tests were used to assess nociceptive responding to thermal and inflammatory stimuli, respectively. Mass spectrometry was performed on post-mortem spinal cord, prefrontal cortex and amygdala to measure endocannabinoid levels and RT-qPCR was used to measure key genes in the endocannabinoid system. Data were analysed with repeated measures ANOVA or two-way ANOVA followed by Fisher's LSD post-hoc analysis or Student's t-test. $P < 0.05$ was considered statistically significant and all data are mean±S.E.M.

Neither strain displayed stress-induced differences in thermal responding compared to non-stressed controls. SD rats exposed to ten days of swim-stress exhibited enhanced late phase formalin-evoked nociceptive behaviour (Composite pain score: 1.1±0.1 vs. 0.6±0.1, $P < 0.05$) compared with controls. In contrast, WKY rats exposed to swim-stress showed reduced late phase formalin-evoked nociceptive behaviour (Composite pain score: 0.1±0.1 vs. 0.6±0.3, $P < 0.01$), versus controls. No significant changes in endocannabinoid levels or key genes coding for components of the endocannabinoid system were found in WKY rat spinal cord, prefrontal cortex or amygdala. Enhanced formalin-evoked nociceptive behaviour in SD rats was not associated with changes in endocannabinoid levels, CB_1 receptor or fatty acid amide hydrolase (FAAH) expression in the spinal cord. However, swim-stressed SD rats exhibited significantly increased expression of monoacylglycerol lipase (MAGL) (61% increase versus naive, $P < 0.05$), an enzyme involved in the degradation of the endocannabinoid 2-arachidonoylglycerol (2-AG), in the dorsal horn of the spinal cord ipsilateral to formalin injection. Swim stressed SD rats also exhibited a significant reduction in anandamide levels in the left amygdala, compared with non-stressed controls (27.34±1.38 vs 71.37±5.02 nmol/g tissue, $P < 0.05$). There were no other effects of swim stress on levels of endocannabinoids, related lipids or genes in the amygdala or prefrontal cortex, though some within-group differences between concentrations in the left vs right amygdala were noted.

In conclusion, swim-stress in SD rats causes enhanced formalin-evoked nociceptive behaviour that is associated with increased spinal expression of the endocannabinoid-catabolising enzyme MAGL and a reduction in levels of anandamide in the left amygdala. Further studies are required to determine the extent to which these alterations in the endocannabinoid system may contribute to a stress-induced hyperalgesic phenotype.

Reference(s)

[1] Quintero L., Cardenas R., Suarez-Roca H., 2011. Stress-induced hyperalgesia is associated with a reduced and delayed GABA inhibitory control that enhances post-synaptic NMDA receptor activation in the spinal cord. Pain 152 1909–1922.

[2] Butler, R.K., Finn, D.P., 2009. Stress-induced analgesia. Prog Neurobiol 88 184–202.

P.2.035 'Anxious' rats exhibit an enhanced anxiogenic response to acute SSRI treatment and indices of heightened serotonergic transmission

J. Näslund[1]*, E. Studer[1], R. Petterson[1], H. Nissbrandt[1], E. Eriksson[1]. [1]*University of Gothenburg, Department of Pharmacology, Gothenburg, Sweden*

Introduction: Acute administration of serotonin reuptake inhibiting drugs often elicits enhanced anxiety in subjects with anxiety disorders or anxiety-related personality traits but not in non-anxious subjects [1,2], suggesting an enhanced responsiveness to an anxiogenic influence of serotonin in anxious subjects. This observation, in conjunction with a large number of studies suggesting polymorphisms in genes encoding serotonin-related proteins to be associated with anxiety-related personality traits, prompted us to investigate if baseline 'anxiety' in

male adult Wistar rats, as assessed using the elevated plus maze, i) predicts the putatively anxiogenic response to acute administration of an SSRI and ii) is associated with certain biochemical markers of central serotonergic neurotransmission.

Methods: Experiment I: 48 male Wistar rats, aged 10 weeks were tested for five minutes on the EPM. Four weeks later, paroxetine (10 mg/kg, i.p.) was administered. The animals were allowed to return to their cages for one hour before being exposed to an open field paradigm for 40 minutes. Experiment II: 30 male Wistar rats, aged 10 weeks were tested for five minutes on the EPM. Six weeks later, the animals were sacrificed and the brains dissected. A small block of the brain stem, containing the raphe nuclei was homogenised with protein and mRNA extracted. qPCR was performed using Applied Biosystems Custom LDA cards with reactions run in duplicate. TPH2 levels were assayed using SDS-PAGE and western blotting, run in duplicate.

Results: Data are expressed as mean±S.E.M. and analyses pertain to differences between the most 'anxious' third of the animals (according to the EPM) in each experiment as compared to the rest. Gene expression data is relative to the control group (that is, the non-'anxious' animals) whereas protein data is relative to a control sample. Mann–Whitney U tests were used in the behavioural experiment while t-tests were employed in the biochemical experiment. Experiment I: 'Anxious' animals experienced a strong anxiogenic-like effect of paroxetine in the open field test (saline: 184.81±44.28, $n = 8$; paroxetine: 46.88±13.05, $n = 8$; $p = 0.028$), an effect absent in other animals (saline: 198.00±33.40, $n = 16$; paroxetine: 230.74±65.33, $n = 16$; n.s.). Experiment II: 'Anxious' animals consistently exhibited higher expression of genes related to serotonergic transmission in the raphe nuclei; TPH2 (relative expression; 'anxious' animals: 1.74±0.16, $n = 9$; non-'anxious': 1.00±0.18, $n = 19$; $p = 0.004$), SERT (relative expression; 'anxious' animals: 1.70±0.18, $n = 9$; non-'anxious': 1.00±0.20, $n = 19$; $p = 0.011$), as well as higher levels of TPH2 as assayed by western blot (relative expression; 'anxious' animals: 1.70±0.12, $n = 9$; non-'anxious': 1.39±0.10, $n = 20$; $p = 0.011$).

Conclusions: The data obtained support the notion of central serotonergic function being associated with anxiety-like behaviour in rats. Animals assayed as more 'anxious' had a strong response to acute SSRI administration while also differing in measures of central serotonergic function in a way that would be consistent with a higher serotonergic activity. This indicate that further investigation of differences in anxiety-like behaviour between rats of the same strain and batch could help shed some light over the relationship between genetic variation in the serotonin system personality differences in humans, as well as over the nature of the acute, anxiogenic effect of SSRIs.

Reference(s)

[1] Rammsayer T, Netter P (1990). Personality related differences in response to 5-HT uptake inhibition. Int J Neurosci 55(2128079): 99–9106.

[2] Ramos RT, Gentil V, Gorenstein C (1993). Clomipramine and initial worsening in panic disorder: beyond the 'jitteriness syndrome'. J Psychopharmacol 7(22290840): 265–269.

Epigenetics: towards new drug targets

Lectures

S.03.01 The epigenetics of brain disorders and their inheritance – focus on cognition

I. Mansuy[1]*. [1]*Brain Research Institute, University/ETH Zürich, Zürich, Switzerland*

The aetiology and expression of behaviours in mammals are strongly influenced by environmental factors, whether positive or negative. Such factors are particularly critical during early postnatal periods and can exert their influence across the entire life. When positive and favorable, they can facilitate the proper development and expression of behavioural responses, but when adverse and stressful, they can alter behaviour and lead to cognitive disorders and psychopathological symptoms. In particular, traumatic events in early life are strong risk factors for conditions such as attention deficit hyperactivity disorder (ADHD), bipolar depression, and antisocial behaviours. Further, while such disorders can strongly affect the individuals who were directly exposed to the traumatic events, they can also often be inherited by the offspring and similarly affect behavioural responses in first and second generation individuals. The biological processes responsible for the transmission of stress-induced symptoms from parent to offspring remain poorly understood but since they are induced by environmental factors, they have been postulated to involve epigenetic mechanisms. This talk will present an experimental model of traumatic and chronic stress in early life in mice that provides initial evidence for the implication of epigenetic mechanisms in the expression and inheritance of the effects of early stress on behavioural responses. This mouse model shows that chronic and unpredictable maternal separation combined with unpredictable maternal stress during the first two weeks of postnatal life results in the development of multiple pathological symptoms in the offspring. It induces cognitive defects, alters social behaviours and social interactions, and leads to depressive-like symptoms in adult individuals. But at the same time, such traumatic experience also leads to stress resilience and favours behavioural flexibility in some conditions. These symptoms are strong and persist throughout life in the animals exposed to stress. Further strikingly, they are also transmitted to the following offspring across two generations, and through both females and males. At a molecular level, the behavioural symptoms are associated with persistent changes in several targets, including components of the HPA pathway, ion channels, neurotransmitter receptors, and mitochondrial regulators. Initial evidence demonstrates that these molecular alterations correlate with persistent changes in DNA methylation at the promoter-associated CpG island in several genes, and that these changes are present both, in the brain of the offspring and the germline of their fathers [1,2]. Further to DNA methylation, other epigenetic mechanisms including regulation by non-coding RNAs and possibly histone posttranslational modifications may also be involved. These findings suggest that epigenetic processes largely contribute to the impact of negative environmental factors early in life on adult behaviour and its inheritance [3].

Reference(s)

[1] Franklin, T.B., et al., 2011. Influence of early stress on social abilities and serotonergic functions across generations in mice. PLoS One 6(7), e21842.
[2] Franklin, T.B., et al., 2010. Epigenetic transmission of the impact of early stress across generations. Biol Psychiatry 68(5), 408–15.
[3] Bohacek, J., et al., 2012. Transgenerational epigenetic effects on brain functions. Biol Psychiatry (ahead of print).

Posters

P.3.001 Valproic acid treatment prevents the development of deficit in sensorimotor gating in adult prenatally methylazoxymethanol-treated rats

J. Latusz[1]*, E. Bator[1], P. Mordalska[1], K. Wedzony[1], M. Mackowiak[1]. [1]*Polish Academy of Sciences, Laboratory of Pharmacology and Brain Biostructure, Krakow, Poland*

Prenatal administration of methylazoxymethanol (MAM) at embryonic day 17 (E17) is considered as a neurodevelopmental model of schizophrenia [1]. Although the first symptoms of schizophrenia are seen in adults, the

factors cause the risk of this disease might be present in early stage of life. The mechanism of this phenomena is still under investigation, however several data indicate that epigenetic regulation of gene expression during development might contribute to behavioural phenotypes of schizophrenia [2]. Thus, the aim of this study was to investigate whether pharmacological manipulation in epigenetic mechanisms by valproic acid (VA), an inhibitor of histone deacetylases, during pre- and post-puberty might influence the induction of schizophrenic symptoms in adults. The deficit in sensorimotor gating as a one of the psychotic symptoms observed after puberty in MAM-treated animals [1] was chosen to determine which period of postnatal life is critical for appearance behavioural sign of psychosis. Pregnant females were injected ip with 22 mg/kg/ml MAM or saline at E17. The offspring were weaned 21 days after birth and only males were used in experiments. VA was given at the dose 250 mg/kg, sc, twice a day, from 23rd to 29th day (pre-puberty) or by prepulse induced inhibition of acoustic startle response (PPI) in rats at postnatal days 30, 45, 60, 70, 80, 90 and 120 (P30, P45, P60, P70, P80, P90 and P120). Statistically significant decrease in PPI in MAM treated group was found since P70 till P120 [($F(3, 66) = 2.8437$, $p < 0.04$) for P70], but not at P30, P45 or P60 [$F(3,66) = 0.3971$, $p = 0.755$, $F(3, 66) = 1.046$, $p = 0.38$ and $F(3,66) = 1.45$, $p = 0.24$, respectively]. Administration of VA in pre-puberty did not influence the sensorimotor gating process at P30 [$F(3, 84) = 0.17$, $p = 0.92$], P45 [$F(3,84) = 0.713$, $p = 0.55$] and P60 [$F(3,84) = 0.52$, $p = 0.69$], but prevented the appearance of deficit in sensorimotor gating in MAM-treated group measured at P70 [$F(3,84) = 2.8$, $p < 0.4$]. The above effect was transient, since the decrease in PPI in MAM-treated group was observed again at P80 [$F(3,84) = 0.76$, $p = 0.52$]. When VA was given in post-puberty, it blocked the reduction in PPI in MAM-treated group detected at P80 [$F(3,84) = 3.07$, $p < 0.04$], at P90 [$F(3,84) = 5.338$, $p < 0.003$] and P120 [$F(3,84) = 3.311$, $p < 0.03$]. The obtained data indicate that the deficit in sensorimotor gating appears only in post-puberty as it was reported by Moore et al. [1]. VA given pre-puberty only delays appearance of deficit in sensorimotor gating in MAM-treated rats. In contrast, VA administration in post-puberty prevents the deficit evoked by MAM. Thus it is conceivable that inhibition of deacetylation process in post- but not in pre-puberty might block the development of some psychotic symptoms such as impairment in sensorimotor gating.

Reference(s)

[1] Moore, H., Jentsch, J.D., Ghajarnia, M., Geyer, M.A., Grace, A.A., 2006. A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. Biol. Psychiatry 60, 253–264.

[2] Dudley, K.J., Li, X., Kobor, M.S., Kippin, T.E., Bredy, T.W., 2011. Epigenetic mechanism mediating vulnerability and resilience to psychiatric disorders. Neurosc. & Biobeh. Reviews 35, 1544–1551.

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P.3.002 The effect of valproic acid on changes in methylation pattern of histone H3 induced by prenatal MAM administration in mPFC

E. Bator[1]*, J. Latusz[1], P. Mordalska[1], K. Wedzony[1], M. Mackowiak[1]. [1]*Polish Academy of Sciences, Laboratory of Pharmacology and Brain Biostructure, Krakow, Poland*

Several data indicate that epigenetic regulation of gene expression might be involved in schizophrenia aetiology [1]. Our previous study showed the changes in the methylation pattern of histone H3 in the medial prefrontal cortex (mPFC) in neurodevelopmental model of schizophrenia [2] based on the prenatal administration of methylazoxymethanol (MAM) at embryonic day 17 (E17) [3]. Thus, the aim of the present study was to determine whether the repeated valproic acid (VA) administration at pre-puberty can affect the observed changes of histone H3 methylation at lysine 9 (H3K9) at 30 day old (P30) and at lysine 4 (H3K4) at 60- or 70-day-old rats (P60 or P70) receiving prenatally MAM. VA acting as an inhibitor of histone deacetylases can influence the epigenetic mechanism and might affect the methylation process of histone H3. Pregnant females were injected with 22 mg/kg/ml MAM or saline ip at E17. The offspring were weaned 21 days after birth and only males were used in experiments. VA was given at the dose 250 mg/kg, sc, twice a day, from 23rd to 29th day (early adolescence) and the animals were killed at P30, P60 and P70. At the same dose VA was injected from 53rd to 59th day of life (late adolescence) and experiments were performed at P60 and P70. The methylation levels of H3K9me2 and H3K4me3 were studied in nuclear fraction of mPFC using Western blot analysis. It was observed that MAM increased H3K9me2 level at P30 (30%) [$F(1,16) = 6.49$, $p < 0.03$], however VA pretreatment in early adolescence did not have any effect on observed increase in H3K9me2 induced by MAM [$F(1,16) = 1.77$, $p = 0.20$]. MAM as well as VA did not affect the H3K9me2 protein level at P60 [$F(1,16) = 1.2$, $p = 0.29$] or P70 [$F(1,16) = 0.15$,

$p = 0.77$] for both procedures of VA administration. In contrast, H3K4me3 methylation level was decreased in post-puberty at P60 (30%) ($F(1,16) = 19.4$, $p < 0.001$) and also at P70 (35%) [$F(1,16) = 5.12$, $p < 0.04$] in MAM-treated group, but not at P30 [$F(1,16) = 3.99$, $p = 0.069$]. VA given in early adolescence prevented the observed decrease in H3K4me3 protein in MAM-treated group at P60 [$F(1,16) = 6.084$, $p < 0.03$]. Interaction between MAM and VA was insignificant at P70 [$F(1,16) = 1.94$, $p = 0.18$], but post hoc analysis revealed the significant effect of VA administration on MAM-treated group ($p < 0.006$). VA pretreatment in late adolescence did not alter MAM induced decrease in H3K4me3 protein level at P60 [$F(1,16) = 0.43$, $p = 0.52$], but prevented MAM-evoked reduction of trimethylated form of H3K4 at P70 [$F(1,16) = 11.72$, $p < 0.004$]. In addition, the total histone H3 level was measured and there were no differences between groups at any age points. The obtained results indicate that VA administration in adolescence might prevent changes in gene expression in post-puberty controlled by methylation at H3K4.

Reference(s)

[1] Roth, T.L., Lubin, F.D., Sodhi, M., Kleinman, J.E., 2009. Epigenetic mechanism in schizophrenia. Biochimica and Biophysica Acta 1790, 869–877.

[2] Mackowiak, M., Bator, E., Latusz, J., Mordalska, P., Wedzony, K., 2012. The impairment of lysine methylation of histone H3 in the medial prefrontal cortex in the neurodevelopmental model of schizophrenia. FENS Abstract 6, 118.14.

[3] Moore, H., Jentsch, J.D., Ghajarnia, M., Geyer, M.A., Grace, A.A., 2006. A neurobehavioral systems analysis of adult rats exposed to methylazoxymethanol acetate on E17: implications for the neuropathology of schizophrenia. Biol. Psychiatry 60, 253–264.

P.3.003 Chronic early-life stress programs hippocampal neurogenesis and cognitive function: a role for epigenetics?

E.F.G. Naninck[1]*, L. Hoeijmakers[1], M. Engel[1], P.J. Lucassen[1], A. Korosi[1]. [1]*UvA SILS Center for Neurosciences, Structural and functional plasticity of the nervous system, Amsterdam, The Netherlands*

Rationale: Early-life stress (ES) increases vulnerability to psychopathologies and impairs cognitive functions later in life. However, the responsible molecular mechanisms remain elusive. Since ES in humans is common and often cannot be prevented, a better understanding of the mechanisms underlying these lasting effects of ES on brain functioning and disease susceptibility is needed in order to develop therapeutic intervention. Rodent studies have indicated that ES induces lasting changes in hippocampal neurogenesis and structure, parallel to cognitive deficits [1]. We therefore set out to investigate, in mice, if these lasting abnormalities have an early onset, and assess the acute and lasting effects of ES on neurogenesis and on hippocampal epigenetic modifications. In addition, we investigate the lasting effects of ES on cognition and emotion.

Methods: Chronic ES is induced in C57Bl/6 mice by limiting nesting/bedding material in the cage from postnatal day (P) 2 till P9 [2]. Levels of postnatal and adult neurogenesis in the hippocampal dentate gyrus (DG) of the offspring are assessed using immunohistochemistry for proliferating ($Ki67^+$) and differentiating ($NeuroD1^+$/$Calretinin^+$) newborn cells and for their survival ($BrdU^+$). Total histone levels (H3, H2b) as well as active (H3K4me3) and repressive (H3K9me3, H3K27me3) histone modifications are assessed by Western blot of DG tissue. All parameters are measured directly after ES (at P9) and in adulthood (at P150) in ES and control male and female mice. In addition, performance in the elevated plus maze, forced swim task, novel object recognition task, novel object location task and Morris water maze was examined in ES and control mice from P150 onwards.

Results: ES dams show characteristics of chronic stress as was evident from fragmented maternal care and a reduced bodyweight gain. The fragmentation of maternal care subsequently led to chronic ES in the pups, that showed reduced bodyweight gain, reduced thymus weight and elevated basal plasma corticosterone levels at P9. Interestingly, levels of postnatal proliferation ($Ki67^+$) and differentiation ($Calretinin^+$) were increased in the DG of 9-day-old ES mice, indicating an early onset of the lasting changes in hippocampal structure after ES. Furthermore, in ES mice, more $NeuroD1^+$ cells were found in the postnatal migratory stream from the hippocampal subventricular zone to the DG. Expression levels of H3/H2b were elevated in ES mice at P9. In addition, behavioural analysis indicates cognitive impairments in adult ES mice. Further characterisation of the lasting effects of ES on adult neurogenesis and epigenetic regulation is in progress.

Conclusion: Chronic ES alters levels of postnatal neurogenesis already at P9 and impairs hippocampus-dependent cognitive function in adulthood. Unravelling the molecular mechanisms that underlie these persistent ES-induced changes will yield important information on how stressful early-life experiences program the brain. This may have clinical implications for the development of treatments to prevent or repair ES-induced deficits.

Reference(s)

[1] Korosi, A., Naninck, E.F.G., Oomen, C.A., Schouten, M., Krugers, H., Fitzsimons, C., Lucassen, P.J., 2012. Early-life stress mediated modulation of adult neurogenesis and behavior. Behav Brain Res 227, 400–9.

[2] Rice, C.J., Sandman, C.A., Lenjavi, M.R., & Baram, T.Z., 2008. A novel mouse model for acute and long-lasting consequences of early life stress. Endocrinology, 149, 4892–4900.

P.3.004 Possible interplay between BDNF and dynorphin in bipolar disorder: role of epigenetic mechanisms

A. Di Francesco[1]*, C. D'Addario[1], B. Dell'Osso[2], M.C. Palazzo[2], B. Benatti[2], D. Galimberti[3], B. Arosio[4], A.C. Altamura[2], M. Maccarrone[1]. *[1]University of Teramo, Biomedical Sciences, Teramo, Italy; [2]University of Milan, Psychiatry, Milan, Italy; [3]University of Milan, Neurological Sciences, Milan, Italy; [4]University of Milan, Internal Medicine Geriatric Unit, Milan, Italy*

Purpose of the study: Bipolar disorder (BD) is a highly disabling mood disorder determined by the interplay between genes and environmental factors [1]. Among genes potentially implicated in the pathophysiology of BD, the brain-derived neurotrophic factor (BDNF) gene has been extensively investigated and we recently suggested selective changes in DNA methylation of BDNF promoter in subjects with BD type II, highlighting the importance of epigenetic factors in mediating the onset and/or susceptibility to BD [2]. To provide further knowledge on the role of BDNF in the development of BD, we here studied the epigenetic regulation of prodynorphin gene, the precursor of the opioid peptide dynorphin, recently suggested as a downstream effector of BDNF regulation [3].

Methods: We conducted this study on peripheral blood mononuclear cells (PBMCs), accessible cells with a potential use for biomarker discovery in psychiatric disorders, containing the full complement of epigenetic enzymes found in most tissues, including neurons. Genomic DNA and total RNA were isolated from patients with BD on stable pharmacological treatment (BD I: $n = 48$, BD II: $n = 41$) and from healthy controls ($n = 42$), with the specific intent of assessing eventual differences in terms of methylation between bipolar patients and healthy subjects. To assess prodynorhin mRNA abundances and to quantify prodynorphin promoter DNA methylation, we used Real-Time RT-PCR and Real-Time Methylation Specific PCR, respectively.

Results: A significant hypermethylation of the prodynorphin promoter region was specifically found in BD II patients (CT: $17.14 \pm 1.25\%$; BD I: $18.06 \pm 1.26\%$; BD II: $21.55 \pm 1.08\%$, $P < 0.05$ vs CT). Consistently, downregulation of prodynorphin gene expression was observed in BD II but not in BD I patients when compared to controls. Of note, higher levels of DNA methylation were observed in BD subjects on pharmacological treatment with antidepressants ($21.01 \pm 1.23\%$) compared with those exclusively on mood-stabilising agents ($16.89 \pm 1.48\%$; $P < 0.05$). Moreover, we found a significant correlation between BDNF and prodynorphin DNA methylation at gene promoters ($P < 0.05$, Spearman $R = 0.2017$).

Conclusions: Present results provide a new clear correlation between BDNF and prodynorphin alterations at epigenetic level, suggesting their possible interaction in the development of BD. This is also supported by the evidence that mood stabilisers regulate in a similar manner BDNF and prodynoprhin DNA methylation levels and thus providing new insight on the specific mechanism of action of these drugs for the treatment of BD. Moreover, we also suggest a possible role for dynorphin as peripheral biomarker useful for differential diagnosis and, mostly, for prediction of treatment response. Overall, our findings are consistent with the epigenetic theory of major psychoses, supporting the importance of DNA methylation alterations in the aetiology of BD.

Reference(s)

[1] Labrie, V., Pai, S., Petronis, A., 2012. Epigenetics of major psychosis: progress, problems and perspectives. Trends Genet. 28, 427–435.

[2] D'Addario, C., Dell'Osso, B., Palazzo, M.C., Benatti, B., Lietti, L., Cattaneo, E., Galimberti, D., Fenoglio, C., Cortini, F., Scarpini, E., Arosio, B., Di Francesco, A., Di Benedetto, M., Romualdi, P., Candeletti, S., Mari, D., Bergamaschini, L., Bresolin, N., Maccarrone, M., Altamura, A.C., 2012. Selective DNA methylation of BDNF promoter in bipolar disorder: differences among patients with BDI and BDII. Neuropsychopharmacology. 37, 1647–1655.

[3] Logrip, M.L., Janak, P.H., Ron, D., 2008. Dynorphin is a downstream effector of striatal BDNF regulation of ethanol intake. FASEB J. 22, 2393–2404.

P.3.005 Early environment affects activity based anorexia genetic susceptibility and epigenetic programming of neurodevelopmental genes in mice

E. Pjetri[1]*, E.V.S. Hessel[1], H. Oppelaar[1], P.N.E. de Graan[1], B. Olivier[2], M.J.H. Kas[1]. [1]*Rudolf Magnus Institute of Neuroscience, Neuroscience and Pharmacology, Utrecht, The Netherlands;* [2]*Utrecht University, Department of Pharmacology, Utrecht, The Netherlands*

Background: Human studies have shown that the interaction between genetic and environmental factors contribute to adult expression of psychiatric phenotypes, such as anti-social behaviour [1]. Recently, gene–environment interactions have been increasingly reported for a wide range of mental disorders such as schizophrenia, depression, and eating disorders [2]. Anorexia nervosa is a complex eating disorder associated with the largest mortality rate among psychiatric disorders [3]. Various genetic, environmental and developmental risk factors are thought to contribute to this dramatic disease. However, little is known about the developmental trajectory preceding this psychiatric disorder. Systematic longitudinal studies with controlled genetic and environmental background variation are necessary to identify and understand the mechanisms underlying the interplay between environment and genetic background susceptibility for maladaptive behaviour. In the present study, the relationship between maternal environment, genetic background and epigenetic mechanisms was studied using low activity based anorexia (ABA) (C57BL/6J) and high ABA (C57BL/6J-Chr4$^{A/J}$/NaJ) (CSS4) susceptible mouse inbred strains.

Methods: Following early life inter- and intra-strain cross-fostering of the inbred strains, the mice were exposed to ABA model in adulthood (3–4 months old).

The ABA rodent model mimics the paradoxical expression of behavioural hyperactivity in periods of reduced food intake that is also observed in anorexia patients. In this rodent model, animals have unlimited voluntary access to a running wheel throughout the experiment, while food is ad libitum available for a limited period at the same time during several consecutive days. The physical hyperactivity observed during food restriction leads to accelerated body weight loss, reminiscent of the features of the disease. ABA susceptibility is a 'time to an event parameter' and therefore it was analysed using Kaplan–Meier survival analysis with Mantel-Cox Log-Rank test.

To investigate whether fostering was associated with epigenetic modifications, genome wide DNA methylation levels were examined in DNA extracted from hippocampal tissue in cross-fostered and non-cross-fostered CSS4 mice a week after weaning the pups from their mother (postnatal day 35).

Results: Independent of the foster mother genetic background, fostered CSS4 mice were less susceptible to ABA when compared to CSS4 mice raised by their biological mother (same strain foster mother: $\chi^2 = 7.690$, $p = 0.006$; different strain foster mother: $\chi^2 = 7.874$, $p = 0.005$). In contrast, no cross-fostering effects were observed on ABA susceptibility in the low susceptible C57BL/6J strain. Global DNA methylation analysis revealed several differentially hypermethylated genome regions as a function of cross-fostering in CSS4 strain. These hypermethylated regions included genes, such as contactin associated protein-like 2 (Cntnap2) gene and α-1C subunit of the L-type voltage-gated calcium channel (Cacna1c) gene, that have been associated with neurodevelopmental disorders.

Conclusions: Thus, early life events may modulate genetic background susceptibility for adult maladaptive behaviour via epigenetic modifications and provide a basis for studies towards further understanding of neurodevelopmental processes underlying adult behaviour.

Reference(s)

[1] Caspi, A., McClay, J., Moffitt, T.E., Mill, J., Martin, J., Craig, I.W., Taylor, A. & Poulton, R., 2002. Role of genotype in the cycle of violence in maltreated children. Science 297, 851–854.

[2] Campbell, I.C., Mill, J., Uher, R. & Schmidt, U., 2011. Eating disorders, gene–environment interactions and epigenetics. Neurosci Biobehav Rev. 35, 784–793.

[3] Arcelus, J., Mitchell, A.J., Wales, J. & Nielsen, S., 2011. Mortality rates in patients with anorexia nervosa and other eating disorders. A meta-analysis of 36 studies. Arch Gen Psychiatry 68, 724–731.

P.3.006 Epigenetic modifications of bdnf gene at hippocampal level induced by chronic ethanol intake in C57BL/6J mice

E. Stragier[1]*, R. Massart[1], M. Hamon[1], L. Lanfumey[1]. [1]*INSERM UMR 894, Centre de Psychiatrie et Neurosciences, Paris, France*

Purpose of the study: Although alcohol at high dosage is well known to induce neurotoxic effects on hippocampal neurogenesis, chronic voluntary intake of alcohol at moderate dosage has been shown to stimulate dentate gyrus (DG) cell proliferation in C57BL/6J mice [1]. In order to unveil the molecular mechanisms underlying alcohol-induced neuroplasticity, we studied the expression

of several markers involved in cell differentiation and plasticity in the hippocampus of adult male C57BL/6J mice that had been exposed to free alcohol (10% ethanol versus water) consumption for three weeks. In addition, alcohol-induced epigenetic modifications were investigated by quantifying the expression of epigenetic markers such as HDACs, which are implicated in gene transcription control and neuronal maturation. Post-translational modifications of histones (acetylation, methylation), which are known to modulate gene transcription, were also determined in alcohol- versus water-exposed mice.

Methods: qRT-PCR, chromatin immunoprecipitation and immunohistochemistry approaches were used for relevant determinations in the hippocampus of 3 month-old male C57BL/6J mice after a 3-week free consumption of ethanol versus tap water. Paired control mice had only access to water for the same period.

Results: Voluntary alcohol consumption reached a stable level corresponding to 10–12 g of ethanol ingested per day from the 10th day of exposure to three bottles of 10% ethanol and a bottle of tap water. After three weeks of such free ethanol consumption, a significant increase in the expression of both bHLH activator family (transcription factors implicated in neural cell proliferation and maturation) and BDNF (at both mRNA and protein levels), and a concomitant decrease in HDACs, were noted in the hippocampus of ethanol-exposed mice versus paired mice exposed to water. In addition, MeCP2 expression was increased also at both mRNA and protein levels in the DG and CA3 area, and acetylated histone H4 (H4Ac) and trimethylated histone H3 (H3K4me3) were upregulated in these hippocampal subareas in alcohol-exposed mice. Specific analysis of bdnf gene showed alcohol-induced modifications at transcription level associated with clear-cut changes in H3Ac and H3K4me3 in bdnf promoters (Table 1). Changes in BDNF exons expression occurred in parallel with alcohol-induced increase in BDNF protein in the hilus and CA3 area of the hippocampus.

Table 1. Alcohol-induced changes in BDNF exons expression and histone post-translational modifications in bdnf promoters in the mouse hippocampus (+, increase; –, decrease)

BDNF exon	mRNA expression	Promoter	
		H3Ac	H3K4me3
II	+	=	+
III	+	–	+
VI	+	+	=
VIII	–	–	–

Conclusions: Altogether, these results suggest that the increased hippocampal neurogenesis produced by chronic moderate alcohol intake under free choice conditions in C57BL/6J mice [1] is underlain by an up regulation of transcription factors and BDNF. Epigenetic mechanisms involving HDAC and histone modifications at the level of bdnf gene play probably a key role in alcohol-induced BDNF upregulation and downstream neurogenesis.

Reference(s)

[1] Paizanis E., Kelaï S., Renoir T., Hamon M., Lanfumey L. 2007. Life-long hippocampal neurogenesis: environmental, pharmacological and neurochemical modulations. Neurochem. Res. 32:1762–1771.

P.3.007 Stress-induced miRNA changes in depression: peripheral biomarker or pathophysiology?

S. Kalman[1]*, K. Garbett[2], A. Vereczkei[2], R.C. Shelton[2], K. Mirnics[2]. [1]*University of Szeged, Faculty of Medicine, Szeged, Hungary;* [2]*Vanderbilt University, Kennedy Center, Nashville, USA*

Introduction: Depression is the second most common human disorder worldwide (WHO). Genetic predisposition and maladaptive stress response considered to be the most important aetiological factors. microRNAs can influence the metabolism of their target mRNAs, act like fine, 'tuning' regulators of adaptation, cell cycle, and apoptosis and are proved to be especially important in certain (patho)physiologic processes of the central nervous system such as synaptoplasticity and degeneration [1]. Our aim was to answer the following questions: (I.) What is the difference between the microRNA expression of dermal fibroblasts originated from control (CNT) and depressed (D) subjects? (II.) How metabolic stress treatment affects the microRNA expression in the CNT and (III.) D groups? (IV.) How these microRNA profile changes appear on the level of their target mRNAs?

Methods: Primary fibroblast cultures (passage number = 5–10) were initiated from skin biopsies of 17 controls and 17 subjects with major depression. Groups were matched for sex and age. The metabolic stress was evoked by growing subcultured cells in glucose deprived, galactose enriched (GAL); lipid reduced, cholesterol deficient (NL); and standard (STD) medium for one week. Total- and small-RNAs were isolated and the expression levels of more than 1000 miRNAs were measured with miRNome PCR array. We accepted $|\text{ddCt}| \geqslant 0.583$ and $\text{p-value}_{\text{grouped}} \leqslant 0.05$, $\text{p-value}_{\text{paired}} \leqslant 0.05$ as a significant change. The amounts of their suspected target mRNAs were also detected with DNA microarray. The gene was considered to be differentially expressed with the $|\text{ALR}| \geqslant 0.378$ and p-value $\leqslant 0.01$.

Results: As compared to the CNT fibroblasts (75 affected microRNAs), the D samples showed almost three times more microRNA expression changes (214 microRNAs) caused by both GAL and NL metabolic stress treatments. The microRNA profile alterations were more remarkable under NL circumstances. Although higher overlap was detected between the GAL and NL induced microRNA changes in the D group (47 microRNAs, while only 4 microRNAs in the CNT), Pearson correlation showed that the two different stress treatments resulted in very similar microRNA expression pattern in the CNT ($r^2 = 0.51$ and 0.43) but not in the D group. Nearly 10% of the suspected target mRNAs of the most significantly affected microRNAs changed due to metabolic stress treatments in the CNT group.

Conclusion: We suppose that the metabolic stress induced microRNA changes in dermal fibroblasts of CNT subjects are part of the normal cell adaptation, while those found in the D group could be the sign of a genetically determined, pathologic stress response presumed in depression. These results could facilitate the understanding of microRNAs' role in the development of depression and contribute to the mapping of the stress related physiologic and degenerative gene expression changes by setting up a potential new in vitro metabolic stress related depression model and diagnostic tool.

Reference(s)

[1] Serafini, G., Pompili, M., Innamorati, M., Giordano, G., Montebovi, F., Sher, L., Dwivedi, Y., Girardi, P., 2012. The role of microRNAs in synaptic plasticity, major affective disorders and suicidal behavior. Neurosci Res 73(3):179–90.

P.3.008 S-Adenosyl-methionine impairs forced swimming-induced behavioural immobility by inhibiting gene expression in dentate gyrus neurons

E.A. Saunderson[1]*, A.F. Trollope[1], M. Gutierrez-Mecinas[1], A.A. Shaikh[1], H. Spiers[2], J. Mill[2], J.M.H.M. Reul[1]. *[1]University of Bristol, School of Clinical Sciences, Bristol, United Kingdom; [2]King's College London, Institute of Psychiatry, London, United Kingdom*

The consolidation of stress-induced adaptive behaviours, such as the learned immobility response in the forced swim (FS) test, depends on specific epigenetic modifications underlying gene transcriptional responses in dentate gyrus (DG) granule neurons of the hippocampus. In these neurons FS evokes the activation of two interacting signalling pathways, i.e. the glucocorticoid receptor (GR) and the NMDAR/ERK1/2/MSK1-Elk-1 pathways, resulting in phosphorylation of serine10 and acetylation of lysine14 at histone H3 (H3S10p-K14ac) which leads to induction of immediate early genes (IEGs) c-Fos and Egr-1 [1,2]. These molecular responses are critical for the consolidation of the behavioural immobility response [1,2].

The drug and endogenous methyl-donor S-adenosyl-methionine (SAM) impairs the consolidation of the behavioural immobility response [3] suggesting the involvement of histone methylation and/or DNA methylation in gene transcriptional control underlying the behavioural response but this is unknown. Therefore, to understand the mechanism of action of SAM and to gain insight into the involvement of histone/DNA methylation, rats were injected with SAM (100 mg/kg s.c.) 30 minutes before FS (15 min, 25ºC). Twenty-four hours later they were subjected to FS again and immobility behaviour were scored in 10 s bins. SAM had no effect on immobility in the initial FS test (mean±SEM; vehicle: 15.8±1.6 bins, $n = 8$; SAM: 15.0±1.9 bins, $n = 9$; $P > 0.05$ post-hoc Bonferroni test). However, in the retest, immobility in the SAM group was significantly lower (11.4±3.3 bins, $n = 9$) than that in the vehicle group (20.6±2.3 bins, $n = 8$; $P < 0.05$), confirming that SAM indeed impairs the consolidation of the behavioural immobility response [3].

To study whether SAM's effects on behaviour could be explained through effects on FS-induced epigenetic and transcriptional responses in DG neurons, rats were pre-treated with SAM or vehicle and submitted to FS or not (baseline control) and killed 1 h later. Compared to vehicle, SAM evoked a significant decrease in FS-induced c-Fos and Egr-1in DG neurons (Vehicle: c-Fos: 127.1±4.6 neurons $n = 5$, Egr-1: 18.4±2.5 $n = 5$; SAM: c-Fos 82.6±5.0 neurons $n = 6$, Egr-1 8.5±1.2 $n = 5$; both c-Fos and Egr-1: $P < 0.01$). However, SAM had no effect on stress-induced H3S10p-K14ac in DG neurons suggesting that the drug effect was independent of this dual epigenetic mark. Next, we investigated the effect of SAM and FS on the methylation status of histone H3 lysine residues. Chromatin immuno-precipitation (ChIP) analysis revealed that SAM and FS had no significant effects on H3K4me3, H3K9me3 and H3K27me3 at the c-Fos and Egr-1 promoters.

Recently, we have started investigating DNA methylation using bisulfite pyrosequencing and methyl-DNA IP (MeDIP) to assess the cytosine methylation status of CpG islands within the c-fos and egr-1 gene promoters. We found that cytosine methylation of both promoters was very low (<5%) in the DG, the rest of hippocampus, and the neocortex and did not change after FS. Presently we are

studying the effects of SAM on de novo DNA methylation of the gene promoters.

We conclude that SAM impairs the FS-induced behavioural immobility response through inhibition of gene transcription in DG neurons. This drug effect appears to be independent of histone modifications.

Reference(s)

[1] Reul, J.M.H.M., Hesketh, S.A., Collins, A., Gutièrrez-Mecinas M., 2009. Epigenetic mechanisms in the dentate gyrus act as a molecular switch in hippocampus-associated memory formation. Epigenetics 4: 434–439.

[2] Gutièrrez-Mecinas, M., Trollope, A.F., Collins, A., Morfett, H., Hesketh, S.A., Kersanté, F., Reul, J.M.H.M., 2011. Long-lasting behavioral responses to stress involve a direct interaction of glucocorticoid receptors with ERK1/2-MSK1-Elk-1 signaling. Proc Natl Acad Sci USA 108: 13806–13811.

[3] Czyrak, A., Rogoz, Z., Skuza, G., Zajaczkowski, W., Maj, J., 1992 Antidepressant activity of S-Adenosyl-L-Methionine in mice and rats. J Basic & Clin Physiol Pharmacol 3: 1–16.

P.3.009 Differential effects of prenatal stress on serotonin transporter deficient mice: the role of epigenetic programming

K. Schraut[1]*, S.B. Jakob[1], G. Kenis[2], A.G. Schmitt[3], S. Kneitz[4], C.J. Scholz[4], G. Ortega[1], H. Steinbusch[2], D.L.A. van den Hove[2], K.P. Lesch[1]. *[1]University of Würzburg, Psychiatry Psychosomatics and Psychotherapy Molecular Psychiatry, Würzburg, Germany; [2]University of Maastricht, Department of Psychiatry and Neuropsychology, Maastricht, The Netherlands; [3]University of Würzburg, Psychiatry Psychosomatics and Psychotherapy, Würzburg, Germany; [4]University of Würzburg, Laboratory for Microarray Applications Interdisciplinary Centre for Clinical Research (IZKF), Würzburg, Germany*

Prenatal stress (PS) exposure in early life is an environmental risk factor that has been shown to affect fetal brain development and to increase the risk for adult psychopathology. Furthermore, a length-polymorphism in the serotonin transporter gene (5-HTTLPR) has been suggested to modulate the association between stress exposure in early life and the development of psychiatric disorders in later life by rendering short allele carriers more vulnerable to stress. The exact molecular mechanisms underlying this gene–environment (G×E) interaction remain to be elucidated though.

Recently, using a maternal restraint stress paradigm of PS in wild-type (WT) and heterozygous (+/–) 5-Htt deficient mice, we have shown that the long-term behavioural effects of PS are partly dependent on the 5-Htt genotype [1]. In our study, 5-Htt$^{+/-}$ mice, particularly females, appeared to be more vulnerable to PS exposure when compared to WT offspring. Additionally, hippocampal gene expression profiles of the females indicated that the effects of the 5-Htt$^{+/-}$ genotype, PS exposure, and their interaction were mediated by distinct molecular mechanisms. More specifically, MAPK and neurotrophin signalling were regulated by both the 5-Htt$^{+/-}$ genotype and PS exposure, whereas cytokine and Wnt signalling were affected in a 5-Htt genotype × PS manner.

Epigenetic mechanisms such as DNA methylation and histone modifications build the interface between environment and genome and thus have been recently heavily discussed to play a role in the development of psychiatric disorders.

In light of recent findings concerning the role of epigenetic mechanism in the regulation of signalling pathways that mediate depression-like behaviour in rodents, the present study aims to examine the role of DNA methylation in mediating the changes in gene expression observed in our 5-Htt × PS paradigm. For this purpose, genome-wide promoter methylation was assessed in the hippocampus using methylated DNA immunoprecipitation followed by Affymetrix Mouse Promoter 1.0R Array analysis (MeDIP-on-chip). Probe signals from MeDIP and input samples were normalised and MeDIP-input signal log2 ratios calculated. A sliding-window approach was applied to decrease the noise in the experiment readout. Genomic regions enriched by MeDIP were detected by the CMARRT algorithm.

We found that the genotype, the PS exposure and an interaction of both caused changes in the DNA methylation of a number of genes that showed also differential expression in our previous study indicating a possible correlation between DNA methylation and gene expression for those genes. Among them were genes as Fgfr4, Map3k1, Nos1, Mbp1 and Cabin1. The promoter array results are currently investigated in detail using bisulfite treatment and pyrosequencing.

In conclusion, hippocampal gene expression and DNA-methylation profiles of female prenatally stressed 5-Htt$^{+/-}$ mice suggest that distinct molecular mechanisms, including epigenetic programming, might mediate the behavioural effects of the 5-Htt genotype, PS exposure, and their interaction.

Reference(s)

[1] Van den Hove DLA, Jakob SB, Schraut KG, Kenis G, Schmitt AG, Kneitz S, Scholz CJ, Wieschollek V,

Ortega G, Prickaerts J, Steinbusch HWM, Lesch KP (2011) Differential effects of prenatal stress in 5-Htt deficient mice: towards molecular mechanisms of gene × environment interactions. PloS One 6:e22715.

P.3.010 Time-dependent effects of antidepressant treatments on miRNome expression profile in hippocampus of rats

M. Seguini[1]*, D. Tardito[1], A. Mallei[1], G. Racagni[1], M. Popoli[1]. [1]*University of Milan, Department of Pharmacological and Biomolecular Sciences, Milan, Italy*

In the past few years, it has become clear that beyond the traditional transcriptional mechanisms, gene expression is also regulated by microRNAs (miRs), small non coding RNAs with a key role in post-transcriptional regulation of gene expression. MiRs have recently been implicated in a variety of human diseases, including neuropsychiatric disorders. Moreover, a role for miRs in the pharmacotherapy of mood disorders has been recently suggested. It has been indeed shown that some miRs and their effectors are modulated by the mood stabilisers lithium and valproate and that fluoxetine (FLX, a selective serotonin reuptake inhibitor-SSRI) modulates miR-16 expression [1,2]. Moreover, a recent clinical study reported changes in blood expression of 30 miRs after 12 weeks of treatment with the SSRI escitalopram in depressed patients [3]. Aim of the present study was to verify whether treatment for different time length (3, 7, 14 days) with two antidepressants (ADs) characterised by different primary mechanism of action (FLX, and desipramine, DMI, a tricyclic AD with predominantly action on the noradrenaline reuptake) modulate miRNome expression in rat hippocampus.

To this aim, total RNA including miRNAs was isolated from each hemi-hippocampus and reverse transcribed. Quantitative Real Time PCR (qRT-PCR) amplification was carried out using TaqMan Array rodent MicroRNA A+B Card Set v3.0 using the ddCT method.

A total of about 450 miRs were detected in all samples. The analysis of the miRNome expression showed evidence for significant effects of AD treatments at the different time points assessed. After 3 days of treatment, FLX down-regulated the expression of 8 miRs and DMI up-regulated the expression of 9 miRs. A more pronounced effect was found after 7 days of treatment; indeed, at this time point FLX modulated 35 miRs (28 up-regulated and 7 down-regulated), whereas DMI down-regulated the expression of 15 miRs. Interestingly, 8 of them were up-regulated by both ADs. Finally, after 14 days of treatment FLX modulated the expression of 4 miRs (1 up-regulated and 4 down-regulated) and DMI down-regulated the expression of 18 miRs. A bioinformatic analysis allowed the identification of putative miRs targets and signalling pathways modulated by the identified miRs. The predicted target genes of miRs are mainly involved in pathways related to neuronal brain function, (such as synaptic and neuronal plasticity, neurotransmission, etc) and many of them have been previously associated to both depression pathophysiology and to the mechanism of action of ADs.

Following different criteria (i.e., genes involved in neuroplasticity modulation and candidate genes for mood disorders according to the more recent evidence available), a number of putative miR target genes have been selected for validation studies by means of mRNA/protein expression studies.

The results of this work, showing that AD treatments induce early and significant modifications in the expression of hippocampal miRs, could be of help to better clarify the mode of the action of current ADs and to identify new putative targets for the development of novel drugs with greater efficacy and a more rapid onset of action.

Reference(s)

[1] O'Connor R.M., Dinan T.G., Cryan J.F., 2012. Little things on which happiness depends: microRNAs as novel therapeutic targets for the treatment of anxiety and depression. Mol Psychiatry 17(4), 359–376.

[2] Dwivedi Y., 2011. Evidence demonstrating role of microRNAs in the etiopathology of major depression. J Chem Neuroanat. 42(2), 142–156.

[3] Bocchio-Chiavetto L., Maffioletti E., Bettinsoli P., Giovannini C., Bignotti S., Tardito D., Corrada D., Milanesi L., Gennarelli M., 2012. Blood microRNA changes in depressed patients during antidepressant treatment. Eur Neuropsychopharmacol Epub ahead of print.

Clinical neuropsychopharmacology

Lectures

S.04.01 Disrupted cerebral connectivity and brain disorders: linking circuits to genes

A. Meyer-Lindenberg[1]*, H. Tost[1], E. Bilek[1]. [1]*University of Heidelberg, Zentralinstitut für Seelische Gesundheit Department of Psychiatry and Psychotherapy, Mannheim, Germany*

In the past decade, imaging genetics has evolved to a highly successful neuroimaging discipline with a variety of sophisticated research tools. To date, several neural systems mechanism have been identified that mediate genetic risk for chronic disabling mental conditions linked to common candidate and genome-wide-supported variants. In particular, the examination of intermediate connectivity phenotypes has recently gained increasing popularity. We give an overview of the scientific methods and evidence that link indices of neural network organisation to healthy human behaviour and the genetic susceptibility for mental illness, following our recent review [1].

The human brain is conceptualised as a complex network of functionally specialised cell populations that interact with each other in a spatially and temporally coherent fashion, and shape physiological and pathological behaviours, as well as their own coupling patterns, by means of neurochemical processes and interconnecting axonal fibres. The anatomical properties of these networks are highly heritable, and shaped by a multitude of genetic variants, some of them conferring risk for psychiatric disorders by affecting the functional integrity of regulatory circuits controlling complex behavioural phenotypes such as cognitions and emotions. In the past decade, the massive technological advances in neuroimaging and molecular genetics facilitated the implementation of a new research strategy ("imaging genetics") that allows for the identification of the neurobiological effects of susceptibility genes.

Historically, imaging genetics studies have focussed on the analysis of single genetic variants that were chosen based on candidacy. Candidate gene approaches are hypothesis-driven, and follow a reverse mode of examination, as the genes of interest are pre-selected based on prior evidence that may arise from various empirical sources such as clinical association studies or basic neuroscience research. A particularly noteworthy contribution to the functional imaging genetics field is the work by Egan and coworkers [2], which examined the effects of a functional variant in the catechol-O-methyltransferase gene (COMT $Val^{108/158}$Met, rs4680) on frontal lobe function in patients with schizophrenia, their unaffected siblings, and healthy volunteers. In all three groups, the carriers of the high dopamine catabolising variant (Val/Val) were characterised by greater activity in the prefrontal cortex during working memory performance. However, while thousands of imaging genetics studies have been published to date, only few reports in psychiatric neuroimaging aimed to characterise the neural correlates of interaction effects within or between genes. In own prior work, we established a method to extend the reach of imaging genetics to the evaluation of the effects of ambiguous haplotypes on prefrontal cortex function and structure. By applying this method to COMT, these analyses showed that the neural effects of the $Val^{108/158}$Met polymorphism are modified by other functional variants and haplotypes in COMT in a pattern that is consistent with complex non-linear effects of extracellular dopamine on prefrontal cortex efficiency and structural integrity. In addition, using fMRI working memory data, several other studies provided biological validation of previously reported effects of gene epistasis on risk for schizophrenia (see e.g. the first report of a three-way interaction by Nicodemus and coworkers [3]). In other studies, significant interaction effects between COMT $Val^{108/158}$Met and other dopamine or glutamate regulating variants such as AKT1 on prefrontal cortex function were demonstrated.

While candidate gene approaches are facing mounting criticism in imaging genetics due to the limited evidence for an association of the variants with the disease phenotype itself, the investigation of risk variants identified in genome-wide association studies at appropriate statistical thresholds has gained much popularity in the recent past. A particularly interesting case is a genome-wide significant single nucleotide polymorphism (rs1344706A) in the gene encoding for the zinc finger protein 804A (ZNF804A), a genetic variant conferring risk for schizophrenia and possibly bipolar disorder. Using a well-established working memory paradigm, we have recently

demonstrated that healthy carriers of rs1344706 risk genotypes do not exhibit differences in regional activation per se, but display pronounced gene dosage dependent alterations in the functional coupling of the DLPFC and hippocampus a finding that mirrors the coupling deficits observed in schizophrenia patients). Subsequent analyses provided evidence for task-specific effects of rs1344706A on prefrontal–hippocampal circuit interaction, thereby pointing to a domain-specific configuration of the genetic risk architecture of schizophrenia. Very recent work by the Weinberger group has further extended these results and provided evidence for this connectivity feature as an intermediate phenotype for schizophrenia.

On a more complex level, the structural and functional composition of neural circuits can be described with graph theoretical metrics. In graph theory, the topology of large-scale cortical networks can be quantified by a graph consisting of a number of nodes (i.e., brain regions) that are connected by edges (i.e., interconnecting fibre tracts or indices of structural or functional coupling). These phenotypes have been shown to be heritable, and the contribution of individual genetic variants to these topological features should be examined. Also, the conceptual expansion of imaging genetics to non-genetic risk factors such as social status and urban environment will further expand our knowledge on the systems-level mechanisms of psychiatric disease. Ultimately, these efforts are expected to pioneer a new generation of evidence-based diagnosis and treatment strategies for these chronic disabling mental conditions.

Reference(s)

[1] Tost, H., E. Bilek, Meyer-Lindenberg, A., 2009. Brain Connectivity in Psychiatric Imaging Genetics. Neuroimage 62, 2250–60.

[2] Egan, M.F., T.E. Goldberg, B.S. Kolachana, J.H. Callicott, C.M. Mazzanti, R.E. Straub, D. Goldman, and D.R. Weinberger. Effect of Comt Val108/158 Met Genotype on Frontal Lobe Function and Risk for Schizophrenia. [In eng]. Proc Natl Acad Sci U S A 98, no. 12 (Jun 5 2001): 6917–22.

[3] Nicodemus, K.K., J.H. Callicott, R.G. Higier, A. Luna, D.C. Nixon, B.K. Lipska, R. Vakkalanka, et al. Evidence of Statistical Epistasis between Disc1, Cit and Ndel1 Impacting Risk for Schizophrenia: Biological Validation with Functional Neuroimaging. [In eng]. Hum Genet 127, no. 4 (Apr 2010): 441–52.

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Posters

P.4.001 Clinical implications of polarity index of drugs in maintenance treatment of bipolar disorder: a naturalistic study

D. Popovic[1]*, E. Vieta[1]. [1]*Hospital Clinic de Barcelona, Neuroscience, Barcelona, Spain*

Purpose of the study: Predominant polarity, defined as at least twice as many episodes of one pole over the other, is among the strongest predictors of recurrence into a specific episode in Bipolar Disorder (BD), and should be considered when implementing maintenance therapy. Our group has recently developed the Polarity Index (PI), a metric indicating antimanic versus antidepressive potential of drugs [1]. The purpose of this study was to determine the role of PI in clinical decision-making. Secondary aim was to assess differences between predominantly manic and depressed patients, with a special focus on their pharmacological treatment.

Methods: The study sample was composed of 604 patients aged $\geqslant$18, with BD I or II, who signed an informed consent, enrolled in the systematic prospective follow up study of the Bipolar Disorders Program of the Hospital Clinic of Barcelona, Spain. Patients who fulfilled criteria for either Manic (MPP) or depressive (DPP) were compared regarding socio-demographic, clinical and therapeutic characteristics.

The PI, a numeric expression of the efficacy profile of a given drug, derives from Number Needed to Treat (NNT) for prevention of depression and NNT for prevention of mania ratio, as emerging from the results of randomised placebo-controlled trials [1,2]. Drugs with $PI > 1$ have stronger antimanic prophylactic properties, while those with $PI < 1$ are more effective for preventing depressive episodes than the manic ones. The PI of drugs for maintenance treatment of BD was: 12.09 for risperidone, 4.38 for aripiprazole, 3.91 for ziprasidone, 2.98 for olanzapine, 1.39 for lithium, 1.14 for quetiapine, and 0.40 for lamotrigine [1]. PI of valproate (0.49) and oxcarbazepine (0.62) may not be reliable due to the failure of their maintenance trials and therefore were not accounted for.

PI for patients' current treatment was calculated as mean value of all prescribed drugs in each patient.

Results: 257/604 (43%) of patients with BD I or II presented predominant polarity. 143 patients (55.6%) fulfilled criteria for DPP and 114 (44.4%) for MPP.

Total PI, as well as Antipsychotics' PI and Mood Stabilisers' PI were higher, indicating a stronger antimanic action, in MPP (Table 1).

MPP presented higher prevalence of BD I, male gender, younger age, age at onset and at first hospitalisation, more hospitalisations, primary substance misuse and psychotic symptoms. DP correlated with BD II, depressive onset, primary life events, melancholia and suicide attempts. The prescription of first generation antipsychotics and second generation antipsychotics olanzapine and risperidone was significantly more frequent among MPP patients, whilst use of lamotrigine, selective serotonin reuptake inhibitors, serotonin–norepinephrine reuptake inhibitors, tricyclic antidepressants and benzodiazepines was more prevalent amongst DPP patients.

Conclusions: The results of this naturalistic study confirm the usefulness of the PI. In this large sample, clinical differences among these groups justify differential treatment approach. The PI appears to be a useful way to operationalise what clinicians do for maintenance therapy in BD.

Table 1.

	Polarity Index								
	Manic (n = 114)		Depressive (n = 143)		p	MW U	p	KS Z	p
	Mean	SD	Mean	SD					
AP+MS	3.68	3.19	2.22	2.36	0.001*	3385.5	0.000*	2.069	0.000*
AP	6.78	4.68	4.77	4.53	0.044*	1281.5	0.006*	1.425	0.035*
MS	1.31	0.23	1.14	0.38	0.001*	2679.0	0.001*	1.429	0.034*

AP = Antipsychotics, MS = Mood Stabilisers, MW = Mann–Whitney, KS = Kolmogorov–Smirnov.

Reference(s)

[1] Popovic, D., Reinares, M., Goikolea, J.M., Bonnin, C.M., Gonzalez-Pinto, A., Vieta, E., 2012. Polarity index of pharmacological agents used for maintenance treatment of bipolar disorder. Eur Neuropsychopharmacol. 22(5), 339–346.

[2] Popovic, D., Reinares, M., Amann, B., Salamero, M., Vieta, E., 2011. Number needed to treat analyses of drugs used for maintenance treatment of bipolar disorder. Psychopharmacology (Berl). 213(4), 657–667.

P.4.002 Genetic differences in drug-metabolising enzymes: can they be used to predict antidepressant treatment response?

K. Hodgson[1]*, K.E. Tansey[1], R. Uher[1], O.S.P. Daivs[1], K.J. Aitchison[1], P. McGuffin[1]. *[1]King's College London, MRC SGDP Institute of Psychiatry, London, United Kingdom*

Purpose of the study: Response to antidepressant medication varies greatly between patients [1]. One factor which may contribute to this variability is the rate at which the drugs are metabolised into inactive compounds, a process performed by the cytochrome P450 family of enzymes. Common genetic differences have been observed in two of the key enzymes (CYP2D6 and CYP2C19) involved in the metabolism of many antidepressants, and these polymorphisms have been linked to variation in rates of drug metabolism. This study examines whether circulating serum levels of antidepressant are influenced by cytochrome P450 genotype in depressed patients and, furthermore, whether treatment outcomes can be predicted by either cytochrome P450 genotype or circulating levels of antidepressant.

Methods used: This study used data from GENDEP (Genome-based Therapeutic Drugs for Depression), a multi-centre pharmacogenetic project following a large sample of depressed patients who received one of two antidepressants for a period of twelve weeks. The two antidepressants studied were the selective serotonin reuptake inhibitor, escitalopram and the tricyclic antidepressant, nortriptyline. These drugs are metabolised into inactive compounds via different pathways, and so analyses were performed separately for each drug. Circulating serum levels of antidepressant (escitalopram or nortriptyline) and primary metabolite (desmethylcitalopram or 10-hydroxy-nortriptyline) were measured after eight weeks of treatment using achiral high-performance liquid chromatography. Data was available from 560 patients (314 were receiving escitalopram and 246 were receiving nortriptyline). Common variants in the genes encoding the cytochrome P450 enzymes CYP2D6 and CYP2C19 were genotyped using the micro-array based Roche AmpliChip CYP450 assay. Treatment response was measured using percentage change from baseline on the Montgomery-Asberg Depression Rating Scale (MADRS). Information was also available regarding other medication that patients were taking during the trial, so the effect of cytochrome P450 enzyme inhibiting medications could also be considered.

Summary of results: Cytochrome P450 genotype predicted circulating levels of both escitalopram ($b = -4.23$, 95% CI = –5.67 to –2.79, $p = 8.56 \times 10^{-9}$) and nortriptyline ($b = -32.18$, 95% CI = –45.27 to –19.08, $p = 1.46 \times 10^{-6}$), as well as the primary metabolite of nortriptyline, 10-hydroxy-nortriptyline ($b = 16.60$, 95% CI = 5.36 to 27.84, $p = 0.0038$). Other medications known to have an effect on the cytochrome P450 enzymes that were being taken concurrently with the antidepressants had no impact on this relationship. Nonetheless, in this sample, cytochrome P450-inhibiting comedication predominantly consisted of the use of oral contraceptives, which are weak inhibitors of the drug-metabolising enzymes. Neither cytochrome P450 genotype, nor circulating levels of drug were predictive of response to antidepressant treatment.

Conclusions: This study investigated the effect of genetic variation in cytochrome P450 genotype on rates of antidepressant metabolism, and the potential relationship to treatment outcomes for two drugs; escitalopram and nortriptyline. These results suggest that, whilst genetic variation in cytochrome P450 enzymes does influence circulating levels of antidepressant for both of these drugs, the variability that is seen in response to treatment is not the result of individual differences in rates of drug metabolism.

Reference(s)

[1] Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L et al. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am J Psychiatry 2006; 163: 28–40.

P.4.003 Publication pressure and burn out among Dutch medical professors: a nationwide survey

J. Tijdink[1]*, Y.M. Smulders[1], A.C.M. Vergouwen[2].
[1]*Free University Medical Centre (VUmc), Internal Medicine/Psychiatrist, Amsterdam, The Netherlands;*
[2]*St. Lucas Andreas Hospital, Psychiatry, Amsterdam, The Netherlands*

Background: Publication of scientific research papers is important for professionals working in academic medical centres and is a key activity in academia. There appears to be increasing focus on quantitative output measures, where quantitative measures of scientific output determine status and prestige, and serve to rank universities as well as individuals. Excessive emphasis on Impact Factors and Hirsch indices could therefore generate a type of pressure that adversely influences quality of science and personal well-being of scientists [1–3].

How this pressure influences professionals' perception of science and their personal well-being is unknown. This manuscript addresses publication culture and its consequences for science and personal well-being in the Netherlands, as seen through the eyes of our academic leaders.

Methods: We performed an online survey inviting all medical professors (n = 1206) of the 8 academic medical centres in The Netherlands to participate. They were asked to fill out 2 questionnaires, besides their demographic data; a Scientific Culture Questionnaire and the Maslach Burnout Inventory. The scientific culture questionnaire contained 24 statements, the responses to which were scored on a 5-point Likert scale. The statements of the scientific culture questionnaire was divided into 3 broad domains; (1) pressure to publish personally experienced by the respondent; (2) publication pressure in general terms in the academic work place, as perceived by the respondent; (3) publication pressure relating to the scientist's position and status (e.g. promotion, re-appointment, etc.).

Results: In total, 437 professors completed the questionnaires. Among them, 54% judge that publication pressure 'has become excessive', 39% believe that publication pressure 'affects the credibility of medical research' and 26% judge that publication pressure has a 'sickening effect on medical science'. The burn out questionnaire indicates that 24% of medical professors has signs of burn out. All 3 subdomains of the publication culture questionnaire were strongly and significantly associated with scores on all 3 subscales of the burnout questionnaire. Correlations were generally strongest for the emotional exhaustion subscale.

Conclusion: A substantial proportion of medical professors believe that publication pressure has become excessive, and are developing a cynical view on the validity of medical science. Publication pressure may have adverse effects on medical science. In the respondents' opinion, publication pressure can adversely affect validity and reliability of the medical literature. These perceptions are statistically correlated to emotional exhaustion and other burn out symptoms. The main limitation is the possibility of participation bias: Those experiencing high pressure could either preferentially participate or be reluctant to do so. Among those who participated, there may still be a taboo on personal pressure and burn out, causing respondents to downplay the severity and personal impact of publication pressure. Further research should address the effects of publication pressure in more detail and identify alternative ways to stimulate the quality of medical science.

Reference(s)

[1] Fanelli D. Do pressures to publish increase scientists' bias? An empirical support from US States Data. PLoS One. 2010 5(4): e10271.
[2] West CP, Hubschka MM, Novotny PJ, Sloan JA, Kolars JC, Habermann TM, et al. Association of perceived medical errors with resident distress and empathy. JAMA. 2006 296(9):1071–1078.
[3] Young NS, Ioannidis JPA, Al-Ubaydli O. Why current publication practices may distort science. PLoS Med 2008 5(10): e201.

P.4.004 Early onset of lithium prophylaxis as possible good prognostic factor for staging bipolar disorder

S. Brioschi[1]*, L. Franchini[1], C. Colombo[1], E. Smeraldi[1]. [1]*Ospedale San Raffaele, Neuroscienze Cliniche, Milano, Italy*

A previous study [1] conducted at our centre, the Clinic for Mood Disorders of S. Raffaele Hospital in Milan, reported that beginning lithium therapy within the first ten years of illness predicts better preventive outcomes than beginning prophylaxis later, both in major depression, recurrent and bipolar patients. The aim of the present study was to confirm these results only considering bipolar patients, and to evaluate clinical markers that may be associated to the response to the stabilisation therapy. In particular, we analysed the role of the initiation time of maintenance therapy on the control of recurrence, and, therefore, of the outcome of Bipolar Disorder.

Two hundred fourteen subjects (84 males, 130 females) affected by Bipolar Disorder receiving a stabilisation therapy (lithium salts, anticonvulsants, or antipsychotics) were studied. Patients were recruited from the ambulatory centre of the Mood Disorder Unit of S. Raffaele Hospital in Milan. Clinical data were collected interviewing the patients through the NIMH Life Chart method. We recorded the time of onset of the preventive therapy and divided the sample into three groups: an 'Early group', including patients who initiated the preventive therapy within the 5th year from the onset of illness; a 'Late group', including patients who initiated the preventive therapy between the 5th and 10th year from the onset of illness; and a 'Very Late group', including patients who initiated the preventive therapy after the 10th year from the onset of illness. The efficacy of the preventive therapy was evaluated calculating the gradient between the recurrence index before and after starting this treatment. Multiple logistic regression analysis was used to determine the factors that influence the response to the preventive therapy.

Patients received, as main stabilisation treatment, lithium salts (65%). The percentage of responders was of 77% during a maintenance treatment period of 4.5 years. The only variables significantly associated with the outcome of preventive therapy and, therefore, with the control of the illness progression were: the use of lithium salts as a first treatment choice ($P = 0.02$), starting the preventive therapy within 5 years of the illness onset ($P < 0.0001$), and the high recurrence index of the illness before treatment ($P < 0.0001$). The presence of psychotic manifestations turned out to be the only factor that negatively influenced the response to the preventive therapy ($P = 0.03$).

The present study confirmed the importance of an early intervention in Bipolar Disorder, indicating that starting lithium therapy within the first five years of illness is more effective than treatments delivered later in the illness course. Finally, our data suggest that the onset time of lithium therapy is a new prognostic element; indeed, an early onset could improve patient's long-term prognosis, while a late onset is associated with a progression of the Bipolar Disorder. Referring to the staging models proposed recently [2], we suggest that the time of initiation of maintenance therapy is a clinic crucial information for staging patients with Bipolar Disorder and, therefore, giving an appropriate preventive treatment.

Reference(s)

[1] Franchini, L., Zanardi, R., Smeraldi, E., Gasperini, M., 1999. Early onset of lithium prophylaxis as a predictor of good long-term out come. Eur Arch Psychiatry Clin Neurosci 249, 227–230.

[2] Vieta, E., Reinaires, M., Rosa, A.R., 2011. Staging bipolar disorder. Neurotox Res 19(2), 279–85.

P.4.005 Effects of cigarette smoking on schizophrenia treatment with olanzapine

K. Zoric[1]*, N. Zivkovic[1], G. Djokic[2]. [1]*Neuropsychiatry Hospital "Dr Laza Lazarevic", acute male psychosis (M department), Belgrade, Serbia;* [2]*Neuropsychiatry Hospital "Dr Laza Lazarevic", Department of Scientific Research, Belgrade, Serbia*

Introduction: Co-morbidity of schizophrenia and cigarette smoking is a well-known phenomenon. Patients with schizophrenia have elevated rates of cigarette smoking compared to the general population, and great difficulty in smoking cessation. Smoking rates (78–88%) in patients with schizophrenia are much higher than the normal population. Excessive cigarette smoking, as it well known, may reduce plasma levels of antipsychotics, including olanzapine up to 50%.

Objective: The goal of this study was to investigate effects of smoking in olanzapine treatment of schizophrenia and to determine does the number of smoked cigarettes during the day affect olanzapine efficacy.

Methods: This prospective clinical study included 127 patients (57.4% males, 42.6% females) with schizophrenia diagnosed by ICD-10 criteria, aged 18–51, which were divided into four groups. Control group (39 patients) and experimental groups: group E1, 1–20 cigarettes/24 h (34 patients), group E2, 21–40 cigarettes/24 h (28 patients) and group E3, more than 40 cigarettes/24 h (26 patients). Patients were observed for one year period in hospital

and out hospital conditions (as outpatients). All patients were assessed by Positive and Negative Symptom Schedule Scale (PANSS) at baseline, after six months and after one year. Data were processed with SPSS for Windows. Kruskal–Wallis and Mann–Whitney tests for independent samples and Wilcoxon test for relationed samples, were used in statistical analyses.

Results: No significant differences were found among four groups for PANSS score at baseline ($p = 0.515$). PANSS score reduction at study endpoint, after one year, is statistically significant in all four groups ($p = 0.001$). Rate of PANSS score reduction after one year is highest in non-smokers group 63.5%, and lowest in group of heavy smokers (40+ cigarettes/24 h) 40.8%. There is a statistically significant difference in PANSS score reduction between group of non-smokers and all three smokers groups ($p < 0.001$). There is a statistically significant difference in PANSS score reduction between E1 and E2, and E1 and E3 group ($p < 0.001$). There was no difference in PANSS score between group E2 and group E3 after one year ($p = 0.384$). There is a statistically significant difference in olanzapine average daily dose after one year between group of non-smokers and all three smokers groups ($p < 0.001$). There is a statistically significant difference in olanzapine average daily dose between E1 and E2 ($p = 0.053$), and E1 and E3 group ($p < 0.001$). There was no statistically significant difference in olanzapine average daily dose between group E2 and group E3 after one year ($p = 0.179$).

Conclusions: The results of our study suggest that cigarette smoking have significant effect on olanzapine therapy of schizophrenia. Olanzapine therapy is most efficient in non-smokers group with most prominent PANSS score reduction. Excessive smoking more than 20 cigarettes per day significantly reduces effects of olanzapine therapy in schizophrenia. Average daily dose of olanzapine is significantly lower in non-smokers group than in all three groups of smokers.

Reference(s)

[1] Haselmo, T., Eikeseth, P., Tanum, L., Molden, E., Refsum, H., 2006. The effect of variable cigarette consumption on the interaction with clozapine and olanzapine. European Journal of Clinical Pharmacology 62 (12): 1049–1053.

[2] Lowe, E.J., Ackman, M.L., 2010. Impact on tobacco smoking cessation on stable clozapine or olanzapine treatment. Ann Pharmacother 44 (4): 727–732.

[3] Cole, M.L., Trigoboff, E., Demler, T.L., Opler, L.A., 2010. Imapact of smoking cessation on psychiatric in patients treated with clozapine or olanzapine. J Psychiatr Pract 16 (2): 75–81.

P.4.006 Reward processing in unaffected siblings of schizophrenia patients: a functional magnetic resonance imaging study

M. de Leeuw[1]*, M. Vink[1], R.S. Kahn[1]. [1]*Rudolf Magnus Institute of Neuroscience, Psychiatry, Utrecht, The Netherlands*

Purpose of the study: Schizophrenia is characterised by a failure in dopaminergic signal transduction. Reward processing is dependent on adequate dopaminergic transduction in mesolimic and mesocortical pathways and may therefore be affected in schizophrenia [1,2]. Reward processing can be dived into two sub-processes: anticipation and the outcome of reward, whereas reward anticipation is thought to be primarily mediated by dopaminergic neurons. Despite abnormal dopamine function in schizophrenia, functional MRI studies in patients during reward anticipation have shown conflicting results: hypo- and hyperactivation of the ventral striatum have been reported. This inconsistency may be due to several (illness-related) factors of which the use of dopamine antagonists is probably the most important one. The goal of the current study is to investigate reward (anticipation) processing using fMRI in siblings of schizophrenia patients who share on average 50% of their ill siblings' genes without having illness-related confounds such as the use of medication.

Methods used: Twenty-nine unaffected siblings of schizophrenia patients and 29 matched controls performed a monetary delayed incentive task during scanning. The task consisted of 72 trials, each lasting 6 seconds on average (range 3–10 s). At the beginning of each trial, a cue was presented signalling a potentially rewarding (a smiling face, 'reward anticipation') or nonrewarding (a non-smiling face, 'neutral anticipation') trial. Following this cue, a target (exclamation mark) was presented to which subjects had to respond by pressing a button as fast as possible. Feedback on performance was given (either a reward or no reward). Subjects could win €1 when they responded within the time limit. Subjects were rewarded in only 50% of the reward trials. fMRI scans were acquired on a 3.0 T scanner using a 2D-EPI sequence (TR: 1600 ms, TE: 23 ms) and preprocessed and analysed using SPM5.

Summary of results: Siblings and controls earned the same amount (€15). Despite this equal performance, siblings showed hypoactivation of the right anterior insula during the reward anticipation (reward anticipation minus neutral anticipation). An ROI analysis, with 11 predefined regions known to be involved in reward processing, revealed hypoactivation bilaterally in the ventral striatum in siblings compared with controls. During the outcome of reward (feedback reward minus feedback neutral) the ROI

analysis showed hyperactivation bilaterally in the oribital frontal cortices (OFC) in siblings compared to controls.

Conclusions: Our finding of ventral striatum hypoactivation during reward anticipation and OFC hyperactivation during reward outcome in siblings is consistent with the notion of impaired dopamine functioning in the fronto-striatal network typically associated with schizophrenia. Moreover, this study supplies further evidence that deficiencies in fronto-striatal pathways mediated by dopminargic signal transmission may be a core deficiency underlying cognitive processes in schizophrenia [3]. Future experiments should clarify what genes may be responsible for these phenotypic anomalies.

Reference(s)

[1] Ziauddeen, H. & Murray, G.K., 2010. The relevance of reward pathways for schizophrenia. Current opinion in Psychiatry 23, 91–6.

[2] Barch, D.M. & Dowd, E.C., 2010. Goal representations and motivational drive in schizophrenia: the role of prefrontal–striatal interactions. Schizophrenia Bulletin 36, 919–34.

[3] Vink, M., Ramsey, N.F., Raemaekers, M., Kahn, R.S., 2006. Striatal dysfunction in schizophrenia and unaffected relatives. Biological Psychiatry 60, 32–9.

P.4.007 Associations of antiepileptic drugs with anxiety and depressive symptoms in paediatric epilepsy: a pilot study

D. Stevanovic[1]*, J. Jancic[2]. [1]*General Hospital Sombor, Department of Psychiatry, Sombor, Serbia;* [2]*Clinic for Neurology and Psychiatry for Children and Youth University of Belgrade School of Medicine Serbia, Department of Neurology, Belgrade, Serbia*

Purpose: Psychopathology in epilepsy is generally considered either to be caused by the epilepsy (ictal, post-ictal or inter-ictal disorders) or to be a comorbidity [1]. Although it is widely accepted that psychopathological manifestations in a person with epilepsy are multifactorial – even a two-way relationship between epilepsy and psychopathology has been proposed, the role of antiepileptic drugs (AEDs) has attracted significant research attention. This has led to the discovery of both beneficial and adverse effects of AEDs on emotion, cognition, and behaviour [2]. Different mechanisms of action have been proposed for the psychotropic effects of AEDs. In particular, the enhancement of gamma-aminobutyric acid (GABA) inhibitory neurotransmission has sedating/anxiolytic/depressiogenic effects, and blocking glutamate excitatory neurotransmission has activating/anxiogenic/antidepressive effects [2]. Studies of psychopathology in paediatric epilepsy in relation to AEDs are scarce, especially with respect to specific symptoms such as anxiety and depressive symptoms. The aim of this study was to investigate associations of AEDs with anxiety and depressive symptoms among children with epilepsy.

Methods: The present analysis was performed on data from 54 children with epilepsy (mean age 14.06±2.74 years) who had participated in our previous study [3]. Anxiety symptoms were assessed using the Screen for Child Anxiety Related Emotional Disorders (SCARED), while depressive symptoms were assessed using the Mood and Feeling Questionnaire (MFQ). Considering that psychopathology in epilepsy emanates from an interplay between various factors, we created a generalised linear model to evaluate associations of AEDs with anxiety and depressive symptoms while taking into account other major factors as well (i.e., age, type of epilepsy, seizure frequency, and epilepsy duration). Separate models were tested for anxiety and depressive symptoms.

Results: Nine children were taking carbamazepine (CBZ), 25 valproate (VPA), 5 lamotrigine (LTG), and 15 were taking two or three AEDs (VPA, LTG, CBZ, oxcarbamazepine, primidone, clobazam, and clonazepam). Age, type of epilepsy, seizure frequency, epilepsy duration, AED, depressive symptoms, and interaction among these variables accounted for 66.9% of the variations in anxiety symptoms ($F = 6.99$, $p < 0.0001$). The same set of variables, with anxiety instead of depressive symptoms, accounted for 60.7% of the variations in depressive symptoms ($F = 5.28$, $p < 0.0001$). Only taking two/three AEDs was associated with significantly lowered anxiety symptoms ($t = -3.06$, $p = 0.004$), accounting for 18.6% of additional variance.

Conclusions: Although it has previously been reported that VPA could be associated with increased depressive symptoms and LTG with increased anxiety symptoms [2], this preliminary study found no association between CBZ, VPA or LTG monotherapy and either type of symptoms among children with epilepsy. However, taking two or three AEDs was associated with significantly lowered anxiety symptoms; this could be an indirect effect of greater sedation by AEDs enhancing GABA inhibitory neurotransmission.

Considering that this cross-sectional study included a small number of children, data from prospective studies including more children – especially with newly diagnosed epilepsy – are needed to investigate all associations of AEDs with anxiety and depressive symptoms.

Reference(s)

[1] Krishnamoorthy, E.S., Trimble, M.R., Blumer, D. 2007. The classification of neuropsychiatric disorders in epilepsy: a proposal by the ILAE Commission

on Psychobiology of Epilepsy. Epilepsy behav 10, 349–53.
[2] Mula, M., Monaco, F. 2009. Antiepileptic drugs and psychopathology of epilepsy: an update. Epileptic Disord 11, 1–9.
[3] Stevanovic, D., Jancic, J., Lakic, A. 2011. The impact of depression and anxiety disorder symptoms on the health-related quality of life of children and adolescents with epilepsy. Epilepsia 52, e75–8.

P.4.008 Lithium-regulated genes in lymphoblastoid cells from bipolar-affective patients

S. Kittel-Schneider[1]*, M. Hilscher[1], S. Schreck[1], C.J. Scholz[1], A. Reif[1]. [1]*University of Würzburg, Dept of Psychiatry, Würzburg, Germany*

Introduction: Bipolar affective disorder affects up to 1% of the population worldwide. The underlying molecular pathomechanisms however still remain unclear and the exact mode of action of the current pharmacological treatment options is still elusive. Lithium still is considered to be the first-line mood stabiliser. However, response is not uniform across the board, as only ca. 10% of all bipolar patients can be considered full lithium responders [1]. Unfortunately, there is no possibility to predict the lithium response in the individual patient before initiating treatment. The aim of our study was therefore to investigate molecular mechanisms of lithium action by studying the expressional profile of lymphoblastoid cell lines generated from bipolar patients.

Methods: EBV-immortalised lymphoblasts from bipolar patients (n = 10) and healthy controls (n = 10) were incubated with either lithium chloride or vehicle (LiCl, c = 1 mMol/l) for 3 weeks. After 1, 2 and 3 weeks of treatment cells were harvested and mRNA was isolated. We conducted a microarray study to detect expressional changes upon lithium treatment. Next, we selected the most promising differentially regulated genes in terms of lithium-associated or disorder-associated pathways on the basis of the microarray results. Also, we added 'circadian genes' based on literature and peripheral gene expression profiles (http://www.ebi.ac.uk/arrayexpress/). The microarray results were then verified via quantitative real time PCR. The PCR results were normalised against two housekeeping genes (HPRT1, TBP1) using LinReg and GeNorm software. The statistical analysis of the data was carried out using SPSS statistical software.

Results: AIF1 and ANP32E expression were decreased after treatment for 3 weeks in bipolar patients. EPHB1 expression was reduced in bipolar patients on baseline level compared to healthy controls, but not influenced by lithium-treatment. PLEKHA2 expression was significantly increased after 3 weeks of lithium incubation, yet more prominently in bipolar patients. KCNK1 showed a lower baseline level in the bipolar cells and lithium treatment lead to an increase of the expression. Regarding the genes which are part of the molecular clockwork, DBP was significantly decreased in expression by lithium treatment, less prominent expressional changes were seen in PER1, PER2 and NR1D2.

Discussion: These results suggest that there is a complex interaction of immuno-modulatory effects (AIF1) of lithium and effects regarding ion channels (KCNK1). Furthermore, genes which seem to play a role in synapto- (ANP32E) und neurogenesis (EPHB1) were identified as lithium-regulated in this peripheral cell model. Additionally, we could show that lithium regulates the expression of genes of the molecular circadian clockwork (DBP, PER1, PER2 and NR1D2), which are in part regulated via GSK-3 beta. In summary, we describe interesting new lithium-regulated genes and we could also identify differences in baseline gene expression between bipolar patient and healthy control cells. These findings could prove useful in the future development of biomarkers for diagnosis and treatment response in bipolar affective disorder.

Reference(s)

[1] Kessing, L.V., et al., 2011, Valproate v. lithium in the treatment of bipolar disorder in clinical practice: observational nationwide register-based cohort study. Br J Psychiatry. 199(1): p. 57–63.

P.4.009 Age at onset and cognitive impairment in schizophrenia: an ecological cross-sectional study with stabilised patients

A. Caldiroli[1]*, M. Buoli[1], E. Caletti[1], R.A. Paoli[1], S. Zago[2], A.C. Altamura[1]. [1]*Ospedale Maggiore Policlinico Fondazione IRCCS Ca' Granda, Psychiatry, Milano, Italy;* [2]*Ospedale Maggiore Policlinico Fondazione IRCCS Ca' Granda, Neurosciences, Milano, Italy*

Purpose of the study: It is widely accepted that schizophrenic patients have global cognitive impairment which is influenced by different clinical variables [1]. Among them, a longer duration of untreated psychosis (DUP)/ duration of illness (DUI) and substance abuse show more evidence to be involved in cognitive deficits; longer DUI was found to correlate with impairment on psychomotor processing speed, verbal fluency and verbal learning [2]. Furthermore, an interesting study has shown a relationship between an early age at onset and pronounced deficits on Digit Symbol Coding and Tower of London [3]. Purpose of the present study is to detect if some clinical variables can

be predictive of cognitive impairment in schizophrenics with particular attention on age at onset.

Methods: Thirty-five clinically stabilised schizophrenic patients (twenty-nine males and six females) were recruited from community services afferent to the Department of Psychiatry (University of Milan). All patients included into the study had to be diagnosed as schizophrenics by SCID-I, to show a Positive and Negative Syndrome Scale (PANSS) score <50 and to be treated with an antipsychotic monotherapy or a combined antipsychotic treatment. They were assessed by the Brief Assessment of Cognition in Schizophrenia (BACS) and an executive/social cognition battery including the Multiple Errands Test for Use in Hospital Settings (MET-HV), the Hotel Task, the Iowa Gambling task, the Reading The Mind in the Eyes Test and the Faux Pas Test. Informations about clinical variables derived from clinical charts and from interviews with the patients and their relatives. Binary logistic models were performed to find an eventual association between continuous clinical variables and cognitive test failures. In addition the total sample was divided in groups according to dichotomous variables (gender, diagnostic sub-type and substance abuse) and the presence of cognitive deficits was compared between groups by χ^2 tests.

Summary of results: An earlier age at onset was found to be predictive of frontal cognitive impairment (Tower of London $p = 0.022$, $OR = 0.709$). In addition female gender was more probably associated with mistakes at MET-HV ($\chi^2 = 4.80$, $p = 0.05$, $phi = 0.40$) and Hotel Task ($\chi^2 = 5.25$, $p = 0.04$, $phi = 0.4$) than male one. Finally, cannabis abusers showed more frequently deficits on verbal fluency ($\chi^2 = 9.35$, $p = 0.04$, $phi = 0.52$) and executive functioning (Tower of London) ($\chi^2 = 11.67$, $p = 0.02$, $phi = 0.58$) than alcohol/cocaine abusers.

Conclusions: Female patients with an early age at onset and cannabis abuse seem to have the worst cognitive profile among schizophrenics. These data illustrate the importance of age at onset on the course of the disorder, as happens for other psychiatric disorders (e.g. obsessive–compulsive disorder) and confirm that early onset population need a higher intensity of care in light of the more severe cognitive impairment. Limits of the study include the small sample size and the differences in the pharmacological treatment between the patients, which could be confounding factors in the interpretation of the data. Prospective studies with larger samples are warranted to confirm the result of this naturalistic study.

Reference(s)

[1] Gur, R., 2011. Neuropsychiatric aspects of schizophrenia. CNS Neuroscience & Therapeutics 17, 45–51.

[2] Bajs, M., Janovic, S., Bajs, M., Dordervic, V., Jevtovic, S., Radonic, E., Kalember, P., 2011. Correlation of cognitive functions with some aspects of illness, treatment and social functioning in recurrently hospitalized schizophrenic patients. Coll Antropol 35, 39–44.

[3] Rajji, T.K., Ismail, Z., Mulsant, H., 2009. Age at onset and cognition in schizophrenia: meta-analysis. BJP 195, 286–293.

P.4.010 Association of personality features with lithium prophylactic response

D. Dembinska-Krajewska[1]*, S. Kliwicki[1], M. Chlopocka-Wozniak[1], J. Rybakowski[1]. [1]*Poznan University of Medical Sciences, Department of Adult Psychiatry, Poznan, Poland*

Purpose of the study: Association of personality features with response to given psychotropic drug has not been frequently studied so far. Lithium is still a cornerstone for the long-term therapy of bipolar disorder. Clinically, the best response to lithium is associated with episodic clinical course, complete remission, bipolar family history and low psychiatric co-morbidity, however, a specific personality profile for the best lithium response has not been estimated. Such a possibility has recently occurred with an advent of new temperamental scales, specifically designed for bipolar illness, and an ability to quantitatively assess lithium prophylactic response. The aim of the present study was to find a possible relationship between the effectiveness of prophylactic lithium efficacy measured by Alda scale [1] and the scores of the Temperament Scale of Memphis, Pisa, Paris, and San Diego Autoquestionnaire (TEMPS-A), as well as the scores of the Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE) scale, in a group of long-term lithium-treated patients.

Methods used: The study was performed on 70 patients with bipolar mood disorder (21 male, 49 female), aged 31–82 (59±12) years, recruited from the outpatients in Department of Adult Psychiatry, Poznan University of medical Sciences. They have been receiving lithium for at least 5 years (5–37, mean 15+8) years. The course of illness was assessed retrospectively based on the results of Alda scale and scored in the range of 0–10. The TEMPS-A questionnaire 110 questions version has been used, assessing five temperament domains: depressive, cyclothymic, hyperthymic, irritable and anxious. Previously, the TEMPS-A scale has been validated in a group of 521 Polish college students [2]. The short version of O-LIFE scale has been used measuring such dimensions of schizotypy as unusual experiences, cognitive disorganisation, introvertive anhedonia, and impulsive nonconformity. Using this scale we have previously

found that bipolar patients obtained significantly higher scores on schizotypy compared with control subjects and we found an association between the features of schizotypy and creativity in bipolar patients [3].

Summary of results: The mean score on the scale Alda was in the whole group 5.9+2.7, similar in male and female patients. As to five temperaments of TEMPS-A, the response to lithium significantly positively correlated with hyperthymic temperament scores ($r = 0.31$, $p = 0.009$), and negatively with anxious ($r = -0.27$, $p = 0.022$), cyclothymic ($r = -0.26$, $p = 0.032$), and depressive ($r = -0.23$, $p = 0.052$) temperament scores. As to the O-LIFE scales, the response to lithium demonstrated a significant negative correlation with cognitive disorganisation ($r = -0.24$, $p = 0.025$). Correlation with unusual experiences and introversion and anhedonia other was also negative, and with impulsive nonconformity positive in men and negative in women, but not statistically significant.

Conclusions: The results of the study indicate that temperamental features of hypomania (hyperthymic temperament) and lack of cognitive disorganisation predict the best results of lithium prophylactic response. They are consistent with primarily antimanic lithium efficacy, its weaker effect in rapid cycling and co-morbid anxiety and a lack of antipsychotic effect.

Reference(s)

[1] Grof, P., Duffy, A., Cavazzoni, P., Grof, E., Garnham, J., MacDougall, M., O'Donovan, C., Alda, M. 2002. Is response to prophylactic lithium a familial trait? J Clin Psychiatry 63, 942–947.

[2] Borkowska, A., Rybakowski, J.K., Drozdz, W., Bielinski, M., Kosmowska, M., Rajewska-Rager, A., Bucinski, A., Akiskal, K.K., Akiskal, H.S. 2010. Polish validation of the TEMPS-A: the profile of affective temperaments in a college student population. J Affect Disord 123, 36–41.

[3] Rybakowski, J.K., Klonowska, P. 2011. Bipolar mood disorder, creativity and schizotypy: An experimental study. Psychopathology 44, 296–302.

P.4.011 Clinical features and drug-induced side effects in early versus late antidepressant responders

C. Fabbri[1]*, A. Marsano[1], M. Balestri[1], A. Serretti[1].
[1]*University of Bologna, Department of Biomedical and NeuroMotor Sciences, Bologna, Italy*

Early antidepressant response has been demonstrated as the result of a true antidepressant effect [1] and a predictor of subsequent stable response [2], but few data about the features of this pattern of response compared to the late one were reported.

With the purpose to study the clinical profile of early response/remission compared to late response/remission, two independent major depressive disorder (MDD) samples (the Sequenced Treatment Alternatives to Relieve Depression or STAR*D level 1 $n = 1922$ and an Italian sample $n = 171$) were investigated. MDD diagnosis was ascertained according to DSM-IV-TR criteria in both samples and depressive symptomatology was assessed biweekly by the Quick Inventory of Depressive Symptomatology Clinician Rated (QIDS-C) in the STAR*D while weekly by the Hamilton Depressive Rating Scale (HDRS) in the Italian sample. Patients were all treated with citalopram in the STAR*D while in a naturalistic setting in the Italian sample (66.67% SSRIs, 23.40% SNRIs, 2.92% TCAs, 2.92% NaSSAs, 2.92% SARIs, 1.17% other antidepressants). Anxiolytics, sedative hypnotics, and other medications for concomitant general medical conditions were the only additional medications allowed in both samples. Antidepressant induced side effects were evaluated according to the Patient-Rated Inventory of Side Effects (PRISE) in the STAR*D and Dosage Record & Treatment Emergent Symptom scale (DOTES) in the Italian sample at each postbaseline visit.

The primary outcome was to identify clinical-demographic predictors of early (2^{nd} week) versus late (4^{th}–6^{th} weeks) antidepressant response (reduction in HDRS or QIDS-C score of at least 50%) and remission ($HDRS \leqslant 7$ or $QIDS\text{-}C \leqslant 5$). Given the hypothesis that early and late response may be determined by differences at pharmacokinetic/pharmacodynamic level, the secondary outcome was to evaluate possible differences in antidepressant induced side effects.

The distribution of variables of interest was compared between early versus late responders/remitters by logistic regression models with baseline symptom severity as covariate. A Bonferroni correction for multiple comparison was applied. The R software served for the analysis (cran.r-project.org/).

In the STAR*D, higher levels of baseline core depressive symptoms according to the Bech subscale [3] were associated with early response ($p = 0.00017$), as well as lower baseline insomnia ($p = 0.003$) and higher functioning ($p = 0.001$) according to the Work and Social Adjustment Scale (WSAS). The absence of anxious MDD showed a trend of association with early response ($p = 0.038$) and remission ($p = 0.026$). In the Italian sample a trend of higher anxious MDD rate ($p = 0.19$) and lower baseline quality of life ($p = 0.078$) according to the World Health Organization Quality of Life Scale (WHOQOL) were observed in late remitters.

In the STAR*D late responders reported higher levels of antidepressant induced side effects, especially difficulty in sleeping ($p = 5.68 \times 10^{-13}$), with a similar trend in the Italian sample ($p = 0.09$).

Thus, early antidepressant response may represent a phenotype characterised by higher core depressive symptoms, lower insomnia, absence of anxious MDD, higher functioning at baseline and lower antidepressant induced side effects. The identification of early versus late antidepressant responders at the beginning of the treatment may be useful in order to guide therapeutic choices in clinical settings.

Reference(s)

[1] Taylor, M.J., Freemantle, N., Geddes, J.R., Bhagwagar, Z. 2006. Early onset of selective serotonin reuptake inhibitor antidepressant action: systematic review and meta-analysis. Arch Gen Psychiatry 63, 1217–23.

[2] Szegedi, A., Jansen, W.T., van Willigenburg, A.P., van der Meulen, E., Stassen, H.H., Thase, M.E. 2009. Early improvement in the first 2 weeks as a predictor of treatment outcome in patients with major depressive disorder: a meta-analysis including 6562 patients. J Clin Psychiatry 70, 344–53.

[3] Bech, P., Gram, L.F., Dein, E., Jacobsen, O., Vitger, J., Bolwig, T.G. 1975. Quantitative rating of depressive states. Acta Psychiatr Scand 51, 161–70.

P.4.012 Effects of second generation antipsychotics on cognitive domains measured with the Matrics Consensus Cognitive Battery in early psychoses

I. Montalvo[1]*, M. Creus[1], A. Gutiérrez-Zotes[1], L. Ortega[1], R. Monseny[1], T. Feliu[1], J. Franch[1], E. Vilella[1], J. Labad[1]. *[1]HPU Institut Pere Mata IISPV Universitat Rovira i Virgili, Early Psychosis Unit & Research Department, Reus, Spain*

Introduction: Cognitive impairments, which are present at early stages of psychoses, are related to low functional outcomes [1]. Second generation antipsychotics may have different effects on cognition, although this remains uncertain [2]. The purpose of the present study was to compare the effect of different second generation antipsychotics on the cognitive performance in patients with a first episode of psychosis.

Methods: We included 54 subjects (46.3% women; mean age 24.5 years) with a psychotic disorder with a duration of illness <5 years, attending the Early Psychosis Program from Reus (Tarragona, Spain). Severity of psychotic symptoms was assessed with the PANSS. Cognition was assessed with the MATRICS Consensus Cognitive Battery (MCCB) [3], that explores seven cognitive domains: Speed of Processing (SOP), Attention/ Vigilance (AV), Working Memory (WM), Verbal learning, Visual Learning, Reasoning and Problem Solving (RPS) and Social Cognition (SC), and the Overall Composite Score. Current antipsychotic treatment was requested. Of all patients, 10 were not receiving antipsychotic drugs, 36 were on monotherapy (17 risperidone/ paliperidone, 13 olanzapine, 6 aripiprazole) and 8 on polytherapy. Antipsychotic drug doses were transformed into equivalents of chlorpromazine (mg/day). Biperden and benzodiazepine (diazepam equivalents) doses were registered.

Statistical analyses were performed with SPSS v.17.0. ANOVA was used to compare MCCB T-scores for each cognitive domain by antipsychotic groups. A multiple linear regression analysis was used to explore the relationship between antipsychotic doses and neurocognitive domains after excluding those subjects on polytherapy. For each cognitive domain, T-scores (age and gender corrected) were considered the dependent variable. Antipsychotic drugs were included in the equation as three independent variables (risperidone/paliperidone, aripiprazole and olanzapine), as well as biperden and diazepam doses. All these variables were included in each model with the enter procedure. Standardised beta coefficients will be shown.

Table: MCCB Cognitive Domains (T-scores) by antipsychotics

	1. No anti-psychotics	2. Risperidone/ Paliperidone	3. Olanzapine	4. Aripiprazole	5. Polytherapy	ANOVA P-value	Significant post-hoc tests (Bonferroni adjustment)
SOP	37.8 (14.2)	33.4 (7.7)	44.1 (9.2)	33.8 (10.1)	19.9 (11.3)	**<0.001**	1 > 5; 3 > 5
AV	36.3 (8.6)	33.0 (7.4)	43.8 (9.8)	38.0 (8.2)	30.0 (6.2)	**0.004**	3 > 2; 3 > 5
WM	35.7 (8.9)	38.4 (9.0)	37.5 (7.5)	37.8 (8.1)	32.9 (13.3)	0.696	
Verbal	45.1 (10.2)	42.6 (5.5)	43.6 (9.5)	39.7 (7.3)	35.5 (3.9)	0.089	
Visual	34.8 (13.0)	40.4 (9.3)	45.9 (8.5)	33.3 (9.9)	22.1 (11.2)	**<0.001**	2 > 5; 3 > 5
RPS	37.5 (8.9)	42.9 (10.1)	47.7 (9.6)	42.5 (6.3)	34.8 (6.0)	**0.031**	
SC	39.4 (11.4)	37.3 (11.1)	39.0 (13.0)	43.8 (11.4)	42.8 (5.6)	0.747	
Composite	29.6 (11.8)	31.7 (7.3)	37.7 (8.4)	30.0 (10.9)	25.5 (4.5)	0.117	

Data are Mean (SD).

Results: The mean (SD) positive, negative and general PANSS scores of the sample were 10.2 (3.8), 14.5 (5.4) and 24.9 (7.4) respectively. There were no significant differences in PANSS scores between groups.

MCCB T-scores by antipsychotic groups are described in Table. Subjects with olanzapine performed better than those subjects on polytherapy in SOP, AV and visual learning. In AV, those subjects on olanzapine performed better than those with risperidone/paliperidone.

In the multiple regression analyses, adjusted by diazepam and biperden, olanzapine was significantly associated to a better performance in SOP ($B = 0.383$, $p = 0.032$), visual learning ($B = 0.394$, $p = 0.029$), and RPS ($B = 0.477$, $p = 0.007$). Risperidone/paliperidone were associated with a poorer cognitive performance in AV ($B = -0.400$, $p = 0.041$). We found no significant

differences related to the Overall Composite Score, although olanzapine showed a trend towards significance (B = 0.356, p = 0.062).

Conclusions: Olanzapine shows a better cognitive profile than other second generation antipsychotics. SOP, AV, visual learning and RPS seem to be more clearly affected by antipsychotic treatment.

Reference(s)

[1] Fett AK, Viechtbauer W, Dominguez MD, Penn DL, Van os J, Krabbendam L., 2011. The relationship between neurocognition and social cognition with functional outcomes in schizophrenia: a meta-analysis. Neurosci. Biobehav. Rev. 35 (3), 573–588.

[2] Woodward ND, Purdon SE, Meltzer HY, Zald DH., 2007 A meta-analysis of cognitive change with haloperidol in clinical trials of atypical antipsychotics: dose effects and comparison to practice effects. Schizophrenia Research. 89, 211–224.

[3] Rodriguez-Jimenez R, Bagney A, Garcia-Navarro C, Aparicio AI, Lopez-Anton R, Moreno-Ortega M, Jimenez-Arriero MA, Santos JL, Lobo A, Kern RS, Green MF, Nuechterlein KH, Palomo T. 2012. The MATRICS consensus cognitive battery (MCCB): Co-norming and standardization in Spain. Schizophrenia Research. 134(2–3), 279–84.

P.4.013 Modulation of brain structure by catechol O-methyltransferase Val^{158}Met polymorphism in chronic cannabis users

A. Batalla[1]*, C. Soriano-Mas[2], M. Torrens[3], J.A. Crippa[4], S. Bhattacharyya[5], L. Blanco-Hinojo[6], B.J. Harrison[7], M. Farré[3], J. Pujol[6], R. Martín-Santos[1]. *[1]Hospital Clinic IDIBAPS CIBERSAM, Department of Psychiatry, Barcelona, Spain; [2]Bellvitge University Hospital-IDIBELL CIBERSAM, Department of Psychiatry, Barcelona, Spain; [3]IMIM (Hospital del Mar Medical Research Institute)-INAD-Parc de Salut Mar, Human Pharmacology and Clinical Neuroscience and Disorders by Use of Substance, Barcelona, Spain; [4]University of Sao Paulo, Neuroscience and Cognitive Behavior Department, Ribeirao Preto, Brazil; [5]King's College London Institute of Psychiatry, Department of Psychosis Studies, London, United Kingdom; [6]Institut d'Alta Tecnologia-PRBB, CRC Hospital del Mar, Barcelona, Spain; [7]Melbourne Neuropsychiatry Centre, Department of Psychiatry, Melbourne, Australia*

Background: Neuroimaging studies have shown that chronic consumption of cannabis may result in alterations in brain morphology [1]. Recent work focussing on the relationship between brain structure and the catechol-O-methyltransferase (COMT) gene polymorphism suggest that functional COMT variants may affect brain volume in schizophrenia patients, subjects at risk for psychosis and in healthy individuals [2]. Furthermore, epidemiological and experimental studies have shown that val allele carriers may be more sensitive to the longer term effects of cannabis as well as the acute effects of delta-9-tetrahydrocannabinol [3]. Nevertheless, there are no previous studies published that have examined the influence of COMT polymorphism on brain morphology in subjects chronically exposed to cannabis.

Aims: The aim of the present study was to explore the influence of COMT Val^{158}Met functional polymorphism on four key regions: the prefrontal cortex, neostriatum (caudate-putamen), anterior cingulate cortex (ACC), and the hippocampus–amygdala complex, in a group of early-onset chronic cannabis users compared with non-using control subjects. We hypothesized that COMT Val^{158}Met functional polymorphism would be associated with brain morphological deficits in cannabis users relative to healthy controls, with dose-dependent associations between volume brain variations and val allele dosage.

Methods: We selected 29 chronic cannabis users who began use cannabis before 16 years of age and matched them to 28 healthy volunteers in terms of age, educational level and intelligence quotient. Participants were male, Caucasians aged between 18 and 30 years. All were assessed by a structured psychiatric interview (PRISM) to exclude any lifetime Axis-I disorder according to DSM-IV. COMT genotyping was performed and structural magnetic resonance imaging data was analysed by voxel-based morphometry (VBM).

Results: The results showed a significantly influence of the COMT polymorphism in bilateral ventral caudate nucleus volume in both groups but in an opposite direction: more copies of val allele was associated with lesser volume in chronic cannabis users and more volume in controls. An opposite pattern was observed for the left amygdala; the greater number of copies of val allele was associated with increased volume in chronic cannabis users and decreased volume in controls. There were no effects of COMT genotype on volumes of the whole brain or the other selected regions. Irrespective of genotype, we also identified a significant positive correlation between caudal rectal gyrus-subgenual cingulate cortex volume and the number of joints consumed. Finally, we reported an almost significant grey matter volume increase in the postcentral gyrus of the left hemisphere in chronic cannabis users.

Conclusions: Our findings support recent reports of neuroanatomical changes associated with cannabis use and, for the first time, reveal that these changes may be influenced by the COMT genotype. Further prospective,

longitudinal research is needed to examine the gene–environment influence and the mechanisms of long-term cannabis related brain impairment.

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Reference(s)

[1] Martin-Santos, R., Fagundo, AB., Crippa, J.A., Atakan, Z., Bhattacharyya, S., Allen, P., Fusar-Poli, P., Borgwardt, S., Seal, M., Busatto, G.F., McGuire, P., 2010. Neuroimaging in cannabis use: a systematic review of the literature. Psychol Med 40, 383–98.

[2] Honea, R., Verchinski, B.A., Pezawas, L., Kolachana B.S., Callicott, J.H., Mattay, V.S., Weinberger, D.R., Meyer-Lindenberg, A., 2009. Impact of interacting functional variants in COMT on regional gray matter volume in human brain. Neuroimage 45, 44–51.

[3] Estrada, G., Fatjó-Vilas, M., Muñoz, M.J., Pulido, G., Miñano, M.J., Toledo, E., Illa, J.M., Martín, M., Miralles, M.L., Miret, S., Campanera, S., Bernabeu, C., Navarro, M.E., Fañanás, L., 2011. Cannabis use and age at onset of psychosis: further evidence of interaction with COMT Val158Met polymorphism. Acta Psychiatr Scand 123, 485–92.

P.4.014 Fear conditioning and context learning in relation to treatment outcome: a study in patients with panic disorder and social phobia

P. Duits[1]*, J.M.P. Baas[2], I.M. Engelhard[1], M.A. van den Hout[1], D.C. Cath[1]. [1]*Utrecht University, Clinical and Health Psychology, Utrecht, The Netherlands;* [2]*Utrecht University, Experimental Psychology, Utrecht, The Netherlands*

Anxiety disorders are among the most prevalent psychiatric disorders. Results from single-cue fear conditioning studies point to modest increases in fear acquisition and fear extinction in patients with anxiety disorders [1]. However, results from discrimination studies have provided inconsistent support so far. First purpose of our study was to further investigate differences between patients with anxiety disorders in fear conditioning. We added a safety cue and a safe context to our fear conditioning paradigm to measure context learning and inhibition of fear as well. The second purpose of our study relates to the state of the art treatment in anxiety disorders: exposure with response prevention. Within this behavioural treatment, fear extinction mechanisms are at the core of the procedure. However, the exact relationship between speed of fear acquisition and extinction and subsequent treatment effect in patients with anxiety disorders is still unclear. Therefore, our second study aim was to investigate the predictive value of fear acquisition and extinction mechanisms in patients with anxiety disorders on treatment outcome.

Patients with panic disorder (n = 15) and social phobia (n = 15) who finished their exposure treatment in the past years, were included in the present study. An age-, gender- and education-matched control group (n = 20) was also included. Fear learning mechanisms were studied in an experimental laboratory test consisting of three phases. Within the task, participants watched movie clips in which two different environments (contexts) were visited for every experimental block. During the first phase, one specific cue (the increase in background illumination) was be followed by the administration of a mild electrical shock to the wrist in one of the contexts, but not the other. After six blocks in which participants may spontaneously learn the association between the light in the shock context and the shock, specific instructions about when the shock can in fact be administered were provided (this second phase consisting of 5 blocks). The last phase of the experiment consisted of four more blocks without the shock reinforcements. During all phases, subjective fear, awareness of the light-shock association and fear potentiated startle to all conditions of the experiment (i.e., the two contexts and the presence / absence of the light cue therein) were assessed. Treatment outcome had been assessed at time of treatment by means of widely used general and disorder specific questionnaires.

Contrary to our expectations, the conditioning task demonstrated no differences in fear acquisition, extinction and context learning between patients and healthy controls. These results suggest that pathological fear mechanisms represent a state characteristic at time of an anxiety disorder being present rather than an underlying trait, since the patients already finished their anxiety treatment at the time of participation in the present study.

Reference(s)

[1] Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., & Pine, D.S. (2005). Classical fear conditioning in the anxiety disorders: A meta-analysis. Behaviour Research and Therapy 43, 1391–1424.

P.4.015 Long term therapy with methylphenidate induces modest effects on growth in ADHD children

C. Balia[1]*, A. Anedda[1], F. Granitzio[1], S. Carucci[1], A. Zuddas[1]. [1]*Cagliari University Hospital, Dept Biomedical Science, Cagliari, Italy*

Background: Although stimulants are the most effective medication for Attention Deficit Hyperactivity Disorder (ADHD), poor growth is a common concern, especially with children already on the lower growth percentiles. Studies providing longitudinal data indicate a reduction in both height and weight gain: these effects are usually minimal, but there is substantial variability with some children completely unaffected, whereas others shows significant growth suppression [1].

Objectives: To evaluate whether long term immediate release methylphenidate (IR-MPH) therapy (one or two years) interferes with the growth of ADHD children and to assess whether the effects on growth are related to the length of the treatment or to the daily dose.

Methods: Growth parameters were collected from 90 ADHD aged 6 to 14, enrolled at one of the site of the Italian National Register for ADHD. All patient were on IR-MPH and with a minimum follow-up of 12 months. 65 were Drug Naïve (DN), 25 were already on MPH since 1–3 years prior to enrollment in the Registry (PR). Weight, height, BMI, height Z-score and BMI Z-score were recorded at each follow-up visit (baseline and after 6, 12, 18, 24 months). Growth velocity SDS and height deficit were calculated after 12 and 24 months.

Data Analysis: Categorical data were analysed using contingency tables (χ^2), continuous variables were compared by one-way ANOVA. Repeated measures ANOVA was performed for height and BMI z scores at baseline, 6, 12, 18, 24 month follow up and for height velocity SDS at 12 and 24 months.

Results: At baseline Height Z-scores of the entire sample was -0.33 ± 0.98, BMI Z-score was equal to 0.19 ± 1.14. During the 24 months in the study, subjects gained in absolute values of height and weight. Height Z-score showed a significant decrease only from T12 to T24 ($p = 0.05$). BMI Z-score decreased significantly at T12 ($p < 0.001$) remaining essentially unchanged at T24. Height deficit was about 0.5 cm at 12 months and 1.3 cm after 24 months. MPH dose/kg/day changed from 0.49 ± 0.21 mg when starting medication, to 0.68 ± 0.24 at T12 and to 0.75 ± 0.25 at T24. No significant differences were found on growth parameters at baseline when stratifying between DN and PR. As in the total sample, in both groups a significant decrease in BMI Z-score from baseline to T12 ($p < 0.001$) and in height Z-score between T12 and T2 ($p = 0.05$) was found. No changes in growth velocity from baseline to the different times considered, were observed, neither in DN nor in PR.

Discussion: The findings of the present study suggest that the effects of MPH on growth are relatively small and unlikely to be of clinical concern for this population. Expected and actual deficit in growth should be considered in the context of the benefits the patient receives from the medication. In the present sample the height deficit appears to be more related to the maximum pro/die dose rather than to the length of therapy. More research is needed to better elucidate the mechanism of growth suppression and to implement specific treatment strategies for ADHD children.

Reference(s)

[1] Pliszka, S.R., Matthews, T.L., Braslow, K.J., Watson, M.A., 2006. Comparative Effects of Methylphenidate and Mixed Salts Amphetamine on Height and Weight in Children With Attention-Deficit/Hyperactivity Disorder. J Am Acad Child Adolesc Psychiatry 45, 520–526.

P.4.016 Regional differences of SERT occupancy in major depression: an in vivo PET study using [^{11}C]DASB

P. Baldinger[1]*, G.S. Kranz[1], M. Savli[1], W. Wadsak[2], D. Haeusler[2], A. Hahn[1], M. Mitterhauser[2], C. Philippe[2], S. Kasper[1], R. Lanzenberger[1]. [1]*Medical University of Vienna, Dept of Psychiatry and Psychotherapy, Vienna, Austria;* [2]*Medical University of Vienna, Dept of Nuclear Medicine – PET Center, Vienna, Austria*

Objective: The blockage of serotonin transporters (SERT) responsible for serotonin reuptake from the synaptic cleft into the presynaptic neuron is the primary mechanism of action of selective serotonin reuptake inhibitors (SSRIs). One might assume that the selective affinity of an SSRI for SERT and hereby its antidepressant effectiveness might be similar throughout the brain. However, SERT activity mediated via various factors may differ between regions as SERT is a priori not equally distributed in the brain. Furthermore, as SERT internalisation process and thereby its availability in a distinct area depends on its activity, SERT occupancy by an SSRI might equally vary throughout the brain [1]. Here, we investigated whether SERT occupancy by escitalopram/citalopram is equally distributed in brain areas known to play a role in major depression using positron emission tomography (PET).

Methods: 19 outpatients (13 females; age 42.3 ± 7.8 years (mean$\pm$SD)) suffering from major depressive disorder (17-item HAMD $\geqslant$ 16, no pharmacological

treatment 3 months prior scanning) were included in this pooled longitudinal study. Subjects received oral doses of either escitalopram (10 mg/day, 10 subjects) or citalopram (20 mg/day, 9 subjects) and underwent three [^{11}C]DASB PET scans: before treatment (PET1), 6 h following the first SSRI dose (PET2) and 6 h after the last dose (PET3), which was administered daily for a minimum of 3 weeks (24.73±3.3 days) as described previously [2]. Quantification of SERT binding potential (BP_{ND}) was performed using MRTM2. PET images were spatially normalised to a template in MNI space using SPM8. SERT BP_{ND} was computed using as regions of interest (ROI) approach, where ROIs were selected from a customised template based on the AAL atlas. Cerebellar grey was used as reference region. Using SPSS median SERT occupancy values across subjects for each of the 27 ROIs were tested against overall occupancy (= median of all 27 ROIs). One-sample Wilcoxon Signed-Rank Tests were performed using median = 68.13 for PET2 and median = 71.50 for PET3.

Results: Injected doses, masses, and specific activities for [^{11}C]DASB did not differ between groups and time points. Regarding PET2, SERT occupancies significantly differ from overall median values in middle temporal gyrus ($T^* = -3.59$, $p < 0.001$), subgenual cingulate cortex ($T^* = 3.78$ $p < 0.001$), amygdala ($T^* = 3.82$, $p < 0.001$), nucleus accumbens ($T^* = 3.66$, $p < 0.001$), median ($T^* = 3.78$, $p < 0.001$) and dorsal raphe nucleus ($T^* = 3.82$, $p < 0.001$). For PET3 this was the case for inferior temporal gyrus ($T^* = -3.37$, $p = 0.001$), supramarginal gyrus ($T^* = -3.37$, $p = 0.001$), middle temporal gyrus ($T^* = -3.59$, $p < 0.001$), subgenual cingulate cortex ($T^* = 3.58$, $p < 0.001$), amygdala ($T^* = 3.82$, $p < 0.001$), nucleus accumbens ($T^* = 3.54$, $p < 0.001$), median ($T^* = 3.82$, $p < 0.001$) and dorsal raphe nucleus ($T^* = 3.82$, $p < 0.001$). All results survive Bonferroni correction for multiple comparisons.

Conclusions: SERT occupancy was shown to vary throughout the cortex in several subcortical and cortical brain areas, e.g. the subgenual cingulate cortex and the amygdala, brain regions known to be involved in the pathogenesis of depression. This is in accordance with previous preclinical studies showing that SSRI concentrations differ between brain regions and might therefore impact on occupancy values in a various degree [3] cortically and subcortically. This region-specific modulation by escitalopram and citalopram might be of major clinical relevance in the treatment of major depression.

Reference(s)

[1] Meyer H.J., Wilson A.A., Sagrati S., Hussey D., Carella A., Potter W.Z., Ginovart N., Spencer E.P., Cheok A., Houle S., 2004. Serotonin Transporter Occupancy of Five Selective Serotonin Reuptake Inhibitors at Different Doses: An [11C]DASB Positron Emission Tomography Study. Am J Psychiatry 161:826–835.

[2] Lanzenberger R., Kranz G.S., Haeusler D., Akimova E., Savli M., Hahn A., Mitterhauser M., Spindelegger C., Phillipe C., Fink M, Wadsak W., Karanikas G., Kasper S., 2012. Prediction of SSRI treatment response in major depression based on serotonin transporter interplay between median raphe nucleus and projection areas. Neuroimage Nov 1;63(2):874–81.

[3] Kugelberg F.C., Apelqvist G., Carlsson B., Ahlner J., Bengtsson F., 2001 In vivo steady-state pharmacokinetic outcome following clinical and toxic of racemic citalopram to rats. British Journal of Pharmacology 132, 1683–1690.

P.4.017 Post training noradrenaline reuptake inhibition modulates fear memory consolidation and abolishes long term fear responses in humans

J. Almeida[1]*, J. Tulen[1], F. van der Veen[2], S. Kushner[1].
[1]Erasmus MC, Department Psychiatry, Rotterdam, The Netherlands; [2]Erasmus University, Department Psychology, Rotterdam, The Netherlands

Background: Although memory enhancement for significant events such as life threatening experiences is an important factor in survival, traumatic events can lead to psychological disturbances, including posttraumatic stress disorder (PTSD). After a given event, formation of its correspondent memory undergoes a process termed consolidation [1]. This raises the possibility that pharmacological intervention in the aftermath of trauma could forestall the development of PTSD and other anxiety disorders [2].

Here we aimed to delineate the precise effects of acute differential modulation of noradrenaline (NA) and serotonin in memory consolidation processes. For this purpose we combined a differential pavlovian fear conditioning (FC) paradigm with the administration of a single dose of the selective NA reuptake inhibitor (SNRI) atomoxetine and the selective 5-HT reuptake inhibitor (SSRI) citalopram.

Methods: Ninety healthy volunteers were recruited and assigned to a differential pavlovian FC protocol, involving two conditional stimuli (CS+ and CS−) where only the former was paired with a shock. Afterwards subjects took a pill (atomoxetine vs citalopram vs placebo) in a double-blind RCT design. Blood samples were taken before conditioning and at t = 1 h and t = 3 h. A day later participants underwent extinction training in which the two CS were repeatedly presented without the shock.

Six months later participants were submitted to reinstatement followed by re-extinction training to examine the long term effects of the intervention. Skin conductance responses were obtained at all occasions for fear memory measure.

Results: Sixty-two subjects showed successful fear acquisition on day 1 and were included in the analysis (placebo = 23; citalopram = 21; atomoxetine = 18). No differences were found between the 3 groups (two-way ANOVA regarding main effects of group and time; non-significant). Testing on day 2 revealed no differences concerning CS+ responses between treatment groups ($F_{2,61} = 1.93$). However there was a significant main effect of group concerning CS− responses ($F_{2,61} = 10.07$; $p < 0.05$). Follow-up t-tests of the first two CS− trials showed significantly lower responses for the atomoxetine group ($t = 2.43$, $t = 2.14$; both $p < 0.05$). MHPG (3-methoxy-4-hydroxyphenylglycol, a NA metabolite) absolute change levels correlated significantly with mean CS+ and CS− difference in the first three trials for the atomoxetine group ($R^2 = 0.28$; $p < 0.05$), with no correlation for the placebo group.

Six months afterwards, 37 participants (placebo = 14; citalopram = 10; atomoxetine = 13) were located and reassessed. Average first CS+ responses between groups showed significance (Kruskal–Wallis; $p < 0.05$). Follow-up testing revealed significantly lower CS+ responses for the atomoxetine group.

Conclusion: This study shows that memory consolidation can be influenced by SNRI administration **after** acquisition, lowering CS− responses on the short term, with an apparent similar effect in the CS+ on the long term.

The findings suggest that the emotional salience of a noxious stimuli conferred by NA could in part stem from the relative downgrading of the concomitant/temporal adjacent stimuli rather than elevation of its absolute value. As generalisation of abnormal responses to similar yet unrelated trauma cues is one of the cardinal features of PTSD this study might offer new insights into its prophylaxis in the aftermath of trauma. Additionally it adds weight to the assumption that memory formation is an ongoing dynamic process that continues long after the events that led to its generation.

Reference(s)

[1] Pitman, R.K., Rasmusson, A.M., Koenen, K.C., Shin, L.M., Orr, S.P., Gilbertson, M.W., et al., 2012. Biological studies of post-traumatic stress disorder. Nat Rev Neurosci. 11, 769–87.

[2] Bush, D.E., Caparosa, E.M., Gekker, A., Ledoux, J., 2010. Beta-adrenergic receptors in the lateral nucleus of the amygdala contribute to the acquisition but not the consolidation of auditory fear conditioning. Front Behav Neurosci. 4, 154.

P.4.018 General error monitoring system dysfunction in obsessive compulsive disorder patients: an event-related potential study

L. Carmi[1]*, U. Alyagon[2], A. Zangen[2], R. Dar[3], J. Zohar[1].
[1]Sheba Medical Center, Pychiatry, Ramat Gan, Israel; [2]Ben Gurion University, Life Science–Neuroscience, Beer-Sheva, Israel; [3]Tel Aviv University, Psychology, Tel Aviv, Israel

Introduction: Enhanced Error Related Negativity (ERN) signal after making mistakes is more frequent in OCD patients compared to healthy participants and was found to be generated by theta band [1]. Nevertheless, it is not clear whether this hyperactive ERN signal reflects a hyperactive monitoring system that is over-sensitive to punishment cues or a dysfunction of a more general and less affective monitoring system. The latter manner would imply a system which **continuously** seeks out erroneous information in the inner or outer environment, regardless of a mistake being made. Indeed, normal participants have shown enhanced theta activity merely by **identifying** erroneous content [2].

To test the hypothesis of a general hyperactive monitoring system we tested whether OCD patients show enhanced theta activity after identifying erroneous content as compared to controls. As the ERN enhancement is correlated with OC symptom severity, we hypothesized that reduction of the hyper theta activity in OCD patients, via Deep Trans Cranial Magnetic Stimulation (dTMS), will be correlated with a decrease in symptom severity.

Method: We used the Stroop task to elicit ERN and an arithmetic task [2] to elicit theta activity in response to erroneous content. In our interim analysis, Nine OCD patients and nine healthy controls were tested. In the arithmetic task, participants were requested only to indicate whether a solution of a simple equation is correct or incorrect. The OCD group went through five weeks of inhibitory blind dTMS treatment (active vs. sham). EEG measurements were taken at baseline, after the first day of treatment and at the end of treatment course. The OC symptom severity was evaluated by YBOCS.

Results: A non-phase locked wavelet analysis revealed a significant interaction of group and condition [$F(2,32) = 5.23$ ($p < 0.01$), $p.e = 0.24$]. A relative power increase in low frequency bands (delta, theta and alpha) were shown in the OCD group compared to healthy control after merely identifying wrong solutions. In contrast,

no differences were found when correct solutions were presented. Activity had a midline frontal locus (Afz electrode), similar to previous findings, suggesting ACC as a possible source [3].

The YBOCS scores (Table 1) revealed that 40% of the real dTMS treatment group (5 patients) reached remission criteria (reduction of 35% from baseline score) after 5 weeks of treatment. No response was obtained in the sham group.

Table 1. YBOCS scores at baseline, middle and end of treatment in OCD group

Subject	Baseline	End (5th week)
Active 1	24	28
Active 2	30	31
Active 3	27	17*
Active 4	29	27
Active 5	24	8*
Sham 1	27	26
Sham 2	29	26
Sham 3	22	22
Sham 4	22	20

*Indicates remission.

Conclusion: Over-monitoring condition is expressed in OCD patients not only when an error is committed (ERN), but also upon observing an error. Moreover, a reduction of this hyper activity (via dTMS) was associated with reducing OC symptom severity (as measured in the YBOCS scores). These interim findings may imply a cognitive endophenotype basis for the bias attributed to OCD patients in particular and OCD spectrum in general.

Reference(s)

[1] Cohen, M.X., Elger, C.E., Ranganath, C., 2007. Reward expectation modulates feedback-related negativity and EEG spectra. Neuroimaging 35, 968–978.
[2] Tzur, G., Berger, A., 2007. When things look wrong: theta activity in rule violation. Neuropsychologia 45, 3122–3126.
[3] Luu, P., Tucker, D.M., Makeig, S., 2004. Frontal midline theta and the error related negativity: Neuro physiological mechanisms of action regulation. Clinical Neurophysiology 115, 1821–1835.

P.4.019 Insight and recovery in schizophrenic patients: an observational study

D. Cannavò[1]*, C. Concerto[1], E. Battaglia[1], O. Bianchini[1], E. Aguglia[1]. [1]*University of Catania, Psychiatry, Catania, Italy*

Purpose of the study: Progress in therapeutic options for schizophrenia has revived long-term expectations for researchers, practitioners and patients. At present, definitions of therapeutic outcome include both maintained symptomatic remission and appropriate functioning in a conceptual framework that targets patient's recovery as the ultimate goal. Paradoxically, insight in schizophrenic patients is associated with positive outcomes such as better compliance and recovery, negative outcomes such as depression, hopelessness, low self-esteem and quality of life [1,2]. The aim of this study was to investigate the correlation between insight and recovery in schizophrenic patients according to criteria for both symptomatic and functional remission.

Methods used: We designed an observational study of 24 months of duration; visits were scheduled at baseline, 12 and 24 months. We included 70 patients (15 patients treated with olanzapine, 15 with risperidone, 15 with aripiprazole, 15 with haloperidol and 10 with ziprasidone) affected by paranoid schizophrenia according to DSM IV TR criteria who changed pharmacological therapy for different reasons (inefficacy, presence of side effects or contraindications) or naive patients who started pharmacological treatment. Rating scales used were SAI (Schedule for the Assessment of Insight) for the assessment of insight, PANSS (Positive and Negative Syndrome Scale) for psychopathological picture, Short Form questionnaire (SF-36) for quality of life, Psychological General Well-Being Index (PGWBI) for well-being and Global Assessment of Functioning scale (GAF) to rate the social, occupational and psychological subjective functioning.

Results: After 2 years, 50% of the subjects achieved symptom remission and 25.5% had adequate social functioning for 2 years or more. Only 12% of subjects met full recovery criteria for 2 years or longer. Patients in the remission group showed a significant better outcome during follow-up on all PANSS subscales (positive, negative, and general symptoms subscales), a significant higher level in social functioning and an improvement in the subjective perception of well-being. No differences were found in terms of quality of life between groups. For what concerns pharmacological antipsychotics treatment, although there were no significant differences in patients distribution about symptomatic remission/functioning levels, patients treated with second generation antipsychotics (olanzapine, risperidone, aripiprazole, ziprasidone) that met the symptomatic remission/functioning criteria were higher compared to those treated with typical antipsychotics (haloperidol) in monotherapy. all patients in recovery showed an improvement in insight levels, especially for patients treated with second generation antipsychotics. Recovery after 2 years was predicted by female sex, higher age, pre-morbid social adaptation and low level of negative symptoms at baseline.

Conclusions: The results indicate that only a small proportion of patients affected by schizophrenia achieve recovery and suggest that social functioning, medication

adherence, type of antipsychotic and improvement in insight levels are important predictors for recovery [3]. Therefore more sensitive instruments and a larger sample are necessary to confirm these results.

Reference(s)

[1] Cavelti M, Kvrgic S, Beck EM, Rüsch N, Vauth R. 2012. Self-stigma and its relationship with insight, demoralization, and clinical outcome among people with schizophrenia spectrum disorders. Compr Psychiatry. 53(5):468–79.
[2] Kurtz MM, Tolman A., 2011. Neurocognition, insight into illness and subjective quality-of-life in schizophrenia: What is their relationship? Schizophrenia Research 127, 157–162.
[3] Stefanopoulou E, Lafuente AR, Saez Fonseca JA, Huxley A., 2009. Insight, global functioning and psychopathology amongst in-patient clients with schizophrenia. Psychiatr Q 80, 155–165.

P.4.020 Allopurinol for mania − a randomised trial of allopurinol vs. placebo as add-on treatment in manic bipolar patients

S. Burshtein[1]*, M. Weiser[1], A.A. Gershon[1], G. Marian[2], N. Vlad[3], I.G. Grecu[4], E. Tocari[5], A. Tiugan[6], M. Hotineanu[7], J.M. Davis[8]. *[1]Sheba Medical Center, Psychiatry C, Ramat Gan, Israel; [2]Spitalul Clinic de Psihiatrie Obregia, Psychiatry, Bucuresti, Romania; [3]Spitalul de Psihiatrie Botosani I, Psychiatry, Botosani, Romania; [4]Spitalul Clinic Judetean Mures-Sectia Clinica Psihiatrie I, Psychiatry, Targu Mures, Romania; [5]Spitalul Judetean Vrancea Sectia Psihiatrie Focsani, Psychiatry, Focsani, Romania; [6]Sp. Clinic de Urgenta Militar "Dr. Stefan Odoblegea", Psychiatry, Craiova, Romania; [7]Spitalul Clinic de Psihiatrie Chisinau, Psychiatry, Chisinau, Moldova Rep. of; [8]University of Illinois, Psychiatry, Chicago, USA*

Objective: An emerging body of evidence supports a role for dysfunctional purinergic neurotransmission in mood disorders. Adenosine agonists have been shown to have properties similar to those of dopamine antagonists; there is a well characterised interaction between adenosine and dopamine receptors in the ventral striatum, and increasing adenosinergic transmission has been demonstrated to reduce the affinity of dopamine agonists for dopamine receptors. Allopurinol increases adenosine levels in the brain, hence is hypothesized to reduce the symptoms of mania.

Two randomised, placebo-controlled trials administering add-on allopurinol to manic patients showed significantly greater improvements in YMRS scores for drug compared to placebo [1,2], while a more recent, relatively small, add-on study showed negative results [3]. Based on these data, our objective was to examine the efficacy of allopurinol as add-on treatment to mood stabilisers and/or antipsychotics in manic bipolar patients.

Methods: We performed a large, well powered, multicentre, 6-week randomised, placebo-controlled trial of allopurinol added to mood stabilisers and/or antipsychotics in 180 bipolar patients in an acute manic episode.

Results: Of the 180 participants enrolled in the study, 90 were randomised to allopurinol and 90 to placebo. Baseline YMRS scores were 24.6 in the placebo group (SD = 5.6), and 25.4 in the allopurinol group (SD = 5.3) ($t = -1.02$, $p = 0.31$). Completion rates were similar, with 74/90 (82.2%) completing the 6 week study in the allopurinol group and 75/90 (83.3%) in the placebo group ($\chi 2 = 0.04$, $p = 0.84$).

Overall, both the treatment and placebo groups improved significantly. Mean total YMRS score improved by 11.0 points in the allopurinol group (SD = 8.65, $t = -11.83$, $p < 0.001$, effect size 1.612) and 10.6 points in the placebo group (SD = 8.56, $t = -11.48$, $p < 0.001$, effect size 1.497) by the end of the study. Other clinical outcome measures such as CGI-BP and PANSS showed similar improvements. However, no significant between-groups differences were detected in change of YMRS score ($t = 0.28$, $p = 0.78$), or in the secondary outcome measures at study's end.

There was no difference in the percent of patients who responded to treatment (defined as showing at least 50% improvement in YMRS score) between the two groups, with 37.8% responders in the allopurinol group and 38.6% responders in the placebo group ($p = 0.92$).

The frequency of adverse events was also similar between the two groups (38.9% for the placebo group and 41.1% for the allopurinol group, $p = 0.88$).

Limitations: None of our patients received lithium. However, the side effects of lithium and its' narrow therapeutic index, made the use of lithium less common and therefore our study results reflect the common current clinical practice. Another limitation of our study is the variety of antipsychotic treatments on which we added on allopurinol. This is also an outcome of the current clinical practice for acute in which there is no single 'gold standard' medication.

Conclusions: The findings of this large, well-powered study do not support allopurinol as a treatment for acute mania.

Reference(s)

[1] Akhondzadeh S, Milajerdi MR, Amini H, Tehrani-Doost M. Allopurinol as an adjunct to lithium and

haloperidol for treatment of patients with acute mania: a double-blind, randomized, placebo-controlled trial. Bipolar Disord. 2006; 8:485–9.
[2] Machado-Vieira R, Soares JC, Lara DR, Luckenbaugh DA, Busnello JV, Marca G, et al. A double-blind, randomized, placebo-controlled 4-week study on the efficacy and safety of the purinergic agents allopurinol and dipyridamole adjunctive to lithium in acute bipolar mania. J Clin Psychiatry. 2008; 69:1237–45.
[3] Fan A, Berg A, Bresee C, Glassman LH, Rapaport MH. Allopurinol augmentation in the outpatient treatment of bipolar mania: a pilot study. Bipolar Disord. 2012; 14:206–10.

P.4.021 Genome-wide association analysis and pathway analysis in predicting antidepressant treatment response

N. Antypa[1]*, A. Drago[1], A. Serretti[1]. [1]*University of Bologna, Department of Biomedical and NeuroMotor Sciences, Bologna, Italy*

Pharmacogenetic research seems to be a promising path towards achieving personalised treatment, however, the link between genetic risk variants and complex mental disorders such as depression is far from direct. Genomewide association (GWAS) studies on antidepressant efficacy have yielded poor results that fail to reach genomewide significance [1] and are hard to replicate. A possible reason is that antidepressant response can be influenced by a multiplicity of sociodemographic and environmental factors, which possibly interact with genetic variation [2]. For example, the patient's quality of life has been previously found to be an independent predictor of acute antidepressant treatment response [3].

The aim of the present study was twofold. Firstly, we included the patients' level of quality of life as a psychosocial predictor of response in a GWAS model of treatment response to citalopram, in a large group of depressed patients from the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial. Secondly, since a number of candidate genes have emerged from prior gene–environment (G×E) interaction studies on depression, we were interested in investigating whether such possibly putative cluster of genes is likely to represent a causal pathway in predicting treatment response.

We investigated a sample of 1426 depressed patients from the STAR*D trial: 774 responders and 652 non-responders and a subset of 418,865 single nucleotide polymorphisms (SNPs) were assessed. We examined whether genetic variations interact with the patients' levels of Quality of Life (QoL) to predict antidepressant response, after controlling for demographic characteristics, depression severity and population stratification. As a second step, we conducted a pathway analysis, which explored whether candidate genes (that have emerged from prior G×E interaction studies) represent a biological pathway in predicting treatment response.

The GWAS model yielded one SNP associated with response, the rs520210 SNP of the NEDD4L gene ($p = 3.64 \times 10^{-8}$; OR: 0.76). We found that responders were most likely to be carriers of the minor allele (A), compared to non-responders, especially those reporting low QoL upon entering the trial. Other genes among the top findings include FKBP1A, SLC37A1, ASTN1 and TNFRSF10B. The pathway analysis showed that SNPs associated with treatment response at a p level <0.001 were more frequently found within the pathway in comparison to elsewhere in the genome. The HTR2A gene contained the most significant markers that predicted response.

Our findings point to one possible target gene, namely, the NEDD4L (18q21.31), which is an ubiquitin protein ligase, thereby playing a role in the protein recycling and house keeping activities of the cell. Other genes among our top hits are also proposed for further independent replication. Our pathway results provide further support on the role of the HTR2A gene in influencing antidepressant response. Despite methodological and statistical challenges, taking into account distinctive predictors of response, along with genetic factors, could be a promising avenue for future research.

Reference(s)

[1] Garriock HA, Kraft JB, Shyn SI, Peters EJ, Yokoyama JS, Jenkins GD et al. 2010. A genomewide association study of citalopram response in major depressive disorder. Biol Psychiatry 67, 133–138.
[2] Malhotra AK. 2010. The pharmacogenetics of depression: enter the GWAS. Am J Psychiatry 167, 493–495.
[3] Pyne JM, Bullock D, Kaplan RM, Smith TL, Gillin JC, Golshan S et al. 2001. Health-related quality-of-life measure enhances acute treatment response prediction in depressed inpatients. J Clin Psychiatry 62, 261–268.

P.4.022 Neural correlates of generalised anxiety disorder without comorbid depression: preliminary data from a functional MRI study

K. Hilbert[1]*, U. Lueken[1], K. Beesdo-Baum[1].
[1]*Technische Universitaet Dresden, Institute of Clinical Psychology and Psychotherapy, Dresden, Germany*

Background and Aims: Few functional neuroimaging studies on Generalised Anxiety Disorder (GAD), which is characterised by excessive and uncontrollable worries about a variety of situations accompanied by physical symptoms, are available so far. These studies reported perturbations in the amygdala, ventrolateral prefrontal cortex (VLPFC), anterior cingulate cortex and middle frontal gyrus. But as GAD samples mostly include patients with comorbid depression, it remains unclear whether the reported neural correlates result from GAD alone [1]. Preliminary data from an ongoing study is reported. We aimed to validate the neural correlates of GAD reported so far by investigating emotional processing under two different attentional states in a GAD sample without comorbid depression.

Methods: N = 22 medication-free subjects (n = 10 GAD patients without depression and n = 12 healthy controls), diagnosed by a standardised clinical interview and carefully matched on age (mean = 32.09, range: 21-57), sex (17 female), and level of education (16 high-school equivalent), participated in an event-related functional magnetic resonance imaging (fMRI) session. Using an emotional processing paradigm that has been applied successfully in a more complex form before [1], subjects viewed 16 fearful and 16 happy faces, each presented for four seconds and repeated four times in randomised order. Attention was varied by instructing the subjects to rate either their internal fear or the nose-width of the faces online during the scanning on a five-point likert scale. Blood oxygen level dependent (BOLD) fMRI data were collected on a 3-T scanner and preprocessed and analysed with SPM8 (whole-brain analysis and region-of-interest, $p < 0.001$ uncorrected).

Results: For the contrast fearful versus happy faces, GAD patients compared to healthy controls showed increased activation in the parietal cortex and decreased activation in the ventrolateral prefrontal cortex, middle frontal gyrus and middle cingulate gyrus during ratings of internal fear. When rating the nose-width of the faces, increased activation in the parietal cortex, temporal cortex, orbitofrontal cortex and ventrolateral prefrontal cortex and decreased activation in the inferior frontal operculum was found in GAD patients for the same contrast.

Conclusions: Present results indicate a failure of brain areas such as the prefrontal cortex, middle frontal gyrus and middle cingulate gyrus to regulate emotional processing in GAD. These processes seem to be modulated by the attentional focus of the subjects. Results thus confirm previous findings and indicate the existence of GAD-specific neural alterations. Furthermore, a robust hyperactivation of the parietal cortex was found in GAD patients, possibly reflecting emotional memory processes during stimulus perception [2] present in GAD samples. However, no differential activation was found in the amygdala, possibly due to limited statistical power. If replicated, these results may prove relevant for the validator-based classification of the disorder and the ongoing nosological discussion. Future studies should employ comparisons of non-comorbid GAD samples with depression and other anxiety disorders to refine knowledge on GAD-specific neural correlates and determine similarities and differences between GAD and related disorders.

Reference(s)

[1] Beesdo, K., Lau, J.Y.F., Guyer, A.E., McClure-Tone, E.B., Monk, C.S., Nelson, E.E., Fromm, S.J., Goldwin, M.A., Wittchen, H.-U., Leibenluft, E., Ernst, M., Pine, D.S., 2009. Common and distinct amygdala-function perturbations in depressed vs anxious adolescents. Arch Gen Psychiatry 66(3), 275–285.

[2] Murty, V.P., Ritchey, M., Adcock, R.A., LaBar, K.S., 2011. Reprint of: fMRI studies of successful emotional memory encoding: a quantitative meta-analysis. Neuropsychologia, 49(4), 695–705.

P.4.023 Habenular nuclei in different phases of major depressive disorder: a magnetic resonance imaging volumetric study

J. De Diego-Adeliño[1]*, M. Carceller[1], M. Serra-Blasco[1], Y. Vives-Gilabert[2], B. Gómez-Anson[3], D. Puigdemont[4], E. Álvarez[4], V. Pérez[4], M.J. Portella[4]. [1]*Hospital Sant Pau CIBERSAM UAB, Dept. of Psychiatry, Barcelona, Spain;* [2]*UAB, Port d'Informació Científica (PIC), Barcelona, Spain;* [3]*Hospital Sant Pau CIBERNED UAB, Neuroradiology, Barcelona, Spain;* [4]*Hospital Sant Pau CIBERSAM UAB, Psychiatry, Barcelona, Spain*

Purpose of the study: Habenular nucleus (HbN) is an epithalamic structure involved in stress-response, anxiety and reward-processing. It receives frontolimbic and hippocampal afferences and also projects inhibitory

fibres to the brainstem monoaminergic nuclei, thus it might play an important role in the pathophysiology of Major Depressive Disorder (MDD) [1]. Prior studies [1–3] have reported functional and volumetric abnormalities in MDD, however, habenular volume has been hardly compared between different stages of depressive disease leaving apart processes related with chronicity and treatment resistance.

We aimed to investigate whether habenular volume differed between patients with MDD in distinct stages of the illness and healthy controls. We hypothesized that chronic/treatment-resistant depression would associate smaller habenular volumes.

Methods: A cross-sectional structural neuroimaging with a 3-T Philips Achieva scanner was conducted. Sample was composed by 61 outpatients with MDD (DSM-IV) in different stages of illness (First-episode, n = 21; Remitted-recurrent MDD, n = 20; Chronic/treatment-resistant MDD, n = 20) and 34 healthy controls. All participants were right-handed and groups were comparable in age, sex and educational level. HbN were manually segmented by two researchers blinded for the clinical data. Inter-rater kappa coefficients were 0.83 ($p = 0.006$) and 0.8 ($p = 0.01$) for right and left habenula, respectively. Total, grey matter (GM) and white matter (WM) habenular volumes were calculated for each participant.

Results: Total habenular volume did not show significant differences among groups. A significant group effect was observed in right habenular WM volume ($F = 2.895$; $df = 3,91$; $p = 0.039$), but not for the rest of comparisons. Post-hoc t-test showed that patients with a first-episode had higher WM volume in the right habenula as compared to healthy controls ($p = 0.046$) and to chronic patients ($p = 0.03$). Similar findings were seen for the left HbN, but the differences did not reach statistical significance.

Conclusions: Although no differences in total habenular volumes were found across the groups, our findings of abnormalities in WM volume provide some evidence for the potential involvement of the habenula in MDD. Particularly, we observed higher habenular WM volumes in patients with a first-episode than in healthy controls and patients with chronic/treatment-resistance MDD. This WM hypertrophy could be related with the habenular hyperactivity described among patients with depression by functional MRI studies [2] and might be part of the neural substrate of MDD in early stages. By contrast, the smaller WM volumes observed in later phases of the illness, especially in chronic patients, suggest that changes in WM volumes within this structure might play a role in the treatment response. In fact, HbN has been targeted for deep brain stimulation in treatment-resistant MDD [1]. Because of the cross-sectional design of the present study, it would remain unclear whether small WM habenular volume would be a vulnerability marker for treatment-resistance or whether a progressive atrophy would occur along the course of the illness (perhaps due to an overuse of the structure).

The conclusions should be taken cautiously because patients were on medication. Additionaly, though reliability inter-rater coefficients were good, habenular nucleus is a little structure highly attached to surrounding nuclei, which could reduce the likelihood of finding further differences.

Reference(s)

[1] Henn, F.A., 2012. Circuits, cells, and synapses: toward a new target for deep brain stimulation in depression. Neuropsychopharmacology 37, 307–108.

[2] Roiser, J.P., Levy, J., Fromm, S.J., Nugent, A.C., Talagala, S.L., Hasler, G., Henn, F.A., Sahakian, B.J., Drevets, W.C., 2009. The effects of tryptophan depletion on neural responses to emotional words in remitted depression. Biol Psychiatry 66, 441–50.

[3] Savitz, J.B., Nugent, A.C., Bogers, W., Roiser, J.P., Bain, E.E., Neumeister, A., Zarate, C.A. Jr, Manji, H.K., Cannon, D.M., Marrett, S., Henn, F., Charney, D.S., Drevets, W.C., 2011. Habenula volume in bipolar disorder and major depressive disorder: a high-resolution magnetic resonance imaging study. Biol Psychiatry 69, 336–343.

P.4.024 Using positron emission tomography to investigate microglial activation in alcohol dependence: preliminary findings

N.J. Kalk[1]*, Q. Guo[2], R. Cherian[3], D. Erritzoe[4], A. Waldman[5], K. Dar[3], R.N. Gunn[2], D.J. Nutt[1], E.A. Rabiner[2], A.R. Lingford-Hughes[1]. *[1]Imperial College London, Centre for Neuropsychopharmacology, London, United Kingdom; [2]Imanova Limited, Imaging, London, United Kingdom; [3]Central North West London NHS Foundation Trust, Addiction Psychiatry, London, United Kingdom; [4]Imperial College, Centre for Neuropsychopharmacology, London, United Kingdom; [5]Imperial College, Medicine, London, United Kingdom*

There is pre-clinical evidence for microglial activation in alcohol dependence and withdrawal which may relate to alcohol-induced cognitive impairment [1]. An increase in microglia was found in human brain tissue from alcoholics [2]. Microglial activation can be measured in humans in vivo using Positron Emission Tomography (PET) tracers that bind to the 18kDa Translocator Protein (TSPO) which has relatively low constitutive expression in the brain but is richly expressed in activated microglia. We are investigating whether there is microglial activation in

male human alcoholics, shortly after detoxification, using [C-11]PBR28 PET in a group comparison study.

13 healthy controls and 7 alcoholics were recruited. [11-C]PBR28's affinity for the TSPO varies according to the presence of a single nucleotide polymorphism on one or both alleles (3). Therefore, participants were genotyped prior to scanning to facilitate interpretation of PET data. Dynamic PET data were acquired for 90 minutes following bolus injection of 331.9±15.27 MBq of [11-C]PBR28. Arterial plasma activity was measured and corrected for the presence of metabolites.

PET data were co-registered to the participant's structural MRI scan which facilitated the application of an in-house anatomical atlas via spatial normalisation to define regions of interest (ROIs): cortical grey matter, the anterior cingulate cortex, hippocampus, thalamus and midbrain. ROIs were applied to the dynamic PET data to generate time activity curves. A two tissue compartmental model with a fixed blood volume (5%) was used to estimate regional volumes of distribution (V_T). Two-way ANCOVAs were performed to examine the effect of genotype and disease status (alcohol dependent vs healthy) in each ROI. Age was included as a co-variate, as TSPO binding is known to increase with age.

Data from 13 healthy control participants and 5 patients are reported. The average age of the alcoholic group was 49±10 (n = 5) and the healthy group was 54±10 (n = 13). The average time from detoxification in the patient group was 25±9 days. Four healthy controls, and four alcoholics, were dependent on nicotine. Two alcoholics reported a history of cocaine use, but none met the criteria for dependence on cocaine or other illicit drugs.

Mean cortical V_T in the healthy control group was 5.0±1.35 vs. 4.7±2.0 in the alcoholic group (CC genotype), and 3.31±0.79 in the healthy group vs 3.3±0.36 in the alcoholic group (CT genotype). The ANCOVA found a significant effect of age across all regions studied (cortical grey matter $F = 7.331$, $p = 0.018$; anterior cingulate cortex $F = 5.297$, $p = 0.040$; thalamus $F = 5.972$ $p = 0.030$, hippocampus $F = 6.151$ $p = 0.028$; midbrain $F = 5.811$, $p = 0.031$) and an effect of genotype approaching or reaching significance in the cortex and thalamus (cortical grey matter $F = 3.617$, $p = 0.080$; thalamus $F = 5.740$, $p = 0.032$) findings which replicate previous work. No significant effect of group was found, but this may reflect insufficient power. Further recruitment is ongoing.

Reference(s)

[1] Obernier, J.A., White, A.M., Swartzwelder, H.S., Crews, F.T., 2009. Cognitive deficits and CNS damage after a 4-day binge ethanol exposure in rats. Pharmacol Biochem Behav 72(3): 521–32.

[2] He, J., Crews, F.T., 2008. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Exp Neurol 210(2): 349–58.

[3] Owen, D.R., Yeo, A.J., Gunn, R.N., Song, K., Wadsworth, G., Lewis, A., Rhodes, C., Pulford, D.J., Bennacef, I., Parker, C.A., StJean, P.L., Cardon, L.R., Mooser, V.E., Matthews, P.M., Rabiner, E.A., Rubio, J.P., 2012. An 18-kDa translocator protein (TSPO) polymorphism explains differences in binding affinity of the PET radioligand PBR28. J Cereb Blood Flow Metab 32(1): 1–5.

P.4.025 Effects of deep brain stimulation of prelimbic and infralimbic areas of the prefrontal cortex of the rat

L. Jimenez-Sanchez[1]*, L. Pérez-Caballero[2], A. Castañé[1], X. López-Gil[1], L. Campa[1], M. Galofré[1], E. Berrocoso[3], A. Adell[1]. *[1]IIBB-CSIC-CIBERSAM, Department of Neurochemistry and Neuropharmacology, Barcelona, Spain; [2]University of Cadiz-CIBERSAM, Neuropsychopharmacology Research Group Department of Neuroscience (Pharmacology and Psychiatry), Cadiz, Spain; [3]University of Cadiz-CIBERSAM, Neuropsychopharmacology Research Group Psychobiology Area Department of Psychology, Cadiz, Spain*

Neuroimaging studies have shown a hyperactivity of subgenual cingulate region (Cg25) in major depression [1]. Further, normalisation of this activity has been associated with effective clinical response to a variety of antidepressant treatments. Deep brain stimulation (DBS) also reduced the hyperactivity of Cg25, and has proved efficacious for treatment-resistant depression patients [1]. In spite of its therapeutic action, little is known about the precise mechanism of DBS. In the present work, we have used in vivo microdialysis to examine the effects of 1-hour DBS (130 Hz frequency, 200 μA amplitude and 90 μs pulse width) applied to prelimbic (PrL) or infralimbic (IL) areas of the prefrontal cortex (PFC) on the release of serotonin (5-HT), dopamine (DA), noradrenaline (NA) and glutamate (Glu) in the entire PFC of male Wistar rats. Neuronal activation throughout the brain after PrL or IL DBS was also examined by c-Fos immunohistochemistry. The antidepressant-like effect of DBS was measured using the forced swim test (FST).

The results show that DBS of the IL region exerts an antidepressant-like action in the FST (measured by a decrease in immobility and an increase in swimming and climbing) that is associated with increased releases of 5-HT, DA, NA and Glu in the PFC. In addition, the effects

of IL DBS on cortical glutamate (but not on 5-HT), as well as its antidepressant effect in the FST, are dependent upon the activation of AMPA receptors, inasmuch as they were prevented by the systemic administration of the AMPA receptor antagonist, NBQX. However, DBS of the PrL displayed a depressogenic-like action in the FST measured as increased immobility and decreased swimming and climbing. Further, PrL DBS reduced the release of DA, NA and Glu in the PFC. On the other hand, important differences in the immunolabelling of c-Fos were observed after 1-h DBS of PrL or IL regions. Thus, after IL DBS, c-Fos expression was higher in the basolateral nucleus of the amygdala, and mediodorsal and centromedial thalamic nuclei, and lower in the habenula and shell of the nucleus accumbens compared with DBS PrL.

To determine whether the actions of IL DBS were due to a local effect in the IL subdivision of the PFC or to the connections between IL and subcortical areas of the brain, a cortical transection was performed. The results showed that IL DBS potentiated the antidepressant-like effects of the cortical transection in the FST, thus suggesting that a local action of DBS is responsible of its rapid antidepressant-like effect.

We conclude that the early antidepressant-like action of DBS of the IL region of the PFC would depend on the activation of AMPA receptor-dependent glutamatergic transmission. The increase of monoaminergic transmission in the PFC and the activity of subcortical projection areas would play an important role in the long-term antidepressant-like effect of IL DBS.

Reference(s)

[1] Mayberg, H.S., Lozano, A.M., Voon, V., McNeely, H.E., Seminowicz, D., Hamani, C., Schwalb, J.M., Kennedy, S.H., 2005. Deep brain stimulation for treatment-resistant depression. Neuron 45, 651–660.

P.4.027 Six-month follow-up study of repetitive transcranial magnetic stimulation in the treatment of resistant major depressive disorder

C. Concerto[1]*, D. Cannavò[1], F. Magnano SanLio[1], R. Ricceri[2], G. Lanza[2], E. Aguglia[1]. *[1]University of Catania, A.O.U. Policlinico-Vittorio Emanuele U.O.P.I. of Psychiatry, Catania, Italy; [2]University of Catania, A.O.U. Policlinico-Vittorio Emanuele Department of Neuroscience, Catania, Italy*

Purpose of the study: Over the last years Transcranial Magnetic Stimulation (TMS) has been investigated to study motor pathways and motor cortical excitability in healthy subjects and in patients with neurological disorders. Several clinical studies have been focussed on the efficacy of Repetitive Transcranial Magnetic Stimulation (rTMS) in the treatment of Resistant Major Depression which is also the psychiatric field where rTMS has received more indications and approvals worldwide. In the treatment of pharmaco-resistant major depression, interventions strategy include combination of antidepressant drugs with different mechanisms of action and augmentation of somatic treatments to pharmacological therapy. In this regard, the use of rTMS as add-on treatment truly represents one of the major foci in clinical research.

Objectives: Object of this study was to evaluate the long-term effect on depressive symptoms and frontal lobe abilities of rTMS as add-on therapy in the treatment of drugs resistant major depression [1].

Methods: A sample of fifteen drug resistant depressed outpatients meeting the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision criteria for nonpsychotic major depressive disorder were assigned to receive fifteen sessions of high frequency rTMS stimulation of the left dorsolateral prefrontal cortex. Active rTMS was performed five days per week for four weeks consecutively. Each session of rTMS was delivered as follows: 10 Hz, four-second-train duration, a 26-second inter-train interval, for a total of 3000 pulses per session lasting 37.5 minutes. The intensity of the magnetic stimulus was set at 120% of the resting motor threshold. During the course of rTMS, the patients were taking their medication which included Selective Serotonin Reuptake Inhibitors (SSRI), Tricyclic Antidepressant and Atypical Antipsychotic Drugs. Their doses were required to remain stable in the four weeks preceding the trial and for its entire duration. Patients were followed up for six months to determine the long-term effect of rTMS as add-on therapy. Outcome measures for the evaluation of depressive symptoms consisted of Hamilton Depression Rating Scale 21-item (HAM-D) and the Montgomery Asberg Depression Rating Scale (MADRS). A neuropsychological battery for the evaluation of different frontal lobe abilities included the Frontal Assessment Battery and the Stroop Color Word Test interference. Effectiveness data was gathered at baseline (T0), at the end of treatment (T1) and 6 months after the end of treatment (T2) [2].

Results: Results from the present study showed statistically significant mood improvements as indexed by a reduction of more than 40% on the Hamilton Rating Scale for Depression and on Montgomery Asberg Depression Rating Scale at the end of treatment and six months after treatment. Frontal Assessment Battery and the Stroop Color Word Test interference outcome scores

didn't significantly differ at treatment end and after six months.

Conclusion: Our findings demonstrate the antidepressant effect of adjunctive Repetitive Transcranial Magnetic Stimulation treatment. Moreover this study confirms good tolerability of rTMS and the maintenance of the antidepressant effect six months after the treatment, as shown by a decrease in HAM-D and MADRS [3].

Reference(s)

[1] Sackeim, H.A., 2001. The definition and meaning of treatment-resistant depression. J Clin Psychiatry 62, 10–17.
[2] Giupponi, G., Pycha, R., Dell'Osso, B., Pompili, M., Walpoth, M., Hausmann, A., Di Pauli, J., Erfurth, A., Conca, A., 2009. Neurophysiological and neuropsychiatric aspects of transcranial magnetic stimulation. Clinical Neuropsychiatry 6, 234–235.
[3] Avery, D.H., Holtzheimer, P.E., Fawaz, W., Russo, J., Neumaier, J., Dunner, D.L., Haynor, D.R., Claypoole, K.H., Wajdik, C., Roy-Byrne, P., 2010. A controlled study of repetitive transcranial magnetic stimulation in medication-resistant major depression. Biol Psychiatry 59, 187–194.

P.4.028 Neural correlates of stress-related hypothalamic–pituitary–adrenal axis activity in neuroticism

A. Boehringer[1]*, F. Lederbogen[1], H. Tost[1], L. Haddad[1], A. Meyer-Lindenberg[1]. [1]*Central Institute of Mental Health, Systems Neuroscience in Psychiatry, Mannheim, Germany*

Neuroticism, the stable tendency to react with negative emotional responses to threat, frustration, or loss, is associated with blunted hypothalamic–pituitary–adrenal (HPA) axis responses to stress [1]. Dysfunctional interactions between the perigenual anterior cingulate cortex (pACC) and the amygdala have been identified as a neural correlate of neuroticism related personality traits [2]. The amygdala is implicated in HPA axis activation and the pACC inhibits amygdala function. Changes in pACC–amygdala brain circuitry may therefore account for the relationship between neuroticism and HPA axis dysfunction. Here, we tested this hypothesis in a sample of healthy individuals using an established social stress paradigm and functional magnetic resonance imaging (fMRI).

Thirty-two (16 males, mean±SD 43.6±14.4 years) healthy participants were exposed to a psychosocial stress task (Montreal Imaging Stress Task, MIST) while their brains were scanned using fMRI. During this task, participants were asked to solve mental arithmetic task under time pressure and negative social evaluative feedback from the study investigators. HPA axis responses (as assessed by salivary cortisol) as well as cardiovascular and subjective stress responses to the MIST were measured repeatedly. At the neural level, effects of stress on brain activity as well as functional connectivity between the pACC and the amygdala were assessed.

At the group level, the MIST induced significant increases in HPA axis and cardiovascular activity, as well as subjective feelings of stress (all $P < 0.001$). None of these stress measures was significantly associated with neuroticism. In line with previous work, variability in the endocrine stress response was high, allowing to split the study group into participants who reacted with a substantial increase in cortisol (cortisol responders, $n = 18$) and others who did not respond (cortisol non-responders, $n = 13$) using a cut-off of 2.5 nmol/l increase in cortisol. Cortisol responders to the stressor showed lower stress related activity in the perigenual anterior cingulate cortex (pACC) than cortisol non-responders, suggesting a role of the pACC in HPA axis inhibition ($P < 0.05$, family-wise error corrected (FEW) for pACC and amygdala volume). Deactivation of the pACC within the cortisol responder group was less pronounced in individuals high in neuroticism ($P < 0.05$, FEW for pACC and amygdala volume), indicating that high neuroticism was associated with less release of inhibitory tone of the pACC on the HPA axis. Moreover, rostral pACC–amygdala functional connectivity was higher in high neuroticism cortisol responders ($p < 0.05$ FEW corrected for amygdala volume), suggesting that changes in pACC–amygdala brain circuitry may contribute to altered HPA axis control in individuals high in neuroticism.

Here, we identify a neural correlate of altered HPA axis control in cortisol responders high in neuroticism. The presented data provide further evidence for a specific role of pACC in the neural processing of social stress and propose a neural mechanism by which altered pACC–amygdala functional interaction translates into an increase in inhibitory control of the HPA axis activity in individuals with maladaptive emotional personality characteristics. These findings may improve our understanding of the well-established association between neuroticism, stress, and various mental diseases [3].

Reference(s)

[1] Oswald et al., Neuropsychopharmacology 2006; 31: 1583–91.
[2] Pezawas et al., Nat Neurosci 2005; 8: 828–34.
[3] Lahey American Psychologist 2009; 64: 241–56.

P.4.029 Antioxidant status and fatty acids in adolescents with Asperger syndrome and first episodes of early-onset psychosis

M.G. Moron-Nozaleda[1]*, M. Álvarez-Blázquez[1], C.M. Díaz-Caneja[1], E. Dulin[2], C. Moreno[1], M.C. Guisasola[3], C. Arango[1], M. Parellada[1]. *[1]Hospital General Universitario Gregorio Marañón, Child and Adolescent Psychiatry Department, Madrid, Spain; [2]Hospital General Universitario Gregorio Marañón, Biochemistry Department, Madrid, Spain; [3]Hospital General Universitario Gregorio Marañón, Experimental Medical and Surgery Unit, Madrid, Spain*

Purpose of the study: Evidence suggests that oxidative stress-mediated cell membrane pathology and impairment of lipid metabolism may be involved in the physiopathology of autism spectrum disorders and schizophrenia [1,2]. Several studies have reported significantly reduced levels of polyunsaturated fatty acids (PUFAs) in plasma and red blood cell membranes from patients with schizophrenia and autism [3]. The aim of this study was to explore the differences in oxidative status and plasma PUFAs as indicators of cell membrane integrity in two groups of adolescents, one with Asperger syndrome (AS) and another with a first episode of early-onset psychosis, and compare them with healthy controls.

Methods: Twenty-four adolescents with AS (mean age 12.7±2.5 years, 95.8% male), 24 with a first episode of psychosis (mean age 15.9±1.2 years, 66.7% male) and 23 controls (mean age 13.1±3 years, 87% male) participated in the study. Inclusion criteria: age 7–17 years; diagnosis of first episode early onset psychosis or AS. Exclusion criteria: mental retardation, neurological disorders, history of head trauma and pregnancy. Fasting venous blood samples were collected into EDTA evacuated tubes. After immediate centrifugation, plasma and whole blood aliquots were transferred into cryogenic tubes and stored frozen at −80ºC. PUFAs were analysed in plasma using a gas chromatograph. Plasma Total Antioxidant Status (TAOS) was measured with a TAOS Assay Kit. For the statistical analysis, because a number of measured variables did not come for normal distribution, they were analysed using non-parametric tests (Kruskal–Wallis and Mann–Whitney).

Summary of results: PUFA composition in plasma and TAOS levels are shown in Table 1. The plasma TAOS was statistically significantly lower in the AS group compared with the healthy controls and with the psychosis patients. No differences were found in total PUFAs or any PUFA individually, except for eicosapentaenoic acid in psychosis. However, significant differences were found in total phospholipids both in AS and psychosis as compared with the controls. The AA/DHA ratio (arachidonic acid n = 6/docosahexaenoic acid n = 3) was significantly higher in AS.

Conclusion: Reduced antioxidant defence, pro-oxidant imbalance and abnormal cell membrane composition are found in AS. Abnormal lipid composition may also contribute to the pathophysiology of psychosis. Further research will assess the role of drugs that act at the oxidative pathway in these disorders.

Table 1. PUFA composition in plasma and TAOS levels

	Asperger	Psychosis	Control	p			
				Between groups	Asperger vs Control	Asperger vs Psychosis	Psychosis vs Control
Total PUFAs	519.28±182.9	559.05±206.43	490.57±206.21	0.183	0.088	0.584	0.158
Total PL	0.56±0.20	0.68±0.48	0.39±0.39	0.036	0.041	0.184	0.030
AA/EPA	0.98±1.58	0.79±0.71	0.57±0.36	0.591	0.596	0.389	0.399
AA/DHA	5.6±2.16	4.68±2.47	3.86±1.12	0.016	0.004	0.162	0.184
AA	4.94±1.05	4.58±1.35	4.42±1	0.484	0.259	0.360	0.853
EPA	7.48±1.93	6.89±2.05	8.72±2.27	0.109	0.239	0.340	0.038
DHA	1.03±0.53	1.32±1.00	1.22±0.47	0.156	0.099	0.097	0.797
TAOS	1.13±0.21	1.28±0.28	1.26±0.25	0.017	0.043	0.007	0.312

Results are shown as mean±SD. PL: Phospholipids; AA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

Reference(s)

[1] Parellada M, et al., Plasma antioxidant capacity is reduced in Asperger syndrome, Journal of Psychiatric Research (2011), doi: 10.1016/j.jpsychires.2011.10.004.

[2] Akyol O, Herken H, Uz E, Fadillioglu E, Unal S, Sogut S, et al. The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients. The possible role of oxidant/antioxidant imbalance. Progress in Neuropsychopharmacology & Biological Psychiatry 2002; 26: 995e1005.

[3] Ming X, Stein TP, Brimacombe M, Johnson WG, Lambert GH, Wagner GC. Increased excretion of a lipid peroxidation biomarker in autism. Prostaglandins, leukotrienes and essential fatty acids 2005; 73: 379e84.

P.4.030 Bright light dose correlates with change in striatal serotonin transporter binding in healthy Scandinavians

B. Mc Mahon[1]*, A.S. Andersen[1], L. Feng[1], K.K. Holst[1], M.K. Madsen[1], S. Lehel[2], M.M. Herth[2], P. Iversen[3], L. Hasholt[4], G.M. Knudsen[1]. [1]*Copenhagen University Hospital Rigshospitalet, Neurobiology Research Unit and Center for Integrated Molecular Brain Imaging, Copenhagen, Denmark;* [2]*Copenhagen University Hospital Rigshospitalet, PET and Cyclotron Unit, Copenhagen, Denmark;* [3]*Copenhagen University Hospital Hvidovre Hospital, Center for Integrated Molecular Brain Imaging and Danish Research Centre for MR, Copenhagen, Denmark;* [4]*Copenhagen University, Department of cellular and Molecular Medicine, Copenhagen, Denmark*

Background: Lack of daylight is prominent at high latitudes and this may be perceived as an environmental stressor associated with a high frequency of seasonal affective disorder that can be treated with bright light therapy. We and others have in cross-sectional studies observed season dependent fluctuations in the serotonin transporter (SERT), with high striatal binding around winter solstice and low binding around summer solstice. In addition, we have identified a genotype-dependent interaction with these environmental stressors with SERT binding being dependent on the carrier status of the 5-HTTLPR promoter polymorphism [1]. This gene*environment paradigm predicts the SERT fluctuations with a negative correlation between SERT binding and daylight minutes in carriers of the short 5-HTTLPR allele (S-allele), but less so in homozygote carriers of the long allele (L-allele). The aim of the present study was to examine the correlation between the intensity of bright light and change in striatal binding potential (BP_{ND}) and if any such change was depending on 5-HTTLPR carrier status. We hypothesize that there is a negative correlation between change in striatal SERT BP_{ND} and lux dose and that this correlation is more prominent in carriers of the S-allele.

Method: Seventy healthy males were pre-screened for inclusion and based on their 5-HTTLPR triallelic carrier status they were invited for participation. Of these, 24 healthy male volunteers (mean age: 24±4.5, range 18–36 years and mean minutes of daylight at baseline: 449±25, range 421–504 min) participated in the study. Exclusion criteria were significant medical history, known retinal pathology and the use of photosensitising medications. Eight L_a-homozygotes and 16 S-allele carriers was exposed to a bright light intervention at varying degrees (mean intensity: 4112±2851, range 131–10.971 lux) for three weeks. Post intervention all subjects received a visit where the distance and angle to the light source was measured. In addition, a semi-structured interview was conducted to assess compliance, subjective effects and daily outdoor activities. The intensity of all biolamps was individually determined with respect to distance and angle with an Elma 1335 luxmeter. The intensity and spectrum of the biolamps will be further characterised by the Department of Photonics Engineering DTU and the data are considered preliminary.

Results: Participants were investigated with MRI and [^{11}C]DASB on an HRRT PET scanner (mean inj. dose: 589±29 MBq, range 421–609) before and after intervention. Quantification was done with MRTM2. A significant positive correlation was found in striatum (slope 2.501e-005 ±1.027e-005, R square: 0.2203) for all subjects. When subdividing subjects with respect to 5-HTTLPR genotype status we saw a tendency for a positive correlation in the S-allele carrier group (slope: 2.328e-005 ±1.224e-005, R square: 0.21) but not in the L-allele homozygote group (slope: 3.221e-005 ±2.296e-005, R square: 0.28).

Conclusion: Contrary to the summer down regulation of SERT, we found that a smaller amount of bright light up regulated the SERT suggesting that the relationship between bright light dose and SERT change might not be explained by a linear model.

Reference(s)

[1] Kalbitzer, J., Erritzoe, D., Holst, K.K., Nielsen, F.A., Marner, L., Lehel, S., Arentzen, T., Jernigan, T.L., Knudsen, G.M., 2010. Seasonal changes in brain serotonin transporter binding in short 5-HTTLPR-allele carriers but not in long-allele homozygotes. Biol Psychiatry 67, 1033–9.

P.4.031 Effects of mental distress on cognitive functioning in patients admitted for cardiac rehabilitation after acute coronary events

J. Burkauskas[1]*, J. Brozaitiene[1], R. Bunevicius[1]. [1]*Lithuanian University of Health Sciences, Institute of Behavioral Medicine, Palanga, Lithuania*

Introduction: Impaired cognition increases mortality in CAD patients, even in cases of mild impairment [1]. Thus, it is important to investigate and determine factors associated with cognitive functioning in this particular population. Our recent findings in patients indicate that patient-oriented outcomes including health related quality of life [2] and fatigue [3] are associated with mental

distress factors such as depression and anxiety. The question of how mental distress might be specifically associated with cognitive functioning received less of attention. The aim of our study was to investigate an association of cognitive functioning with mental distress in CAD patients.

Methods: This study was conducted over a 3-year period examining CAD patients two weeks after acute myocardial infarction or unstable angina attending cardiac rehabilitation programme. 539 patients participated in the study; 386 (72%) men and 153 (28%) women with a mean age of 58 years (SD = 9). Participants were evaluated for heart functional class, following the guidelines provided by the New York Heart Association. Mini Mental State Examination (MMSE) was used to asess global cognitive functioning. Digit Span Test and Digit Symbol Test were used to assess auditory attention, mental flexibility, psychomotor performance and incidental learning. Trail Making Test A and B was used to measure perceptual speed, task switching and executive control. Mental distress factors were evaluated using the Beck Depression Inventory-II (BDI-II) to measure depressive symptoms, the State-Trait Anxiety Inventory (STAI) to measure state anxiety (STAI-S) and trait anxiety (STAI-T), and Type-D Scale (DS-14) to measure Type-D personality characteristics.

Results: In univariate regression analysis almost all mental distress, socio-demographic and clinical characteristics were significantly associated with different cognitive functions. After adjusting for gender, age, education, New York Heart Association functional class, MMSE scores remained associated with DS-14 ($\beta = -0.144$, $p < 0.01$); Digit Span Test Backward recall of digits, with STAI-S ($\beta = -0.120$, $p < 0.01$). Digit symbol test raw scores were associated with BDI-II ($\beta = -0.115$, $p < 0.01$), as well as with STAI-S ($\beta = -0.084$, $p = 0.03$). Similarly, time which individuals took to complete the Digit Symbol Test was associated with BDI-II ($\beta = 0.145$, $p < 0.01$), and STAI-S ($\beta = 0.110$, $p < 0.01$). Significant associations remained between Trail Making Test A scores and STAI-S scores ($\beta = 0.113$, $p < 0.01$) as well as between Trail Making Test B scores and STAI-S scores ($\beta = 0.103$, $p = 0.04$), while Trail Making Test B-A scores remained associated with STAI-T scores ($\beta = -0.119$, $p < 0.03$).

Conclusions: In non-demented CAD patients two weeks after acute cardiac events cognitive functioning is associated with mental distress independently from cardiac functional class, gender, age and education. Specifically, Type-D personality worsens global cognitive functioning. The presence of symptoms of depression and higher levels of situational anxiety negatively affects test completion time and psychomotor performance. Higher levels of situational anxiety also results in decrease of perceptual speed, mental flexibility and task switching while higher levels of trait anxiety improves executive control.

Reference(s)

[1] Shavelle, R., Paculdo, D., Strauss, D., Kush S., 2009 Cognitive Impairment and Mortality in the Cardiovascular Health Study. J Insur Med. 41(2), 110–116.

[2] Staniute, M.J., Brozaitiene, J., Bunevicius, R., 2011 Effects of Social Support and Stressful Life Events on Health-Related Quality of Life in Coronary Artery Disease Patients. J Cardiovasc Nurs. [Epub ahead of print].

[3] Bunevicius, A., Stankus, A., Brozaitiene, J., Girdler, S.S., Bunevicius, R., 2012 Relationship of fatigue and exercise capacity with emotional and physical state in patients with coronary artery disease admitted for rehabilitation program. Am Heart J. 162(2), 310–316.

P.4.032 Neuroinflammation in temporal cortex in schizophrenia patients

T.F. van der Doef[1]*, M. Yaqub[2], M.G. Bossong[1], R. Boellaard[2], A.D. Windhorst[2], A.A. Lammertsma[2], R.S. Kahn[1], B.N.M. van Berckel[2]. *[1]University Medical Center Utrecht, Psychiatry, Utrecht, The Netherlands; [2]VU University Medical Center, Nuclear Medicine and PET Research, Amsterdam, The Netherlands*

Background: There is increasing evidence that neuroinflammation is associated with schizophrenia. Neuroinflammation is characterised by the activation of microglial cells. Increased expression of the translocator protein is a biomarker for microglial activation and can be measured in vivo using the positron emission tomography ligand (R)-[^{11}C]PK11195. The purpose of this study was to compare the regional distribution of (R)-[^{11}C]PK11195 binding in patients with schizophrenia to that in healthy controls.

Methods: (R)-[^{11}C]PK11195 binding potential (BP_{ND}) was assessed in ten patients with schizophrenia and ten age-matched healthy controls. Psychopathology was measured using the Positive and Negative Syndrome Scale (PANSS). All patients received atypical antipsychotic drugs. Dynamic (R)-[^{11}C]PK11195 scans were acquired using an ECAT EXACT HR+ scanner.

PET data were analysed using receptor parametric mapping (RPM), a basis function implementation of the simplified reference tissue model (SRTM). Supervised cluster analysis (SCVA) algorithm was used to extract the reference tissue input function from the dynamic PET scans [1,2]. We used a supervised cluster method with

four kinetic classes (SCVA4), which is a revised version of the supervised cluster analysis approach of Turkheimer et al. [1,2]. The outcome measure was (R)-[^{11}C]PK11195 binding potential.

Subsequently, grey matter regions of interest (ROIs) were delineated on a T1-weighted structural MRI scan using an automatic procedure, resulting in the following regions: frontal, temporal, parietal and occipital cortex, and cerebellum. Regional values of BP_{ND} were obtained by projecting the ROIs onto the parametric (R)-[^{11}C]PK11195 BP_{ND} images.

Multivariate analysis of variance (MANOVA) was used to test for differences in binding potential between patients and controls with group as the between-subjects factor and region of interest as the within-subjects factor.

Results: MANOVA showed an overall significant effect of group ($F(5) = 3.7$, $p = 0.03$). Schizophrenia patients showed increased (R)-[^{11}C]PK11195 binding potential in the temporal cortex ($F(1) = 6.1$, $p = 0.02$) (Table 1). There were no significant differences in mean (R)-[^{11}C]PK11195 binding potential in the other areas tested (Table 1). Patients with schizophrenia had minimal to moderate symptoms of the disease at the time of PET scanning (PANSS total score = 52.5±9.5).

Table 1. Regional (R)-[^{11}C]PK11195 BP_{ND} values

Region	Mean BP_{ND} ±SD		General Linear Model	
	Controls	Schizophrenia	F	p
Frontal	0.11±0.09	0.10±0.06	0.05	0.82
Temporal	0.03±0.08	0.11±0.08	6.06	0.02*
Parietal	0.15±0.11	0.13±0.06	0.20	0.66
Occipital	0.33±0.13	0.35±0.14	0.10	0.75
Cerebellum	0.16±0.08	0.15±0.13	0.06	0.82

*$p \leqslant 0.05$.

Conclusions: An increase in (R)-[^{11}C]PK11195 binding potential was found in the temporal cortex in schizophrenia patients, suggesting focal microglial activation. This in turn provides preliminary evidence for neuroinflammation in the temporal cortex in schizophrenia during the first years of the illness. This may provide an explanation for progressive tissue loss in this area in schizophrenia. Further studies are warranted to assess anti-inflammatory treatment in this disease.

Reference(s)

[1] Turkheimer, F.E., Edison, P., Pavese, N., Roncaroli, F., Anderson, A.N., Hammers, A., Gerhard, A., Hinz, R., Tai, Y.F. and Brooks, D.J., 2007. Reference and target region modeling of [11C]-(R)-PK11195 brain studies. J Nucl Med. 48, 158–167.

[2] Yaqub, M., van Berckel, B.N., Schuitemaker, A., Hinz, R., Turkheimer, F.E., Tomasi, G., Lammertsma, A.A. and Boellaard, R., 2012. Optimization of supervised cluster analysis for extracting reference tissue input curves in (R)-[(11)C]PK11195 brain PET studies. J Cereb Blood Flow Metab. 32, 1600–1608.

P.4.033 Cerebrospinal fluid biomarkers of brain injury in bipolar disorder

J. Jakobsson[1]*, E. Pålsson[1], C.J. Ekman[2], C. Sellgren[3], A.G.M. Johansson[2], H. Zetterberg[1], K. Blennow[1], M. Landén[1]. *[1]The Sahlgrenska Academy University of Gothenburg, Department of Psychiatry and Neurochemistry, Göteborg, Sweden; [2]Karolinska Institutet, Department of Clinical Neuroscience, Stockholm, Sweden; [3]Karolinska Institutet, Department of Medical Epidemiology and Biostatistics, Stockholm, Sweden*

Purpose of the study: Bipolar disorder is a common psychiatric disorder characterised by recurrent episodes of mania/hypomania and depression. Structural imaging studies suggest that bipolar disorder is associated with morphological abnormalities of the brain. The most recurrent finding is that patients with bipolar disorder have lateral ventricular enlargement and increased rates of deep white matter hyperintensities [1]. Furthermore, several studies suggest that structural brain changes are associated with a decline in cognitive function. We previously investigated a neurodegenerative component of bipolar disorder by analysing several cerebrospinal fluid (CSF) biomarkers for neurodegenerative processes in bipolar patients and healthy controls [2]. No major alterations were observed between patients and controls thus excluding neurodegenerative processes related to these biomarkers (T-tau, P-tau, and amyloid beta). There are, however, other established CSF biomarkers reflecting injuries in different cells/structures in brain. These biomarkers include: neurofilament light chain (NF-L), Heart-type fatty acid binding protein (H-FABP), calcium binding protein S100B, and myelin basic protein (MBP) [3]. In this study the relation between CSF biomarkers of brain injuries and bipolar disorder were investigated. The aim was to test the suggested neuroprogressive component in bipolar disorder and to investigate possible associations between CSF biomarkers and disease severity, ongoing medications, and/or cognitive functions.

Methods: 137 patients were recruited from the St. Göran bipolar project, enrolling patients from the bipolar unit at the Northern Stockholm Psychiatric Clinic, Stockholm, Sweden. 86 age and gender matched healthy, population-based controls were randomly selected by Statistics Sweden (SCB). Cerebrospinal fluid sampling

(lumbar puncture) was performed when the participants were in a stable euthymic mood. The cerebrospinal fluid concentrations of the different biomarkers were measured using ELISA. Linear regression (using age and gender as covariates) was used to analyse patient–control differences in CSF biomarker concentrations. P-values less than 0.05 were regarded as significant (two-tailed).

Results: The CSF concentrations of NF-L and MBP were higher in patients when compared with healthy controls (NF-L: $b = 0.166$, $t = 3.578$, $df = 1$, $p < 0.001$; MBP: $b = 0.102$, $t = 12.023$, $df = 1$, $p = 0.044$). There were no differences in the concentration of S100B and h-FABP between patients and controls (S100B: $b = 0.046$, $t = 0.742$, $df = 1$, $p = 0.459$; h-FABP: $b = 0.095$, $t = 1.584$, $df = 1$, $p = 0.115$). We also observed several associations between CSF biomarkers and medications at the time of CSF sampling and performances at neuropsychological tests.

Conclusions: We conclude that the large subject population in the current study is suitable for finding potential biomarkers for bipolar disorder as well as identifying different subgroups of bipolar disorder. Thus, the findings in this study have the potential of increasing the understanding of neuroprogression in bipolar disorder as well as giving indication of the biological effects of different medications. Future studies will give emphasis to the identification of associations between cerebrospinal fluid biomarker concentrations and alterations in brain structures, genetic variations, and cognitive functions.

Reference(s)

[1] Kempton MJ, Geddes JR, Ettinger U, Williams SC, Grasby PM. Meta-analysis, database, and meta-regression of 98 structural imaging studies in bipolar disorder. Archives of general psychiatry. 2008;65(9):1017–32. Epub 2008/09/03.

[2] Jakobsson J, Zetterberg H, Blennow K, Ekman C, Johansson AGM, Landén M. Altered concentrations of amyloid precursor protein metabolites in the cerebrospinal fluid of patients with bipolar disorder. Neuropsychopharmacology. 2012;In press (accepted October 2012).

[3] Olsson B, Zetterberg H, Hampel H, Blennow K. Biomarker-based dissection of neurodegenerative diseases. Prog Neurobiol. 2011. Epub 2011/04/29.

P.4.034 Glucose abnormalities in newly diagnosed, medication-naïve patients with bipolar disorder, mania, and psychosis

C. Garcia-Rizo[1]*, B. Kirkpatrick[2], E. Fernandez-Egea[3], C. Oliveira[4], I. Grande[4], J. Undurraga[4], E. Vieta[4], M. Bernardo[4]. *[1]Hospital Clinic of Barcelona, Dept. of Psychiatry, Barcelona, Spain; [2]College of Medicine and Scott & White Healthcare, Dept. of Psychiatry, Texas, USA; [3]Cambridgeshire and Peterborough NHS Foundation Trust, Department of Psychiatry, Cambridge, United Kingdom; [4]Hospital Clinic of Barcelona, Department of Psychiatry, Barcelona, Spain*

Severe mental disorders, such as schizophrenia and major depression have been associated with increased mortality, which is due in part to an increased risk of diabetes and cardiovascular disease. Some evidence suggests that patients with schizophrenia and major depression have an increased risk of these disorders independently of medication and other confounding factors. The association of these problems with bipolar disorders has received less attention. The prevalence of Type 2 diabetes mellitus (T2DM) in bipolar disorders ranges from 8% to 17% [1], a threefold increase compared to the general population. Bipolar patients with co-morbid T2DM may have a more severe course of the psychiatric illness and refractoriness to treatment. However pharmacological treatment, including both antipsychotic agents and mood stabilisers, may confound this relationship.

We test the hypothesis that abnormal glucose tolerance is present in patients with bipolar disorder at the onset of the disease. Newly diagnosed drug-naïve patients with DSM-IV bipolar I disorder were recruited at the time of their first clinical contact for psychotic symptom and underwent a two-hour glucose tolerance test. We recruited a control group that was matched to these patients with respect to age, gender, ethnicity, body mass index (BMI), smoking habit (average number of cigarettes per day), cortisol values and socioeconomic status of the family of origin. The two matched groups were compared using the non-paired Student's t-test, or the χ^2 test for comparisons of proportions. Smoking and BMI were log-transformed in order to approximate a normal distribution. Baseline glucose values were compared with the Mann Whitney U, due to a skewed distribution.

Fasting measures of glucose metabolism were very similar for the two groups. Baseline glucose concentrations (mg/dL[SD]) were 92.6[17.4] for the bipolar group and 85.5[6.5] for the control group ($p = 0.275$). Baseline insulin concentrations (mU/L[SD]) were 8.9[4.0] for the bipolar group and 10.8[5.9] for the control group ($p = 0.295$).

Cortisol values were also similar in both groups, mean value 18.8 μg/dL for patients and 19.1 μg/dL for controls ($p = 0.906$).

In contrast, the bipolar patients had a higher mean two hour glucose (2HG) value compared with matched controls (respective means, mg/dL[SD], of 145.9[16.9] vs. 84.8[27.8]; $p < 0.001$). Two patients (29%) but only one control subject (2%) met criteria for impaired fasting glucose ($p = 0.037$). Six patients (86%) and two controls (4%) met criteria for impaired glucose tolerance ($p < 0.001$).

We found that newly diagnosed, medication-naïve patients with bipolar disorder, manic, with psychotic features had higher 2HG concentrations than did matched control subjects. These differences could not be attributed to confounding by BMI, gender, age, psychotropic medications, cortisol concentration, socioeconomic status, ethnicity, smoking or drugs that affect glucose tolerance. Our results are consistent with the proposals that bipolar disorder is a multi-systemic disease [2], or a syndrome of accelerated aging, and with the concept of allostatic load as applied to bipolar illness [3].

Reference(s)

[1] Newcomer, J.W., 2006. Medical risk in patients with bipolar disorder and schizophrenia. J Clin Psychiatry 67 Suppl 9, 25–30; discussion 36–42.

[2] Leboyer, M., Soreca, I., Scott, J., Frye, M., Henry, C., Tamouza, R., Kupfer, D.J., 2012. Can bipolar disorder be viewed as a multi-system inflammatory disease? J Affect Disord 141, 1–10.

[3] Vieta, E., Popovic, D., Rosa, A.R., Sole, B., Grande, I., Frey, B.N., Martinez-Aran, A., Sanchez-Moreno, J., Balanza-Martinez, V., Tabares-Seisdedos, R., Kapczinski, F., 2012. The clinical implications of cognitive impairment and allostatic load in bipolar disorder. Eur Psychiatry Apr 23 [Epub ahead of print].

P.4.035 Clinical improvement and plasmatic concentrations of fluoxetine in major depressive disorder (MDD), obsessive–compulsive disorder (OCD) and generalised anxiety disorder (GAD)

A. Blazquez[1]*, S. Mas[2], M.T. Plana[1], A. Lafuente[2], I. Méndez[1], L. Lázaro[1]. *[1]Hospital Clinic of Barcelona, Department of Child and Adolescent Psychiatry and Psychology, Barcelona, Spain; [2]University of Barcelona, Department of Anatomic Pathology Pharmacology and Microbiology, Barcelona, Spain*

Introduction: Fluoxetine is a useful drug in the treatment of major depression disorder (MDD), obsessive compulsory disorder (OCD) and generalised anxiety disorder (GAD) in child and adolescent populations [1].

Despite its efficacy, 30–40% of the patients do not respond to treatment [2].

Aim: To evaluate if clinical improvement is related to fluoxetine plasma levels at 8 and 12 weeks after staring treatment in a sample of adolescents diagnosed of MDD, OCD or GAD.

Methods: The study was conducted at the Child and Adolescent Psychiatry and Psychology Service of the Institute of Neurosciences at the Hospital Clinic in Barcelona. The period of recruitment was from June 2011 to October 2012. All the subjects met DSM-IV diagnostic criteria for MDD, OCD or GAD made by an experienced child psychiatrist. All subjects began fluoxetine treatment at the initial phase of the study (week 0). Patients and their parents were interview at week 0 with the Spanish version of the semi-structured diagnostic interview K-SADS-PL (Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version) in order to assess current and past psychopathology. Information about severity of illness was obtained at week 0 through the CDI (children's depression inventory), OCI/CV (obsessive–compulsory inventory-child version), SCARED (the screen for child anxiety related emotional disorders), CGI (the clinical global impression) and GAF/CGAS (global assessment of functioning) scales. In patients with OCD, CYBOCS (children's Yale-Brown obsessive compulsive scale) was also administered. To assess clinical improvement CDI, SCARED, OCI, CGI and GAF/CGAS scales were administered at weeks 8 and 12. UKU (Udvalg for Klinske Undersogelser) scale was administered to assess side effects of treatment.

All data were analysed using SPSS 18.0 for Windows. Spearman's rank correlation coefficient was used to assess relationship between two variables. Statistical significance was set at $p < 0.05$.

Results: The sample included 50 patients (34% males and 66% females). The mean age was 14.72 years (DS 1.86). A 76% of the patients were diagnosed with MDD, 12% OCD, 12% of GAD.

At week 8 the mean dose of fluoxetine was 23.44 mg/day (DS = 10.37) and the mean of plasmatic concentrations of fluoxetine was 82.38 ng/ml (DS = 83.443). No significant linear correlation between plasmatic concentrations of fluoxetine and side effects (measured by UKU scale) was found (Spearman's rank correlation coefficient = 0.27; $p = 0.852 > 0.05$).

At week 12 the mean dose of fluoxetine was 25.32 mg/day (DS = 13.32) and the mean of plasmatic concentrations of fluoxetine was 109.90 ng/ml (DS = 129.029). A significant linear correlation between plasmatic concentrations of fluoxetine and side effects was

found (Spearman's rank correlation coefficient = 0.311; $p = 0.028 < 0.05$). The most common side effects were somnolence and asthenia. Also was found a significant correlation between plasmatic concentrations of fluoxetine and punctuation at CGI scale (Spearman's rank correlation coefficient = 0.294; $p = 0.038 < 0.05$).

Conclusions: At week 8 no relation between clinical improvement/side effects and plasmatic concentrations of fluoxetine was found, but at week 12 side effects were related to plasmatic concentrations of fluoxetine and also to clinical improvement measured by CGI scale.

Reference(s)

[1] Wilens TE, Cohen L, Biederman J, Abrams A, Neft D, Faird N et al. Fluoxetine Pharmacokinetics in pediatric patients. J Clin Psychopharmacol. 2002; 22 (6):568–75.

[2] Findling RL, McNamara NK, Stansbrey RJ, Feeny NC, Young CM, Peric FV, et al. The relevance of pharmacokinetic studies in designing efficacy trials in juvenile major depression. J Child Adolesc Psychopharmacol. 2006; 16 (1–2):131–45.

P.4.036 Corticotropin-releasing hormone and serotonin − the neuropsychopharmacology of fear learning deficit from rodents to humans

I. Heitland[1]*, L. Groenink[2], E.Y. Bijlsma[2], R.S. Oosting[2], J.L. Kenemans[1], J.M.P. Baas[1]. *[1]Utrecht University, Experimental Psychology & Psychopharmacology, Utrecht, The Netherlands; [2]Utrecht Institute of Pharmaceutical Sciences, Psychopharmacology, Utrecht, The Netherlands*

Background: From an evolutionary perspective, the acquisition of fear responses enables organisms to respond appropriately to predictors of aversive events. In the laboratory, this process is often modelled by classical fear conditioning procedures in which an originally neutral conditioned stimulus (CS; e.g., a light) is repeatedly paired with an unconditioned aversive stimulus (UCS; e.g. an electrical shock). During the course of this learning process, conditioned fear responses develop towards the threat cue. As a consequence, absence of the CS may come to signal periods of safety. However, if this contingency is not learned, threat remains unpredictable. This can result in chronic states of maladaptive anxiety in the context in which the CS is presented [1]. Literature suggests that this learning deficit plays a crucial role in the pathogenesis of human anxiety disorders [2], and large interindividual variability in fear learning deficits has been reported [1]. As of yet, however, it remains largely unknown which neurotransmitter systems are involved in human fear learning deficits. Recently, preclinical studies have shown that the corticotropin-releasing factor (CRF) [3] and serotonin (5HT) are interactively involved in adaptive fear learning. Here, we aim to translate this preclinical evidence to the human realm.

Methods: 150 healthy medication-free human subjects completed a well-established cue and context fear conditioning procedure in a virtual reality environment [1]. As in preclinical studies, fear potentiation of the startle reflex (FPS) was measured to assess fear conditioning. During the paradigm, we assessed both uninstructed fear acquisition and instructed fear expression. Due to the paucity of pharmacological administrations with regard to the CRF system, we chose to investigate innate variability in both the corticotropin releasing hormone system and the serotonin system. Therefore, all participants were genotyped for polymorphisms located within regulatory regions of the corticotropin releasing hormone receptor 1 (CRHR1 – rs878886) and the serotonin transporter (5HTTLPR). These polymorphisms have previously been linked to panic disorder (CRHR1 [rs878886] G-allele) and anxious symptomology and personality (5HTTLPR short allele), respectively.

Results: G-allele carriers of CRHR1 (rs878886) showed no acquisition of fear conditioned responses (FPS) to the threat cue in the uninstructed phase, whereas fear acquisition was present in C/C homozygotes. Moreover, carrying the risk alleles of both rs878886 [G-allele] and 5HTTLPR [short allele] was associated with increased FPS to the threat context during this phase. After explicit instructions regarding the threat contingency were given, the cue FPS and context FPS normalised in all genotype groups.

Conclusion: The present results indicate that genetic variability in the corticotropin-releasing hormone receptor 1, especially in interaction with the 5HTTLPR, is involved in the acquisition of fear in humans. Fear expression in the contrary was not associated with genetic variability in both systems. These results translate prior animal findings to the human realm.

Reference(s)

[1] Baas, J.M., van Ooijen, L., Goudriaan, A., Kenemans, J.L., 2008. Failure to condition to a cue is associated with sustained contextual fear. Acta Psychol (Amst) 127, 581–592.

[2] Grillon, C., 2002. Associative learning deficits increase symptoms of anxiety in humans. Biol Psychiatry 51, 851–858.

[3] Bijlsma, E.Y., van Leeuwen, M.L., Westphal, K.G., Olivier, B., Groenink, L., 2011. Local repeated corticotropin-releasing factor infusion exacerbates

anxiety- and fear-related behavior: differential involvement of the basolateral amygdala and medial prefrontal cortex. Neuroscience 173, 82–92.

P.4.037 HMGB1 signalling alters T-cell functioning in response to redox status in depressed patients: effect on glucocorticoid receptor function

J. Rybka[1]*, A. Cattaneo[2], K. Kedziora-Kornatowska[3], D. Kupczyk[1], J. Kedziora[1]. *[1]Nicolaus Copernicus University Collegium Medicum, Department of Biochemistry, Bydgoszcz, Poland; [2]Fatebenefratelli Giovanni di Dio, Genetics Unit, Brescia, Italy; [3]Nicolaus Copernicus University Collegium Medicum, Department and Clinic of Geriatrics, Bydgoszcz, Poland*

It is well known that inflammation plays a pivotal role in depression pathogenesis and treatment response, also interacting with other biological systems, including glucocorticoid receptor (GR) functionality and redox-oxidative functions [1]. One molecule, named High-Mobility Group protein B1 (HMGB1), is an important mediator of redox status and also an effective stimulus triggering inflammation and GR function.

In this study we aimed to investigate more in detail the role of HMGB1 as a mediator of inflammatory response in depression. In addition to measuring HMGB1 we studied pro- and antioxidant status alongside with T cells phenotype and also proinflammatory cytokine profiles in depressed patient. Moreover, we have also investigated the GR response in relation to oxidative and inflammatory status.

Methods: Blood samples were collected from 15 patients diagnosed with recurrent depressive disorder (rDD) and from 19 healthy controls. We measured several markers related to oxidative-redox status including plasma hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in red blood cells. Furthermore, activities of antioxidant enzymes superoxide dismutase (SOD1), glutathione proxidase (GPx1), glutathione reductase (GSHR) in red blood cells and heme oxygenase (HO1) levels in plasma were assessed in this study.

IL-2, IL-6, IL-8, IFN-g, IL-17, HMGB1, neopterin and cortisol were assayed immunoenzymatically in sera. Surface phenotype expression of T regulatory and T effector cells were investigated by means of flow cytometry. The GR response was measured by dexamethasone-induced inhibition of IL-6 produced by LPS-stimulated peripheral blood.

Results: We observed pro- and antioxidant imbalance in depressed patients expressed by significant changes in the enzyme activities. In particular we found increased levels of H_2O_2 ($p < 0.01$), increased concentrations of lipid peroxidation product MDA ($p < 0.001$) and altered activities of antioxidant enzymes as expressed by decreased activities of SOD1 ($p < 0.05$), GPx1 ($p < 0.01$) and HO1 ($p < 0.01$) and increased activity of GSHR ($p < 0.001$).

Inflammatory status of depressed patients was reflected by increased concentration of IL-6 ($p < 0.05$) and decreased concentration of IL-17A ($p < 0.01$). Interestingly, alterations in cytokine levels where accompanied by increased Treg/Teffector cells ratio ($p < 0.01$). Moreover immune activation and oxidative stress in depressed patients were confirmed by elevated neopterin levels ($p < 0.001$).

We also revealed a distinct role of oxidative potential of the blood as measured by H_2O_2, MDA, SOD1, GPx1, HO1, GSHR in modulating HMGB1 levels ($p < 0.01$) and HMGB1 proinflammatory activity ($p < 0.001$). Furthermore, HMGB1 signalling was observed to alter cytokine profile, specifically IL-6/IL-17A balance ($p < 0.01$) and Treg/Teffector cells ratio ($p < 0.05$) in depressed patients.

Finally, we also found in depressed patients significant correlations between glucocorticoid responses and redox and inflammatory parameters such as MDA, IL-17A and IFN-g (all $p < 0.05$).

Conclusions: Our data suggest that the immunomodulatory role of HMGB1 might be related to the change of its activity induced by redox imbalance in depressed patients. Moreover, the shifted immune balance alongside with prooxidative environment modulates glucocorticoid response in these patients.

Reference(s)

[1] Rybka, J., Kedziora-Kornatowska, K., Kedziora, J., Kucharski, R., 2009. Immunosenescence and late life depression. Centr Eur J Immunol 34 (4), 271–275.

P.4.038 Effect of neuregulin-1 gene functional variant and environmental factors on alcohol use disorder

M. Vaht[1]*, E. Kiive[1], K. Laas[1], J. Parik[2], T. Veidebaum[3], J. Harro[1]. *[1]University of Tartu, Department of Psychology, Tartu, Estonia; [2]University of Tartu, Institute of Molecular and Cell Biology, Tartu, Estonia; [3]National Institute for Health Development, Tallinn, Estonia*

Neuregulin-1 is a signalling protein that affects neuronal development, synaptic plasticity, neuronal survival, glutamatergic neurotransmission, and glial functioning. A functional promoter polymorphism in the neuregulin-1

gene (NRG1) – SNP8NRG243177/rs6994992; (C/T) in the type IV promoter region of NRG1 – has been found to affect brain structure [1]. Subjects with C/C genotype were found to have higher white and also grey matter volume in several brain regions, for example inferior, middle and superior frontal gyri. Thus, the effects of NRG1 on psychiatric disorders are of interest.

Purpose of the study: We aimed at examining the effect of NRG1 genotype and different environmental factors on psychiatric disorders in population-representative sample of young adults.

Methods: We used data of the older cohort of the longitudinal Estonian Children Personality Behavior and Health Study that at age 25 went through M.I.N.I. interview for lifetime psychiatric disorders. Data on diagnosis were available on 501 subjects, 221 of them male, 280 female. The rationale and procedure of sample formation have been described elsewhere [2]. NRG1 genotype frequencies were in HWE: C/C – 38%, T/T – 12%, C/T – 50%.

Table 1. NRG1 × environment effects on AUD in females

	NRG1 × AUD			
	C/C		T-allele	
	No AUD	Aud	No AUD	Aud
Stressful life events, 18 yrs	2.5±2.4	6.3±3.8	2.5±2.5	5.0±2.2
Victim of rape/attempted rape, 18 yrs	18%	58%***	14%	0%
Stressful life events, 25 yrs	3.8±2.7	6.0±2.6*	4.2±2.8	3.6±2.7
Relationship problems, 25 yrs	58%	94%*	60%	60%
Depressiveness, MADRS, 18 yrs	11.1±6.1	19.5±7.7*	11.3±6.5	10.5±5.7
Depressiveness, MADRS, 25 yrs	8.0±6.1	12.1±7.8	8.6±6.4	8.2±5.0
Aggressiveness, 18 yrs	2.2±1.2	1.5±0.8**	1.8±1.0	3.3±1.0

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ – difference from T-allele carriers with AUD.

Results: The NRG1 genotype had no effect on prevalence of anxiety or affective disorders. By age 25, 74 (33%) males and 21 (8%) females had suffered from alcohol use disorder (AUD). In females, subjects with C/C genotype were approximately 5 times more likely to have AUD by the age of 25 than T-allele carriers ($df = 2$, $\chi^2 = 14.2$, $p = 0.001$): 15% of females with C/C genotype and 3% of T-allele carriers had had AUD. In males, no effect of NRG1 genotype on lifetime AUD was found ($df = 2$, $\chi^2 = 0.1$, $p = 0.946$). Neuroticism was not affected by NRG1 genotype either, however, it was higher in females with AUD. Females with C/C genotype and AUD had experienced more stressful life events, especially relationship problems by the age of 25 (Table 1). Already by the age 18 they had more likely been victims of rape or attempted rape, and they had higher depressiveness scores. Interestingly, they had also exhibited less aggressive behaviour as reported by teachers. The effect of NRG1 genotype on AUD was not affected by the age of drinking the first half a dosage of alcohol. However, there was a tendency towards more frequent use of alcohol in females with C/C genotype and AUD at the age of 18.

Conclusion: NRG1 genotype significantly affects the risk of developing alcohol use disorder in females, and the relationship is affected by environmental conditions.

Reference(s)

[1] Barnes, A., Isohanni, M., Barnett, J.H., Pietiläinen, O., Veijola, J., Miettunen, J., Paunio, T., Tanskanen, P., Ridler, K., Suckling, J., Bullmore, E.T., Jones, P.B., Murray, G.K., 2012. Neuregulin-1 genotype is associated with structural differences in the normal human brain. Neuroimage 59, 2057–2061.

[2] Tomson, K., Merenäkk, L., Loit, H.-M., Mäestu, J., Harro, J., 2011. The relationship between serotonin transporter gene promoter polymorphism and serum lipid levels at young age in a longitudinal population-representative study. Prog Neuropsychopharmacol Biol Psychiatry 35, 1857–1862.

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