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NEWMOOD

New molecules in mood disorders: a genomic, neurobiological and systems approach in animal models and human disorder.

Integrated programme

Priority 1: "Life Sciences, Genomics and Biotechnology for Health"

Final Activity Report

(for the full duration - 5 years May 2004-October 2009)

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Publishable Executive Summary

NewMood – New molecules for Mood disorders.

Overview

The Consortium and Objectives

NewMood is a major collaboration between 13 clinical and basic science groups in 10 EU countries which addresses the compelling need to discover:

- New molecular mechanisms in the causation of depression.
- New molecular mechanisms of effective drug-treatment.

Map of the NewMood Co-ordinators, Principal Investigators and Centres

Co-ordinators
Bill Deakin
Manchester
Laurence Lanfumey
Paris
Renato Corradetti
Firenze

Principal Investigators
Rafael Maldonado
Barcelona
Klaus-Peter Lesch
Würzburg
Harry Steinbusch
Maastricht
Gyorgy Bagdy
Budapest
Jaanus Harro
Tartu
Artur Swiergiel
Warsaw
Tomas Hökfelt
Stockholm
Trevor Sharp
Oxford
Paul Kelly
Edinburgh
Joaquin del Rio and Rosa Tordera
Navarra

The need

Depression is common and affects twice as many women as men. In the global burden of disease survey, it is the fourth most prevalent cause of disability, greater than e.g. cardiovascular disease. Gender, social and familial factors increase risk for depression, but little is known about how these influences work in the brain, least of all at the molecular level. Newer antidepressant treatments still act in the same way as 30 years ago and their downstream molecular actions are obscure. At most 65% of new episodes respond to drug-treatment and chronic, treatment-resistant depression is a major health burden.

Research effort NewMood was supported by the EC's Framework 7 (FP7) with a total of 7.2m euros over 5 years (May 2004 – October 2009) and involved a total of 4,611 person-months effort (1,985 funded by NewMood and 2,626 funded from other sources).

Section 1 - Project Execution

Objectives

The over-arching objectives were to identify

- New molecular mechanisms in the causation of depression, specifically new candidate genes for diagnosis, prognosis and treatment choice.
- New molecular mechanisms of effective drug-treatment, specifically new molecular targets for antidepressant drugs and novel candidate drug treatments.

The major objective of NewMood is in its acronym - to identify new molecules for mood disorders. At the heart of the NewMood strategy are gene-expression studies which aim to identify genes whose expression is changed (how much they are turned on or off) in a number of animal models of vulnerability to depression. The candidate genes are then confirmed in humans principally by demonstrating that allelic variation (the activity of the two genes inherited one from each parent) in candidate genes is associated with variation in biomarkers for vulnerability such as emotionality and a bias to detecting negative information. Further validation could be sought by determining whether the genes overexpressed in the brain in animal models are also overexpressed in human post-mortem brain from patients with depression.

In humans, vulnerability to depression appears to act by sensitizing individuals to the triggering effect of adverse life events especially in the setting of current chronic social adversity. One endophenotypic marker for vulnerability is the personality trait of neuroticism which has a heritability of about 0.5 and is known from prospective studies to greatly amplify the risk of depression following a life event, especially in the setting of chronic social adversity. Adversity early in life such as neglect, parental loss or abuse, is also a vulnerability factor that acts to increase the potency of life events. NewMood has attempted to model genetic vulnerability using genetically modified (GM) mice. Maternal separation (MS) and chronic mild stress (CMS) model early and late psychosocial stress. Giving up struggling in an inescapable situation and reduced preference for sucrose model respectively the hopelessness and anhedonia of the depressed state. Combining these translational measures with the NewMood expression array data and with human SNP associations offers a unique opportunity to rapidly validate the new candidate genes identified through the array studies.

NewMood has established a comprehensive translational dataset in rodents and humans that has yielded a multilevel integrated account of the pathogenesis of depression (table 4). The Illumina array gene expression data has taken the account to its deepest molecular level, the ultimate task of NewMood, securely tied to a superstructure of carefully validated behaviours through intervening brain processes. Where possible at each level the processes have been translated to and from humans and rodents. The superstructure is important in validating the molecular translation: we can say not only that the same genes affect vulnerability in rodents and humans but also that they do so through similar processes. We have reached this state for several genes – the CB1 cannabis receptor, the 5-HT transporter, Tryptophan hydroxylase 2, 5-HT_{1A} and $5HT_{1B}$ receptors and Brain-Derived Neurotrophic Factor (BDNF). The Major Achievements section describes the CB1 and BDNF translations, which we believe offers a new hypothesis-testing way to proceed in detecting molecular mechanisms in depression. The Major Achievements section begins at the molecular level by asking whether NewMood found new molecules for mood disorders using microarrays to detect changes in gene expression and then describes some of the notable findings at selected levels of table 4.

Summary of original objectives and progress toward them

1. To define the common behavioural/psychological endophenotypes of genetic and acquired vulnerability to depression which
   - are shared by a range of animal models
   - occur in humans
   - are reversed by antidepressant drugs

   These objectives have been met in full

2. To directly identify new candidate genetic mechanisms of depression by identifying changes in brain microarray gene expression which
   - underlie the behavioural endophenotypes across several models
   - occur in human post-mortem brain
   - are reversed by antidepressant drugs

   New candidate molecular genetic mechanisms have been generated from array studies carried out by a single service provider to a uniform protocol in three brain areas in quadruplicate pools of 3 animals in 9 core models. First analyses of all the models have been carried out using a unified analysis pipeline and some confirmatory qPCR studies have been successful but these are not regarded as obligatory in our view. A single analysis of frontal cortex samples has been carried out on the GM mouse models and further combined analyses of other regions and in rats will be carried out. In several cases, array data on the effect of chronic stress and of 14 days antidepressant treatment on control and experimental animals have been accumulated but these analyses are at a preliminary stage. All data will be made available on the NewMood website. Studies in human post-mortem brain confirmed changes in DLGAP1 protein and neurotrophins but further studies were not possible within the available time and budget.

3. To identify candidate molecular mechanisms of vulnerability based on underlying neurobiology including (hypothesis-testing approach to identifying new genes).
   - changes in distributed brain system networks and physiology which are common to animal and human endophenotypes
   - identifying changes in neurotransmitter and hormonal function, including mechanisms of 5HT sensitisation and cortisol hypersecretion, which underlie endophenotypic components of vulnerability to depression

   Many of these objectives have been met. Regional brain metabolism was mapped in all the models using cytochrome oxidase and in some cases using 2-deoxyglucose. The latter was useful for mapping metabolic responses to drug probes of 5-HT receptor function in the models but neither method proved very sensitive to mapping activity of networks. This objective was well met using fMRI in humans. All of the models were in general well characterised in term of the core dataset in animals. In humans novel imaging methods for probing 5-HT and cortisol feedback were developed and used.

4. To identify candidate molecular mechanisms of vulnerability through understanding trophic and apoptotic influences of 5HT and of antidepressants on development and adaptation of cortical networks and circuitry.

   Apoptosis was not a major focus of study. 5-HT, antidepressants, BDNF and neurogenesis have been major foci of study in the animal models and the human cohorts. Cortical networks and circuitry have been important in human fMRI studies but have been less amenable to extensive study in the models although some cytoarchitectonic changes were described in some models.

5. To validate new candidate molecules by:
    - determining whether altering their expression in animals induces component endophenotypes of depression defined in 1 & 3 above
    - identifying single nucleotide and functional polymorphisms in the gene in humans and determining their association with endophenotypic markers and with depressive disease

    A number of new GM models were developed including the technically difficult Tph2 knockout. The emphasis has been on double GM models such 5-HTT / BDNF or 5-HTT / Tph2 modified animals. Candidate genes from array studies have emerged too late to stimulate new GM models within the time period.

6. Create a new tool for research in depression: the NEWMOOD 'Depression' microarray to:
    - validate candidate gene-expression signatures detected
    - validate new animal models of depression
    - detect entirely new molecules with targeted efficacy against depression and its component processes
    - lead to the design of chip of human polymorphisms for molecular diagnosis, prognosis and selection of most appropriate drug treatment

    The last of these was clearly overambitious in the absence of a major clinical trial component to NewMood. Similarly, detecting new antidepressant molecules was not feasible without a major collaboration with industry and a library of molecules. However, two new principles of enhancing the effect of SSRIs by blocking local and long-feedback control the raphe have emerged from NewMood discoveries. In general the objectives were superseded by the use of the Illumina whole genome bead arrays.

7. Define a human pharmaco - fMRI signature for antidepressant efficacy

    NewMood has developed potential fMRI signatures for antidepressants and also for vulnerability to depression based on modification of responses to emotional stimuli and on changes in connectivity during such tasks. They need validation with noradrenaline acting antidepressants. However, one fMRI model successfully detected the novel effective agent agomelatine for Servier in healthy human volunteers.

8. Disseminate new and harmonised standards of behavioural research in animal models of depression vulnerability

    Many new harmonised technologies and techniques have spread through the participating laboratories. The main route of dissemination will continue to be via conference proceedings and publications in peer-reviewed journals.

Contractors involved

NewMood involved 13 partners in 10 EC countries:

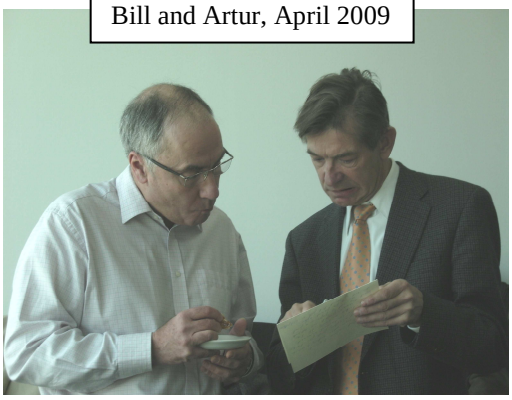
Table 1. List of NewMood Partners, locations and duration of involvement

Partiner Number	Participant name	Institute	Short name	Country	Project month	
					Start	End
1	Bill DEAKIN	University of Manchester	UNIMAN	UK	1	66
2	Laurence LANFUMEY	INSERM, Paris	INSERM	France	1	66
3	Peter LESCH	University of Würzburg	UWAR	Germany	1	66
4	Raphael MALDONADO	University Pompeu Fabra, Barcelona	UBAR	Spain	1	66
5	Jaanus HARRO	University of Tartu	UTAR	Estonia	1	66
6	Harry STEINBUSCH	University of Maastricht	UMAA	Netherlands	1	66
7 *	Gyorgy BADGY	National Institute of Psychiatry and Neurology, Budapest	NIPN	Hungary	1	44
8	Paul KELLY	University of Edinburgh	UEDIN	UK	1	66
9	Trevor SHARP	University of Oxford	UOXF.BW	UK	1	66
10	Tomas HOKFELT	Karolinska Institute, Stockholm	KISTO	Sweden	1	66
11	Renato CORRADETTI	University of Florence	UNIFI	Italy	1	66
12	Joaquín DEL RIO and Rosa TORDERA	University of Navarra, Pamplona	UNAV	Spain	1	66
13	Artur SWIERGIEL	Institute of Genetics and Animal Breeding, Jastrzebiec	IGHZ	Poland	1	66
14 *	Gyorgy BAGDY	Semmelweis University, Budapest	SE	Hungary	36	66

* In 2007, due to the reorganization of the healthcare system in Hungary and the closure of the National Institute of Psychiatry and Neurology (NIPN), Prof. Gyorgy Bagdy was forced to relocate his research group to Semmelweis University (SE). While this caused considerable disruption to Prof Bagdy and his research group, it did not result in any significant delay in the research programme. In effect, Partner 7 (Prof. Bagdy at NIPN) was replaced by Partner 14 (Prof. Bagdy at SE), however the planned work remained the same.

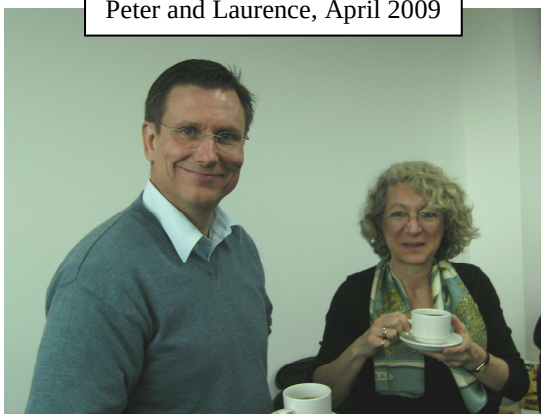
Partner 1: Bill Deakin, MB PhD (male) Professor of Psychiatry in the Neuroscience and Psychiatry Unit: with over 25 years experience in experimental clinical and basic psychopharmacology research, focusing on the normal psychological functions of 5-HT and how they become disturbed in depression, anxiety and impulsivity, including fMRI and phMRI. He was responsible for co-ordinating the project as a whole.

Bill and Artur, April 2009



Partner 2: Laurence Lanfumey, PhD (female): has more than 20 year experience in research on CNS neurotransmission, using electrophysiological and pharmacological approaches. She was responsible for the electrophysiological, neuroendocrinological and neuropharmacological investigations and coordinated the activities of all the co-workers in her team.

Peter and Laurence, April 2009



Partner 3: Klaus Peter Lesch, MD (male) Professor of Psychiatry and Psychotherapy at the Department of Psychiatry and head of the Laboratory of Molecular & Clinical Psychobiology, he was responsible for overseeing all aspects of the research activities, data collection and analysis in his team.

Rafael and Rosa, April 2009

Partner 4: Rafael Maldonado MD, PhD (male) Professor of Pharmacology, has significant expertise in the behavioural study of affective disorders and drug dependence processes, making major contributions to the understanding of neurobiological mechanisms involved by using classical pharmacological approaches as well as generating knockout mice.

Partner 5: Jaanus Harro, MD, PhD (male) Professor of Psychophysiology has over 20 years experience of different methods used in experimental and clinical neurosciences, particularly the development of behavioural techniques in laboratory animals, psychopharmacology of neuropeptides, and receptor biochemistry. His team takes a multi-disciplinary approach.

Trevor and Jaanus, April 2009

Partner 6: Harry Steinbusch, PhD (male) Professor of Neuroscience, has 27 years experience in the field of functional neuroanatomy, micropharmacology and animal behaviour in the rat brain, using micropharmacological approaches to focus on the hypothesis that peri-, pre- or postnatal stress triggers the initiation of early life depression and late-life neurodegeneration.

Partner 7 / 14: Gyorgy Bagdy, PhD, DSc, (male) has 27 years experience in research in preclinical and clinical neuro- and psychopharmacology and

neuroendocrinology. He focussed on EEG, neuroendocrine and behavioural pharmacological studies in combination with surgical and morphological techniques, plus human genetic polymorphism studies.

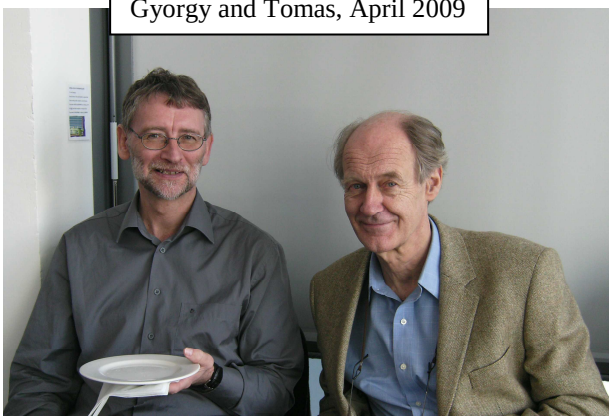
Harry and Renato, April 2009

Paul Kelly PhD, (male) is a Systems Neuroscientist and former Head of Medical Faculty Animal Facilities. With twenty years experience of using quantitative autoradiographic brain imaging techniques, he oversaw the management of animal resources/welfare for all *in vivo* work and directed the LCMRglu/LCBF autoradiography and functional studies.

Trevor Sharp, PhD (male) has a special interest in the application of neurochemical, electrophysiological and molecular techniques to understand the function of 5-HT receptor subtypes in the CNS, and their role in the therapeutic effect of antidepressant drugs. Non-clinical and clinical researchers work and train side by side in key areas relevant to mood disorders.

Tomas Hökfelt, **MD, PhD** (male) Professor in Histology has thirty years of experience in the histochemical analysis of the nervous system, including immunohistochemistry at the light and ultrastructural level and *in situ* hybridization, as well as ordinary electron microscopy. He has mapped many molecules related to synaptic and non-synaptic signalling in rat and mouse brain.

Gyorgy and Tomas, April 2009



Renato Corradetti, M.D. (male) Professor of Pharmacology has 20 years experience in electrophysiological recording of synaptically- and receptor-stimulated responses in neurones. He has devoted much work in developing multi-disciplinary approaches which combined electrophysiology with neurochemical techniques in the brain tissue used for recordings.

Joaquin and Paul, April 2009

Joaquín Del Río, PhD, (male), Professor and Director of the Division of Neuroscience has more than forty years experience in neuropharmacology, including the development of new animal models of depression, and neurochemical / molecular studies on the mechanism of action of antidepressants and pharmacological development of new synthetic drugs.

Rosa Tordera, PhD, (female) Researcher / Associate Professor, supervised various neurochemical, molecular and behavioural aspects of the research. In close collaboration with Prof. Del Rio, she also took responsibility for the project in Pamplona for the two final years.

Artur H. Swiergiel, PhD, DSc, (male) has over 20 years experience in developing behavioural models of responses in stress in a number of species, including transgenic mice, and knowledge of methods typical for behavioural pharmacology, plus experience with neurochemical methods (HPLC, microdialysis, voltammetry).

Work Performed

The work was coordinated through a set of 7 research Work-Packages (Table 2 - WPs), plus one for management and dissemination, integrated with 5 Activities (Table 3).

Table 2. List of NewMood Workpackages		
WP No.	**Workpackage title**	**Workpackage leader**
1	Behaviour	Rafael Maldonado
2	Genomics animal and human	Klaus-Peter Lesch
3	Neurochemistry of neurotransmission and hormone function	Trevor Sharp
4	Neurochemistry of cell responses and gene expression: NewMood chip array	Laurence Lanfumey
5	Cyto and immunohistochemistry	Tomas Hokfelt
6	Neurophysiology	Renato Corradetti
7	Functional imaging Human and animal	Bill Deakin
8	Review, Assessment and Dissemination	Bill Deakin
	Activity title	**Activity leader**
1	Models of Vulnerability Factors in Depression – NewMood 1000 array	Rafael (behaviour), Laurence (array studies), Bill (imaging)
2	The Neurotransmitter Endophenotype of Vulnerability	Renato
3	The Neuroendocrine (stress-hormone) Endophenotype of Vulnerability	Peter (neurogenesis), Paul /Gyorgy (HPA)
4	Human Studies	Ian Anderson
5	Effects of Anti-depressants on Behavioural, Neurotransmitter and Stress-hormone Endophenotypes	Bill Deakin

Table 3. Summary of Activities

Activity 1	Activity 2	Activity 3	Activity 4	Activity 5
1.1 Reward, aversion and cognition • Open-field exploration • Tail suspension (mice) • Learned immobility (rats & mice) • CMS anhedonia 1.2 Regional brain function • Cytochrome oxidase (rats & mice) • 2DG	2.1 Monoamine cells • Raphe & locus dialysis • Raphe & locus: tryptophan & tyrosine hydroxylase mRNA autoreceptor mRNA CRF receptor ish CRF immuno Galanin peptide and receptors NPY and receptors 2.2 Monoamine release • Hippocampal dialysis • Forebrain 5-HTT & NAT binding 2.3 Beyond the synapse • 5-HT1A & 5-HT2C binding • NAα1 &2 binding	3.1 Molecular markers HPA • Gluco & mineralo- corticoid receptor mRNA: • Hippocampus & PVN • CRF mRNA PVN 3.3 Neurogenesis • BrdU cell counts	4.0 Community cohort • Genetic vulnerability Family history 5-HT polymorphisms • Developmental vulnerability Early life adversity Personality • Acquired vulnerability Acute life events Chronic difficulties Tryptophan availability 4.1 Neurobiology of reward, aversion and cognition • reward tasks (gambling), • stress & mood induction • affective bias memory & face emotion identification	5.1 Antidepressants • Reversal 1.1 & 1.2 by SSRI & NARI
Illumina microarrays changes confirmed by qPCR & in-situ hybridisation				

Section 2 - Project objectives and major achievements

Major scientific achievements

Table 4. NewMood translational dataset in rodents and humans

Processes	***Human measures***	***Rodent models and measures***
Genetic / molecular:	Family history. SNPs haplotypes. PM brain, ISH, qPCR	GM mice Rat strains Microarrray, ISH, qPCR
Developmental adversity: Neglect, Abuse, Loss	CEQ self-ratings.	Prenatal stress Maternal separation
Brain development: Impaired frontal regulation of limbic system	Voxel-based morphometry. Connectivity analysis.	Measure neurogenesis & cytoarchitecture. Regional brain metabolism maps.
Compromised 5HT system	Citalopram pharmaco-mri. Citalopram modulation of tasks.	Partial 5HT depletion model. 5HT functional measures e.g. cell firing, neurochemistry, rat pharmaco mri
Info processing biases of vulnerability: Perceptual distortion Emotion dysregulation Impaired reward	Core processes in recovered depressives (i.e. vulnerable): computer task performance task-evoked fMRI reponses.	Core behaviours pre-stress (see below)
Personality/ behavioural traits.	Big 5 Personality Q. (neuroticism, agreeableness…) Impulsivity Qs. Rumination Qs.	Stable individual differences: High/low Exploration High/low Sociability High/low Pleasure chirps
Chronic psychosocial stress: Lack of food, shelter, social contact	Self – report.	Chronic mild / ultra mild stress
Abnormal HPA function	GR SNPs. AM cortisol response to waking.	GR-i model GR and CRF expression Challenge studies
Acute stress	Life events Q Loss, humiliation, threat…	Learned immobility TST, FST
Depressed state: Emotional activation, anxiety anhedonia. Cognitive impairment: Executive and mnemonic.	Currently depressed patients: Symptoms. Core processes - performance and fMRI. CANTAB computer tests.	Core behaviours post-stress: Fearfulness, helplessness anhedonia. Set-shifting, social & object recognition.

Abbreviations: CEQ, childhood experiences questionnaire; FST, forced swim test; GM, genetically modified; GR, glucocorticoid receptor; ISH, in-situ hybridisation; Q, questionnaire; TST, tail suspension test.

One starting point for NewMood was to was to compare 4 genetically modified (GM) mouse models of vulnerability to depression beginning with genes in four systems most directly implicated in human studies of depression: 5-hydroxytryptamine (5-HT), the hypothalamico-pituitary-adrenal system (HPA; stress hormone system), glutamate and cannabinoid signalling. Altered 5-HT function and impaired negative feedback control of the hypothalamico-pituitary-adrenal system (HPA) have been described in depression for several decades. More recent interest has focussed on glutamate and cannabinoid systems as candidate mechanisms for depression, largely on the basis of antidepressant drug effects and the ability of the cannabis receptor antagonist rimonabant to precipitate depressive illness.

The mouse models were selected according to the pre-determined behavioural criteria and harmonised procedures of the NewMood project (WP1) - that they all show learned immobility in the tail suspension or forced swim tests, and preferably increased anxiety-like behaviour in the light-dark emergence or open field tests and anhedonia in the sucrose preference test using harmonised procedures. The four GM models selected comprised three knock-outs; 5-HT transporter (HTT-/-), vesicular glutamate transporter (VGlut -/+), cannabis 1 receptor (CNR1 -/-) and an antisense knock down of the glucocorticoid receptor (GR-i). A uniform method of brain dissection and mRNA extraction was used. Quality control and hybridisation was carried out by a service company using Illumina arrays. We used a standard NewMood pipeline for analysis of the 24,000 genes.

The models converged in showing changes in expression of genes (WP 4) in various formally defined functional categories including cellular metabolism, protein handling, mitochondrial function, ribosomal proteins but most specifically in 36 genes in the 'synapse' category and others in the MAPK signalling pathway. The greatest changes in expression across the models were in activity-related cytoskeletal associated protein (Arc; gene 1 below). Partner 9 had previously shown that this gene is influenced by antidepressant treatment and Partner 13 has subsequently shown it is upregulated after chronic mild stress. The changes in genes in the synapse category (genes 1-3 below) are implicated not only in glutamate and GABA signalling but also in long-term changes in connectivity that underpin learning and memory. The MAPK pathway is also associated with plastic changes in neuronal development and connectivity and it is responsive to brain-derived neurotrophic factor (BDNF). The ability to modify neuronal connectivity is thought to be an important part of resilience to adversity and in long-term recovery from depression. Some of the ribosomes showing altered expression are concerned with the local synthesis of proteins such as Arc.

Table 5. Dysregulated genes common to four mouse models

No	Gene	CB1	VGlut1	5HTT	Gri
1	activity regulated cytoskeletal-associated protein	0.58	1.46	1.21	2.23
2	lin-7 homolog b (C. elegans)	1.23	1.26	1.16	0.88
3	synaptic vesicle glycoprotein 2 a	1.26	0.71	1.36	1.14
4	erythroid differentiation regulator 1	1.38	1.36	1.40	1.26
5	quininoid dihydropteridine reductase	0.72	0.88	0.68	0.87

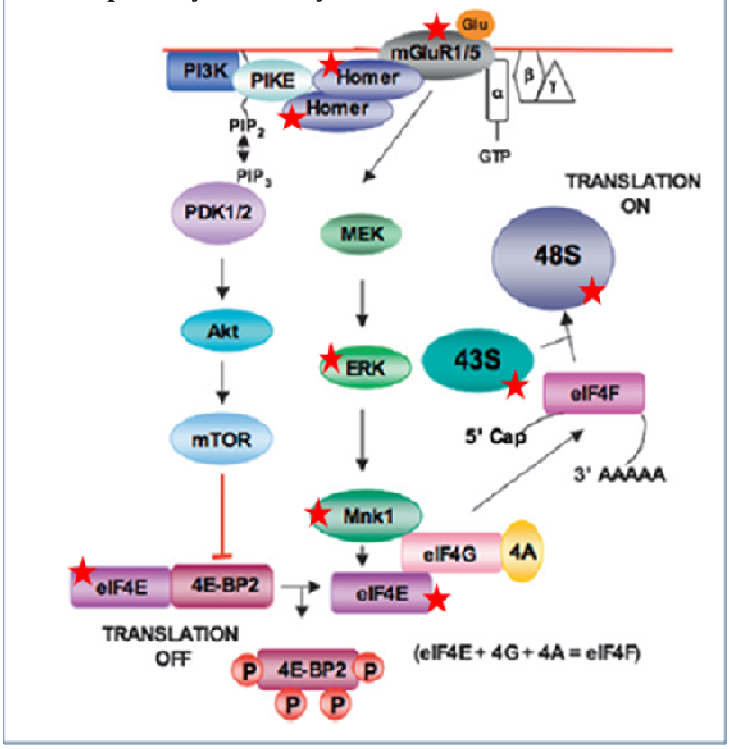
The numbers in the table are fold changes – the ratio of expression in the GM animals to the wild type controls. Red denotes increased expression, blue decreased.

As reported in the last PAR some genes were up-regulated in all 4 models albeit to a varying extent. The greatest changes were seen in the edrd1 gene (gene 4 above). This initially unpromising and little known gene that influences the development of red blood cells, turned out to have shown major changes in expression in association with anxiety-like behaviour in various

mouse strains. This may point to a new direction in the pathogenesis of depression once more is known about its role in the brain.

A combined array analysis of the rat models of acquired vulnerability (prenatal stress, 5-HT depletion and individual differences in behaviour) will be carried out but individual analyses show some striking affinities with each other and with the mouse models. For example, the low-exploratory rats that are susceptible to depression-like behaviour (Jaanus Harro, Tartu) and adults that were prenatally stressed (Gunter Kenis, Maastricht) show changes in expression of glutamate and GABA receptors, the long-term-potentiation category, MAPK signalling and ribosomal proteins.

Genes dysregulated in the GM models in the MTOR and MAPK pathways shown by red stars

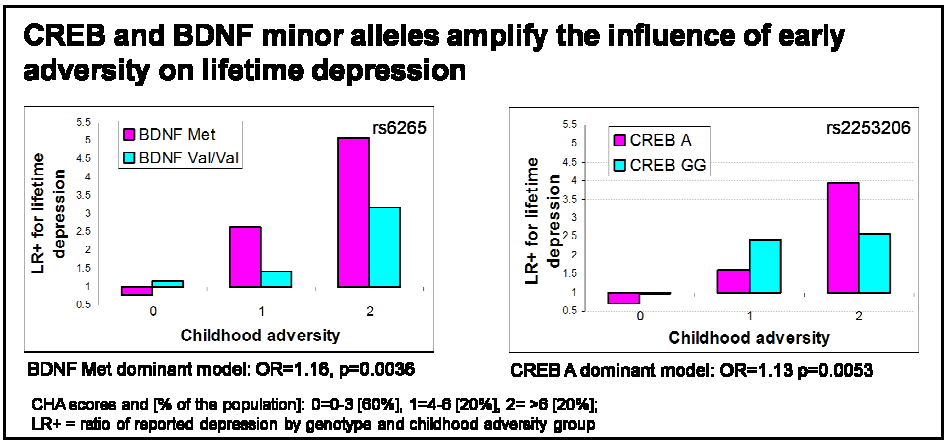


Several array studies in individual mouse and rat models are in various stages of publication and the combined analysis of the GM mouse models outlined above are in preparation for the special NewMood issue of European Neuropsychopharmacology. However, the work with the very extensive NewMood array results will continue for some considerable period and be available to the wider community through the NewMood website, presentations and publications.

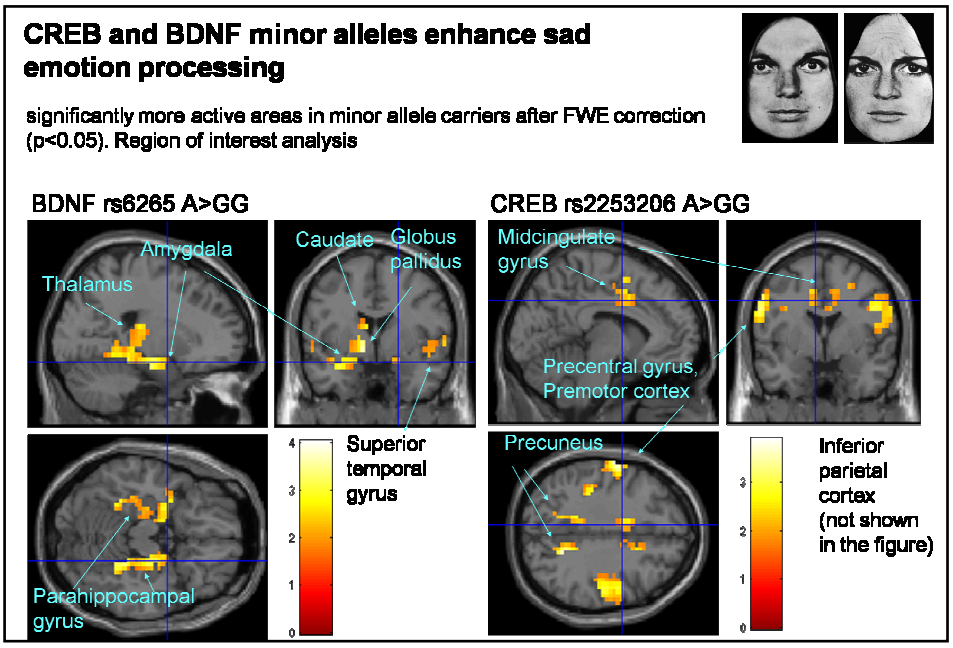
Developmental adversity and vulnerability to depression in animals and humans

The experience of adversity in early life through neglect and abuse is a major risk factor for depression. This was modelled in various ways in the NewMood models. Rat pups separated for periods from their mother, show increased susceptibility to chronic mild stress-induced learned helplessness in adulthood. Partner 13 showed this was associated with changes in Arc and glutamate function using microarray and other molecular methods. Partner 3 showed that mouse pups rearing by females with poor mothering behaviour was not itself sufficient to induce depression and anxiety-like behaviour when the pups reached adulthood. However when 5-HTT -/- pups were reared by poor mothers they showed depression-like behaviour; a demonstration of a gene x environment vulnerability interaction in an animal system. This was associated with persistently increased expression of BDNF in their hippocampi, a possible epigenetic effect in which early experience produces a long-term change in gene expression.

In humans, developmental adversity was measured by including the self-rated Childhood Experiences Questionnaire (CEQ) in the assessments completed by the community cohort of 1500 individuals recruited and genotyped by Partner 1. Instead of seeking association on a gene by gene basis, they determined genetic variations (single nucleotide polymorphisms; SNPs) at 9 locations in the CREB-BDNF-TrkB neurotrophin pathway. Importantly, these genes are quite distinct genetically with different chromosomal locations but they converge in promoting neuronal differentiation and survival through the MAPK pathway discussed in the previous section. Partner 1 showed in human post-mortem brain that the SNPs had functional effects in that they affected protein levels in human post-mortem brain. No associations with current depressive symptoms were found. However, 8 of the 9 SNPs significantly amplified the effect of early psychosocial adversity (CEQ-rated) in increasing the risk of depression (see figure).



In a subset of the cohort, people with no prior history of depression who had risk CREB and BDNF variants had increased brain responses when viewing faces with sad expressions during a functional magnetic resonance imaging (fMRI) session.



These findings suggest that the neurotrophin system affects the development of emotion processing systems in the brain and that early adversity such as parental neglect or abuse modifies this vulnerability to increase the risk of later depression. We conclude that genome-wide association studies of depression that do not include information about known psychosocial risk factors, greatly reduce the possibility of detecting genetic effects in depression. Furthermore, the hypothesis-testing pathway approach, validated by functional effects on brain emotion processing, would appear to be a powerful way to test insights from animal models of vulnerability

Compromised 5-HT systems in depression

NewMood has led to major new insights into the local and long-feedback control of 5-HT function (Workpackage 3) and into 5-HT mechanisms of depression in humans and animals.

Local control of 5-HT cells in the midbrain raphe. Partner 11 showed that non-impulse-dependent release of 5-HT from dendrites in the raphe, which is determined by availability of tryptophan in the diet controls the firing-rate of cells. Local 5-HT acts on dendritic 5-HT_{1A} receptors. These autoreceptors mediate inhibition of 5-HT cell firing early in antidepressant treatment and as they desensitise during continued treatment, the firing rate returns to normal. This increases release from terminals and allowing a greater accumulation of 5-HT in the synapse by inhibition of uptake. This is thought to account for the delay in onset of the full therapeutic effect of uptake inhibitors. Blockade of 5-HT_{1A} autoreceptors could thus hasten the onset of antidepressant effect. Unfortunately, current 5-HT_{1A} antagonists also block forebrain post-

synaptic receptors that probably mediate antidepressant effects. Partner 2 has important evidence that that pre- and post-synaptic receptors differ their linkage to G-protein mediated signalling and that therefore selective presynaptic antagonists could be developed. Indeed Partner 11 has been experimenting with such an agent.

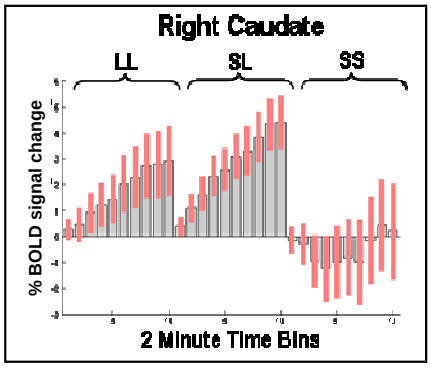
Laurence Lanfumey (Paris) and Renato Corradetti (Florence) have collaborated with the groups with experimental models to disseminate electrophysiological (WP6) and neurochemical (WP3) methods of assessing 5-HT_{1A} autoreceptor functioning. In general autoreceptor function is reduced. This rules out the possibility that excessive autoreceptor restraint of raphe 5-HT cell firing is a general mechanism of compromised 5-HT function in depression. Indeed, the results are more in keeping with the more subtle idea that 5-HT release is enhanced in emotion processing areas in response to acute stress and mediates anxiety responses. In contrast, after chronic stress impairment of 5-HT release occurs in other brain regions as part of the mechanism of learned helplessness. Antidepressant drugs may work by re-balancing different 5-HT systems through their combined effects on terminal 5-HT release, changes in postsynaptic sensitivity and long and local control of the raphe.

Cortical control of the raphe; novel long-feedback mechanisms. Partners 2 and 9 have described and characterised novel and important long feedback control mechanisms that control raphe firing from glutamate cells in prefrontal cortex (WP6). One population is activated by 5-HT_{2A} receptors which project to local GABA interneurones that inhibit 5-HT cells in the raphe. Another population probably activated by 5-HT_{2C} receptors, projects to the lateral habenula which sends extrinsic inhibitory projections to the raphe. Partner 9 has shown that 5-HT_{2C} antagonists by blocking long feedback inhibition of the raphe, increase 5-HT accumulation in the synapse following treatment with serotonin selective reuptake inhibitors. These findings demand clinical trials to establish whether 5-HT_{2C} selective antagonists enhance antidepressant efficacy.

Compromised 5-HT function in depression in humans. Whether 5-HT function is compromised in depression continues to elude decisive demonstration in humans. Ian Anderson and colleagues in Manchester have developed intravenous citalopram challenge pharmacoMRI as a way of probing 5-HT function in living humans; citalopram abruptly increases synaptic 5-HT and MRI is used to follow the brains response (WP7). Patients with a history of depression show evidence of reduced responses in subcortical structures such as the caudate nucleus but some regions of prefrontal cortex show enhanced responses. Impaired subcortical responsiveness may indicate that vulnerable people who are currently well nevertheless have impaired subcortical 5-HT function and better frontal regulation of limbic emotion processing centres. However, this pattern could also indicate a mechanism of remission in which frontal cortex is better able to control stress-related 5-HT release. This group is vigorously continuing to investigate these fundamental issues using UK MRC funding obtained on the basis of NewMood data. Parallel studies in animals by Trevor Sharp in Oxford have used citalopram or fenfluramine challenge phMRI and also 2 deoxyglucose imaging as used by Paul Kelly in Edinburgh, to show changes in response to stress and antidepressant treatment, and in the different animal models.

Compromised 5-HT function in animals. A number of groups have studied the behavioural consequences of experimentally impairing 5-HT function using a variety of techniques including drug–induced partial depletion with the recreational drug ecstasy (MDMA; Paris, Budapest and Edinburgh groups) and p-chloroamphetamine (PCA; Tartu). In general these animals show depression-like disturbances of sleep and learned helplessness in some circumstances but do not display the full anhedonia/anxiety/helpless syndrome. Peter Lesch (Würzburg) has developed a GM mouse lacking the 5-HT synthetic enzyme Tph2. Remarkably, their 5-HT neurones appear to develop normally but are completely empty of 5-HT. Their behaviour is also remarkably normal in the absence of stress but further studies are needed to determine their vulnerability to depression-like response to chronic stress.

Role of the 5-HT transporter. Much research world-wide has been generated by the seminal observation of Peter Lesch's group that genetic variation in the activity of the 5-HT transporter was related to the personality trait of neuroticism. This finding was confirmed and elaborated by Gyorgy Bagdy's group in their Hungarian population. That the s-allele is the less active form of the transporter has been directly demonstrated in humans by the Manchester group. They showed that the citalopram-challenge phMRI responses were clearly reduced in homozygous ss-allele carriers (see fig).



The most compelling evidence that the less active s-allele is associated with emotionality comes from Würzburg and other groups using fMRI; the amygdala, a key centre for integrating anxiety responses, is more responsive to emotional images in humans who carry the s-allele. The role of the transporter in depression is more controversial. The 5HTT-/- knockout mouse has been extensively studied by many NewMood groups together with its complement, the 5HTT over expressing mouse and this has generated many insights. Broadly speaking the results are compatible with the idea that raised synaptic 5-HT (5-HTT-/-) is associated with anxiety-like behaviour but that helplessness may be more associated low 5-HT in the 5-HTT o/e mouse in which the greater number of uptake sites removes 5-HT from the synapse. However, increased helplessness compared to control animals can be induced in both strains. It seems likely that other changes are needed to reveal the depression phenotype in animals and humans such as those revealed by NewMood - early adversity, altered brain development, and changes in glutamate/GABA and neurotrophin pathways. The studies described in WP3 indeed show that other risk genes such as the CB1 receptor and Tph2 interact with 5-HTT variants to influence emotionality and impulsiveness at the level of personality and at a neural systems level of emotion information processing.

Information processing biases and vulnerability to depression

The core translational processes which may be closer to genetic mechanisms (endophenotypes) than symptoms in humans are impaired reward processing, increased anxiety and impaired tolerance of stress. The extensive behavioural characterisation of the animal models is described in WP1. In humans, much use has been made of the ready activation of the amygdala, detected by fMRI, when viewing emotional faces (see previous section). This appears to be a genuine endophenotypic marker for convergent influences of several genes described in WP3 on unconscious emotion processing. These effects can be detected with much smaller samples than those needed to detect genetic influences on human symptoms or questionnaire scores. The Manchester group have recruited a group of abut 100 people currently well but with two or more past episodes of depression. This remitted depressed (RD) group who are vulnerable to depression, have been compared a similar number of people who have never been depressed. The vulnerable group showed a greater ability to detect negative emotion in faces using performance measures and this was paralleled by greater coupling between visual and emotion processing areas in the brain when viewing faces. However, in emotion processing regions, fMRI responses were reduced in the RD group and this may be a process that sustains remission. NewMood has been dramatically successful in demonstrating emotion-information processing biases that underpin genetic and acquired vulnerability to depression.

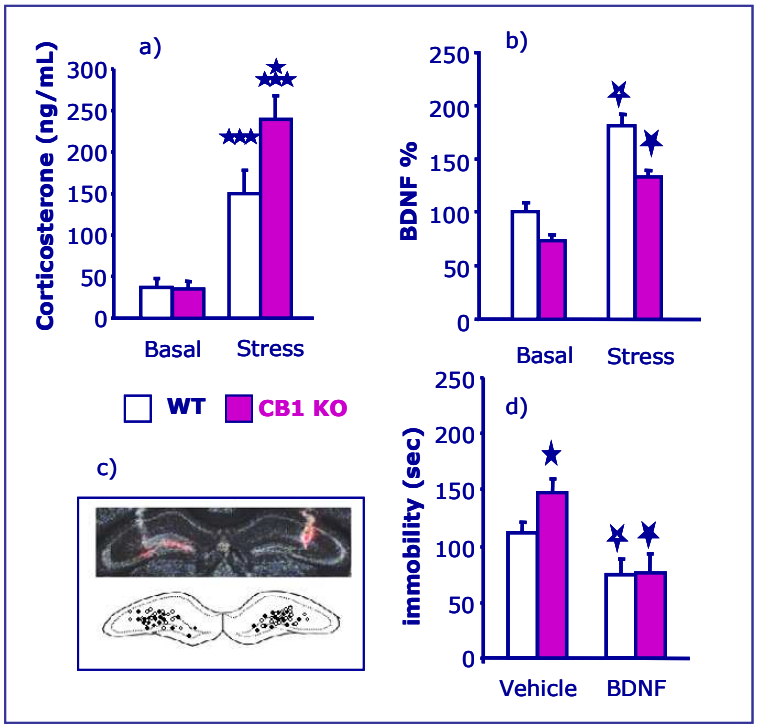
Depression involves rather general impairments of intellectual functioning ('cold-cognition') – reasoning, memory and decision-making. The RD group show persisting cognitive impairments. Jaanus Harro (Tartu) and Trevor Sharp (Oxford) have been able to bring this under experimental control in animals. Animals with depression-like behaviours after repeated social defeat show

impaired performance in tasks involving attention and memory. These measures may be useful in finding drugs that improve cognition in depression.

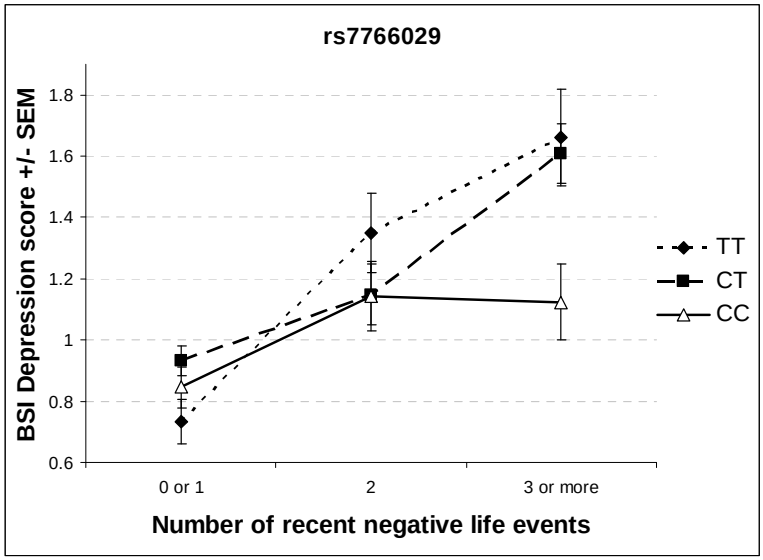
Acute and chronic psychosocial stress

The chronic mild stress (CMS) and ultra-mild stress paradigms have been used extensively by NewMood to reveal vulnerability to stress both in the GM and acquired vulnerability models. These stressors involve repeated exposure to unpredictable mild 'hassles' such as unchanged bedding, delayed feeding and disrupted light-dark cycling. The animals show signs of anhedonia and lose their preference for drinking sweetened water. They more rapidly develop helpless behaviour in response to the acute stress of inescapable swim stress or tail suspension. In the human cohorts several groups (Budapest, Maastricht, Manchester and Würzburg) obtained self-ratings of stressful life-events and determined their influence on mood in people with variants in the genes disrupted in the animal models. A very good example of this translation is provided by the NewMood studies of the CN1 receptor.

Rafael Maldonado and colleagues generated the CB1 knockout mouse model and showed their susceptibility to repeated swim stress. The GM animals develop immobility more rapidly and they show exaggerated stress hormone responses (fig a). They showed an impaired ability to mount a BDNF response to the stress (fig b) and replacement of the BDNF by local injection (fig c) reversed the deficit (fig d).



In a direct translation, the Manchester group genotyped their community cohort for variants of the CB1 receptor. Several SNPs and combinations of SNPs predicted current depressive symptoms but this was almost entirely attributable to the group that had experienced life-events. Possession of the risk variants of the CB1 receptor increased susceptibility to develop depression in the face of life-stress (see fig). Some of this effect may be mediated by the personality trait of neuroticism since 1% of the variance in this personality trait was accounted for by CB1 genetic variants.



Summary
NewMood has identified through its gene-expression studies new leads in the search for understanding the pathogenesis of depression. The focus is very much on molecular mechanisms of synaptic plasticity. The partners work together well and continue to collaborate. Much more will emerge from the rich molecular dataset in identifying molecular signatures of pathogenesis and the effect of antidepressants on the changes in the experimental models. Large collections of DNA together with phenotypic information have been accumulated in human cohorts and these clinical datasets will continue to be used to test emerging molecular insights from the animal models. In addition much has been learned about fundamental mechanisms of the control of 5-HT function and the role of neurotrophic mechanisms in adaptation to stress.

Section 3 – Workpackage 5-year Summary

Table 6. List of all Milestones throughout the project

All the Milestones have been passed successfully.

No.	Milestone Name	WP no.	Due date	Actual date
1	Uniform method of brain dissection agreed. (DVD made by Partners 6 and 2; available to all consortium members)	4	15	15
2	Selection of rodent models for more detailed behaviour, neurotransmitter and neuroendocrine endophenotypic analysis	3	18	18
3	New genomic targets for mechanisms and treatment identified, (Preliminary results of functional SNP-based association/linkage study of a appropriate candidate gene in depression)	2	18	18
4	Decisions on models to be taken forward for detailed analysis of mechanisms (Selection of the models to be used for further studies)	2	18	18
5	Identification of new genetic and developmental models from molecular developmental & array studies.	4	24	24
6a	Data lock on collaborative community study (Level I) [Decided to delay data-lock until month 48 to allow inclusion of participants recruited at Levels 2 and 3.]	7	24	48
6b	Completion of motivation-reward and rumination fMRI studies (Level 3).	7	24	48
7	Decisions on detailed studies of mechanisms in selected models	All	30	30
8	Decisions on new Activities on basis of promising findings e.g. role of vulnerability models in addiction.	1	30	30
9	Selection of parameters and models for antidepressant effects to be studied	All	30	30
10	First results of antidepressant effects on gene-expression.	4	30	42
11	Completion of sample from community study (Level 1) in Manchester	7	30	30
12	Evaluation of use of 2-dimensional statistical parametric mapping of group differences applied to autoradiographic brain sections.	7	36	36
13	Decision on viability of peptide release experiments using molecular biological approaches	3	36	36
14	Assessment of the utility of arachidonic acid imaging as an adjunct to GTPϒγS,	3,7	36	36
15	Decision on studied monoamine neurochemical endophenotypes for inclusion in characterization of all models	3,5,6	36	36
16	Neurogenesis studies complete in core models	5,2	36	36
17	Completion of neuropsychological study (Level 2)	7, 2	36	42
18	Decision on the influence of a reduced expression of VGLUT1 on 5-HT cell body function	6,3	42	54
19	Completion of motivation-reward and rumination fMRI studies (Level 3).	7	42	42
20	Decisions on detailed studies of mechanisms in selected models	All	42	42
21	Selection of parameters and models for antidepressant effects to be further studied in Activity 5	All	42	42
22	Identification of changes in gene expression shared in several models from NewMood Bioinformatics Analysis Pipeline	4	48	48
23	Decision on whether *in vivo* rat brain MRS measurements of GABA/glutamate are achievable. [The decision was made that these measurements are not achievable]	3	48	48

No.	Milestone Name	WP no.	Due date	Actual date
24	Effectiveness of antidepressant treatment at reversing the decreased neurogenesis induced by maternal separation and chronic mild stress	4, 5	48	48
25	Identification of abnormalities of HPA feedback regulation shared by several models	3	48	48
26	Completion of direct citalopram phMRI in controls, depressed and remitted patients.	7	48	54
27	Decision on feasibility of Level 2 study in Budapest	7	48	48
28	BDNF, TPH1, TPH2, GR genotypes determined and related to behavioural measures in Longitudinal TWIN-study	2, 4, 7	48	54
29	NewMood Genelist for depression and antidepressant efficacy presented [The original Milestone 29 "Design of NewMood chip" is virtual and has been superseded by the NewMood Genelist]	2, 4, 5	54	60
30	Decisions on final detailed studies of mechanisms in selected models	All	54	54
31	Selection of parameters and models to be refined in core models	All	54	54
32	Genotyping completed for a group of subjects with well characterised brain responses to face emotion and emotional processing tasks	2, 4, 7	54	54
33	Correlation of the behavioural depression endophenotype of CB1 knockout mice with patterns of regional brain activity (cytochrome oxidase) and with respect to serotonin patterns (5HT release and transporter)	1, 3, 4, 5, 6	60	66
34	Validation replication of genes from the expression arrays (e.g. by RT-PCR or *in situ* hybridisation)	4	60	60
35	Correlation of the serotonergic deregulation of CB1 knockout mice with the differential gene expression of the $5HT_{2C}$ receptor	4	60	66
36	Determination of changes induced by chronic antidepressants on gene expression in CB1 knockout mice and validation by RT-PCR	4	60	60
37	The effect of galanin and galanin agonists/antagonists on depression-like behaviour and on expression of markers in dorsal raphe 5-HT neurons will be presented	1, 5	60	60
38	Integrated understanding of the role of HPA dysfunction across mouse and rat models.	3, 4, 5	60	66
39	Integrated understanding of raphe control mechanisms in core behavioural phonotype of depression in mouse and rat models	1, 3, 4, 5, 6, 7	60	66

Table 7. List of all Deliverables throughout the project

All the Deliverables have been accomplished. (Details on the achievement of each research Deliverable, is given in the relevant WP Deliverable document. Details of the achievement of the dissemination Deliverables is covered by the separate document - PUDK 2004-09).

Del. No.	Deliverable Name	WP no.	Due date	Actual date
			Project month	
1	Initial core behavioural data-set complete.	**1**,8	18	18
2	Initial NEWMOOD-1000 microarray data-set.	1,**4**	18	24
3	Two new mutant mice models ready for Core Data–set evaluation	**2**	18	36
4	Publications describing relation between behaviour/ neurochemistry /hormone. Total >25	All	18	18
5	First association study in study participants	2,**7**	18	18
6	Analysis of initial microarray data-set complete in 4 mouse models.	1,3,**4**	24	48
7	First conditional 5HT knock-outs available.	**2**	24	36
8	Core data-set of molecular HPA parameters across models	**1**,2,3,4,5	24	36
9	Micro-array identifies first genes common to animal models	**4**	24	60
10	Modified 18-month plan	8	18	N.A.
11 & 32b	Completed data-set on neurotransmitter endophenotype across several models.	**3**, 5,6	30	36
12	MCPP or citalopram pMRI and modulation of reward, aversion and cognition completed.	**7**	30	48
13	Initial analysis of data in collaborative community study (Level 1)	**7**	30	42
14 & 28a	Two Level III studies (fMRI, pMRI studies in participants) completed	**7**	30	48
15 & 31a	Three NEWMOOD reviews published (behaviour, 5HT regulation, neurogenesis)	All	36	60
16	Design of NEWMOOD 'Depression' chip agreed [The use of the Illumina whole genome chip has lead to this Deliverable becoming void]	All	36	N.A.
17	Review of molecular basis of changes in neurogenesis in NewMood models of vulnerability	**5**	30	48
18 & 31b	Illumina array microarray data-set in Core Models	1, 2, **4**	36	48
19	Identification of first candidate genes from NewMood / Illumina array	**4**	36	48
20	First array data from human post-mortem brain	**2**, 7	36	36
21	Long-loop and local control of 5-HT cell activity: physiology	5,**6**,7	36	36
22	Long-loop and local control of 5-HT cell activity: models of vulnerability	3,5,**6**	36	36
23	Neurophysiological responses in selected models	**3**, 4, 5, 6	36	36
24	Cellular responses in selected models	1, 4, **5**, 6	36	48
25a	Communications to international scientific meetings on behaviour/ 5HT regulation / neurogenesis neurophysiology/ neurochemistry/hormone	All	60	60
25b & 30a	Completed data on neurotransmitter regulation in the maternal separation model	**3**, 4, 6	42	48
26	Review of insights from NewMood models on control HPA in stress	**3**, 4	36	42
27a	Initial analysis of gene-environment data in collaborative community study (Level 1)	2,4, **7**	36	48
27b & 32a	Citalopram pMRI measuring 5-HT sensitivity complete in 15 controls and 15 remitted depressed subjects	**7**	42	48
28b	Brain maps of comparative oxidative metabolism in vulnerability to depression	**7**	42	60

Del. No.	Deliverable Name	WP no.	Due date	Actual date
			Project month	
29b	Changes in the acute effects of SSRI antidepressants in partial 5HT depletion models	**1**	42	42
30b	Completion of the pharmacological profiling of brain function that contributes to the imaging core data set in modified 5-HTT function models	**7**	42	48
31a & b	(see 15 and 18)			
32a & b	(see 27 and 11)			
33	Communications to international scientific meetings, publications	All	36	36
34	Changes in hippocampal gene expression in vulnerability models replicated in samples from depressed humans	**4**	48	60
35	Brain maps of 5-HT receptor function in modified 5-HTT function models	**7**	48	48
36 & 45	Characterization of sleep parameters in the rat model of partial serotonergic lesion during damage and partial recovery [Duplicate]	**6**	54	54
37	Validation of animal imaging model of 5-HT_{2C} long-loop feedback	**3**, 7	48	60
38	Role of the NPY system in neurogenesis will be available, including the effect of selective deletion of the two receptors	**5**	48	48
39	First association data in resistant depressed patients	**2**	48	60
40	Determination of effect of SSRI/5-HT_{2C} antagonist combinations on gene expression	**4**	48	60
41	The effect of galanin and galanin agonists/antagonists on depression-like behaviour and on expression of markers in dorsal raphe 5-HT neurons will be reported	**5**	48	48
42	Key results from neuropsychological study (Level 2, 300 subjects) available	**7**	54	54
43	Tryptophan hydroxylase mapping in raphe of core models presented	**5**	54	60
44	Microarray data on 5-HTT over-expressing and knock-out mice	**4**	54	60
45	(see 36)			
46	Behavioural data set on the effects of chronic social defeat	**1**	60	60
47	Molecular account of CB1 behavioural, monoamine and neuro-endocrine phenotype	**1**, 4	60	60
48	Data on transcript levels of galanin and galanin and its receptors in various brain regions	3, **5**, 6	60	60
49	Behavioural data-set for the effects of habenula lesions on 5-HT_{2C} mediated behaviour	**1**	60	60
50	Role of different G-protein-coupled receptors on the production of endocannabinoids relevant to depressive behaviour	**1**, 2, 4	60	60
51	Local and long-loop regulatory mechanisms of 5-HT system function	3, 5, **6**, 7	60	60
52	Brain maps of 5-HT receptor function in core mouse models	**7**	60	60
53	Effects of serotonergic damage in pregnant females on the development of the serotonergic system and behavioural phenotype of the offspring in adulthood	**1**, 3	60	60
54	Role of the CCK system in neurogenesis will be published	**5**	60	60
55	Association study and/or haplotype analysis of traits related to mood in the Hungarian cohort	**2**	60	54
56	Communications to international scientific meetings, publications	All	48	48

Workpackage 1 – Behaviour

1. Objectives

i. To determine the effects of classic and new antidepressants in animals exposed to acute and chronic behavioural models of depression
ii. To characterize emotional-like behaviour in genetically modified mice.
iii. To define the responses induced by classic and new antidepressants in mice with target mutations.

2. Progress towards the Objectives

Activity 1: Behavioural endophenotype of models of genetic, developmental and acquired vulnerability to depression.

The major objective of Activity 1 is the identification of core behavioural endophenotypes (anhedonia, fearfulness, helplessness) which occur in animal models of genetic and acquired vulnerability (1.1) in order to identify shared changes in regional brain activation (1.2) and gene expression (1.3) which mediate the influence of vulnerability on the core processes. Firstly, the behavioural characterisation of the Core Models was carried out and, secondly, it was performed the identification of changes in regional brain activation (1.2) and gene-expression (1.3), which are common to the models and which relate to particular component processes such as anhedonia or anxiety which vary in degree and sometimes direction, in the different models.

Further to the switching to the comprehensive Illumina array system, the identification of the first genes which respond to stress, to vulnerability and to treatment with antidepressant drugs has been completed in the last period year. These findings will be rapidly validated in humans because: i) all DNA has been collected; ii) volunteers and patient samples have been evaluated clinically and for endophenotypic markers of vulnerability (Activity 4) including fMRI; and iii) we have established our statistical analysis methods. Thus, as soon as a genotypic data is available, associations with disease and endophenotypic markers will rapidly follow.

Table 8. Core Models - behavioural endophenotyping

Genetic vulnerability	**Species**	**Partner**	**Anhed**	**Anxiety**	**Help-less**	**Reversal by ADs**	**Array done**
Cannabis receptor 1 knockout (CB1-/-)	Mouse	4,2	+	+	+	Yes	Yes
Glucocorticoid receptor antisense (GR-i)	Mouse	2	ns	ns	+	Yes	Yes
5-HT transporter models • 5-HTT knockout (5-HTT-/-) • 5-HTT overexpressing (5-HTT o/e)	 Mouse Mouse	 2,3,4,8 8,9	ns	ns/+ –	+/- +		 Yes Yes
Vesicular glutamate transporter (VGLUT1+/–)	Mouse	12	+	+	+	Yes	Yes
High/low swim test analgesia (HA/LA) [HA is the 'depressed' endophenotype]	Mouse	13	+	+	+	Yes	Yes
High/low exploration (novelty response) (HE / LE) [LE is the 'depressed' phenotype]	Rat	5	+	+	+	resistant	Yes
Acquired vulnerability							
Partial 5-HT depletion (PCA) + CMS	Rat	5	+	+	+	Yes	Yes
Maternal separation (MS)	Rat (♂) Rat (♀)	12	+ ns	+ ns	+ +	Yes Yes	Yes Yes
Prenatal stress (PS)	Rat	6	cms	+	+	Yes	Yes
Chronic Social Defeat (SD) [non-resilient]	Rat	5,9	ns	+	+	unknown	Yes

Anhed = anhedonia; Helpless = helplessness; ADs = Antidepressants; CMS = chronic mild stress; model vs wild-type (HE vs LE, HA vs LA): ns = no significant difference; + = increase; – = decrease;

1. *Models of genetic & constitutional vulnerability*

1.1. *Core models*

Cannabis receptor 1 knock-out mice (CB1-/-) ***Partner 4 (Barcelona)***

The behaviour of CB1-/- mice has been extensively characterised and they show a consistent depressive endophenotypic pattern; they show an increased response to acute and chronic stress exposure in behavioural models of anhedonia (chronic unpredictable mild stress), anxiety (elevated plus maze and lit/dark box) and helplessness (tail suspension test, TST, and forced swimming test, FST). Furthermore, they have altered corticosterone release after stress exposure and decreased expression of brain derived neurotrophic factor (BDNF) in the hippocampus in basal conditions and after acute or chronic exposure to stress, in comparison with control animals (Activity 3). In order to elucidate its regulatory mechanism, the expression of cAMP responsive element binding protein (CREB), and its activation (levels of phosphorylated CREB, pCREB) in the hippocampus of CB_1 knockout mice were studied. No differences in the activation of CREB (ratio pCREB/CREB) were found in basal conditions when compared CB1-/- mice to wild-type littermates. However, chronic stress induced an increase in the activation of CREB in wild-type animals that was not observed in CB1-/- mice, indicative of a possible mechanism of differential response to stress between genotypes. On the other hand, chronic treatment with fluoxetine (a selective serotonin reuptake inhibitor) ameliorates the depressive-like behaviour shown by CB1-/- mice in the TST and induced an increase in BDNF levels in the hippocampus of CB1-/- animals. In order to further investigate the role of CB1 cannabinoid receptor in the control of stress responses that are altered in CB1-/- mice, human recombinant BDNF was directly administered in the hippocampus of those animals, with a complete reversion of the depressive-like. These results suggest that CB1 cannabinoid receptor would not be involved in the BDNF related mechanisms of response to stress. However, this receptor seems to play a crucial role in the control of the HPA axis since the lack of CB1 cannabinoid receptor induces an increased response to stress and hippocampal alterations (Aso et al., 2008). The effects induced by nicotine, physostigmine and scopolamine were studied in CB1-/-and wild-type mice in the active avoidance paradigm. In addition, the effects of the pretreatment with the CB1 receptor antagonist rimonabant were evaluated on the responses induced by nicotine in the active avoidance and the object recognition tasks in wild-type mice. Nicotine did not modify the performance of CB1-/- and wild-type mice in this model, whereas scopolamine impaired the performance in both genotypes. Physostigmine increased the active avoidance performance in wild-type but not in CB1-/- mice. Rimonabant did not modify the performance in the active avoidance test, given alone or co-administered with nicotine. In contrast, nicotine enhanced the performance in the object recognition task but this response was attenuated by rimonabant co-administration. The different responses induced by nicotine in the active avoidance and object recognition task might be consequence of the distinct neurobiological substrate and cognitive responses evoked in these behavioural models. The present findings demonstrate that the effects of nicotine and physosotigmine are attenuated in the absence of CB1 receptor activity. However, scopolamine effects are independent from CB1 receptor activity. The cognitive responses induced by rimonabant in the active avoidance paradigm were different to those observed in CB1-/- mice. Moreover, we have demonstrated the correlation of the BDNF impairment with the behavioural endophenotype of CB1 knockouts since the local microinjection of BDNF in the hippocampus reversed the increased despair behaviour exhibited by the mutant mice in the Tail Suspension Test. These results highlight the important role played by neurotrophic factors on the manifestation of the depressive-like phenotype (see, Aso et al., 2008).

Glucocorticoid receptor antisense mice (GR-i) ***Partner 2 (Paris)***

The GR-i mouse is a genetic model of vulnerability to environmental factors such as stress and is of special relevance to assess how HPA axis dysfunction can contribute to trigger behavioural deficits associated with depression.

- No difference in saccharine intake was measured in GR-i versus their wild-type controls.
- GR-i mice exhibited a higher immobility score (+100%) than their wild-type controls, when recorded for a 6 min TST session.
- No changes was seen in anxiety behaviour has been analyzed using elevated plus maze.

The effects of stress on these parameters are under experimentation. Some of these data have been presented in different meetings (FENS, Païzanis et al, 2006, Society for Neuroscience, Païzanis et al, 2006) and a manuscript is currently in preparation for submission.

Serotonin transporter knock-out mice (*5-HTT-/-)* ***Partner 2 (Paris)***

In contrast to GR-i mice, 5-HTT-/- mice exhibited a lower immobility (-71.0 %) compared to WT control in the TST. This "anti-depressant-like" behaviour is in line with low immobility obtained after a fluoxetine treatment (-56.7 %) in C57BL/6J mice (on which these 5-HTT mice have been backcrossed). No modification in the *preference of sweet solution* was found in 5-HTT-/- mice. On the CD-1 genetic background, we confirmed, to a certain extent, that mutant mice lacking the 5-HTT exhibited enhanced responses to anxiogenic conditions. Thus, compared to their WT littermates, 5-HTT-/- mutant mice showed a decrease in the ratio of central/peripheral activity in the open-field test, which indicates an increased anxiety-like behaviour. However, 5-HTT-/- mutants exhibited no signs of increased anxiety in the light-dark box or the elevated plus-maze tests. As previously described for other genetic backgrounds, we observed a general reduction in exploratory locomotion for 5-HTT-/- mice with CD-1 background.

Mice over-expressing the human serotonin transporter gene (5-HTT o/e) ***Partner 9 (Oxford)***

A commonly occurring polymorphic variant of the human 5-HT transporter (5-HTT) gene, that increases 5-HTT expression, has been associated with reduced anxiety levels in human volunteers and patient populations. However, it is not known whether this linkage between genotype and anxiety relates to variation in 5-HTT expression, and consequent changes in 5-HT transmission. We have investigated this hypothesis by measuring the neurochemical and behavioural characteristics of a mouse genetically engineered to over-express the human 5-HTT. These transgenic mice showed the presence of 5-HTT mRNA in the midbrain raphe nuclei, as well as a 2-3 fold increase in 5-HTT binding sites in the raphe nuclei and a range of forebrain regions. The transgenic mice had reduced regional brain whole tissue levels of 5-HT and, in microdialysis experiments, decreased brain extracellular 5-HT which reversed on administration of the 5-HTT inhibitor, paroxetine. Compared to wild-type mice, the transgenic mice exhibited a low anxiety phenotype in a variety of anxiety tests including the elevated plus maze and hyponeophagia tests. Furthermore, in the plus maze test the low anxiety phenotype of the transgenic mice was reversed by acute administration of paroxetine, suggesting a direct link between the behaviour, 5-HTT overexpression and low extracellular 5-HT. ***Partner 9*** has extended the characterisation of the behavioural phenotype of this mouse model using anxiety paradigms (including conditioned fear) and measures of stress and learned helplessness (forced swim test). The data demonstrate that 5-HTT overexpressing mice have a low anxiety phenotype across a range of anxiety tests including conditioned fear, and demonstrate increased immobility in the forced swim test. Effects on sleep are described in WP6. *In toto*, these findings demonstrate that associations between increased 5-HTT expression and anxiety can be modelled in mice and may be specifically mediated by decreases in 5-HT transmission. ***Partner 9*** has also extended the behavioural characterization of these animals, to include the study of appetitive processes (satiety sequences) and spatial working memory (water maze). In both respects, the 5-HTT overexpressing mice were not different from wildtype controls. One implication of these data is that variation in 5-HTT expression does not have a significant impact on satiety. *Partner 9* commenced collaborate studies with ***Partner 3***

(Würzburg), investigating the behaviour of the 5-HT transporter overexpressing mice in comparison with 5-HT transporter knock out mice (especially, decision-making and effort based reward tasks). ***Partner 9***, in collaboration with ***Partner 2*** (Paris), studied sleep architecture in the 5-HTTo/e mice (WP1/6) and obtained preliminary evidence of exhibited lower levels of REM sleep, a phenotype opposite to that previously observed in 5-HTT-/- mice.

Vesicular glutamate transporter heterozygous knock-out mice (VGLUT1+/-) ***Partner 12 (Pamplona)***

The vesicular glutamate transporter (VGLUT1) is the major isoform in cerebral cortex and hippocampus, where it is selectively located on synaptic vesicles of excitatory glutamatergic terminals. We investigated the possible influence of a down regulation of the VGLUT1 transporter in anxiety, depressive-like behaviour and learning. The behavioural phenotype of adult VGLUT1 heterozygous knockout mice (male and female, C57/BL/6) was compared to wild type mice. VGLUT1+/- mice displayed normal spontaneous locomotor activity, increased anxiety in the light-dark exploration test and depressive-like behaviour in the forced swimming test. In the novel object recognition test, VGLUT1 heterozygous mice showed normal short-term but impaired long-term memory. These results, suggest that a presynaptic alteration of the glutamatergic transmission leads to a behavioural phenotype resembling some aspects of neuropsychiatric disorders (Tordera et al., 2006). Moreover, adult male VGLUT1+/- and wild type (WT) mice (C57BL/6) were exposed to unpredictable repeated mild stressors for six weeks. Anhedonic-like behaviour was evaluated by weekly monitoring of sucrose intake and a battery of behavioural tests was performed over the week after the end of stress (Elizalde et al., submitted). VGLUT1+/- mice exposed to CMS showed a higher vulnerability for anhedonic behaviour compared to WT mice. Moreover, VGLUT1+/- control mice showed lower levels of sucrose intake compared to WT. One week after the end of stress, both VGLUT1+/- control and CMS mice showed depressive behaviour compared to their corresponding WT groups. However, no significant differences across genotypes were observed in the CMS-induced anxious behaviour (elevated plus maze) and impaired recognition memory (novel object recognition). Repeated antidepressant treatment with imipramine reversed the CMS-induced anhedonic-like behaviour from the 5th week until the end. Imipramine showed also anti depressant activity in the FST both in control and CMS exposed mice and reverted recognition memory deficit (*Activity 5*). In summary, VGLUT1+/- control mice show a behavioural depressive-like profile (Tordera et al., 2007), which is comparable to that shown by WT exposed to CMS. Moreover, VGLUT1+/- mice exposed to CMS showed a higher vulnerability for anhedonic and depressive behaviour compared to WT, effects that were fully reverted by chronic imipramine, thus confirming its validity as a transgenic model of depression.

High and Low swim test analgesia mice (HA/LA) ***Partner 13 (Warsaw)***

Over the last 22 years mice have been selected for the magnitude of analgesia induced by 3-min swimming in 20°C water (swim stress-induced analgesia, SSIA) and developed high analgesia (HA) and low analgesia (LA) lines of mice (Panocka et al., 1986). HA mice, as compared with LA mice, are much more responsive to the analgesic effects of morphine and selective agonists of mu, delta and kappa opioid receptors and display a significantly higher level of mu-opioid receptor mRNA in the nucleus raphe magnus. The lines differ in a number of physiological responses, including anxiety- and depression-like behaviours. Partner 13 investigated whether there are differences between the mouse lines with respect to changes in sweetened milk intake induced by restraint. As a decreased intake of sweet solutions is considered a measure of anhedonia, a cardinal symptom of depression, the role of interaction between the genetically determined activity of the opioid system and stress in inducing anhedonia was studied. Restraint strongly depressed drinking and HA mice drank significantly less than LA mice. Moreover, HA/LA animals do not show major differences in response to CMS although the CMS procedure was shown to be reliable. As a link between alcoholism and depression has been suggested we

studied the effects of chronic mild stress (CMS) on the voluntary intake of 8% ethanol in the mouse lines displaying high (HA) or low (LA) swim stress-induced analgesia. Normally, 8% ethanol is aversive to rodents. We found that LA mice with the low opioid system activity exposed to CMS manifested greater ethanol intake than under no stress conditions. No such effect of CMS on ethanol consumption was observed in HA mice that display the enhanced opioid system activity. We conclude that CMS imposed on individuals with a genetically determined low opioid activity may favour the development of ethanol abuse and, possibly, susceptibility to depression under stress.

Low vs. High Exploration rats (HE/LE) - ***Partner 5 (Tartu)***

Low or high exploration in a novel environment appears to be trait-like characteristic in individual animals and is associated with features of the depression endophenotype. We have characterised this model further regarding behaviour, biochemistry (see Activity 2 and 3) and sensitivity to chronic variable stress. LE-rats (the "depressed" phenotype) did not differ in new home-cage activity which contrasts to their low activity in the exploration box and elevated plus-maze. In contrast to stronger sensitization to amphetamine in LE-rats, no difference was found between LE- and HE-rats in cocaine-induced place preference, but in LE-rats the extinction of preference upon repeated testing was faster, which is similar to their faster extinction of conditioned fear. Chronic variable stress elicited a larger decrease in sucrose intake in the LE-rats; this effect was also more persistent as in the HE-rats partial adaptation to this effect of stress. We conclude that LE-rats are more vulnerable to chronic stress and have, besides, higher anxiety, a different cognitive profile which may contribute to their passive strategy in fear-provoking novel environments. An alternative test was developed, and further used (see Activity 3.2), for predicting the LE/HE phenotype, as preliminary experiments demonstrated the effect of CRF_1 receptor blockade in LE-HE-rats being influenced by previous experience of the testing environment.

1.2. *Other models of genetic & constitutional vulnerability*

We have also analysed and behaviourally characterised other models of genetic & constitutional vulnerability of depression. After the full behavioural characterisation of these models, they were not included in the list of core models (see above). The main additional models that we have characterised in this project are briefly described below.

Galanin and galanin receptor overexpressing mice - ***Partner 10 (Stockholm)***

Galanin is co-expressed with 5HT in neurones of the dorsal raphe nucleus. Previously reported experiments (WP5) indicate that galanin receptors (GalR) interfere with 5HT release and that the galanin receptor is a target for antidepressant drug development. Mice modified to overproduce galanin or one of its receptors show evidence of increased immobility in the forced swim test. We have continued the analysis of our transgenic mice, now focusing on the line overexpressing GalR2 under the PDGF-B promoter with an EGFP construct. They exhibit a decreased immobilization time in the forced swim test. No certain difference between wild-type and transgenic was observed in the elevated plus maze test or in the activity box. Histochemical analysis indicates overexpression of GalR2 in particular in cortical areas with high expression in the retrosplenial cortex as well as in ventral cortical areas, whereas no signal was observed in the hippocampus. This confirms our and others findings that the GalR2 is 'antidepressive' and further underlines that a GalR2 agonist is a potential treatment of depression.

Individual differences in sociability & reward - ***Partner 5 (Tartu)***

LS/HS rats: animals with persistently different sociability. We have characterised this model regarding sensitivity to chronic variable stress. In contrast to LE- vs. HE-rats, in which case the more anxious and "depressed" LE-rats have higher anhedonic response to stress, the initially less

anxious HS-rats were more sensitive to stress than LS-rats as measured by the decrease in sucrose intake. HS-rats also had a higher number of defecations during immobilization stress than LS-rats. Conclusively, animals with high trait sociability are more vulnerable to stress in conditions of single housing, suggesting that social behaviour may in such animals serve as a protective factor against negative emotionality. The effects of chronic variable stress on reward, aversion and cognition have been studied in this vulnerability model. HS-rats, while less anxious/depressed at baseline, are more sensitive, in terms of reduction in sucrose intake, to the anhedonia-eliciting effect of chronic stress (Tõnissaar *et al, Neuroscience* 2008, 152: 867-876). This may be, however, because of their dependence on social interactions is higher and the experiments were carried out with single housed animals. The initially more anxious LS-rats develop during chronic variable stress a number of behavioural changes that resemble increased impulsivity and may reflect alterations in cognitive processing.

LC/HC rats: animals with persistently different expression of positive emotions. HC animals have high levels of 50 kHz ultrasonic vocalizations (USVs) associated with pleasurable states. We have extended their behavioural characterization in both male and female animals, and also compared the sensitivity to chronic variable stress. An individually stable and characteristic level of 50-kHz chirps was reached after two weeks of manipulation in three independent experiments. 22-kHz USVs, revealing negative affective states, were less frequent and did not correlate with 50-kHz USVs. Further behavioural tests were always carried out after the animals had reached adulthood (60 days). HC-animals of both sexes had less anxiety in the elevated plus-maze and foot-shock induced freezing in the fear-conditioning paradigm, as compared to not handled controls. In contrast, in the exploration box test the HC-rats are less active. Chronic variable stress regime reduced sucrose preference more in the LC-rats, this effect being significant only in females. Conclusively, the low positive emotionality LC-rats appear to be more anxious but have stronger approach behaviour, and female LC-rats are more prone to anhedonia elicited by chronic stress.

Reward sensitivity – persistent differences in sucrose intake. Measurement of sucrose intake or preference is in widespread use for predicting sensitivity to rewards, but limited information is available about the consistency of individual sucrose intake or preference. We have measured this in free-feeding rats during both the dark and light phases. Altogether eight two-bottle tests were carried out intermittently during light and dark phase. Intake and preference of sucrose during the dark phase was significantly higher compared to the light phase, and sucrose intake, but not preference was individually very consistent across different tests, especially during the dark phase. Thus, sucrose intake is an individually stable trait, especially when measured during the dark phase, and animals with different reward sensitivity as measured by sucrose intake should be further examined in tests of aversion and cognition. Furthermore, this phenotype is related to the function of dopamine D_2 receptors in the nucleus accumbens (see Activity 2.3). Animals with low baseline sucrose intake become more anxious after stress as shown in the open field test whereas sucrose intake became similar in the high and low intake animals during chronic exposure to stress.

Hypomorphic tryptophan hydroxylase-2 mice (TPH2) - ***Partner 3 (Würzburg)***

TPH2 hypomorphic mice have been generated with reduced 5-HT synthesis. They showed decrease anxiety-like behaviour which was dependent on maternal behaviour. In addition, preliminary results in heterozygous 5HTT+/- mice carrying one hypomorphic $TPH2^{447R}$ allele and exposure to adverse rearing conditions revealed penetration of a latent anxiety-like phenotype. The identification of non-synonymous polymorphisms in the murine *Tph2* allowed us to generate a mouse strain with reduced 5-HT synthesis and to study epigenetic programming in a mouse model. These Tph2 hypomorphic mice showed decreased anxiety-like behaviour which was dependent on maternal behaviour. In addition, preliminary results in heterozygous *HTT*+/-

mice carrying one hypomorphic Tph2^{447R} allele and exposed to adverse rearing conditions revealed penetration of a latent anxiety-like phenotype (Carola et al., in preparation).

Nitric oxide synthase-I (NOS-I) knockout mice - ***Partner 3 (Würzburg)***

Nitric oxide (NO) in the brain is formed by nitric oxide synthase-I (NOS-I). A considerable body of evidence implicated NO in fear conditioning, stress response and depression-like behaviour of animals: pharmacological inhibition of NOS-I, for example, has antidepressant and anxiolytic properties. *NOS3* (the gene for the so-called "endothelial isoform") knockout mice likewise show an antidepressant phenotype. Mice in which the *Nos1* gene has been partially deleted (to a remaining expression level of ca. 6%) by disruption of exon 2 show overtly aggressive behaviour; however, specific behavioural assessment with regards to depression-like behaviour has not yet been systematically undertaken, and neither has the neuronal expression profile of these animals been investigated. The results of our study indicate, in conclusion, despite convincing pharmacological evidence, Nos1 knockout mice did *not* display anti-depressant behaviour. In contrast, they showed only subtle abnormalities in activity and cognition. Expression profiling with the NewMood chip revealed several regulated genes, some of which will undergo further investigation by quantitative RT-PCR and *in situ* hybridisation. The model will serve as a positive control for the selected models of depression vulnerability.

Arginine vasopressin deficient (Brattleboro) rats - ***Partner 7/14 (Budapest)***

Increased sucrose preference, decreased helplessness and decreased anxiety have been found in Brattleboro rats in basal conditions compared to +/- controls. These data provide evidence for decreased depression-like behaviour in these animals. The effects of chronic mild stress (CMS) were characterized in Brattleboro rats. CMS decreased food and water intake, increased immobility and anxiety in the elevated plus maze in both Brattleboro and control rats. Some behavioural and physiological (body weight change and corticosterone secretion) effects of CMS were altered in Brattleboro rats, and these data together with those found at baseline conditions in acute challenges led us to the conclusion that vasopressin indeed is an important factor in the mediation of the behavioural effects of chronic stress. That means, in general, that these animals show less behavioural signs of depression compared to +/- controls. We concluded that arginine vasopressin deficiency is a protective factor against the development of depression.

New TRKC overexpressing mouse ***Partner 3 (Würzburg)***

Accumulating evidence suggest that neurotrophins participate in the pathophysiology of mood disorders. We have studied the role of such molecules by means of transgenic mice overexpressing the full-length neurotrophin-3 receptor TrkC (TgNTRK3) in the central nervous system. TgNTRK3 mice show increased anxiety-like behaviour and enhancement of panic reaction in the mouse defence test battery, along with an increase in the number and density of catecholaminergic (tyrosine hydroxylase positive) neurons in locus coeruleus and substantia nigra. Furthermore, treatment of TgNTRK3 mice with diazepam significantly attenuated the anxiety-like behaviours in the elevated plus maze. These results provide evidence for the involvement of TrkC in the development of noradrenergic neurons in the central nervous system with consequences on anxiety-like behaviour and panic reaction. Thus, changes in TrkC expression levels could contribute to the phenotypic expression of panic disorder through a trophic effect on noradrenergic neurons in the locus coeruleus. The elevated NT3- TrkC tone via overexpression of TrkC in the brain may constitute an underlying molecular/neuronal mechanism for the expression of anxiety.

5-HTT (S) / BDNF (B) double knockout mice. ***Partners 2 (Paris) and 3 (Würzburg)***

A colony of 5-HTT / BDNF (SB) double knockout mice has been established. With the first mice we obtained, immobility using TST in heterozygous S+/-B+/- mice and compared to their respective WT controls (S+/+B+/-, S+/-B+/+, S+/+B+/+) was measured. Immobility in TST was

measured in S+/-B+/- heterozygous mice compared to their respective wild-type controls (S+/+B+/-, S+/-B+/+, S+/+B+/+). No modification of despair like behaviour was observed in these heterozygous mice. Future experiments have to be done using homozygous 5-HTT and heterozygous BDNF (S-/-B+/-) mice, and their controls, as soon as the mutated colony will be large enough to handle these experiments. Studies on 5-HT_{1A} regulation in SB double knockout mice in comparison with their respective controls (single knockout mice) are being carried out using *in vitro* electrophysiological approaches.

2. *Models of acquired vulnerability*

2.1. *Core models*

Partial 5-HT denervation + chronic mild stress - ***Partner 5 (Tartu)***

This model has been fully characterised and analysis of the effect of specific stressors and their order of presentation has been carried out to develop a procedure with controlled degree of adaptive response to repeated stressors in the chronic variable/mild stress paradigm. The model has also been characterised for regional brain metabolism (see 1.2).

Maternal separation (MS) in rats - ***Partner 12 (Pamplona)***

In a widely used model of depression, the forced swimming test, MS produced a significant increase in immobility both in males and females. Sucrose intake (sucrose consumption / rat weight) was also reduced in male, not in female, MS rats as compared with controls, a sign of anhedonia. In the plus-maze, male MS rats, but not female rats, showed increased anxiety-like behaviour. Consequently, neonatal MS in the rat can be considered as an animal model of vulnerability to development of a depression-like syndrome, although there are some differences in the endophenotype of male /female MS rats (manuscript in preparation). MS male rats exhibited a significant cognitive impairment in two different learning tasks (Aisa et al., 2008). In the acquisition phase of the Morris water maze task, there was no significant effect of rearing in the latency to find the platform. However, rearing produced a statistically significant impairment in the retention phase. Time spent by animals searching for the platform was significantly lower in the MS group compared to control rats. In the novel object recognition test, MS rats showed a learning impairment. None of the experimental groups differ in their spontaneous locomotor activity in an open-field.

Prenatal stress & gender differences - ***Partner 6 (Maastricht)***

Exposure to physical and/or psychological stress in pregnancy has been implicated in the pathogenesis of mood disorders in offspring. We studied the increased vulnerability to depression in later life in the prenatally-stressed offspring of Fischer 344 rats. In those studies we found that the impact of prenatal stress heavily depends on the genetic background, i.e. stain, of rats used (Van den Hove et al. 2005, see also activity 1.2). Therefore, we also examined the effects of prenatal stress on adult anxiety- and depression-related behaviour in Sprague-Dawley male and female rats. Prenatal stress was associated with increased anxiety, in male, but not female adult offspring. Likewise, depression-related behaviour was increased in male prenatally-stressed rats only. Male offspring further showed increased basal plasma corticosterone levels, whereas both prenatally-stressed males and females failed to show an adequate response to stress with lower stress-induced corticosterone levels as compared to controls. Female hippocampal weight was relatively higher after prenatal stress, which may explain the absence of behavioural effects of prenatal stress in this gender. Thus, surprisingly, prenatal stress probably affects the male offspring more than the female offspring with respect to affective behaviour. These studies test the hypothesis that psychological stress in pregnancy increases vulnerability to mood disorders in later life possibly by stress hormone effects or by epigenetic mechanisms. In addition, we investigated the effect of chronic mild stress (a 'second hit') during adult life on anxiety- and

depression-related behaviour in both prenatally-stressed and control rats. Prenatal stress increased anxiety-related behaviour (elevated zero maze) and depression-related behaviour (forced swim test). Acute stress-induced plasma corticosterone secretion was also increased. Interestingly, we found an age-related gender-specific effect of prenatal stress, i.e. females are more vulnerable at a younger age. Depression-like symptoms in the sucrose intake test and forced swim test were increased after chronic mild stress.

Social defeat in rats - ***Partners 9 (Oxford) and 5 (Tartu)***

Social defeat is a major natural stressor in animals and a source of depression, anxiety, low self esteem, and social withdrawal in humans (e.g. bullying). The model is based on exposure of animal to social defeat and continuous contact with aggressor, and reportedly produces long lasting social aversion, anhedonia, loss of biological rhythms, reduced feeding and weight loss. In experiments to date in Oxford (PhD student from Tartu, Ms Kadri Kõiv), intruder rats demonstrated submissive behavioural postures and ultra-sonic vocalisations in response exposure to an aggressive resident rat. Chronically exposed intruder rats showed reduced weight gain and reduced sucrose consumption. While the Oxford group has attempted to increase the aggressive behaviour of residents by housing them in close neighbourhood with female animals, the Tartu group has used pharmacological means, the apomorphine sensitization aggressiveness. Analysis of other behavioural and physiological measures (social aversion, anxiety, learned helplessness, plasma corticosterone etc) are ongoing. The outcome of these experiments will help determine whether regional brain tissue from the social defeat rats (which is collected) is subject to microarray analysis.

2.1. Other models of acquired vulnerability

We have also analysed and behaviourally characterised other models of acquired vulnerability of depression. After the full behavioural characterisation of these models, they were not included in the list of core models (see above). The main additional models that we have characterised in this project are briefly described below.

Repeated MDMA ***Partner 2 (Paris)***

As in the case of alcohol abuse, MDMA (ecstasy) is known to drive severe depressive disorders, particularly in the young population. In order to further explore the mechanisms which are linked to this risk, we treated for 4 days mice with MDMA and explored different parameters 21 days after the treatment. TST experiments did not reveal any despair behaviour after this delay, although in this model, such as in the alcohol model, the alteration of the 5-HT system can be observed (see activity 2). In addition, and in collaboration with Partner 4 (Barcelona), MDMA self administration behaviour was investigated in 5-HTT knock-out mice. These experiments showed that MDMA self-administration was abolished when the 5-HTT was inactivated. We also evaluated the delayed effects of MDMA exposure on the 5-HT system, using *in vitro* and *in vivo* approaches in both 5-HTT wild-type and knock-out mice. Acute MDMA in vitro application on slices of the dorsal raphe nucleus (DRN) induced concentration-dependent 5-HT release and 5-HT cell firing inhibition. Four weeks after MDMA administration (20 mg/kg, b.i.d for 4 days), a 2-fold increase in the potency of the 5-HT_{1A} receptor agonist ipsapirone to inhibit the discharge of DRN 5-HT neurons and a larger hypothermic response to 8-OH-DPAT were observed in MDMA- compared to saline-treated mice. In addition, long term MDMA treatment also induced increased immobility duration in the forced swim test suggesting a depressive-like behaviour induced by MDMA treatment. All these effects were abolished in 5-HTT -/- knock-out mice (Renoir et al., 2008).

MDMA partial 5HT lesion. ***Partners 7/14 & 8 (Budapest and Edinburgh)***

Behavioural phenotyping has been continued after long-term partial lesion of the serotonergic system in rats. Increased impulsivity, motor activation, without differences in peaceful social interaction and overt aggressive behaviour were observed. These data provide evidence that a partial decrease in 5-HT content in the neocortex provides a unique behavioural profile with some but not other symptoms similar to depression in humans (Kirilly et al., 2005; Ando et al., in press) (WP1) (collaboration: Partner 7/14 and Partner 8).

Prenatal stress; offspring of MDMA treated mothers. ***Partners 7/14 & 8 (Budapest & Edinburgh)***

Body weight at birth and thereafter is decreased in offspring of mothers treated three times with a single low dose of MDMA over gestation. Behavioural effects are have been analysed.

Prenatal mercury neurotoxicity - ***Partner 10 (Stockholm)***

Initial experiments have focused on the effect of an environmental contaminant, methal mercury, given to pregnant female mice. Using a novel behavioural analysis system, the Intellicage, offspring were found to have lower sucrose preference than matching controls and longer immobility time in the forced swimming test, suggesting that the system can monitor depression-like behaviour (Onishchenko et al., 2006).

Bacterial endotoxin sickness behaviour mode - ***Partner 13 (Warsaw)***

Lipopolysaccharide (LPS) evokes an adaptive sickness behaviour in rodents together changes in stress hormones and serotonergic and noradrenergic function. However, LPS injection had no differential effects in the high (HA) or low (LA) swim stress-induced analgesia strains on various measures of emotional reactivity including the acoustic startle reflex and prepulse inhibition despite the fact that LPS produced robust sickness behaviour, as evidenced by a decrease in locomotion and body weight, and an increase in corticosterone concentration.

Comparison of different stressors and responses in rats and mice - ***Partner 13 (Warsaw)***

The effects of chronic footshock or restraint on the behavioural responses to acute stressors and corticotropin-releasing factor (CRF) were studied in rats and mice. In rats (male Harlan Sprague-Dawley rats) chronic footshock decreased acute footshock-induced freezing and ultrasonic vocalization (measures of anxiety), but it increased context conditioned freezing and ultrasonic vocalization. Intracerebroventricular (icv) corticotropin-releasing factor (CRF) increased conditioned freezing and vocalization but only in non-chronically stressed rats (the finding may suggest desensitization of CRF receptors by chronic stress). In rats, in the forced swim test (FST), chronic footshock did not induce consistent effects, although there was a trend for increased immobility (floating). By contrast, chronic restraint consistently decreased floating. Intracerebroventricular (Icv) CRF increased floating, an effect akin to that observed after chronic footshock. In adult male CD-1 mice (like in rats), chronic footshock significantly increased the time spent floating in the FST. In mice, in the tail suspension test (TST), chronic footshock did not alter immobility. Thus chronic footshock induced depression-like activity in the FST, but acute footshock or restraint immediately before testing induced antidepressant-like effects in both the TST and the FST. In mice, icv CRF consistently decreased immobility in the TST and the FST, with a significant effect at the 100 ng dose, thus mimicking the effects of the acute footshock or restraint. CRF depressed activity in the open field, so these effects are unlikely to reflect a change in overall activity. Therefore, chronic footshock increased immobility in the FST in both species, whereas icv administration of CRF produced opposite effects in the FST. It is concluded that although many behavioural and neurochemical responses in stress are similar in both species, caution should be exercised in extrapolating the results of experiments with CRF from rats to mice and vice versa (Dunn and Swiergiel, 2008).

3. *Revealing vulnerability: stress paradigms*

The concept of vulnerability implies a trait which may be hidden until circumstances reveal it. A full valuation of rodent models of vulnerability requires that some environmental manipulation is applied to reveal the predisposition. Two partners are standardising chronic mild and ultra mild stress paradigms (see below) as one approach to revealing vulnerability. The next section describes another approach using 5HT depletion.

Behavioural phenotype of mice exposed to chronic mild stress (CMS) - ***Partner 12 (Pamplona)***

Adult male C57BL/6 mice were exposed to unpredictable repeated mild stressors applied for 6 weeks. Anhedonic behaviour was evaluated by weekly monitorization of sucrose intake. Paroxetine (10 mg/kg) or saline were daily administered for the last three weeks of the CMS and treatment was continued for two weeks thereafter. A battery of behavioural tests, including motor activity, compulsivity (marble burying), anxiety (light-dark exploration test), depression (forced swimming test: FST) and memory test (novel object recognition), was carried out over the last week of the CMS and one month after the last stress. Mice exposed to CMS showed anhedonia (decrease in sucrose intake) from the third week until the end of the procedure. However, the effect of paroxetine could not be evaluated due to an intrinsic effect of this antidepressant on sucrose intake. Over the last week, mice exposed to CMS displayed depressive behaviour (increased immobility time in FST) and impaired memory in the object recognition test. Repeated paroxetine produced an antidepressant-like effect in the FST in control mice but failed to prevent the depressive-like behaviour and memory deficits in CMS mice. One month after the end of the test, saline treated CMS mice still showed increased immobility time in the FST. The depressive-like behaviour was reversed in paroxetine treated CMS mice. These results suggest that this CMS procedure leads to a long term behavioural profile that parallels the chronic symptoms of depression.

Chronic Ultra Mild Stress (CUMS) - ***Partner 2 (Paris)***

The chronic ultra mild stress we are applying on mice is an adaptation of the chronic mild stress protocol described originally by P. Wilner. This chronic ultra mild stress induced after a three week session, in both C57BL/6J mice and DBA/2J mice, a reduction in *TST* immobility (respectively -27.7 and -61.5%), further corroborating the increase in impulsivity already described after a similar stress applied in normal and healthy mice. In addition, chronic ultra mild stress induced by itself a decrease in saccharine intake (~-60 and -65% for C57BL/6J and DBA/2J, respectively), suggesting that CMS could induce anhedonia. Despair behaviour was measured using TST after a four week ultra-mild stress session, in the two mouse strains. In both C57BL/6J and DBA/2J mice, stress induced a reduction of immobility (respectively, ~-25 and -50%). The technique may be a more sensitive way of detecting vulnerability.

5HT depletion/Acute Tryptophan Depletion (ATD) challenge in mice - ***Partner 6 (Maastricht)***

Acute Tryptophan Depletion (ATD) is a way of depleting the circulation of tryptophan and removing the precursor for 5HT synthesis from the brain. In humans, symptoms of depression can be evoked by ATD but only where there is pre-existing vulnerability – e.g. previous illness, genetic risk, and 5-HTT risk allele. The ATD challenge method has been fully established in rats, but not in mice. In this study we used the chronic mild stress (CMS) paradigm in the C57BL/6J mouse as a model of depression to test the hypothesis that CMS mice would be more sensitive to ATD. CMS increased plasma baseline CORT levels and decreased body weights after 3 weeks of stress, whereas anxiety and depression-like behaviour was not affected. After 6 weeks of CMS, anxiety was still not affected, yet depression-like behaviour was increased. ATD also appeared to increase immobility in control mice. Male C57BL/6J mice were orally administered (2x15ml/kg, 60 min interval) with a protein-carbohydrate mixture, either with (TRP+ group) or without (TRP-

group) TRP. One hour after the first administration a 64% depletion of plasma TRP levels was found in the TRP- group, which was still 64% one hour after the second administration. This indicates that the ATD is at its lowest level. In contrast, TRP levels remained the same in the TRP+ group. However, 5-HT levels were not affected in the hippocampus. Yet, 5-HIAA was decreased in the TRP- group, indicating that 5-HT turnover is decreased, probably as a compensatory mechanism. ATD did not have a differential effect in CMS mice, possibly due to a ceiling effect.

Chronic alcohol in mice - ***Partner 2 (Paris)***

The purpose of this study was to evaluate whether alcohol consumption during the "adolescent" period could modify the spontaneous alcohol preference at adult age in two mouse strains, C57BL/6J and DBA/2J, with marked differences in native alcohol preference. To this aim, 3-4 week-old (PND 26) male C57BL/6J and DBA/2J mice were subjected to an intragastric ingestion of various doses (1, 2.5 and 5 g/kg) of ethanol for 5 or 15 days. They were then exposed to a progressive ethanol intake procedure, in a free choice paradigm (3-10% alcohol in water, versus tap water, 21 days) at adult age (8-10 week-old). In C57BL/6J mice, the early ethanol administration did not significantly modify their spontaneously high alcohol consumption at adult age, except when ethanol 1 mg/kg was administered for 5 days (PND 26 to PND 30), which caused a slight decrease (-23.5%) of alcohol consumption. Conversely, DBA/2J mice, which normally have no preference for alcohol, drank significantly more alcohol when they have been given ethanol during the "adolescent" period (between +33% and +185%, depending on the dose and duration of early ethanol administration). These results show that an early ethanol exposure can exert a permissive control on alcohol intake. However, the differences between C57BL/6J and DBA/2J mice indicate that such modulations of alcohol intake are under genetic control, which will be further studied using gene targeted mutated mice.

Extra-dimensional (*ED) shifting in rats.* ***Partner 9 (Oxford)***

Impairments in executive function are strongly linked to the cognitive deficits of psychiatric disorders such as major depression. Executive function is dependent on the prefrontal cortex and can be modelled in animals and humans by measuring the ability to shift attention from one dimension of a complex perceptual stimulus to another, the extra-dimensional (ED) shift. In the last reporting period, Partner 9 developed a model of ED shifting in rats (WP1) and used functional imaging methods (Fos expression) to show that this cognition caused prefrontal cortex activation (WP5 and WP7). The effect of set shifting of pharmacological challenges which target 5-HT receptors, and especially the 5-HT_6 receptor subtype which is implicated in the modulation of cognition in recent studies was investigated. Experiments showed that a selective 5-HT_6 agonist (WAY 181187) facilitated extra-dimensional (ED) set shifting and this effect was blocked by pretreatment with a selective 5-HT_6 antagonist (SB 399885). The agonist had no effect on other non-ED phases of the task (e.g. intra-dimensional set shifting) and enhanced ED set shifting even when administered after the attentional set had been acquired, thereby ruling out impairments in attentional set formation. Moreover, the agonist evoked Fos expression in the prefrontal cortex. These findings comprise the first evidence that ED set shifting can be enhanced by 5-HT, and specifically through activation of 5-HT_6 receptors.

Activity 5: Testing classic and new molecules on the molecular gene expression targets, identified through Activities 1-4.

5.1 Effects of SSRI and NARI treatments

Effects on behavioural, neurotransmitter and neuroendocrine endophenotypes

Core model. GR-i mice - ***Partner 2 (Paris)***

The effects of agomelatine, a novel antidepressant with melatonin receptor agonist and 5-HT_{2C} receptor antagonist properties, has been evaluated in GR-i mice and compared with the reference compound fluoxetine. Both antidepressants reduced immobility in the TST. However, this effect was reached after acute treatment in the case of fluoxetine (30 mg/kg) and only after chronic administration (50 mg/kg, 21 days) in the case of agomelatine (Paizanis et al., 2009). Both antidepressants raised BDNF mRNA expression in the hippocampus of the transgenic mice, restoring mRNA expression up to wild-type levels. However agomelatine, but not fluoxetine, increased GR expression in the hippocampus of GR-i transgenic mice, so that GR mRNA levels in these mice were the same than in wild-type mice (Païzanis et al, 2009).

Core model. Maternal separation (MS) in rats - ***Partner 12 (Pamplona)***

The effect of chronic treatment with two antidepressants (fluoxetine and venlafaxine) on the behavioural deficit induced by maternal separation has been studied. Acute administration of venlafaxine, but not fluoxetine, was able to revert the depressive-like phenotype (increased immobility in the Porsolt forced swim test) and cognitive deficits (novel object recognition test). Chronic (2 weeks) administration of both antidepressants was able to revert the depressive-like phenotype (increased immobility in the Porsolt forced swim test) and cognitive deficits (novel object recognition test). In addition, on the basis of the effects of MS on cognition and the effects of stress on aging, we hypothesize that MS in rats may contribute to cognitive deficits associated to aging. Therefore, the effects of MS on the behavioural phenotype of aged rats (18-24 months old) have been studied. In close analogy with adult rats (3 months old), we have found a depressive-like phenotype (increased immobility time in the Porsolt forced swim test) and cognitive deficits (novel object recognition test) in aged rats.

Core model. HE- and LE- rats - ***Partner 5 (Tartu)***

While the effects of citalopram on behavioural changes elicited by partial serotonergic denervation, chronic variable stress, and their combination have been reported (Tõnissaar *et al*, 2008), similar experiments in other models have lagged behind the schedule, but several are now in progress. Partner 5 has focused on the reversibility of the defining phenotype in the LE/HE-model. It was found that this phenotype is resistant to chronic stress, and that different methods of antidepressant treatment, from intraperitoneal imipramine to reboxetine given by osmotic minipumps, can not substantially change the LE-phenotype. Long-term experiments attenuate the differences in behavioural tests of anhedonia and helplessness and anxiety between LE- and HE-rats, so the efficacy of antidepressants is clouded by the "placebo response" as is the case in clinical studies. We have now strong evidence that the LE-rats can indeed be made similar to HE-rats in terms of the defining "depressed" phenotype, using a hypothesis based approach that combines classic and novel psychopharmacological and neurobiological techniques. Thus the LE/HE-model appears as a very promising model to screen for antidepressants with much higher efficacy.

Galanin and depression-related phenotypes - ***Partner 10 (Stockholm)***

The work has focused on the differential role of galanin receptors in the regulation of depression-like behaviour and monoamine/stress related genes in the dorsal raphe and is now published (Kuteeva et al., 2008). Galanin, the GalR1 agonist M617 or the GalR2 antagonist M871

increased, while the GalR2/R3 agonist ARM1896 decreased immobility time compared to controls. Galanin transcript levels were increased in LC, but not in DR, and tyrosine hydroxylase in LC following injection plus swim stress in saline and fluoxotine treated rats. TPH2 were not affected. These results support that GalR1 receptors mediate prodepressive and GalR2 antidepressant effects of galanin. This is indicated by work with a GalR2-OE mouse that shows decreased immobility time in the forced swim test. This mouse is now further investigated in various behavioural paradigms as well as being explored with regard to expression of GalR2, both with regard to levels and anatomical localization.

MDMA-induced partial serotonergic lesion - ***Partner 7/14 (Budapest)***

Chronic SNRI treatments were performed in the partial serotonergic lesion model by Partner 14. Behavioural and HPA parameters were measured and RNA was extracted according to the harmonized methods. Dark-Agouti rats expressed very high baseline anxiety and helplessness compared to other rat strains, but anhedonia measured by sucrose-preference test was normal. MDMA in parallel with a significant (30-40%) loss in 5-HT axons and terminals increased anhedonia, but decreased anxiety and helplessness in these rats. Some of these alterations were reversed by chronic treatment with the serotonin-norepinephrine-selective reuptake inhibitor venlafaxine. RNA measurements were performed and are currently being analysed.

Overview

1. The objectives have been met:
   i) Eight core animal models for genetic and constitutional vulnerability for depression have been fully characterised at the behavioural, molecular and histochemical level.
   ii) Three core animal models for acquired vulnerability to depression have been fully characterised at the behavioural, molecular and histochemical level
   iii) Several non-core models for genetic and constitutional (six) and acquired (six) vulnerability for depression have also been studied.
   iv) Several classical antidepressants and new potential antidepressants, namely galanin agonists and agomelatine, with novel actions, have been studied and shown to reverse the depressive behavioural phenotype of the core models.

2. To better mirror the stress-diathesis features of depression and to model depressive state, six different stress paradigms, including chronic mild stress, 5HT depletion/acute tryptophan depletion, chronic alcohol, social defeat and extra-dimensional shifting have been studied in most of the core models of vulnerability.

3. A number of sex differences in the models are apparent and their molecular basis has been investigated especially in WP 4.

4. The identification of changes in regional brain activation and gene-expression, which are common to the models, particularly in component processes such as anhedonia or anxiety, have been carried out.

Table 9. Deliverables and milestones for Workpackage 1

All the Milestones and Deliverables involved with WP1 have been achieved. For detailed information about the Deliverables, please see separate report ("Deliverables WP 1: Behaviour"). Below is a list of all Deliverables and Milestones to which WP1 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name**	**Date due**	**Actual date**
1	Initial core behavioural data-set complete and database constructed & populated	18	18
8	Core data-set of molecular HPA parameters across models	24	36
29b	Changes in the acute effects of SSRI antidepressants in partial 5HT depletion models	42	42
46	Behavioural data set on the effects of chronic social defeat	60	60
47	Molecular account of CB1 behavioural, monoamine and neuro-endocrine phenotype	60	60
49	Behavioural data-set for the effects of habenula lesions on 5-HT_{2C} mediated behaviour	60	60
50	Role of different G-protein-coupled receptors on the production of endocannabinoids relevant to depressive behaviour	60	60
53	Effects of serotonergic damage in pregnant females on the development of the serotonergic system and behavioural phenotype of the offspring in adulthood	60	60
Deliverable No. (part-contribution)			
2, 4, 6, 15 & 31, 18 & 31, 24, 25a, 33, 56	(various)	18 - 60	18 - 66
Milestone No. (main contributor)	**Milestone name**	**Date due**	**Actual date**
8	Decisions on new Activities on basis of promising findings, e.g. role of vulnerability models in addiction.	30	30
Milestone No. (part-contribution)			
7, 9, 20. 21, 30, 31, 33, 37, 39	(various)	18 - 60	18 - 66

Workpackage 2 - Genomics - animal and human

1. Objectives

The general goal is to enhance the basic understanding of factors controlling:

i. the development of serotonergic (5-HT) neurones and
ii. the morphogenic effects of 5-HT on target cells.

By developing the knowledge base, tools, and resources needed to decipher the function of 5-HT pathway genes and their products (e.g. transcription factors, neurotrophins, signal transducers) their interactions with each other and with environment and their relevance to depression will be explored.

Specific objectives:

- Functional genomics approaches to genes known to be involved in the development of 5-HT related systems. Detection of new genes by comparative genomics.
- Single-Nucleotide Polymorphism (SNP) screening in these genes and functional analysis. Functional SNP-based association/linkage studies in depression and related disorders.
- Generation of mouse models with constitutive, inducible, and combined knockout of selected candidate genes. Phenotyping and gene expression profiling of these models.

A multidisciplinary approach will be applied to basic structural and functional genomics by integrating behavioural and population genetics, gene expression, and transgenic technologies.

2. Progress towards the Objectives

Activity 1. Behavioural endophenotype of models of genetic, developmental and acquired vulnerability to depression.

1.4 Molecular developmental approach to new genes and models of vulnerability

Identification of factors controlling development of 5HT neurones

A series of variants in genes involved in the development and functional activity of the 5-HT system (e.g., SLC6A4/SERT/5-HTT, HTR2A, TPH2, CNR1, BDNF, COMT, NOS1) were tested for association with depression and related disorders as well as depression-associated endophenotypes involving **Partner 1, 3, 6 and 7/14 (Manchester, Würzburg, Maastricht and Budapest)**. Although human personality is characterized by a substantial heritable component, only few functional gene variants have been associated with personality and behavioural traits. Although genetic and pharmacological studies in animals have demonstrated a role for the neuronal isoform of nitric oxide synthase (nNOS, NOS-I) in a wide range of behaviours including aggression, until now no evidence existed for an association between genetic variants of NOS1 and behavioural traits in humans. In comprehensive study it was demonstrated that a novel functional promoter repeat length variation in NOS1 (NOS1 Ex1f VNTR) is associated with traits related to impulsivity, including hyperactive, aggressive, and violent behaviours, in a cohort of more than 3,500 individuals (Reif et al. 2009). Specifically, the short repeat variant is more frequent in adult Attention-Deficit/Hyperactivity Disorder, Cluster B Personality Disorder, and other disorders displaying by impulsive as well as auto- and hetero-aggressive behaviours. It was shown that the short variant is associated with decreased transcriptional activity of the NOS1 exon 1f promoter and alterations in the neuronal transcriptome in human post-mortem brains, including altered expression of RGS4 and GRIN1, genes previously linked to behavioural disorders. Moreover, the short variant is associated with deficits in prefrontal brain activity, including hypo-activation of the anterior cingulate cortex, a structure known to be involved in the processing of emotion and reward in behavioural control. These findings implicate deficits in neuronal signalling via gaseous nitric oxide in the moderation of prefrontal circuits underlying impulsivity-related behaviour in humans.

Cannabinoid receptor 1 (CB1) gene (CNR1) knockout mice are prone to develop anhedonic and helpless behaviour after chronic mild stress. In humans, the CB1 antagonist rimonabant increases the risk of depressed mood disorders and anxiety. These studies suggest the hypothesis that genetic variation in CB1 receptor function influences the risk of depression in humans in response to stressful life events. In a population sample (n=1269 from NewMood Level 1), we obtained questionnaire measures of personality (Big Five Inventory), depression and anxiety (Brief Symptom Inventory), and life events. The CNR1 gene was covered by 10 SNPs located throughout the gene to determine haplotypic association. Variations in the CNR1 gene were significantly associated with a high neuroticism and low agreeableness phenotype (explained variance 1.5 and 2.5%, respectively). Epistasis analysis of the SNPs showed that the previously reported functional 5' end of the CNR1 gene significantly interacts with the 3' end in these phenotypes (Juhasz et al. (2009). Furthermore, current depression scores significantly associated with CNR1 haplotypes but this effect diminished after covariation for recent life events, suggesting a gene x environment interaction. Indeed, rs7766029 showed highly significant interaction between recent negative life events and depression scores. The results represent the first evidence in humans that the CNR1 gene is a risk factor for depression – and probably also for co-morbid psychiatric conditions such as substance use disorders – through a high neuroticism and low agreeableness phenotype. This study also suggests that the CNR1 gene influences vulnerability to recent psychosocial adversity to produce current symptoms of depression.

Seasonal Affective Disorder (SAD), seasonality and increased sensitivity to the fluctuation of seasons in psychological and biological parameters can manifest to varying degrees across the normal population. The serotonin-2A receptor gene has long been suggested as a candidate gene in the background of this phenomenon. Association between genotypes and haplotypes of the HTR2A receptor gene polymorphism rs731779, rs985934 and rs6311 was there fore analyzed in 609 population sample. Seasonality was measured by the self-rating Global Seasonality Scale (GSS) of the Seasonal Pattern Assessment Questionnaire, and SAD by the Seasonal Health Questionnaire (SHQ). GG subjects of rs731779 scored significantly higher on the GSS scale compared to carriers of the T allele. People carrying the GG genotype were six times more likely to manifest winter or summer SAD compared to GT or TT genotype ones (OR=6.47, CI=1.94-21.57), and the chance to have winter-type SAD with GG genotype were almost seven-fold (OR=8.7 CI=2.53-29.74). In the haplotype analysis subjects carrying the G allele of rs731779 scored higher, while presence of the T allele lead to lower scores on the GSS scale. These results suggest that variations in the HTR2A gene play a significant role in the development of seasonality and especially in winter type SAD. The fact that the polymorphisms in questions showed association not only with clinical SAD but also seasonality symptoms in a general population provided evidence for the spectrum nature of this phenomenon.

The possible association of the serotonin-1A (5-HT_{1A}) receptors and impulsivity phenotype was studied. 5-HT_{1A} receptors are known to play a role in impulsivity-related behaviour. The C(-1019)G functional polymorphism (rs6295) has been suggested to regulate the 5-HT_{1A} receptor gene (HTR_{1A}) expression in presynaptic raphe neurons, namely, increased receptor concentration and reduced neuronal firing could be associated with the G allele. Previous studies indicated that this polymorphism was associated with aggression, suicide and several psychiatric disorders, yet its association with impulsivity has rarely been investigated. We studied the relationship between impulsivity and the C(-1019)G polymorphism of the HTR_{1A} in a population sample of 725 volunteers using the Impulsiveness subscale (IVE-I) of the Eysenck Impulsiveness, Venturesomeness and Empathy scale and also the Barratt Impulsiveness Scale (BIS-11). Significant differences between the C(-1019)G genotype groups (GG vs. GC vs. CC) were found. Subjects carrying GG genotype showed significantly higher impulsiveness scores compared to GC or CC carriers for the IVE-I scale (p=0.014), for the Motor (p=0.021) and Cognitive

Impulsiveness (p=0.002) and for the BIS total score (p=0.008) but not for the Nonplanning Impulsiveness (p=0.520) subscale of the BIS-11. These results suggest the involvement of the HTR_{1A} in the continuum phenotype of impulsivity (Benko et al. 2009). It was also found that cyclothymic, depressive and anxious temperaments have a significant role in the explained variance of depressive scores and they are all significantly related to affective family history. A significant difference was determined between groups with positive and negative affective family history in ZSDS and BSI-D score and these effects were eliminated if affective temperaments were entered as covariants. The chance to have any dominant temperament was more than two-fold in the group with positive compared to negative affective family history (OR=2.33). These results suggest that a crucial part of inherited factors of depression is mediated by affective temperaments, (Lazary et al. 2009).

The tryptophan hydroxylase-2 gene (TPH2) codes for the enzyme of 5-HT synthesis in the brain and variation of TPH2 have been implicated in disorders of emotion regulation. In order to extend these studies toward conditions with a high comorbidity of depression, the influence of allelic variation of TPH2 activity on anxiety-related personality traits and disease risk in patients with personality disorders and co-morbid affective disorders was investigated. The genotype of TPH2 polymorphisms was determined in 428 patients with personality disorders and in 336 healthy controls. Individual SNP and haplotype analyses revealed significant differences in genotype frequencies between controls and cluster B as well as cluster C patients, respectively (Gutknecht et al. 2006). In both patient groups, overrepresentation of T allele carriers of a functional polymorphism in the upstream regulatory region of TPH2 (SNP G-703T, rs4570625) which biases the responsiveness of the amygdala (Canli et al. 2005), a structure critically involved in the modulation emotional behaviours, was observed. Furthermore, significant effects of TPH2 variants on anxiety-related traits defined primarily by the TPQ Harm Avoidance were found in healthy individuals. The results link functional TPH2 variants to personality traits related to emotional instability as well as to cluster B and C personality disorders. These findings implicate alterations of 5-HT synthesis in emotion regulation and confirm TPH2 as a susceptibility and/or modifier gene of affective spectrum disorders.

Previous studies have related TPH2 also to depression, schizophrenia, alcoholism, drug abuse, aggression and suicidality. One possible explanation of this is that TPH2 function might relate to a more basic behavioural feature, or endophenotype, involved in many psychiatric disorders. Impaired decision making, attributed to a 5-HT deficit in orbitofrontal cortex (OFC), is one candidate endophenotype for many psychiatric conditions. It was also demonstrated that rapid dietary tryptophan depletion – leading to reduced central 5-HT function – altered decision-making of healthy volunteers. Therefore, the hypothesis whether TPH2 gene variation affects decision making was tested in a large sample using a simple paper-pencil task. The task consisted of choosing between two ‘wheel of fortune-like’ alternatives: ‘A’ which provided a smaller but more likely ‘win’ and ‘B’ which gave a ‘win’ 2 times the amount with lower probability that was systematically varied. A repeated measure design with 4 different probability levels of winning on ‘A’ was used. The probability of winning on ‘B’ at which the subjects valued the two trials equally (indifference point) was determined as a measure of willingness to take a risk. TPH2 haplotypes and Anxiety scores had an independent significant effect on decision making, with more anxious subjects making less risky choices (Juhasz et al. 2009). There was no interaction between TPH2 haplotypes and Anxiety scores. Depression scores had no further effect on decision making keeping anxiety in the model. This study supports the role of TPH2 gene, and the 5-HT system, in risk taking behaviour. This genetic effect is independent of mood state and might be an additive factor contributing to psychiatric disorders.

The 5-HTTLPR of SERT was investigated in several cohorts including samples from the EFPTS (twin) study consisting of a cohort of 260 female twins from a normal population. It was examined whether the 5-HTTLPR polymorphism moderated the association between neuroticism

and depression, and how this may explain previous findings concerning Significant Life Events (SLEs). A neuroticism score was calculated using the Neuroticism-Extraversion subscale of the Eysenck Personality Questionnaire. The findings show that the depressogenic effect of SLEs was significantly greater in women with 2 copies of the s-allele, compared to women with 1 or none. This effect disappeared however after taking into account the effect of SLEs that were conditional on neuroticism. The depressogenic effect of neuroticism was also progressively greater with number of s-alleles, but this was unchanged after taking into account the effect of neuroticism conditional on SLEs (Jacobs et al., 2006). It is therefore concluded that the 5-HTTLPR genotype moderates the way individuals continually respond to, and cope with, mild SLEs and minor environmental experiences in daily life, rather then it merely moderates the relationship between a threatening event and the onset of depression. Thus, genotype x environment interactions in depression may be more productively interpreted by involving mechanisms more proximal to psychological experience itself.

Spielberger's STAI and 5-HT transporter (SLC6A4/SERT) genotype were investigated in a cohort of psychiatrically healthy females also including migraineurs. Migraineurs showed significantly higher state and trait anxiety than non-migraineurs, and also had a significantly higher frequency of the s-allele of the SERT gene. The findings support the hypothesis that high comorbidity of depressive and anxiety disorders and migraine may be influenced by the genetic background, and the common genetic factor is possibly conveyed by allelic variation of SERT expression. In a related study, Zung's Self-Rating Depression Scale was evaluated and 5-HTTLPR was genotyped female subjects with no lifetime and current history of DSM-IV Axis I disorder. Significant differences were found on the Zung SDS and also on the physical-vegetative subscale of the ZUNG SDS according to both phenotype and genotype. Subjects carrying the s-allele scored significantly higher on the Zung SDS and had also significantly higher scores on the physical-vegetative symptom subscale (Gonda et al., 2005). Furthermore, subjects carrying the ss genotype scored highest and subjects carrying the ll genotype scored the lowest on both scales. It is conclude that subclinical depressive symptoms (i.e. DSM-IV subthreshold depression) are associated with the functional polymorphism of SERT. The s allele of the 5HTTLPR gene is associated with a "low mood endophenotype".

In the general population polymorphisms of candidate genes were also studied in relation to affective temperaments and other symptoms related to depression and "low mood" endophenotype. In a subgroup of subjects, the standardized Hungarian version of the TEMPS-A questionnaire and 5-HTTLPR genotype was investigated in a sample of unrelated Caucasian females with no current or lifetime Axis I psychiatric disorders. A significant association was found between the s allele and the TEMPS scores of the depressive, anxious, irritable, and particularly the cyclothymic temperaments; no such association emerged with respect to the hyperthymic temperament. In addition, the s allele of the 5-HTTLPR was significantly associated also with Spielberger STAI, both state and trait anxiety (Gonda et al., 2006; Gonda et al., 2007). The results are in good agreement with earlier studies reporting a strong association between the s allele of the 5-HTTLPR and major as well as subthreshold forms of depression, and extend this association to the normative temperament level. Indeed, these temperaments might best be regarded as proximate behavioural endophenotypes.

Animal studies have linked genetic variation of 5-HTT function to an increased stress reactivity of the hypothalamus-pituitary-adrenal (HPA) axis. While there is also preliminary evidence for such an association in humans, the extent to which an altered HPA axis function is a result of a significant gene-environment interaction is still unknown. The role of the 5-HTTLPR polymorphism in HPA axis activity was therefore investigated in humans who had not yet experienced a significant number of adverse life events so far (Müller et al., 2009). The 5-HTTLPR genotype including the A/G SNP in a sample of 216 three days old newborns. A heel prick was applied as a stressor and endocrine and behavioural stress responses were determined.

The heel prick induced a significant behavioural and endocrine stress response in all newborns. However, the 5-HTTLPR genotype did not explain variance in the endocrine or behavioural stress response profiles. It is concluded that the 5-HTTLPR genotype per se does not affect HPA stress responses without the additional impact of environmental influence.

Impulsivity is a multidimensional personality trait that includes the inability to delay gratification and to act without regard to consequences. It is a characteristic of several psychiatric conditions, such as substance abuse and addiction, attention deficit/hyperactivity (ADHD), and mood disorders, as well as a feature of suicide attempts. COMT gene variations have been implicated in a wide range of psychiatric disorders but their role in impulsivity has not yet been directly assessed. Our aim was to investigate the function of the COMT gene in impulsivity by applying haplotypic association analysis in a population study design. We recruited 1267 participants from Manchester (UK) and a replication sample of 942 subjects from Budapest (Hungary) (Level 1 subjects). Impulsivity was measured using impulsiveness items from the Impulsiveness-Venturesomeness-Empathy Scale (IVE) that has been validated in both languages. For the analysis we calculated a continuous weighted dimension score (sum of item scores divided by the number of items completed). The COMT gene was covered by four htSNPs (rs933271, rs740603, rs4680, rs4646316) located throughout the gene. To remove the influence of multiple testing we used a permutation test, randomly grouping the sample 1000 times. All the SNPs were in Hardy-Weinberg equilibrium in both populations. In the Manchester sample the COMT gene showed significant association (permutated $p = 0.003$) with impulsivity explaining 1.29% of the variance in this personality factor. We successfully replicated this finding in the Budapest sample, where the COMT gene also associated with impulsivity (permutated $p = 0.038$) and explained 1.19% of the variance. Five common haplotypes represented 77.85% of the Manchester and 76.21% of the Budapest population. All haplotypes showed the same direction of effect (risk or protective) in both populations although the most significant effect was associated with the TGGC haplotype (protective; $p = 0.0008$) in the Manchester population and to the CGAC haplotype (risk; $p = 0.0018$) in the Budapest population. The results offer the first evidence that the COMT gene is significantly associated with self reported impulsivity in two independent European populations. However, there was no evidence for major effects of the Val108/158Met (rs4680) non-synonymous polymorphism in the COMT functional variations, as both alleles (G/A) can be seen in protective as well as in risk haplotypes. This observation might explain previous, inconsistent findings related to the Val108/158Met polymorphism in different psychiatric disorders, (Juhasz et al. 2009).

Several studies addressed the role of gene x environment (GxE) interactions in depression with particular focus on stress sensitivity, mediation of HPA-axis reactivity, neuroticism, and reward experience involving **Partner 1, 3, 6 and 14**. The EFPTS (twin) study investigated whether the 5-HTTLPR polymorphism moderated the association between neuroticism and depression, and how this may explain previous findings concerning Stressful Life Events (SLEs). It was found that the 5-HTTLPR genotype moderates the way individuals continually respond to, and cope with, mild SLEs and minor environmental experiences in daily life, rather then it merely moderates the relationship between a threatening event and the onset of depression (Jacobs et al. 2006). In addition, the influence of the (-1019)CG polymorphism in the promoter of the 5-HT1A gene on HPA-axis reactivity to social stressors in daily life was assessed. Negative affect (NA) was a weighted measure on different emotional items including anxiety and mood, which were measured on a 7-point likert scale. It was found that the G/G genotype of the 5-HT1A(-1019)CG polymorphism, which is a risk factor for major depression, is associated with increased cortisol levels in response to social stress. This polymorphism also moderated the effect of social stress on negative affect (NA) (Wichers et al. 2008a). Whether the effect of social stress on NA is mediated by cortisol (in interaction with 5-HT1A genotype) is still subject of future studies.

From the experience sampling method (ESM) data, a measure for positive affect (PA) was constructed. Reward experience was then defined as the effect of the (positive) appraisal of an event on PA. The more pleasant the event was experienced, the greater an effect it showed on PA. However, the ability to experience reward was mediated by the COMTval158met polymorphism, in that subjects with more 'met'-alleles showed higher reward experience (Wichers et al. 2008b). It has been shown that subjects with a genetic liability to develop depression do not show increased stress sensitivity if they have the ability to experience PA as well at the time of the stressor. We were already able to demonstrate that the BDNFval66met polymorphism mediates stress sensitivity: thus, 'met'-allele carriers display higher stress sensitivity than 'non-met' carriers. Interestingly, the ability to experience PA reduced the increased stress-sensitivity in 'met'-allele carriers (Wichers et al. 2008c).

Activity 2: Functional states of monoamine regulation in rodent models of vulnerability – the neurotransmitter endophenotype.

2.1 Regulation of monoamine cell-body function in raphe and locus coeruleus

To uncover the molecular substrates of the gene x environment (GxE) risk factor, a mouse model was established in which a heterozygous null mutation in the 5-Htt gene moderates the effects of poor maternal care on adult anxiety and depression-related behavior. Biochemical analysis of brains from these animals identified distinct molecular substrates of the gene, environment, and GxE effects. Mice experiencing low maternal care showed deficient GABA-A receptor binding in the amygdala and 5-Htt heterozygous knockout mice showed decreased 5-HT turnover in hippocampus and striatum (Carola et al. 2008). Strikingly, levels of BDNF mRNA in hippocampus were elevated exclusively in 5-Htt heterozygous knockout mice experiencing poor maternal care, suggesting that developmental programming of hippocampal circuits may underlie the 5-HTT x E risk factor. These findings demonstrate that 5-HT plays a similar role in modifying the long-term behavioural effects of rearing environment in diverse mammalian species and confirms BDNF as a molecular substrate of this risk factor.

In several follow-up studies attempts are being made to identify the molecular mechanisms underlying GxE risk factor in order to improve understanding individual differences in resilience to stress. This was done in a mouse model of the 5-HTT x stress risk factor. Wild-type and heterozygous 5-Htt knockout mice were subjected to chronic psychosocial stress. 5-Htt genotype did not affect physiological and behavioural consequences of stress as measured by changes in body temperature and weight and home cage locomotion. However, heterozygous 5-Htt knockout mice experiencing high levels of stress showed significantly increased social avoidance toward an unfamiliar male in a novel environment when compared to wild-type littermates (Bartolomucci et al. 2010, in press). Heterozygous 5-Htt knockout mice exposed to stress also showed significantly lower levels of serotonin turnover than wild-type littermates selectively in the frontal cortex, a structure known to control of fear and avoidance responses and implicated in susceptibility to depression. In another experiment concerning social experiences, males of all three 5-Htt genotypes were provided with a winner or a loser experience in a resident-intruder paradigm on three consecutive days (Heiming et al. 2009). Anxiety-like behaviour and exploration were recorded in the dark-light, elevated plus-maze and open-field test. To non-invasively assess adrenocortical activity, corticosterone metabolites were determined from feces. The main findings were: Repeated social experience, irrespective of winning or losing, elevated levels of anxiety-like behaviour and decreased exploration. In losers a distinct effect of genotype occurred, with homozygous knockout males showing more anxiety-like behaviour and less exploration than the other genotypes. In winners no genotype-dependent variation was found. Genotypes did not differ in basal stress hormone secretion. There was, however, a main effect of social experience with higher activation of the stress hormone system in losers than in winners. This effect was strongest in the heterozygous genotype. Finally, a study was aiming at to elucidation of the

effects of a threatening environment during early phases of life on anxiety-like (ANX) and exploratory behaviour (EXP) in adult mice, varying in serotonin transporter (5-Htt) genotype (Jansen et al. 2009). For this purpose, pregnant and lactating 5-Htt+/- dams were repeatedly exposed to olfactory cues of unfamiliar adult males by introducing small amounts of soiled bedding to their home cage. These stimuli signal the danger of infanticide and simulate a threatening environment. Control females were treated with neutral bedding. The offspring (5-Htt+/+, +/-, -/-) were examined for their ANX and EXP. The main results were: (1) a main effect of genotype existed, with 5-Htt-/- showing higher levels of ANX and lower levels of EXP than 5-Htt+/- and wild-types. (2) When mothers had lived in a threatening environment, their offspring showed increased ANX and reduced EXP compared to controls. (3) These effects were most pronounced in 5-Htt -/- mice. By applying a new ecologically relevant paradigm we conclude: If 5-Htt +/- mothers live in a threatening environment during pregnancy and lactation, their offspring behavioural profile will, in principle, be shaped in an adaptive way preparing the young for an adverse environment. This process is, however, modulated by 5-Htt genotype, bearing the risk that individuals with impaired serotonergic neurotransmission (5-Htt-/-) will develop an exaggerated, potentially pathological level of anxiety from gene x environment interactions. These data indicate that the 5-Htt modified mouse serve as a useful animal model for the increased vulnerability to stress reported in individuals carrying the 5-HTTLPR s allele and suggest that dysregulated 5-HT homeostasis in frontal cortical structures plays a role in adaptations to social adversity.

2.3 Molecules beyond the monoamine synapse

Inactivation of 5-HTT by pharmacologically in the neonate or genetically increases risk for depression in adulthood, whereas pharmacological inhibition of 5-HTT ameliorates symptoms in depressed patients. The differing role of 5-HTT function during early development and in adult brain plasticity in causing or reversing depression remains an unexplained paradox. To address this Partner 3 profiled the gene expression of adult 5-Htt knockout (5-Htt -/-) mice and 5-HTT inhibitor-treated mice (Ichikawa et al. 2008). Inverted profile changes between the two experimental conditions were seen in 30 genes. Consistent results of the upstream regulatory element search and the co-localization search of these genes indicated that the regulation may be executed by Pax5, Pax7 and Gata3, known to be involved in the survival, proliferation, and migration of serotonergic neurons in the developing brain. Several factors involved in the survival, proliferation, and migration of serotonergic neurons are likely to regulate downstream genes related to serotonin system in the adult brain.

For generation of a Tph2-deficient mouse (constitutive and conditional/inducible Tph2 knockout - cKO) a targeting construct **Partner 3** designed in which a region of Tph2 is flanked by LoxP sites. Initial characterisation of this new mouse model on the genetic, histological and neurochemical levels indicates that in Tph2 knockout mice raphe neurons are completely devoid of 5-HT, whereas no obvious alteration in morphology and fiber distribution are observed (Gutknecht et al. 2008, Kriegebaum et al. 2010). The findings confirm the exclusive specificity of Tph2 in brain 5-HT synthesis and suggest that Tph2-synthesized 5-HT is not required for serotonergic neuron formation., The reduced 5-HT levels observed in these cKO mice predict an altered serotonergic neurotransmission, which constitutes a new powerful model to be extensively studied in order to elucidate the underlying mechanisms of 5-HT homeostasis, its effect on behaviour and its implication in psychiatric disorders. The generation of a complete and an inducible knockout of Tph2 on the basis of this cKO strategy will provide new insights into the role of 5-HT during development of the nervous system. After further expansion of the colony breeding pairs are being distributed to other NewMood partners.

Dysregulation of the brain 5-HT system, including dysfunction based on genetic variation in tryptophan hydroxylase (TPH) -dependent 5-HT synthesis, has long been implicated in the

etiopathogenesis of a wide variety of neuropsychiatric disorders, although there is presently considerable controversy regarding the differential expression pattern and the specific role of the two isoforms, TPH1 and TPH2. A systematic spatio-temporal analysis of Tph1 and Tph2 expression in murine brain during pre- and postnatal development (from E9.5 to P23) and in mouse and human adult brain (Gutknecht et al. 2009). To assess expression at the transcriptional and translational level in brain as well as in other TPH-expressing tissues serving as internal control, we used complementary methods permitting isoform-specific detection of mRNA (quantitative real time PCR and in situ hybridization) and protein (Western blot and immunohistochemistry). Effects of potential confounding factors on brain TPH isoform content conveyed by non-neural circulating cells were also evaluated. Commencing before E11 during murine development, TPH2 expression was found in the raphe nuclei of both species, as well as in fibres in the deep pineal gland and in the small intestine. In contrast, none of the techniques used identified significant TPH1 expression, neither during murine brain development, nor in mouse and human adult brain. Physiologically meaningful TPH1 expression was nevertheless detected in the pineal gland and small intestine. It was confirmed that in both mice and human brain TPH2 expression is restricted to serotonergic neurons of the raphe nuclei. In the brain, TPH1 expression is confined to the pineal gland but most abundant in the intestine. The findings are relevant to the interpretation of the TPH isoforms' role in brain development and function as well as in the molecular neurobiology of complex behaviour and psychopathology. This also puts the brain-specific TPH2 as a critical regulator of serotonergic transmission into the centre of transgenic mouse model research.

Activity 4: In vivo studies of vulnerability, depression and recovery in humans

4.1 Neurobiology of reward, emotion, and cognition by risk group and depression

A distinct mechanism may involve the control of cellular microstructure underlying neurogenesis and neural plasticity during later life in brain pathways that process emotional information. A promising candidate for such a mechanism is stathmin, which is a cytosolic neuron-enriched phosphoprotein that regulates the formation and stability of microtubules in response to a number of extracellular messengers. It is preferentially expressed in pathways processing contextual fear conditioning and emotional learning, and stathmin-deficient mice exhibit impairment in fear-related innate and learned behaviour. It was therefore hypothesized that in humans the gene coding for stathmin (*STMN1*) influences behavioural responses to fear and anxiety stimuli by way of two common single nucleotide polymorphisms (rs182455, SNP1; rs213641, SNP2) located within or close to the putative transcriptional control region. **Partner 3** used the acoustic startle paradigm and a standardized laboratory protocol for the induction of fear and psychosocial stress in 106 healthy volunteers to investigate the impact of stathmin gene variation on human emotion regulation by behavioral measures of fear and anxiety. We found that *STMN1* genotype interacting with individuals' gender significantly impacts fear and anxiety responses as measured with the startle and cortisol stress response (Brocke et al. 2009). It is conclude that *STMN1* genotype has functional relevance for the regulation of basic fear and anxiety responses also in humans. The study suggests a novel plasticity regulating molecular mechanism underlying human fear and anxiety behaviour and may thus contribute to a better understanding of human fear behaviour at the molecular level.

Several studies also investigated the role of gene x gene (GxG) interactions in depression involving **Partner 1, 3 and 14**. Since functional variants of both the 5-HTT and TPH2 genes, are key regulators of the serotonergic signalling pathway, and modulate amygdala activation during emotional processing. The question was addressed whether these two gene variants modulate each other, using two different cognitive-affective tasks with emotional and neutral facial expressions and word stimuli and fMRI. The study was designed to replicate and extent this initial report in an independent study sample, and use to identify specific neural loci that may

mediate the 5-HTT–TPH2 additive effect. There was evidence for an additive effect of 5-HTT–TPH2 genotype, which was most robust in the putamen, a region rich in both 5-HTT and TPH2 protein, but was also observed in the amygdala at a less stringent threshold, and in other cortical regions (Canli et al. 2008). The additive effect was more robust effect for visuospatial than for verbal stimuli, and more robust for negatively than for positively valenced stimuli. The results confirm and extend the additive effect of two critical genes in the serotonergic regulation of neural processing of affective stimuli, and identify the striatum as a critical site where is epistasis takes place.

Experimental data suggest that the Endocannabinoid System (ECS) can modulate several neurotransmitter systems, including the serotonergic system, which itself plays a significant role in anxiety. However, to date there is no evidence of GxG interactions; indeed genetic studies focusing separately on the two systems provide conflicting data. Thus, the interaction of the promoter regions of the serotonin transporter (SLC6A4) and cannabinoid receptor 1 (CNR1) genes on anxiety was analyzed. Seven hundred and six individuals were genotyped for the 5-HTTLPR in the SLC6A4 promoter and 4 SNPs located in the CNR1 promoter region. Anxiety was measured by the State-Trait Anxiety Inventory (STAI-S, STAI-T), the anxiety subscale of TEMPS-A (TEMPS-Anx), and the Brief Symptom Inventory (BSI-Anx). Significant 5-HTTLPR x CNR1 promoter-promoter interaction was observed using STAI-T ($p=0.0006$) and TEMPS-Anx ($p=0.0013$). The risk of high anxiety scores on BSI-Anx was 4.6-fold greater in homozygous 'GG' rs2180619 in combination with homozygous 'SS' 5-HTTLPR ($p=0.0005$) compared to other genotypes. The effect of previously described "TGC" haplotype in the alternative promoter of CNR1 depended both on the conventional promoter polymorphism and the 5-HTTLPR. Visualized results of likelihood ratio tests for interactions between genetic markers (5-HTTLPR, rs2180619, rs806379, rs1535255, rs2023239) and TEMPS-Anx, STAI-T and BSI-Anx are generated by 'SNPassoc' R-package. Highly suggestive interactions were shown between the 5-HTTLPR of SLC6A4 promoter and rs2180619 of CNR1 promoter on anxiety ($p<0.01$).

The neuroplastic pathway, that consists of the cyclic adenosine monophosphate (cAMP) response element binding protein (CREB1), brain derived neurotrophic factor (BDNF) and its receptor TrkB (NTRK2), plays a crucial role in the adaptation of brain to stress and thus variations of these genes are plausible risk factors for depression. This analysis of the NewMood Level 1, Level 2 and Level 3 Manchester data tested the hypothesis that different genes in the neuroplastic pathway exert similar effects on risk mechanisms of depression using a pathway approach. A total of 1269 Caucasian subjects (Level 1) were used for a genetic association study, subsets of which were interviewed (n=264 from Level 2) and underwent fMRI (n=33 from Level 3) (see WP7). Lifetime depression, current depressive symptoms, and ruminative response style were analysed. Regional BOLD signal changes were also investigated during sad face emotion processing task (see WP7). For BDNF the previously identified non-synonymous Val66Met (rs6265) functional polymorphism and another 5 htSNPs (rs12273363, rs962369, rs988748, rs7127507, and rs1519480) were genotyped to cover the whole gene; of these rs12273363 and rs7127507 had been found in our previous study to influence the hippocampal proBDNF density (Dunham et al. 2009). For CREB1 we genotyped the rs2253206 promoter htSNP that also showed functional effects. For NTRK2 we genotyped 2 htSNPs at the 5' end of the gene (rs1187323 and rs1187326) that significantly influenced the TrkB receptor density in hippocampus. In this study, the major alleles of rs6265 and rs2253206 were significantly associated with rumination and through rumination with current depression severity. However, childhood adversity increased the risk of lifetime depression in the minor allele carriers of rs6265, rs988748 and rs2253206 and in the major allele carriers of five other SNPs. We validated our findings in the interviewed subjects using structural equation modelling. Finally, areas that are more active in depressed subjects based on previous literature, showed significantly increased activation in rs6265 A (66Met) and rs2253206 A carriers to a sad face emotion processing fMRI task (manuscript submitted). Genetic variation associated with reduced function in the CREB1-

BDNF-NTRK2 pathway has multiple, sometimes opposing, influences on risk mechanisms of depression but almost all the SNPs studied amplified the effect of childhood adversity on risk of depression. Differential effects on neural processing of emotion appear to be involved. Investigation of genetic pathways with cognitive and neural intermediate phenotypes may be a productive route to revealing pathomechanism of depression.

Serotonin transporter gene polymorphisms, personality and response to serotonergic and noradrenergic antidepressants was investigated by **Partner 1** and NewMood-associated researchers (R. Tranter, Bangor, Wales). This study examined associations between 5-HTTLPR polymorphisms, baseline harm avoidance (HA) scores and clinical response to the SSRI citalopram and the NaRI reboxetine in primary care patients. A total of 55 Caucasian subjects were recruited. Personality was assessed using the Tri-dimensional Personality Questionnaire (TPQ) before randomisation. Twenty four patients were randomised to citalopram (20 mg) and 31 patients to reboxetine (8 mg), with follow-up over 6 weeks. Baseline and 6 week clinical outcomes were measured using the Clinical Outcomes in Routine Evaluation (CORE). 5-HTTLPR polymorphisms were genotyped from buccal swab samples. Given the small sample the genotype was analysed as ll homozygote and s-carrier. Associations between genotype and baseline HA scores, and between genotype and percentage change in CORE scores over 6 weeks for the two treatment groups were explored using two-way analysis of variance (ANOVA). 5-HTTLPR genotype frequencies did not significantly differ from expected frequencies in a European population. The ll genotype was associated with higher harm avoidance scores $F(1,57) = 8.41$, $p = 0.005$. ANOVA of percentage change in CORE scores over 6 weeks did not show any significant between-subjects effects for medication ($p = 0.17$) or genotype ($p = 0.48$), with a possible interaction between genotype and medication, $F(1,54) = 3.42$, $p = 0.07$ (Tranter et al. 2009). Covarying for HA score the interaction between medication and genotype just missed significance, $F(1,54) = 4$, $p = 0.051$. Post-hoc analysis showed a trend towards the s allele being associated with greater percentage change in total CORE scores on citalopram ($p = 0.08$), but not on reboxetine ($p = 0.43$). There was a significant association between the s allele and percentage change in the functioning subscale of the CORE on citalopram ($p = 0.015$) but not on reboxetine ($p = 0.94$). In this small sample there was a significant association between 5-HTTLPR polymorphisms and HA scores, and a strong trend to association with treatment outcome when HA scores were included as a covariate. The results suggest that the association between 5-HTTLPR polymorphisms and clinical outcome is specific to the SSRI. However, the direction of effect for HA and response to citalopram were in the opposite direction from previous studies in Western populations.

Overview

1) NewMood is increasing the knowledge of the effect of genetic variation on brain activity (psychophysiology and functional imaging) in traits of anxiety and depression as well as in related disorders of emotion regulation through identification of: a) novel candidate genes, b) gene x gene (GxG) interaction, c) gene x environment (GxE) interaction, and d) gender effects.
2) The generation a *Tph2* constitutive and conditional knockout mice has been completed together with advances in behavioural, neurochemical and cytoarchitectural phenotyping.
3) The completion of Level 1 genotyping of over 3000 individuals with self-rated phenotypic features has resulted in the discovery of a number of SNP and haplotype associations of genes in humans corresponding to the genetic mouse models. They include 5-HTT, TPH2, BDNF and CB1 genes. The direct association of CB1 haplotypes with depression is especially striking and provides the first validation of a genetic mouse model in NewMood. Associations with other candidate genes have also been described, notably with CREB1. The

DNA collection provides a valuable resource to screen SNPs for new candidate genes from the NewMood array findings for association with vulnerability to depression.

4) A very large collection of cortisol responses to waking in 100 controls, 100 recovered depressives (i.e. a group with vulnerability to depression) and 30 depressives indicates that common depression is associated with an impaired ability to mount a cortisol response.
5) Collaborations and interactions continue for specific projects involving Partners 2, 4, 5, 6, 7, 8, 9, 11, 13 and 14.

Deviations

No significant deviations from the project work programme have occurred.

Table 10. Deliverables and milestones for Workpackage 2

All the Milestones and Deliverables involved with WP2 have been achieved (partial achievement of deliverable 39). For detailed information about the Deliverables, please see separate report ("Deliverables WP 2: Genomics – animal and human")

Below is a list of all Deliverables and Milestones to which WP2 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name**	**Date due**	**Actual date**
3	Two new mutant mice models ready for Core Data–set evaluation	18	36
7	First conditional 5HT knock-outs available	24	36
20	First array data from human post-mortem brain	36	36
39	First association data in resistant depressed patients	48	66
55	Association study and/or haplotype analysis of traits related to mood in the Hungarian cohort	60	54
Deliverable No. (part-contribution)			
4, 5, 8, 15 & 31a, 18 & 31b, 25a, 27a, 33, 50, 56	(various)	18 - 60	18 - 66
Milestone No. (main contributor)	**Milestone name**	**Date due**	**Actual/ date**
3	New genomic targets for mechanisms and treatment identified, (Preliminary results of functional SNP-based association/linkage study of a appropriate candidate gene in depression)	18	18
4	Decisions on models to be taken forward for detailed analysis of mechanisms (Selection of the models to be used for further studies)	18	18
32	Genotyping completed for a group of subjects with well characterised brain responses to face emotion and emotional processing tasks	54	54
Milestone No. (part-contribution)			
7, 9, 16, 17, 20, 21, 28, 29, 30, 31, 32	(various)	18 - 60	18 - 66

Workpackage 3 - Neurochemistry and hormone function

1. Objectives

The objectives of this work package are to establish and understand a data set of neurochemical and hormonal changes across a range of selected genetic and environmental models of vulnerability depression, to determine and understand the effects of classical and new antidepressant treatments on neurochemical and hormonal function, and finally to determine and understand the effects of classical and new antidepressant treatments on neurochemical and hormonal function in selected genetic and environmental models of vulnerability to depression.

Specific objectives of the study

- To investigate basal and stress-evoked monoamine and amino acid transmitter release in key mood-related brain regions *in vivo*;
- To investigate the function of presynaptic auto- and heteroceptors modulating transmitter release;
- To investigate basal and stress-evoked levels of hormones of the hypothalamic pituitary axis (HPA).

2. Progress towards the Objectives

Activity 2: Functional states of monoamine regulation in rodent models of vulnerability – the neurotransmitter endophenotype.

2.1 Changes in regional brain function & response to stress shared by models

5-HTT overexpressing mice.
Studies by Partners 8 and 9 (Edinburgh, Oxford) assessed regional brain function and response to stress in 5-HTT overexpressing mice. Brain region activity-dependent markers (c-fos, regional glucose utilisation) were similar between wild-type and transgenic mice under basal conditions and in response to acute stress.
Comment: These data indicate that 5-HTT over-expression does not alter responsivity to acute stress, possibly due adaptive changes in 5-HT function (see Activity 2.3).

5-HTT-/- mice.
A similar investigative approach was initiated with 5-HTT knock-out mice by Partners 2 (Paris) and 3 (Würzburg). Acute restraint stress increased c-Fos expression within raphe serotonergic neurons and this was reduced in 5-HTT-/- mice.

VGLUT+/- mice
Studies by Partner 9 (Pamplona) found that VGLUT1+/- mice have increased vulnerability to depression (García-García et al. (2009) Biol. Psychiatry 66:275-82). These mice also showed an increased c-fos response to acute stress (forced swim) localised to raphe 5-HT neurones.

Chronic mild stress
Partner 9 (Pamplona) found that the raphe c-fos response to acute stress increased in rats exposed chronic mild stress, an effect prevented by antidepressant treatment.
Comment: Overall, in several models of depression vulnerability, the responsivity of the 5-HT system to acute stress was elevated. This could be related to findings that increased 5-HT counteracts the deleterious effects of repeated (inescapable) stress.

Tryptophan depletion.
Acute TRP depletion (ATD) reduces 5-HT function, and may increase vulnerability to depression. Partner 6 (Maastricht) investigated ATD in mice and found no decrease brain 5-HT

levels or change in affective behaviour. Studies on the effects of food and stress manipulations ruled out interfering factors.
Comment: These results suggest that ATD in mice does not provide a suitable model of depression vulnerability (Van Donkelaar et al. (2010) *Neurochem Int*. 56:21-34). Species-specific compensatory mechanism may counter ATD depletion.

Cytokine-5-HT interactions
Proinflammatory cytokines lower mood in humans and animal models. Partners 9 (Oxford) and 6 (Maastricht) studied effects of immune system activation on brain 5-HT, focusing on indoleamine 2,3-dioxygenase (IDO), a tryptophan metabolising enzyme. Immune stimulants elevated peripheral but not brain IDO, and did not alter brain tryptophan or 5-HT. However, experiments found effects of immune stimulants in an imaging model of 5-HT function and on expression of 5-HT genes.
Comment: These findings suggest that while immune stimulation does not activate IDO in the brain or decrease brain tryptophan and 5-HT, it has other striking effects on 5-HT function unrelated to IDO.

Partner 13 (Warsaw) studied CNS neurochemical and hormonal effects of cytokines (IL-1) and tested the role of the vagus and cyclo-oxygenase (COX). Vagotomy with COX inhibition prevent changes induced by IL-1 treatment.
Comment: These results add new evidence for a role of the vagus in the neurochemical and endocrine responses to cytokine administration.

2.2 Regulation of monoamine cell-body function in raphe and locus coeruleus

Quantal release of noradrenaline
Partner 10 (Stockholm) studied noradrenaline release from locus coeruleus neurons at the single-cell level using combined electrochemical and electrophysiological analysis of quantal release. Noradrenaline release was strongly modulated by action potential frequency, and somatodendritic release of noradrenaline caused autoinhibition
Comment: These data demonstrate for the first time that in contrast to classic synaptic transmission, quantal noradrenaline release from somata is characterized by distinct properties, including long latency and high sensitivity to action potential frequency (Huang et al, *PNAS* 2007 104:1401-1406).

Effect of 5-HT$_{2C}$ receptor activation on 5-HT transmission
Partner 9 (Oxford) found that 5-HT$_{2C}$ receptors inhibit the firing of raphe 5-HT neurons, via a habenula-dependent mechanism (Sharp et al (2007) TIPS 28:629-36; Quérée et al (2009) Br J Pharmacol. 158:1477-85). Partners 9 and 6 (Maastricht) found a behavioural correlate of this phenomenon. Partner 2 (Paris) found that 5-HT$_{2C}$ agonists inhibited stress-induced but not basal 5-HT release.
Comment: These findings provide new evidence that 5-HT neurones are subject to feedback control by 5-HT$_{2C}$ receptors and that this mechanism is stress-sensitive.

Rats with low and high exploratory traits
Rats with a low exploration trait (LE) have a depressive-like phenotype versus rats with a high exploration trait (HE). Partner 5 (Tartu) studied monoamine release in LE and HE rats with microdialysis. Partners 5 and 9 (Oxford) exchanged visits to harmonise protocols. LE rats had higher 5-HT release in prefrontal cortex but also higher 5-HT reuptake. Dopamine release was higher in HE than LE rats at baseline and after amphetamine, and HE rats had a higher proportion of D_2 (high) receptors.

Comment: The data suggest that the LE trait is associated changes in 5-HT and dopamine release which may link to the differences in behavioural phenotypes (Mällo et al, 2008, *Brain Res* 1194: 110-17; Alttoa et al, 2009, *Synapse* 63:443-6; Harro, 2010, *Pharmacol Ther*. 125:402-22).

High- and low-sociability traits in rats.
Partner 5 (Tartu) monitored monoamine release in rats with a low and high sociability traits (LS and HS, respectively). In microdialysis studies, 5-HT release in prefrontal cortex was higher in LS compared to HS rats. In comparison, a 5-HT releasing agent elicited a similar 5-HT increase in both groups.
Comment: These findings suggest that low presynaptic 5-HT function may be linked to persistent individual differences in sociability.

CB1 knockout mice
CB1 receptor -/- mice are another core model of genetic vulnerability to depression. In microdialysis experiments Partner 4 (Barcelona) found that SSRI-induced increase in extracellular 5-HT in the prefrontal cortex is attenuated in CB1-/- mice.
Comment: These results are some of the first evidence suggesting altered presynaptic 5-HT function in CB1 knockout mice (Aso et al, 2009, *J Neurochem*. 109:935-44).

5-HTT overexpressing mice.
In microdialysis studies Partner 9 (Oxford) found that 5-HTT overexpressing mice had reduced basal and depolarisation-evoked 5-HT release in prefrontal cortex and hippocampus. *In vitro* voltammetric methods were developed to obtain high time resolution measurements of 5-HT release. Studies with Partner 3 (Würzburg) found a marked loss of 5-HT release in the mice, whereas 5-HTT-/- mice had increased 5-HT.
Comment: These experiments are the first to demonstrate striking changes in synaptic 5-HT output in the association with variability in 5-HTT expression (Jennings et al, 2006, J *Neuroscience* 26:8955-64).

High and low susceptibility to stress in mice.
Experiments by Partner 13 (Warsaw) studied monoamine neurochemistry and HPA activity in mice selected for high and low susceptibility to stress (swim-stress evoked analgesia). It was found that stress reliably increased markers of noradrenergic, dopaminergic and serotonergic activity and plasma corticosterone.

2.3 Molecules beyond the monoamine synapse

Postsynaptic 5-HT receptor expression and modulation of forebrain function

5-HTT overexpressing mice.
Partners 8 (Edinburgh), 9 (Oxford) and 3 (Würzburg) studied 5-HT receptor expression in 5-HTT overexpressing mice. 5-HT_{2A} receptor function was increased, and this effect could be mimicked by 5-HT depletion. The mice also had increased 5-HT_4 receptor binding, whereas 5-HT_4 receptor binding was decreased in 5-HTT-/- mice. These changes were also linked to altered availability of 5-HT. Density of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} and 5-HT_{2C} binding was not changed in 5-HTT overexpressing mice (excluding gender effects). 5-HT_{1A} and 5-HT_{2A} receptor mRNA was not altered.
Comment: These studies discovered that variation 5-HTT expression results in adaptation of specific postsynaptic 5-HT receptor subtypes (Jennings et al, 2008, *Neuropharmacology* 54:776-83)

Rats with low/high exploratory and high/low-sociability traits.
Partner 5 (Tartu) measured 5-HT and dopamine receptors in rats with low/high exploratory and high/low-sociability traits. 5-HT_{1A} receptor binding was not changed but rats with low/high

exploratory traits had no changes in dopamine D_1 receptor mediated cAMP accumulation either under basal conditions or following stress.

2.4. Modulation of information processing beyond the monoamine synapse

VGLUT1+/- mice
Partner 12 (Pamplona) studied glutamate systems in VGLUT1+/- mice and found that chronic mild stress increased cortical and hippocampal glutamate in both wildtype and VGLUT1+/- mice. In response to stress, VGLUT1+/- mice showed decreased GABA. In wild type mice chronic mild stress decreased VGLUT1 expression, which fits with findings that antidepressants increase VGLUT1 expression.
Comment: These data demonstrate an impact of stress on cortical glutamate and GABA, and suggest that altered VGLUT1 expression may be a key factor in the causes of depression and treatment (Tordera et al, 2007, *Eur J Neurosci*. 25:281-90).

Activity 3.1: Molecular markers of HPA function in models, following stress and antidepressants.

Olfactory bulbectomy and maternally-deprived rats.
Partner 7 (Budapest) found higher baseline levels of plasma corticosterone in rats with a bilateral olfactory bulbectomy, and rats subject to maternal separation.

Arginine-vasopression (AVP)-deficient Brattleboro rats.
Partner 7 (Budapest) tested the validity of Brattleboro rats as a depression model. Hypothalamic CRH mRNA was increased and CRH sensitivity was reduced, and a tendency for increased basal corticosterone, and increased dexamethasone suppression of corticosterone after restraint stress. Chronic mild stress elevated ACTH and corticosterone in these rats and these responses were less than controls.
Comment: These data indicate that while Brattleboro rats do not have depressive endophenotypic markers, they inform on the role of vasopressin in HPA responses to stress (Mlynarik et al, 2007, *Horm Behav* 51:395-405).

Repeated stress.
Partner 13 (Warsaw) studied the effects of repeated restraint or footshock on HPA responses to acute stressors and found that repeated stress decreased sensitivity of CRF receptors or decreased CRF activation, and attenuated increased plasma ACTH and corticosterone, observed after acute stress (Dunn & Swiergiel, 2008, *Ann N Y Acad Sci*. 1148:118-26; Dunn & Swiergiel, 2008, *Eur J Pharmacol*. 583:186-93.).

5-HTT overexpressing mice.
Partner 8 (Edinburgh) found that 5-HTT over-expressing mice had normal basal and acute stress-evoked levels of circulating ACTH and corticosterone. The sensitivity of 5-HT_2 and 5-HT_{1B} receptor control of HPA axis function was not altered although hippocampal mineralocorticoid and glucocorticoid receptor mRNA was increased.

Maternally separated rats
Partner 12 (Pamplona) found that maternally separated rats had increased in plasma corticosterone levels, a change reduced by treatment with antidepressants.

3.2 Influence of CRF1&2, AVP, GC and MR receptors

Effect of agonists and antagonists on reward, aversion and cognition

Rats with low and high exploratory traits

Partner 5 (Tartu) found that CRF_1 receptor blockade reduced exploration in HE rats when given acutely, but increased exploration when given repeatedly. In contrast, in LE rats the antagonist reduced exploration after both acute and repeated treatment. LE-animals had higher corticosterone than HE-rats. Repeated exploration testing reduced corticosterone in LE- and HE-rats. Consequently, repeated CRF_1 blockade reduced corticosterone in HE-rats, but increased levels in LE-rats.

Comment: These findings suggest that the behavioural effects of CRF_1 receptor blockade are dependent on environmental habituation. The opposite effects of CRF_1 blockade in LE and HE rats suggest differences in their stress coping mechanisms.

Activity 5: Testing classic and new molecules on the molecular gene expression targets, identified through Activities 1-4.

5.1 Effects of SSRI and NARI treatments

Antidepressant augmentation strategies.

Partner 9 (Oxford) studied the effects of antidepressants combined with blockers of 5-HT_1 autoreceptors and 5-HT_2 receptors. In microdialysis experiments, the SSRI/5-HT_1 antagonist, SB 649915-B, increased 5-HT release in cortex, as did the novel SSRI/5-HT_{1A} antagonist, SB-733885-B. The latter also evoked a 5-HT-dependent increase in activity-dependent gene expression. Non-selective 5-HT_2 blockade enhanced SSRI-induced 5-HT release, as did selective 5-$HT_{2A/C}$ antagonists. Combined SSRI/5-HT_{2C} antagonists increased activity-dependent gene expression.

Partner 2 (Paris) found that 5-HT_{2C} receptor function was reduced by chronic SSRIs, as well as in 5-HTT-/- mice, suggesting desensitization of 5-HT_{2C} receptor-mediated feedback by long-term 5-HTT blockade.

Comment: The data suggest that combined blockade of the 5-HTT and 5-HT feedback results in antidepressant augmentation (Boothman et al, 2006, *Neuropharmacology* 50:726-32; Hughes et al, 2007, *Psychopharmacology* 192:121-33).

Galanin.

Partner 10 (Stockholm) studied galanin receptor antagonists. Microdialysis studies showed that GalR3 blockade partially prevented galanin-evoked reduction in hippocampal 5-HT release, as did 5-HT_{1A} receptor blockade. Combined GalR3 and 5-HT_{1A} blockade completely reversed the inhibition of 5-HT release by galanin. GalR3 blockade also reversed galanin-evoked inhibition of raphe 5-HT neuron firing.

Comment: These results indicate that the antidepressant- and anxiolytic-like effects Gal3-selective antagonists may be mediated by attenuation of the inhibitory influence of galanin on 5-HT transmission (Swanson et al, 2005, *PNAS* 102:17489-94).

SSRI effect on glial and neuronal glutamate synthesis – novel C13 methodology.

Partners 1 and 13 (Manchester & Warsaw) probed 5-HT function in rats using non-invasive MRS measurement of glutamate turnover, by tracing 1-^{13}C-glucose and 1,2-^{13}C acetate. SSRI administration stimulated neuronal and glucose oxidation in the cerebrum. Astrocytic substrate utilization was not changed. Absence of change in ^{1}H MR spectra indicated unaffected pool sizes of unlabelled metabolites.

Comment: ^{13}C MRS is a more sensitive method than ^{1}H MRS for detecting changes in neurotransmitter –related glutamate function, and potentially translatable to man.

Overview

Over the past 66 months the research activities of all contributing partners of WP 3 (***Partners 1, 2, 3, 4, 5, 6, 7/14, 8, 9, 10, 12 and 13***) have produced deliverables that focus on the main objectives of this WP, and especially in relation to establishing and understanding a data set of neurochemical and hormonal changes across a range of selected genetic and environmental models of vulnerability depression.

The main objectives of WP3 for this reporting period have been fulfilled in terms of the following discoveries:

1) New knowledge on neurochemical and hormonal changes in genetic and environmental animal models of depression have been obtained by studies: i) tryptophan depletion, ii) chronic mild stress, iii) 5-HTT knockout and overexpressing mice, iv) low exploration and sociability traits, v) Brattleboro rats, vi) VGLUT1 knockout mice, vii) cannabinoid receptor knockout mice, and viii) immune system activation.
2) The finding that activation of 5-HT release by stress appears to be an adaptive coping response to stress.
3) New knowledge of the fundamental mechanisms controlling 5-HT and noradrenaline neurones.
4) New knowledge on adaptive changes beyond the 5-HT neurone in response to variation in 5-HTT expression in terms of 5-HT receptor binding and receptor function as assessed HPA responses to 5-HT drug challenge (Partner 8).
5) New knowledge on the effects of classical and new antidepressant treatments on neurochemical and hormonal function has been obtained by studies: i) Galanin antagonists, ii) SSRI/5-HT_1 autoreceptor antagonist, iii) SSRI/5-HT_2 antagonist combinations.
6) Extensive collaborations and interactions were established for specific projects including Partners 2, 3 & 4, Partners 6 & 8, Partners 8 & 9, Partners 6, 8 & 9, Partners 5 & 9 and Partners 1 & 13.

Table 11. Deliverables and milestones for Workpackage 3

All the Milestones and Deliverables involved with WP3 have been achieved. For detailed information about the Deliverables, please see separate report ("Deliverable WP 3: Neurochemistry of neurotransmission and hormone function")

Below is a list of all Deliverables and Milestones to which WP3 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name** **(All Achieved)**	**Date due**	**Actual date**
11 & 32b	Completed data-set on neurotransmitter endophenotype across several models.	30	36
23	Neurophysiological responses in selected models	36	36
26	Review of insights from NewMood models on control HPA in stress	36	42
25b & 30a	Completed data on neurotransmitter regulation in the maternal separation model	42	48
37	Validation of animal imaging model of 5-HT_{2C} long-loop feedback	48	60
Deliverable No. (part-contribution)			
4, 6, 8, 15 & 31a, 22, 25a, 33, 48, 51, 53, 56	(various)	18 - 60	18 - 66
Milestone No. (main contributor)	**Milestone name**	**Date due**	**Actual date**
2	Selection of rodent models for more detailed behaviour, neurotransmitter and neuroendocrine endophenotypic analysis	18	18
13	Decision on viability of peptide release experiments using molecular biological approaches	36	36
23	Decision on whether *in vivo* rat brain MRS measurements of GABA/glutamate are achievable	48	48
25	Identification of abnormalities of HPA feedback regulation shared by several models	48	48
Milestone No. (part-contribution)			
7, 9, 14, 15, 18, 20, 21, 30, 31, 33, 38, 39.	(various)	18 - 60	18 - 60

Workpackage 4 - Neurochemistry of cell responses and gene expression:

NewMood chip array

1. Objectives

The main objective of the WP4 is to analyse the expression and functional implications of relevant genes and proteins

i. in models of genetic, developmental and acquired vulnerability to depression,
ii. after either classical (antidepressant molecules, ECT) or new approaches to alleviate the affective disorders
iii. in human post-mortem brain.

Studies will be conducted at the cell level to analyse the cellular / molecular mechanisms responsible for the adaptive cell responses both in the animal models and after treatment.

Detailed objectives

Oligonucleotide microarrays: We wanted to investigate which genes were differentially expressed in the models of affective disorders raised by the consortium, and those susceptible to change after classical and new antidepressant treatments. Chips were constructed with the help of both the local facilities and a biotech company, GeneScore, in order to raise a standard gene array (NEWMOOD array) to be used by all the Partners of the consortium. *(Activity 1.3. Changes in gene expression which are shared by models: The NEWMOOD gene array)*

Membrane binding assays, autoradiographic labelling, Western blotting, enzyme mobility shift assay (EMSA), RT-PCR and in situ hybridization histochemistry: to visualize and quantify *receptor binding proteins*, transcription factors (CREB and other) and their encoding mRNAs. In addition, the specific binding of [^{35}S]GTPγS to brain membranes (using antibody capture assay) and brain sections (autoradiography) exposed to selective agonists was measured in order to assess the G-protein coupling status of receptors in selected brain areas *(Activity 2.3 Molecules beyond the monoamine synapse)*

Immunocytochemical methods were used to visualize and quantify the subcellular distribution and the possible internalization of monoamine-, neuropeptide- and HPA-axis-related receptors in the various models of depression and after various treatments *(Activity 2.3 Molecules beyond the monoamine synapse and Activity 3.1 Molecular markers of HPA function in models, following stress & antidepressants).*

2 Progress towards objectives

Activity 1.3. Changes in gene expression which are shared by models: The NEWMOOD gene array

Gene expression was planned to be studied by chips constructed with the help of both the local facilities and a biotech company, GeneScore, in order to raise a standard gene array (NEWMOOD array) to be used by all the Partners of the consortium. However, in October 2006, without prior warning, the SME Genescore was declared bankrupt. The bankruptcy forced the consortium to reconsider its gene-finding strategy in the light of current technology and the marketplace. It was agreed that we should take advantage of the greatly increased possibility of identifying altered function in biochemical pathways afforded by a genome-wide platform. After thoroughly investigating the various options available, the consortium decided that all the laboratory work would use the Illumina platform and be carried out at ServiceXS (Leiden).
The first years of the program has been devoted to the selection of genes for the GeneScore NewMood chip, to the analysis of gene expression using local facilities and to the construction of the standard gene array (NewMood-1000 array) with the help of the biotech company, GeneScore.

Using available commercial microarrays, partners have run pilot gene analysis using tissues from either animal models of affective disorders or animals treated with antidepressants.

- ***Partner 2*** (Paris) (on mutated mouse model, 5-HTT), using a oligonucleotide-based microarrays "Neurotrans" from GeneScore), analysed the differential expression for gene observed with the GeneSore chip by real-time PCR using specific (TaqMan) probes and confirmed that the mRNA encoding prepro-CCK (cholecystokinin) was down-regulated (-30%) in the anterior raphe area of 5-HTT knock-out mice.
- ***Partner 3*** (Würzburg) studied the expression profiling with the NewMood chip in NOS1-/- mice which revealed several regulated genes, some of which will undergo further investigation by quantitative RT-PCR (qRT-PCR) and *in situ* hybridisation as a positive control for the core models. In addition, using genome-wide expression profiling approach in 5-HTT deficient mice, Partner 3 shows the importance of *Pomc1* gene peak expression in the mouse brain at postnatal day three for resilience to stress.
- ***Partner 9*** (Oxford) developed pilot gene array analysis of tissue from rats treated repeatedly with electroconvulsive shocks ECS by hybridizing cDNA probes to nylon membrane arrays, BD Atlas array,
- ***Partner 12*** (Pamplona) analyzed gene expression in maternal separation in Wister rats, using Affimetrix array. Hypothesis-testing Western-blot studies were then carried out on protein expression of the GABA-B receptor and to the α subunit of the GABA-A receptor. The GABA-B receptor protein was decreased in MS rats, which, confirms the changes in gene expression previously found in the rat hippocampus after maternal separation using the Affymetrix microarray. The α subunit of the GABA-A receptor was significantly decreased in the frontal cortex and increased in the striatum of maternal separated rats.
- ***Partner 13*** (Warsaw) using GeneScore array found a different expression among 11 genes in RNA isolated from brains of mice subjected to chronic mild stress (CMS), which produces depression-like behaviour versus mice housed in normal conditions in mice selected for high (HA line) and low (LA line) swim stress-induced analgesia.

In ***Partner 1*** (Manchester) team, a central and single analysis pipeline was developed for all array data at the University of Manchester. This included array data previously obtained in several models using the NewMood 1000 Genescore array.

In the three other years, after the bankruptcy of GeneScore, the consortium shifted to an Illunima array

- ***Partner 1*** set up the NewMood biometric array analysis. To identify changes in gene-expression that are shared across the models, lists of genes showing changed expression within models were compared between them. In addition the LIMMA linear modelling program was used to run analyses of variance for all genes across mouse and across rat models with factors for depressed phenotype, model, brain region, and, stress. The linear modelling approach is a potentially powerful way of detecting changes common to several models and across brain areas resulting in large effective sample sizes; the main effect of phenotype were based on a comparison of 16 vs. 16 arrays for each brain area.
- A combined analysis of array data from the 4 GM mouse strains has been carried and summarised in the Major Achievments section and in more detail in the PAR for the fifth reporting period.
- ***Partner 2*** (Paris) investigated the genome-wide expression in the core model GR-i mice. Genes whose expression was modified in three brain areas known to be involved in mood disorders (anterior raphe area, hippocampus and anterior cortex) of these mutants versus paired wild-type (WT) mice were searched using oligonucleotide-based microarrays (Whole-Genome Expression BeadChips, Illumina). Among the few genes whose expression clearly differed in mutants versus WT mice, the genes encoding Urocortin and

CART were overexpressed in the raphe area, whereas those encoding Arc, Cortistatin and Vamp 8 were upregulated in the three regions.

- ***Partners 2 and 9*** (Paris and Oxford) joined their efforts to analyse gene expression in the 5-HTT -/- and overexpressing mice. Genes whose expression was modified in the anterior raphe area of these mutants versus paired wild-type (WT) mice were searched using oligonucleotide-based microarrays (Whole-Genome Expression BeadChips, Illumina MouseRef-8). Among the genes whose expression clearly differed in mutants vs WT mice, the gene encoding Urocortin (UCN) appeared to be down-regulated (-50%) in the anterior raphe area of 5-HTT-/- mice and, in contrast, up-regulated (+200%) in this same area in 5-HTT+ mice.
- ***Partner 4*** (Barcelona) after completion of the results get with the first NewMood array on CB1 knockout mice, used Illumina microarray chips (24,000 transcripts) in hippocampus, frontal cortex and dorsal raphe nucleus samples from these animals in order to extend the results to the whole genome and to homogenize the experimental conditions to those used in the studies that will be done in other core animal models of depression. The effects produced by chronic antidepressant treatments on genome expression have been also investigated in CB1 knockout mice.
- ***Partner 5*** (Tartu) studied three brain regions selected by NewMood for Illumina array studies, in the models selected as NewMood core models: partial 5-HT denervation by low dose PCA + chronic variable stress, and LE- vs. HE-rats: animals with persistently different behavioural pattern in novelty. The LE/HE-model has so far received more attention as this was selected as one of the four NewMood models. Comparing the HE- and LE-rats, 816 transcripts in the raphe, 809 in the hippocampus, and 602 in the frontal cortex were significantly differentially expressed (pplr < 0.001). There was considerable overlap in the expression profiles between the studied brain areas, as 390 transcripts were changed in the same direction and in common in the raphe, the hippocampus, and the frontal cortex. Of these, 264 genes were downregulated and 156 upregulated in the LE-rats. RNA for Illumina arrays was also run for the social defeat model. Several genes identified overlap with those detected as differentially expressed in previously analysed models.
- ***Partner 6*** (Maastricht) investigated gene expression in relation to prenatal-stress and additional adult stress and showed that hippocampal gene-list contained several over-expressed genes for ribosomal proteins especially in female offspring and several concerned with glutamate neurotransmission. In particular, in females, interesting patterns showed up, suggesting changes in e.g. central insulin signalling, GABAergic and glutamatergic neurotransmission, and synaptic plasticity.
- ***Partner 12*** (Pamplona) analyzed in the VGLUT1+/- model, the prefrontal cortex and revealed changes in the expression of 950 genes (MinPplr <0.001) in VGLUT1+/- mice compared to WT. On the other hand, WT mice exposed to chronic mild stress showed changes in 147 genes. Interestingly, 55 genes were common to the VGLUT1+/- and the CMS models and, also, most of them showed regulations in the same direction. Among these, candidate genes selected for playing an important role on cell survival, apoptosis, redox metabolism, glutamate transmission or cytoskeleton were included.
- ***Partner 13*** (Warsaw) investigated gene expression in mice (both HA and LA) subjected to restraint stress and chronic mild stress and treated with desipramine. Different genes in each brain structures differently expressed in the untreated (naïve) HA and LA mice are involved in regulation of biochemical pathways such as gap junction, long-term potentiation, long-term depression, axon guidance, and neuroactive ligand-receptor interaction. After chronic mild stress, a total number of 49 genes changed their expression in HA and LA mice compared with corresponding control groups that were not subjected to stress procedure in the raphe area, and the results of the analysis suggested that 5-HT5B and orphan Gpr88 receptors may be involved in adaptation to chronic stress.

Activity 2.3 Molecules beyond the monoamine synapse

- ***Partner 1*** (Manchester) has assessed the expression of NMDAR-related genes in the post-mortem hippocampus (Stanley Consortium brain collection) using real-time quantitative PCR (qRT-PCR). These genes included NR1, NR2A, NR2B, NR2C, NR2D, PSD-95, CLCA1, DLGAP1, NOS1 and LRFN1.No changes in gene expression were found for the obligatory NMDAR subunit, NR1, nor associated post-synaptic density protein PSD-95. However, increases in three of the four NR2 subunits, 2A, 2B and 2D were found in depression. Also, an increase in DLGAP1, a molecule known to functionally interact with PSD-95, was found specifically in depression. Contrary to the suggestion by Paul and Skolnick, (2003), no effects of antidepressants on NMDAR subunits were found.
- ***Partners 2 and 12*** (Paris and Pamplona) determined the activity of 5-HT_{1A} receptors *in vitro* by quantitative autoradiography of 5-HT_{1A} receptor-mediated [γ-^{35}S]GTP-S binding using the 5-HT_{1A} receptor agonist 5-carboxamido-tryptamine (5-CT). As a complementary *in vivo* approach, 8-OH-DPAT (induced hypothermia was evaluated. 5-CT induced increase in [γ-^{35}S]GTP-S binding was markedly less in the DRN of VGLUT1+/- mutants compared to WT. Moreover 8-OH-DPAT-induced hypothermia was also significantly attenuated in VGLUT1+/- *versus* WT mice. In addition, higher levels of 5-HT in the brain stem of VGLUT1+/- compared to WT were found. These data suggest that 5-HT_{1A} autoreceptors were desensitised in the heterozygous mice. Impaired feed back mechanisms of control from descending glutamatergic projections over the 5-HT activity in the brain stem are suggested to be involved in this desensitization.
- ***Partner 4*** (Würzburg) in collaboration with ***Partner 2*** (Paris) analyzed the 5-HT system in the core model CB1-/- mice. The lack of CB(1) receptor has been shown to induce a facilitation of the activity of serotonergic neurons in the DRN by altering different components of the 5-HT feedback as well as an increase in 5-HT extracellular levels in the prefrontal cortex in mice. *In vivo* microdialysis experiments revealed increased basal 5-HT extracellular levels and attenuated fluoxetine-induced increase of 5-HT extracellular levels in the prefrontal cortex of CB1 knockout compared with wild-type mice. The lack of CB1 receptor also altered 5-HT receptors related to the 5-HT feedback. Extracellular recordings in the dorsal raphe nucleus revealed that the deletion of CB1 receptor induced a 5-HT_{1A} autoreceptor functional desensitization.
- ***Partner 5*** (Tartu) determined dopamine D_1 receptor function, measured by cAMP accumulation, in striatum and accumbens in LE- vs. HE-rats which had been given either vehicle or cocaine for three weeks. In accumbens, significantly higher D_1 receptor dependent cAMP accumulation was found in HE-rats, and this difference was abolished by repeated cocaine treatment. In striatum, however, both basal cAMP levels and D_1 receptor dependent cAMP accumulation were twice lower in the LE-rats, the "depressed" phenotype. The differences were particularly remarkable because there was no overlap in the data of the groups, and the difference was not abolished by cocaine which did not influence the differences in behavioural phenotype either. Thus, D_1 receptor and adenylate cyclase related mechanisms appear as an important feature in the LE-phenotype. In addition, ***Partner 5*** used the 8-OH-DPAT induced hypothermia to characterize 5-HT_{1A} receptor function in the LE/HE-model, and in the PCA/stress model. The "depressed" LE-rats were less sensitive to the hypothermic effect of 8-OH-DPAT. As these animals also had lower 5-HT_{1A} receptor expression hippocampus in the microarray experiment they might have a more general 5-HT_{1A} receptor functional deficit. Finally, chronic stress increased the hyperthermic stress response and interfered with the recovery to baseline body temperature; both serotonergic autoreceptor activation and serotonergic denervation reduced the acute hyperthermic stress response in rats.
- ***Partner 7/14*** (Budapest) studied the MDMA partial 5HT lesion of the serotonergic system to characterize possible changes in the function of postsynaptic receptors after

long-term decrease in availability of 5-HT. Behavioural and vigilance effects were measured after the use of selective receptor agonists and antagonists as challenges. Basal and agonist-induced specific binding of [35S]GTPgammaS to brain membranes and brain sections were measured in this model by the partner 2 group. Like that observed in the DRN, basal labelling within the hippocampus did not differ. With ***Partner 8*** (Edinburgh), uncoupling of the heterologous G-proteins and subsequent internalization of the receptor after β-arrestin binding was measured for 5-HT_{1B} receptors. The ability of 5-HT_{1B} receptors to bind β-arrestin2 upon stimulation was followed using confocal microscopy and bioluminescence resonance energy transfer (BRET). Interestingly, no detectable interaction between 5-HT_{1B} receptors and β-arrestin2 were found. These data show that serotonin is unable to induce association of the 5-HT_{1B} receptor with β-arrestin2 and suggest that β-arrestins do not participate in regulation of 5-HT_{1B} receptor function.

Activity 3.1 Molecular markers of HPA function in models, following stress & antidepressants).

- ***Partner 4*** (Barcelona) in the core model, the CB1-/- mouse. Evaluated plasmatic corticosterone levels under basal and stressful conditions. The expression of glucocorticoid receptors and the levels of CRF mRNA were alsoevaluated in these mice on different brain structures under basal conditions and after exposure to the different protocols of stress used in previous experiments.
- ***Partner 2*** (Paris) set up the steps to complete the study of the HPA axis in the core model. GR-i mouse. Both GR and MR mRNA quantification was done using qRT-PCR with specific probes, and as expected, GR expression is significantly decreased in GR-i mice, a genetic model of vulnerability to environmental factors, compared to WT. In addition, we showed that chronic treatment with the novel antidepressant, agomelatine, but not fluoxetine, increased GR expression in the hippocampus of GR-i transgenic mice, so that GR mRNA levels in these mice were no longer different from those in WT mice.
- ***Partner 12*** (Pamplona) measured the levels of GC receptors in the hippocampus after chronic treatment with fluoxetine and venlafaxine in the core model. Maternal Separation rats. MS produced a significant decrease in hippocampal GR expression that was reverted by chronic fluoxetine or venlafaxine treatments.

Overview

The main objectives of the WP4 have been reached by the different partners.

1) The bankruptcy forced the consortium to reconsider its gene-finding strategy in the light of current technology and the marketplace Illumina array studies have been completed on all core models The NewMood biometric array analysis pipeline has been successfully implemented.
2) The array data are complemented by expression studies in cortex in response to chronic mild stress and antidepressant drugs.
3) The quantification of several receptor binding proteins and their encoding mRNA, and also specific GTP-γ-S binding has been initiated. The 5-HT1A autoreceptor function has been analyzed in all the core models.
4) Monoamine and HPA-axis-related receptors has been analyzed in various models of affective disorders and after treatment

Table 12. Deliverables and milestones for Workpackage 4

All the Milestones and Deliverables involved with WP4 have been achieved. For detailed information about the Deliverables, please see separate report ("Deliverables WP 4: Neurochemistry of cell responses and gene expression: NewMood / Illumina array"). Below is a list of all Deliverables and Milestones to which WP4 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name** **(All Achieved)**	**Date due**	**Actual date**
2	Initial NEWMOOD-1000 microarray data-set	18	24
6	Analysis of initial microarray data-set complete in 4 mouse models.	24	48
9	Micro-array identifies first genes common to animal models	24	60
16 $	~~Design of NEWMOOD 'Depression' chip agreed~~	~~36~~	~~X~~
18 & 31b	Illumina array microarray data-set in Core Models	36	48
19	Identification of first candidate genes from NewMood / Illumina array	36	48
34	Changes in hippocampal gene expression in vulnerability models replicated in samples from depressed humans	48	60
40	Determination of effect of SSRI/5-HT_{2C} antagonist combinations on gene expression	48	60
44	Microarray data on 5-HTT over-expressing and knock-out mice	54	60
Deliverable No. (part-contribution)			
4, 8, 15 & 31a, 23, 24, 25a, 25b & 30a, 26, 27a, 33, 47, 50, 56	(various)	18 - 60	18 - 66
Milestone No. (sole contributor)			
1	Uniform method of brain dissection agreed. (DVD made by Partners 6 and 2; available to all consortium members)	15	15
5	Identification of new genetic and developmental models from molecular developmental & array studies	24	24
10	First results of antidepressant effects on gene-expression	30	30
22	Identification of changes in gene expression shared in several models from NewMood Bioinformatics Analysis Pipeline	48	48
34	Validation replication of genes from the expression arrays (e.g. by RT-PCR or *in situ* hybridisation)	60	60
35	Correlation of the serotonergic deregulation of CB1 knockout mice with the differential gene expression of the $5HT_{2C}$ receptor	60	60
36	Determination of changes induced by chronic antidepressants on gene expression in CB1 knockout mice and validation by RT-PCR	60	66
Milestone No. (part-contribution)			
7, 9, 20, 21, 24, 28, 30, 31, 32, 33,	(various)	30-60	30-60

$ The original Deliverables number 16 "Design of NEWMOOD 'Depression' chip agreed" was removed when Geenscore was declarded bankrupt and NewMood used the Illumina whole-genome bead-arrays.

Workpackage 5 – Cyto- and immunochemistry

1. Objectives:

The objective is to characterize the neuroanatomical and neurochemical phenotype of various animal models, including genetically modified mice, relevant to depression, in particularly markers related to central 5-HT neurons in the dorsal raphe, and also noradrenaline neurons in the locus coeruleus nucleus.

Specific objectives of the study

- To characterize in particular noradrenergic and serotonergic markers in various rat and mouse depression models as well as in the human brain in relation to controls.
- To characterize expression patterns of auxiliary messengers including nitric oxide, neuropeptides, cytokines and their receptors.
- To establish whether or not cell death and new formation of cells is an important issue in depression.

2. Progress towards the Objectives:

In this summary, we will focus of some of the major advancements achieved within the framework of WP 5: (1) Regulation of the DRN 5-HT neurons and (2) the phenotypic characterization of 5-HT DRN and NA LC neurons in rodents and man (Activity 2), and (3) relation of neurogenesis to depression-like behavior in various core models and other experimental paradigms (Activity 3)

Activity 2: Functional states of monoamine regulation in rodent models of vulnerability – the neurotransmitter endophenotype.

Regulation of monoamine cell-body function in raphe and locus coeruleus

A major effort has been made, in particular by ***Partner 9*** (Oxford) together with ***Partner 6*** (Maastricht), to elucidate how the 5-HT neurons in the dorsal raphe region are controlled, that is defining the feedback pathway(s) from forebrain areas innervated by the 5-HT neurons. Pharmacological and neuroanatomical properties of such pathways involved in the long-loop feedback regulation have been analysed using a combination of *in vivo* electrophysiological, histochemical, neuropharmacological, functional imaging (c-fos), pathway tracing and fMRI approaches. These studies provide strong evidence for the existence of a long-loop, polysynaptic 5-HT feedback pathway, whereby 5-HT_{2A} receptors on prefrontal cortical afferents modulate the firing of dorsal raphe 5-HT neurons. More recently an important input from the lateral habenula nucleus (LHN) has been established, involving both 5-HT_{2A} and 5-HT_{2C} receptors, with local GABA neurons in the periaqueductal grey as critical components in the circuitry. This was established by studying the effect of lesions of LHN on 5-HT_{2C} receptor mediated inhibition of 5-HT cell firing. The circuitry underlying the control of 5-HT neurons in the DRN has been further analysed focusing on 5-HT_{2C} receptors and the LHN activating the GABA neurons in the ventral PAG It was found that LHN lesion dramatically reduced the inhibitory effect of 5-HT_{2C} agonist administration.

This is now a well understood and proven circuitry mechanism in rat. The immunohistochemical analysis of the mouse PAG shows a similarly prominent presence of GABA neurons, but to what extent they are functionally involved in the same circuits as in rat remains to be analysed (Partner 10, Stockholm). Clearly, it is critical to establish the situation in the human brain.

In complementary experiments, ***Partner 9*** also investigated the effect of administration of different 5-HT_{2C} agonists (WAY 161503, Ro 60-0175, mCPP) on fos expression in the LHN. Each 5-HT_{2C} agonist caused a marked increase in Fos expression in this nucleus, and the effect was blocked by a selective 5-HT_{2C} antagonist (SB 242084).

Further mechanisms were explored. Thus, 5-HT neurons in the dorsal raphe can express the IL1-R1 mRNA, and the transcript levels are increased after administration of LPS. There is also evidence that the antidepressant and anxiolytic effects of NK1 antagonists may be exerted via actions on afferents to the dorsal raphe 5-HT neurons. The effect on MDMA on forebrain serotonin nerve terminals has been characterized in detail using anti-sera against tryptophan hydroxylase and the 5-HTT (Partner 7/14). Long-term, partial lesions of the 5-HT system by MDMA treatment caused biphasic effects on 5-HTT. Also the glucose metabolism was affected in the raphe nuclei.

Ongoing experiments by ***Partners 6 and 9*** are studying the interaction between the subthalamic nucleus and raphe. These studies are of major importance for our understanding of the effect of subthalamic stimulation in Parkinson's disease not rarely leading to depression.

Taken together, thanks to the NewMood grant our knowledge of key mechanisms underlying involvement of the 5-HT neurons in mood regulation and the therapeutic effect of antidepressants have been substantially advanced.

The phenotypic characterization of 5-HT DRN and NA LC neurons in rodents and man

The monoamine nuclei locus coeruleus (LC) (noradrenaline, NA) and the dorsal raphe nucleus/complex (DRN)(serotonin, 5-hydroxytryptamine, 5-HT) are key structures in the brain for understanding the pathology and treatment of major depression disorder (MDD). This means that characterization of their neurochemical profile, as well as of their projections and their afferent input is an essential task, that is the circuitry in which they are involved.

Partners 2 and 10 have phenotyped neurons in the DRN/periaqueductal gray (PAG)

In the mouse PAG has been studied with focus on neuropeptides, GABA (glutamic acid decarboxylase, GAD), nitric oxide synthase (NOS) and their relation to 5-HT neurons. The main result is the occurrence of distinct species differences: Whereas many 5-HT neurons in rat express both NOS and galanin, these two molecules were not found in 5-HT neurons in the mouse DR. In fact, with exceptions of single cells expressing a neuropeptide, the mouse 5-HT neurons seem to lack coexisting neuropeptide messenger molecules (of the ones so far studied). However, our data strongly suggest that a population of 5-HT neurons, mainly in the midline, is GABAergic. Moreover, it is highly likely that there is coexpression of the vesicular glutamate transporter 3 (VGLUT3) in many midline 5-HT DRN neurons (data from ABA and literature), as has previously been shown for rat. However, in mouse this coexistence has not been demonstrated at the cellular level, but the strong overlap suggests that many 5-HT neurons release glutamate also in mouse. These results have now been submitted to *J. Comp. Neurol.*, and the revised manuscript has been returned to the journal. The results have been compared with the recently published Allen Brain Atlas (ABA), which provides a fairly complete information on the distribution of some 20,000 transcripts in the PAG (and all other brain areas). Further studies are needed, in particular employing double-*in situ* hybridization using non-radioactive riboprobes for the peptides and the radioactive oligonucleotide probes for TPH2. (Collaboration with Partner 2, Paris)

An important aspect is the phenotypic characteristics of the LC and DRN neurons in humans. The hypothesis-building is mostly based on results from studies of rodents, but these results may not necessarily be relevant for humans. Therefore studies have been carried out on the human post mortem brain with focus on NA and 5-HT synthesizing enzymes, markers for glutamate and GABA neurons and, in particular, neuropeptides and their receptors. The latter group of molecules have recently been considered as novel targets for development of antidepressants. Initially riboprobes for galanin, GalR1 and -R3 receptors have been prepared. Galanin and GalR3 signals have been detected in the LC, and all three markers in the PAG (Le Maître et al., 2008a, b, 2009). So far NOS transcript has not been observed in the DRN, but in LC neurons. We have also carried out qRT-PCR studies on galanin and GalR1-R3, initially on adrenal

pheochromocytoma tumours (Tofighi et al., 2008) and a similar study on pituitary tumors is completed, and will be submitted soon. We also monitored transcript levels in samples from several brain regions obtained from control and depressed (suicide) brains (from The Human Brain Bank in Budapest (***Partner 14*** and Dr. Miklos Palkovits, Semmelweis University).

Taken together, the results show that distinct species differences exist with regard to the galanin system when comparing human, rat and mouse. Whereas galanin peptide is expressed robustly in all three species, GalR1 and -2 are expressed in mouse and art LC, whereas human has GalR3. The rat, but not mouse or human, 5-HT DRN neurons express galanin. In the human, but not in rodents, these 5-HT neurons express GalR3.

Thus, when attempting to identify relevant targets for developing antidepressants, it is important to compare expression patterns in rodents with those in humans.

Activity 3.3 Neurogenesis & apoptosis: stress, monoamine & antidepressant effects

Neurogenesis, with its relation to growth factors, has emerged as a possibly important mechanism involved in etiology and treatment of MDD. Several partners have done frontline research in this area. The results from studies of the core models are particularly important.

Partner 2 (Paris) has studied cell proliferation, differentiation and survival in some of the core models, including the GR-i mouse. They showed that cell proliferation within the hippocampus is modified in some models. Thus, neurogenesis in GR-i mice was significantly decreased when compared to WT mice. In addition, BDNF mRNA expression (analyzed by qRT-PCR) was also reduced in the hippocampus of these mice. Moreover, chronic treatments with a reference antidepressant compound, fluoxetine, or the new antidepressant Agomelatine, promote both granule cell proliferation and cell survival within the dorsal part of the dentate gyrus of GR-i mice. In addition, both treatments raised BDNF mRNA expression in the hippocampus of the transgenic mouse, restoring mRNA expression to a similar level as in WT mice.

The core model 5-HTT-/- was studied by ***Partners 4*** (Barcelona) ***and 2*** with regard to adult neurogenesis, that is under conditions of enhanced 5-HT function on adult neurogenesis. Compared to WT controls, aged 5-HTT-/- mice (~14.5 months) showed an increase in proliferative capacity of adult neural stem cells. In contrast, in vivo analyses of young adult 5-HTT-/- mice (~3.0 months) and cultures of neurospheres from their hippocampus did not reveal significant changes in proliferation of neural stem cells or survival of newborn cells. Thus, the cellular fate of newly generated cells in 5-HTT-/- mice is not different with respect to the total number and percentage of neurons or glial cells from wildtype controls.

The findings indicate that elevation of synaptic 5-HT concentration throughout early development and later life of 5-HTT deficient mice does not induce adult neurogenesis but may influence stem cell proliferation in senescent mice.

Partner 6 has studied the Core Model Prenatal stress (PS) in rats and shown that hippocampal BDNF protein content is decreased in PS rat pups just after birth, but increases within the hippocampus of particularly female offspring. These results indicate that PS can trigger a gender- and region-specific, compensatory effect, in attempt to protect the hippocampus against the deleterious effects of stress. The increased BDNF content may be linked to the relatively high hippocampal weight and relative less affected affective behaviour of prenatal stress in this gender in later life. Male rats exposed to PS and/or chronic mild stress (CMS) displayed changes in the morphology and number of basal spines in the medial prefrontal cortex. CMS had a negative effect on spine densities, particularly on spines of the mushroom type. PS alone did not affect spine densities, but the percentage of mushroom spines was reduced.

Partner 12 (Pamplona) has studied the effect of CMS and antidepressant treatment on neurogenesis and found a short-term and long-term decrease in cell proliferation in the dentate gyrus of the hippocampus 24 h after CMS. Chronic paroxetine (px) treatment reverted the effects of CMS. In long-term studies on mice exposed to CMS and px treatment, cytogenesis but not

neurogenesis was decreased in the dentate gyrus of the ventral hippocampus in CMS mice, suggesting that the generation of glial cells is affected at long-term while the neurogenesis is recovered. Unexpectedly, chronic px treatment significantly decreased both the cytogenesis and neurogenesis in control mice. It is suggested that this decrease could be related to homeostatic mechanisms.

In the maternal separation rat core model Partner 12 showed a significant decrease in cell proliferation in dentate gyrus of hippocampus which was normalized by chronic treatment either with fluoxetine or venlafaxine

Taken together these findings indicate stress-induced gender and region specific, changes in cell proliferation and BDNF levels. Moreover, there are clear morphological changes in response to stress; however, the type of stress is decisive. Also, there may be differences between mild and ultramild stress.

Partner 2 has also initiated a new program regarding the relationship between antidepressant modulation of neurogenesis induced by cerebral focal ischemia in mice. Indeed, there is a comorbidity for stroke and depression, and chronic treatment with antidepressants has been reported not only to alleviate MDD but also post-stroke mood disorders. At anatomical level, a reduction in hippocampus volume is a commun feature in both MD and stroke. However, marked differences have been noted regarding neurogenesis in relevant rodent models of these disorders. A clear decrease in hippocampal neurogenesis has been described in depression-like models whereas experimental stroke caused by transient occlusion of middle cerebral artery (tMCAO) is usually followed by an increase in cell proliferation in both the subgranular (SGZ) and subventricular (SVZ) zones. Furthermore, the peri-infact zone is also depicted to develop neurogenic properties after tMCAO. In depression-like models, antidepressants of various classes can restore neurogenesis at the hippocampal level, probably through induction of neurotrophic factors such as BDNF. Partner 2 has shown, perhaps surprisingly, that chronic paroxetine had apparently no impact on the effects of tMCAO on SVZ cell proliferation (and locomotor performance) in CD1 mice.

Partner 10 has shown that double-cortin (DCx; a marker for immature neurons) is coexpressed with Y1R but not Y2R. There is a marked reduction in DCX-positive cells both in Y1R and Y2R knock-out mice. These findings indicate that neuropeptides may be of significance in depression in view of the now substantiated relation between neurogenesis and antidepressant treatment, as well as the demonstration that some effects of BDNF are mediated by NPY (Howell et al., 2005).

Partner 3 (Würzburg) studied morphogenic effects of 5-HT on target cells, assessing the thickness and neuronal cell density of various cerebral cortical areas in 5-HTT-/-, characterized by elevated extracellular 5-HT levels. Effects on neocortical morphology depended upon the genetic background of 5-HTT-/-. Mixed c129-CD1-C57BL/6J mice display a consistently thinner layer IV, yielding significantly decreased overall thickness in many cortical areas. Instead, mice backcrossed into the C57BL/6J background display increases in supragranular and infragranular layers, which compensate entirely for decreased layer IV thickness, resulting in unchanged, or even enhanced cortical thickness. In general, females display either less of a decrease, or more of an increase in cortical thickness compared to males, depending on genetic background and cortical area. Also significant increases in neuronal cell density are found in 5-HTT-/- with a C57BL/6J background (wt:hz:ko ratio = 1.00:1.04:1.17), and not in mixed c129-CD1-C57BL/6J knockout animals. The findings of this quantitative study show altered neocortical cell density and layer thickness in 5-HTT -/- and provide evidence of 5-HTT gene effects on neocortical morphology, in epistatic interaction with genetic variants at other loci.

Taken together, studies by NewMood partners have greatly advanced our knowledge on possible roles of neurogensis in relation to MDD.

Overview

The Partners in **WP5** have through intense work and fruitful interactions distinctly advanced the knowledge in the field of depression-related behaviour/disease and provided a better basis for the understanding and possible treatment of this type of serious mental disorders. This includes a detailed description of the origin of the afferent inputs and the receptors involved in the control of the ascending 5-HT neurons, which are key target for the major group of antidepressants. A neurochemical phenotyping of transmitters and neuropeptides expressed in the 5-HT neurons has been carried in mouse, to a limited extent also in the core depression models. In addition, messenger molecules have been studied in the human postmortem brain, reporting some interesting species differences, which may be relevant when discussing translational, therapeutic aspects. Finally, a fairly recent idea in the depression field is a possible role of hippocampal neurogenesis as an important process involved in depressive behaviour. Here WP5 has produced a wealth of information in studies of core models and the effect of various antidepressants. The results support the neurogenesis hypothesis, but also point to some inconsistencies.

Table 13. Deliverables and milestones for Workpackage 5

All the Milestones and Deliverables involved with WP5 have been achieved. For detailed information about the Deliverables, please see separate report ("Deliverables WP 5: "Cyto- and Immuno-histochemistry"). Below is a list of all Deliverables and Milestones to which WP5 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name** **(All Achieved)**	**Date due**	**Actual date**
17	Review of molecular basis of changes in neurogenesis in NewMood models of vulnerability	30	48
38	Role of the NPY system in neurogenesis will be available, including the effect of selective deletion of the two receptors	48	48
41	The effect of galanin and galanin agonists/antagonists on depression-like behaviour and on expression of markers in dorsal raphe 5-HT neurons will be reported	48	48
43	Tryptophan hydroxylase mapping in raphe of core models presented	54	60
54	Role of the CCK system in neurogenesis will be published	60	60
Deliverable No. (part-contribution)			
4, 8, 15 & 31, 24, 25a, 33, 48, 11 & 32b, 23, 21, 22, 51, 56	(various)	18 - 60	18 - 66
Milestone No. (main contributor)			
(none)			
Milestone No. (part-contribution)			
7, 9, 15, 16, 20, 21, 24, 28, 29, 30, 31, 33, 37, 38, 39.	(various)	30-60	30-66

Workpackage 6 – Neurophysiology

1. Objectives:

To provide the consortium with the necessary knowledge of neurophysiological properties of several brain nuclei (e.g. dorsal raphe nucleus) and brain cortical areas (e.g. hippocampus, cerebral cortex) involved in depression and anxiety. WP6 is structured to supply novel background information on mechanisms of 5-HT system functioning and focussed information on specific topics relevant to development of animal models and/or effects of treatment with antidepressants. The investigation will develop throughout the entire project lifetime.

Specific objectives of the study:

- to characterise the neurophysiological modifications of synaptic function in selected animal models of vulnerability;
- the alterations in neurotransmission and receptor sensitivity in various brain regions (relevant to depression);
- the adaptive neurophysiological changes produced by treatments.

2. Progress towards the Objectives:

The present summary reports only the principal findings that are considered most relevant to illustrate the progression of the project

For a full account of the results and deliverables obtained by the WP6, please refer to annual Activity and Deliverable Reports

Activity 1. Behavioural endophenotype of models of genetic, developmental and acquired vulnerability to depression.

During the 5 years of project's life, functional neurophysiological studies have been performed in several models of vulnerability.

As activity 1 was directed to the characterization of animal models of genetic, developmental and acquired vulnerability to depression, WP6 contribution to this activity was the study of functional responses in selected animal models where neurophysiological investigation could support the interpretation of behavioural findings and eventually mechanism of drug action.

Therefore, most of the results obtained in a number of *core* mouse models (e.g.: *i)* CB1-/-; *ii)* GR-i *iii)* HA and LA; and mood-pathology relevant mouse models *iv)* Helpless / non-helpless mouse strains; *v)* 5-HT_{2A} (-/-) mice;) have been published as integrated parts of behavioural, neurochemical, and pharmacological investigation on rodent models of vulnerability to depression.

In many cases these studies have been done to answer specific questions and it would be dispersive to mention them in detail here. Exhaustive information can be tracked back in the annual reports and in the description of WP6 deliverables. Thus, only key references are indicated in this summary.

However, some *core* models have been selected for more systematic and thorough investigation and the results obtained deserve mention as they fulfil specific aims of WP6: (a) the mouse core models of vulnerability due to altered 5-HT membrane transporter function: 5-HT transporter knock-out mice (5-HTT-/-), 5-HT transporter heterozygous mice (5-HTT+/-) and mice over-expressing the human 5-HTT gene (5-HTTo/e); (b) the rat core model of partial 5-HT depletion obtained with injection of the selective 5-HT system neurotoxin 3,4-methylene-dioxymethamphetamine (MDMA).

5-HTT-/-, 5-HTT+/- and 5-HTT o/e mice

Sleep disturbances are a common finding in depressed patients. 5-HT is known to play key role in mechanisms controlling sleep/wake patterns, and their alterations in depressed patients.

In 5-HTT-/- mice, ***Partner 2*** (Paris) investigated the consequences of an excess of 5-HT in the synaptic cleft on sleep-wake stages and found that 5-HTT-/- mutants display, at baseline, an increase in rapid eye movement sleep (REMS) amounts (Alexandre et al, 2006). The study was extended to the REM sleep rebound caused restraint stress and interplay between 5-HT and the hypothalamic neuropeptide hypocretin (hcrt). Using polysomnographic recordings Partner 2 found that REM sleep rebound was blunted in 5-HTT-/- mice and that pretreatment with a selective hcrtR1 antagonist restored a robust REM sleep rebound. Altogether, these data show functional interactions between hypocretinergic and serotonergic systems that may be relevant to alteration of sleep and wakefulness homeostasis in depression. Next, ***Partners 2 and 9*** (Oxford) examined 5-HTTo/e mice which have deficient 5-HT release and storage mechanisms and exhibit increased anxiety-like behaviour. In 5-HTTo/e mice wakefulness was increased and REMS episodes were decreased throughout the dark period. In contrast, no significant differences between wild-type and transgenic mice were observed regarding slow-wave sleep. Altogether, these data show that the decrease in extracellular 5-HT levels measured in 5-HTTo/e mice results in changes in the sleep-wake pattern ostensibly opposite to what observed in 5-HTT-/- mice (Rachalski et al., 2010). Using voltammetry *in vivo*, ***Partner 9*** found striking loss of frequency sensitivity of electrically-evoked release of 5-HT at level of serotonergic terminals, both in the 5-HTTo/e and 5-HTT-/- mice. This non-linear relationship between 5-HTT expression and 5-HT signalling predicts abnormal 5-HT transmission with both loss- and gain-of-function 5-HTT gene variants.

Partner 8 (Edinburgh) investigated synaptic plasticity in the dentate gyrus of hippocampal slices from adult male 5-HTT-/- and their wild-type and heterozygote littermates. In 5-HTT+/+ and 5-HTT+/- groups long–term potentiation (LTP) was not significantly different whereas the 5-HTT -/- group showed a significant decrease in the magnitude of potentiation. Post-tetanic potentiation, measured immediately after tetanic stimulation, was comparable across all three groups. When a similar study was performed at dentate gyrus granule cell synapse and CA3/CA1 Schaffer collateral synapse in the hippocampus of both male and female 5-HTTo/e mice, no significant differences in LTP were found either with respect to genotype or with respect to gender. These data suggest that serotonergic systems play a role in synaptic plasticity in the dentate gyrus either directly through its synaptic actions or indirectly through its effects on development, and that certain cognitive processes to which the hippocampus contributes might be disrupted when 5-HT transporter functioning is substantially altered.

Partner 3 (Würzburg) examined the effects of genetically driven loss of 5-HTT function in the 5-HTT-/- mouse on the mediation of fear- and stress-related behaviours, and electrophysiological properties of hippocampus, amygdala, and prefrontal cortex. They demonstrate a specific phenotypic profile of fear- and stress-related deficits in 5-HTT-/- mice, accompanied by morphological abnormalities in amygdala and prefrontal cortex (Wellman et al., 2007).

Furthermore, using patch-clamp whole-cell recordings ***Partner 2*** (Paris) found that 5-HT_{1A} autoreceptor desensitisation in 5-HTT-/- mice reflected alteration in the coupling between G-proteins and GIRK channels (Loucif et al., 2006). Finally, Partner 2 found resilience of 5-HTT-/- mice to several effects produced by MDMA-induced mild lesion of 5-HT system. Notably, the increased immobility duration in the forced swim test (i.e. depressive-like behaviour), the increase in 5-HT_{1A} receptor-mediated *autoinhibition* of DRN 5-HT neurons (see Activity 2) and the larger hypothermic response to 8-OH-DPAT, were abolished (Renoir et al., 2008).

Collectively, the results obtained with 5-HTT-/-, 5-HTT-/+ and 5-HTTo/e mice show that, altogether this set of animals represents a valuable tool for exploring the role of 5-HTT in vulnerability to depression and effectiveness of new drugs.

MDMA-induced selective 5-HT system impairment in Dark-Agouti rats

The investigation has been performed by ***Partners 2 (Paris), 7/14 (Budapest), 8 (Edinburgh)*** **and** ***Partner 11 (Florence).***

Complete 24 hour long recordings of sleep in adolescent animals with MDMA–induced partial depletion of 5-HT performed by ***Partner 7/14*** revealed significant decreases in REM latencies and increased duration of REM in the first hour of the passive sleep phase. This was accompanied by increased delta power off either SWS-1 or in SWS-2, but none of the other parameters used for characterization of NREM were significantly affected. Next, the effects of the selective serotonin reuptake inhibitor (SSRI) antidepressant citalopram were studied on EEG, EMG, motor activity recordings were performed, and effects on vigilance states, including wake, light slow wave sleep, deep slow wave sleep, and paradoxical sleep. Immediate effects of citalopram were similar or even stronger in animals treated with MDMA several weeks earlier. However, the effects lasted only a few hours while the effects persisted much longer in control animals. An attenuation of SSRI-induced *in vivo* effects, but not of those produced by 8-OH-DPAT, suggested that blunted responses to the SSRIs are direct effects of decreased axonal densities rather than direct receptorial changes. This conclusion appears to be confirmed by findings obtained in a collaborative study with ***Partner 2*** on basal and agonist-induced specific binding of [^{35}S]-GTPγS to cell membranes in hippocampal regions. Reduced REM latency is a classic feature of depression and the results add to the evidence that MDMA treatment models some but not all aspects of depression. Consistently, our data provide evidence that a partial lesion of the serotonergic axons and terminals, or hypofunction of the 5-HT system, changes the effects of SSRI antidepressants indicating that alteration in autoreceptor function and adaptive changes relevant to depression and antidepressant treatment, also occur. 5-HT$_{1B}$ and 5-HT$_3$ receptor activation-mediated functional changes of motor activity and sleep-wake parameters were also measured by Partner 7/14, several months after the induction of the lesion, when most areas show complete recovery of axons at least by quantitative measures. Induction of wake by stimulation of 5-HT$_{1B}$ receptors was markedly reduced, but other effects were not affected 6 months after the lesion. 5-HT$_3$ receptor activation-induced changes in the theta power as well as motor activity effects were significantly altered 6 months after the induction of the lesion providing further evidence that several key receptor functions are altered during the recovery period and imply that virtually complete recovery may not necessarily mean a complete recovery of functions (Kirilly et al., 2008)

Alteration of 5-HT system functioning has been associated with impairment of cognitive performances, which also is a trait present in severe forms of depression. ***Partner 11*** (Florence) investigated synaptic plasticity in slices of ventral hippocampus taken from rats with mild 5-HT system impairment produced by repeated administration of MDMA *in vivo*. Since we had demonstrated that endogenous 5-HT released in ventral slices increases the excitability of CA1 pyramidal cells through activation of 5-HT$_4$, but not 5-HT$_6$ or 5-HT$_7$, receptors (Mlinar et al., 2006, 2008), the work focussed on possible changes in 5-HT$_4$ receptor sensitivity and changes in plasticity. Mild depletion of 5-HT *in vivo* produced an *increase* in LTP susceptibility that was not accompanied by changes in 5-HT$_4$ receptor or β_1-adrenoceptor sensitivity in the CA1 region of the hippocampus [Morini et al., (2010) Enhanced hippocampal LTP following MDMA-induced serotonergic impairment in Dark-Agouti rats. European Journal of Neuropsychopharmacology (submitted)]. We concluded that substantial removal of the inhibitory effects of 5-HT does not disrupt the basic hippocampal mechanisms of plasticity relevant for learning and memory, but that functional changes in hippocampal plasticity may be relevant to altered emotional responses.

The offspring of MDMA-treated female rats have also been characterized as a model for the study of the effects of prenatal exposure to synthetic drugs on vulnerability to depression **(*Partner 8*)**. Electrophysiological assessment of 5-HT modulation of dorsal raphe and hippocampal neuronal function in slices from these offspring showed no major alterations and

helped to interpret the changes in regional cerebral function detected with 2DG autoradiography *(see WP 7)*.

In conclusion, the MDMA-induced selective 5-HT system impairment model resulted very useful for the study of the behavioural alterations produced by an impairment of the 5-HT system and provided new knowledge of 5-HT system functioning, mechanisms of its repair after mild lesion, and its role in altered social interaction, sleep, and cognitive functions. The properties and limits of this model, based on anatomical loss of a part of terminals, for future studies of antidepressant drug actions have also been defined.

Activity 2. Functional states of monoamine regulation

Activity 2 was devoted to in-depth and innovative exploration of the functional mechanisms regulating the activity of serotonergic cells in raphe nuclei (local and long-loop feed-back mechanisms), hence the release of 5-HT throughout the brain. Furthermore, the neurophysiological effects of serotonin release onto target cells in hippocampus and other cortical and subcortical areas have been studied.

Altogether, this activity resulted in extremely important new knowledge on 5-HT system functioning which undoubtedly will mark future development of our understanding of the role of serotonin in mood disorders.

Long-loop and local control of 5-HT cell activity

Long feedback regulation

Partner 9 (Oxford) obtained data suggesting the presence of a novel negative feedback mechanism that controls the electrical activity of midbrain 5-HT neurones via postsynaptic 5-HT_2 receptors. The investigation has been extended to the neuroanatomical pathways involved in long-loop feedback regulation of 5-HT neurones using a combination of neuropharmacological, immunocytochemical and pathway tracing approaches. ***Partner 2*** (Paris) and ***Partner 9***, applied *in vitro* electrophysiological methods to model local 5-HT_2 receptor-mediated feedback loops and used transgenic mice (5-HT_{2A} receptor knock-outs) to explore the pharmacology of 5-HT receptor subtypes involved (Boothman et al, 2006a). Electrophysiological *in vivo* recordings have been carried out to investigate the 5-HT_2 receptor subtype involved, and specifically to test for the involvement of the 5-HT_{2C} subtype. (Boothman et al, 2006b; Boothman et al., 2007).

Collectively the results indicated that medial prefrontal cortical neurones projecting to raphe express 5-HT_{2A} receptors and supported a role for the 5-HT_{2C} receptor subtype in postsynaptic feedback control of 5-HT neurones.

Next, ***Partner 9***, in collaboration with ***Partner 6*** (Maastricht), continued to investigate possible behavioural correlates of long-loop feedback, with a focus on the 5-HT_{2C} feedback system. Specifically, we investigated the effect of disrupting 5-HT_{2C} receptor-mediated long-loop feedback on a range of experimental models of 5-HT_{2C} receptor function including hypophagia, anxiety, learned helplessness and hypolocomotion. 5-HT_{2C} receptor-mediated long-loop feedback was disrupted by excitotoxic lesion of the lateral habenula nucleus, since this lesion prevents 5-HT_{2C} receptor agonist-induced inhibition of 5-HT cell firing. Importantly, neuroanatomically-discrete lesions of the lateral habenula nucleus resulted in a marked attenuation of 5-HT_{2C} receptor agonist-induced hypolocomotion, hypophagia and anxiety.

These data provide a direct link between the 5-HT_{2C} receptor-mediated long-loop feedback pathway and some common behavioural effects of 5-HT_{2C} receptor activation (Quérée et al., 2009). Altogether, these data suggest that 5-HT_{2C} agonists activate the lateral habenula nucleus that in turn activates DRN GABA neurones to inhibit 5-HT cell firing. The presence of 5-HT_{2C} receptors on DRN GABA neurones also suggests a local effect of 5-HT_{2C} agonists.

Sub Thalamic Nucleus-raphe interactions

Bilateral stimulation of the subthalamic nucleus (STN) is used clinically to treat Parkinson's Disease, but suffers from a high incidence of affective side effects (Temel et al., 2009). In collaborative work ***Partners 9 and 6*** (Oxford and Maastricht) investigated the effects of stimulation of the subthalamic nucleus (STN) on 5-HT cell firing in the midbrain raphe nuclei with a view to understanding better the high incidence of affective side effects associated with the therapeutic application of STN stimulation in patients with Parkinson's Disease (Temel et al., 2007). STN stimulation at clinically relevant frequencies and low currents, caused a clear-cut inhibition of firing rate in the vast majority of 5-HT neurones tested. This marked effect on 5-HT cell firing may underlie the affective side effects of STN stimulation. A combination of retrograde tracing and VGLUT2 immunocytochemistry (a putative marker of STN outputs) revealed connectivity between STN inputs to the substantia nigra and nigral outputs to the dorsal raphe nucleus. These data identify the substantia nigra as a candidate neural substrate of the effect of high frequency stimulation of the STN on 5-HT cell firing. Collectively, their data provided the first experimental evidence that electrical stimulation of the STN, at parameters used to relieve the motor deficits of Parkinson's disease, causes a striking inhibition of 5-HT neuronal activity which is directly linked to the induction of depressive-like behaviour. This effect may contribute to the psychiatric disturbances observed in patients subject to STN stimulation and provide an experimental basis for their clinical management. The powerful link between the STN and the 5-HT system constitutes evidence for the existence of a novel 'limbic-motor interface' for the integration and processing of sensorimotor, associative and emotional information.

Local physiological regulation - auto and hetero receptor function

The fact that presynaptic (somatodendritic) 5-HT_{1A} autoreceptors inhibit firing of 5-HT neurones is well recognised, but whether this autoinhibitory mechanism is relevant to physiology (and/or pathology) or only to pharmacological treatments has been a matter of debate for long time.

The results obtained in activity 2, greatly contributed to clarify the issue and provided a breakthrough that unequivocally pointed to *altered autoinhibition* as a possible causative mechanism of pathology in autonomic and mood disorders (Audero et al., 2008). The concept has recently been confirmed by an US competing group using a different approach (Richardson-Jones et al., 5-HT1A autoreceptor levels determine vulnerability to stress and response to antidepressants. *Neuron* 65(1):40-52, 2010)

Partner 11 (Florence), in collaboration with ***Partners 2*** (Paris) ***3*** (Würzburg) ***and 6*** (Maastricht), conducted a thorough study of the role of 5-HT_{1A} receptor-mediated inhibition of serotonergic neuron firing by investigating the relationship between 5-HT turnover, level of 5-HT_{1A} receptor expression by serotonergic neurones, autoinhibition, and response to SSRI application, using extracellular and/or whole cell intracellular recordings in brainstem slices containing DRN.

Partner 11 also found that the activity of 5-HT cells in DRN is (auto) inhibited by tonic stimulation of 5-HT_{1A} receptors exerted by 5-HT originating from non vesicular pool(s) and outflowing from 5-HT cell bodies to the extracellular space and that the availability and level of L-tryptophan is crucial for maintaining the physiological degree of autoinhibition (Mlinar et al., 2005).

These data provided background knowledge relevant to the physiological regulation of DRN 5-HT neurone activity by endogenous 5-HT and defined the conditions for future investigations on the functional role of 5-HT neuron autoinhibition in pathology.

The issue was investigated using mutant mice *selectively* and *conditionally* overexpressing 5-HT_{1A} receptors in raphe 5-HT cells (5-HT_{1A} o/e, estimate of overexpression ~8 times greater than littermates). In these mice the level of autoinhibition of 5-HT cells following application of 10 μM Trp was greatly enhanced compared to that of control littermates and mutants in which overexpression was kept repressed. Consistently, the SSRI citalopram produced a decrease in 5-

HT cell firing rate with greater efficacy than in control littermates. Using whole-cell patch-clamp recording, differences in response to 5-HT_{1A} agonists have been characterized in 5-HT cells identified by intracellular injection of Alexa 488 and later processing of the slice for TpH2 immunohistochemistry, in collaboration with ***Partner 3*** (Würzburg).

In vivo, the increased autoinhibition of raphe 5-HT cells is likely to decrease 5-HT tonic release from terminals in projection areas, which correlates with the depression-like behaviour (increase in tail suspension immobility), increased aggression and autonomic disregulation observed in these animals. The correlation between the overexpression of 5-HT_{1A} receptors and the presence of an aggressive phenotype has been investigated in detail and the data showed an involvement of altered autoinhibition of raphe 5-HT cells in the aggressive behaviour and in autonomic disregulation consequences observed in these mice (Audero et al., 2008; Audero et al., 2009)

Therefore, two important consequences of the existence of 5-HT_{1A} receptor-mediated autoinhibition of raphe 5-HT cell firing for physiology and pathology are that: i) the level of 5-HT_{1A} expression and/or their functionality may greatly change the level of autoinhibition, hence the response to antidepressants; ii) the bioavailability of 5-HT, that strictly depends on tryptophan (Trp) bioavailability, will be the major determinant of physiological activity of 5-HT cells.

Therefore, locally released 5-HT exerts a tonic (auto)inhibition that may have a crucial role in physiopathology of depression and mood disorders and in the effects of antidepressant drugs able to modify the level of extracellular 5-HT in raphe nuclei. The results are also crucial for understanding the role that desensitization or blockade of 5-HT_{1A} receptors may have in therapeutic effects of antidepressant drugs.

For years there has been an active search for antagonists selectively acting at raphe 5-HT_{1A} receptors because the use of these compounds may be of value for hastening the response to antidepressant drugs, without greatly impairing the effects of 5-HT at postsynaptic 5-HT_{1A} receptors. In contrast to the desired effects, the currently available antagonists appear devoid of regional selectivity. ***Partner 11*** (Florence) characterised the electrophysiological action two new selective 5-HT_{1A} receptor inverse agonists, Rec 27/0224 and Rec 27/0074, at 5-HT_{1A} (auto) receptors of DRN 5-HT neurones and at (hetero) receptors expressed by CA1 pyramidal cells and demonstrated that 5-HT_{1A} receptor inverse agonists display a considerable degree of cell selectivity and are effective in blocking the effects of SSRIs in the DRN. DRN (Corradetti et al., 2005) A molecular mechanism that could explain the differential sensitivity of 5-HT_{1A} receptors expressed in hippocampal pyramidal cells to inverse agonists has been provided by the parallel study performed by ***Partner 2*** (Paris) who demonstrated a differential coupling of 5-HT_{1A} receptors to specific G proteins in the hippocampus and raphe (Mannoury et al., 2006).

These data provide novel background knowledge for the possible development of new antidepressant drugs.

Overview

Throughout the project, **WP 6** contributed to:

- the assessment of the characteristics of animal models of depression (***Activities 1, 2, 3***);
- the selection of those models to be used for further studies (***Activities 1, 5***);
- the assessment of sensitivity of a several receptors (5-HT_{1A}, 5-HT_{2C}, CB1, β1-adrenoceptors, and galanin) in selected mesencephalic brain nuclei, and cortical areas (***Activities 2, 5***).

During the five years of the project's life WP6 contributed to the achievement of NewMood objectives through the following major results:

A. New knowledge on neurophysiological responses in animal models of depression has been obtained by the studies in a number of core and/or mood-pathology relevant mouse models.

Core mouse models:
(i) 5-HTT expression modified mice [i.e.: 5-HTT-/-, 5-HTT+/- and 5-HTTo/e];
(ii) CB1-/-; (iii) GR-i; (iv) VGlut1(+/-), and (v) HA and LA strains;
Mood-pathology relevant mouse models:
(i) Helpless / non-helpless strains; (ii) 5-HT_{2A} -/-; (iii) galanin o/e; (iv) raphe5-HT_{1A} o/e;
Rat model:
(i) MDMA-induced partial 5-HT depletion

This research has involved ***Partners 2, 3, 7/14, 8, 9, 10, 11.*** (*Deliverables 22, 36, 45 achieved*);

B. Advancement in the understanding of long-loop feedback and local regulation of dorsal raphe 5-HT neurone activity with deep insight has been obtained into the role of:
(i) 5-HT and 5-HT_{1A} receptors; (ii) 5-HT_2 receptors; (iii) CB1 receptors.

Notably, ***three outstanding results*** have been obtained:

1. the circuitry underlying the control of 5-HT neurons in the DRN has been analysed focusing on 5-HT2C receptors and the lateral habenular nucleus activating the GABA neurons in the ventral PAG. This is now a well understood and proven circuitry mechanism in rat.
2. we provided the first experimental evidence that electrical stimulation of the STN, at parameters used to relieve the motor deficits of Parkinson's disease, causes a striking inhibition of 5-HT neuronal activity which is directly linked to the induction of depressive-like behaviour.
3. we obtained the first experimental evidence that altered level of 5-HT_{1A} receptors in the raphe serotonergic cells produces pathological consequences relevant to mood disorders and autonomic disregulation. (***Partners 2, 4, 6, 9, 11***) (*Deliverable 51 achieved*).

Finally, collaborations have been established / are continuing between ***Partners 2 & 7/14*** (Paris & Budapest); ***2 & 4*** (Paris & Barcelona); ***2 & 9*** (Paris & Oxford); ***8, 9 & 6*** (Edinburgh, Oxford & Maastricht), ***11*** (Florence) ***& 2*** (Paris)***, & 6*** (Maastricht) ***& 3*** (Würzburg).

Table 14. Deliverables and milestones for Workpackage 6

All the Milestones and Deliverables involved with WP6 have been achieved. For detailed information about the Deliverables, please see separate report ("Deliverables WP 6: "Neurophysiology"). Below is a list of all Deliverables and Milestones to which WP6 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name** **(All Achieved)**	**Date due**	**Actual date**
21	Long-loop and local control of 5-HT cell activity: physiology	36	36
22	Long-loop and local control of 5-HT cell activity: models of vulnerability	36	36
36 & 45	Characterization of sleep parameters in the rat model of partial serotonergic lesion during damage and partial recovery [Duplicate]	54	54
51	Local and long-loop regulatory mechanisms of 5-HT system function	60	60
Deliverable No. (part-contribution)			
4, 11 & 32b, 15 & 31a, 23, 24, 25a, 25b & 30a, 33, 48, 56	(various)	18 - 60	18 - 66
Milestone No. (main contributor)	(none)		
Milestone No. (part-contribution)			
7, 9, 15, 18, 20, 21, 30, 31, 33, 39	(various)	30-60	30-66

Workpackage 7 – Functional Imaging - Human and Animal

1. Objectives

i) To identify in animals and humans, the distributed brain systems whose functional activity is associated with a) vulnerability to depression, b) changes in reward, aversion and cognitive processing in depression, c) treatment with antidepressant drugs.
ii) To identify at a cellular level the neural pathways mediating responses to aversion and how these responses are changed after repeated stress in animal models of depression.
iii) To use magnetic resonance spectroscopy (MRS) to quantify changes in glutamate metabolism in models of vulnerability and response to treatment in animals and humans.
iv) To use Blood Oxygen Level Dependent (BOLD) magnetic resonance imaging (MRI) to measure the functional neural response to drug challenges of neurotransmitter systems in animals and humans (pharmacoMRI; pMRI).
v) To guide neurochemical and molecular studies of depression to appropriate regions of brain

2. Progress towards the Objectives

Activity 1. Behavioural endophenotype of models of genetic, developmental and acquired vulnerability to depression.

1.2. Changes in regional brain function & response to stress shared by models

Cytochrome oxidase mapping

Partner 5 (Tartu) examined the patterns of oxidative metabolism by cytochrome oxidase (COX) histochemistry and developed a standard operating procedure to be used in the consortium. COX metabolism was studied in different models of depression together with the interaction between model and chronic mild stress (CMS) to test vulnerability x environmental stress mechanisms. CMS increases COX metabolism in brainstem and midbrain regions making this manipulation a useful tool to explore the effects of stress in the brain. The effect of partial 5-HT depletion using parachlorophenylalanine (PCA) was shown to have distinct effects on COX metabolism and to reduce the effect of CMS indicating that stress-mediated changes in COX metabolism are partially dependent on intact 5-HT innervation. In addition the broad upregulation of COX metabolism may be replaced with widespread downregulation under specific stress such as social defeat stress (where maintained hedonic response to sucrose protects against downregulation). The models studied were from***: Partner 5*** (Tartu; High v Low Exploring (HE/LE) rats, High v Low Social (HS/LS) rats and High v Low Positive Emotional Expression (HC/LC) rats), ***Partner 4*** (Barcelona; CB1-/- mice), ***Partner 13*** (Warsaw; HA/LA stress analgesia mice), ***Partner 12*** (Pamplona; maternally deprived rats) and ***Partner 2*** (Paris; GR-i mice). In two other mouse depression models 5-HTT overexpressing mice (5-HTTo/e) from ***Partner 8*** (Edinburgh) and VGLUT1 +/- mice from ***Partner 12*** (Pamplona) changes in COX were not observed so the studies were not taken further. There were larger inter-individual differences making it difficult to find clear findings with the conventional number of observations and in the mouse COX metabolism levels in different brain regions are in strong correlation. LE v HE rats show increased COX in limbic regions and the reverse in cortical regions with no differential response to CMS (possibly due to a developmental time window). In HS/LS rats COX metabolism in striatal regions and anterior supraoptic nucleus varied with sociability but in brainstem and midbrain both extremes varied from a medium group. In the HC/LC model striking sex differences were observed and LC versus HC male, but not female rats were more sensitive to CMS with increased COX metabolism in midbrain and limbic regions. In CB1ko mice COX metabolism was decreased in thalamus and mamilary nuclei. In maternally deprived rats increases in COX were found in midbrain areas and limbic areas. In GR-i mice, 56 out of 101 analyzed regions COX was higher in many brain regions relevant to the stress response.

Studies mapping tryptophan and tyrosine hydroxylase have also been started (Partner 4, Barcelona; Partner 10, Stockholm; Partner 5, Tartu) reflecting the enduring nature of the NewMood collaboration.

2-deoxyglucose (2DG) mapping of current metabolically active brain regions in animal models

Partners 8 (Edinburgh) used 2DG autoradiography to visualise altered patterns of brain metabolism (local cerebral glucose utilization, lCMRglc) in 5-HT depleted (using MDMA) nulliparous female rats, and their subsequent offspring, under normal conditions and conditions of stress and pharmacological challenge. 2DG was also applied to animal models used by other partners, and used to validate animal pharmacoMRI studies. It was not found possible to apply statistical parametric mapping (SPM) to 2DG autoradiographs which would have enabled better comparison with pharmacoMRI studies. With ***Partner 7/14*** (Budapest), a standard MDMA treatment protocol was agreed and standardised. Acute MDMA produces sex-specific alterations in brain metabolism with more brain regions affected in female rats and changes (increases or decreases) more marked in female rats, particularly striking in the dorsal raphe nucleus. MDMA-experienced animals had attenuated effects compared with naïve animal which are consistent with tolerance to locomotor activating effects of the drug and to anhedonia due to decreased activation of reward centres (mesocorticolimbic system).

We now have a comprehensive pharmacological profile of brain function in the two modified 5-HTT mouse models: 5-HTT overexpressing, (5-HTTo/e) and 5-HTT knock-out (5-HTT-/-) and in particular have shown how sex by genotype interactions influence brain function. ***Partners 8*** (Edinburgh) and ***Partner 6*** (Maastricht) studied 5-HTTo/e together with chronic fluoxetine treatment to reveal a gender x genotype interaction and a significant gender x drug treatment interaction. SSRI treatment decreased lCMRglc more in males than female wild-type mice with more marked decreases in 5-HTTo/e in both sexes with a slightly greater effect in females. In collaboration with ***Partner 5*** (Tartu) the only correlation between the 2DG and COX was where there was an increase in lCMRglc. In 5-HTT-/- mice (***Partner 8*** and ***Partner 3***, Würzburg) in contrast there was no alteration of lCMRglc at baseline but 8-OH-DPAT challenge-induced decreases in lCMRglc were almost totally attenuated in female, but not male, 5-HTT-/- mice. ***Partner 8*** has shown that there is no alteration in response to 5-HT_{1B} (CP 94253), and 5-HT_2 (DOI) agonists in both male and female 5-HTT-/- mice.

Partners 8 (Edinburgh), ***6*** (Maastricht) and ***7/14*** (Budapest) carried out studies of the effects of ATD on glucose utilization, blood flow, BDNF expression and behaviour showing effects of ATD to uncouple blood flow from glucose metabolism, lower glucose use after ATD in MDMA-pretreated rats than control rats, but no overall effects on BDNF concentrations although brain TRP levels correlated positively with BDNF in both the prefrontal cortex and hippocampus. MDMA attenuated the effect of citalopram on LCMRglu in thalamus and hippocampus and that of on fluoxetine on improving anxiety.

Other studies have included studies of the DISC1 (Disrupted in Schizophrenia 1) gene and the effects of the 5-HT_2 agonist, DOI (***Partner 8*** and ***Partner 6*** (Maastricht). Partner 8 has investigated basal neurogenesis and differentiation in wild type and 5-HTTo/e male mice using BrdU labelling.

In summary COX metabolism was studied across models depression models and in interaction with CMS and similarities and differences were observed but with no consistent picture across all models. 2DG autoradiography has been used to investigate cerebral metabolism and provided mapping of activity in relation to 5-HT in two mouse modified 5-HTT models and effects in the MDMA-treated rats.

Activity 2: Functional states of monoamine regulation in rodent models of vulnerability – the neurotransmitter endophenotype.

2.3 Molecules beyond the monoamine synapse

2-deoxyglucose autoradiography

Partner 8 (Edinburgh) showed that MDMA-treated rats had fewer and less widespread decreases lCMRglu than untreated rats group after acute citalopram in neocortex, although some areas (e.g. limbic system and sensory nuclei) were unaffected by MDMA-pretreatment. 8-OH-DPAT mostly reproduced the effect of citalopram. ***Partners 6*** (Maastricht), ***7/14*** (Budapest) and ***8*** in collaboration found that in rats pretreated with MDMA a general increase in lCMRglu was found in response to DOI in contrast to the decrease in untreated animals. ***Partner 8*** studied the 5-HTTo/e mouse and found significant drug effects for citalopram and 5-HT_2, 5-HT_{1A} and 5-HT_{1B} agonists and a sex by drug genotype effect; in contrast ATD had no effect

5HT drug-challenge studies – BOLD pharmacoMRI

Partner 1 (Manchester) and ***Partner 9*** (Oxford) have shown that drugs which enhance 5HT function by contrasting methods evoke activations in overlapping regions and that antagonists or synthesis inhibitors block the responses suggesting they indeed reflect actions mediated by 5HT receptors. ***Partner 1*** in collaboration with the Department of Imaging Science and Biomedical Engineering (ISBE) in Manchester used animal pharmacoMRI to show that mCPP (a $5HT_{2C}$ agonist) activated frontal and ventral striatal regions and some activations were blocked by pre-treatment with a selective $5HT_{2C}$ antagonist. ***Partner 9*** showed that acute challenge with the 5-HT releasing agent, fenfluramine, increased c-fos expression (mRNA and immunoreactivity) in a variety of cortical and subcortical regions. In most cases the BOLD response to fenfluramine appears to be blocked in animals pretreated with the 5-HT synthesis inhibitor, p-chlorophenylalanine, 5-HT_{2A} receptor antagonist administration and attenuated by proinflammatory cytokines that have mood lowering effects.

2.4 Modulation of information processing beyond the monoamine synapse

Amino-acid neurotransmission

Partner 9 (Oxford) using fenfluramine pharmacoMRI showed that increases in c-fos expression in cortical and subcortical areas occurred in pyramidal neurons double-labelled with EAAT3, an amino acid transporter. This effect was not seen after 5-HT depletion indicating that 5-HT activates glutamatergic cortical neurons.

Partner 12 (Pamplona) investigated glutamate function in the VGLUT1+/- mouse using ^{13}C-MRS studies. A significant increase of [4-^{13}C] glutamate, derived from [1-^{13}C] glucose was shown in whole brain extracts of VGLUT1+/- mice with no changes in the levels of the other metabolites; this suggest an increase of the neuronal de novo synthesis of glutamate.

In summary the effects of 5-HT neurotransmission has been studied using 2DG and pharmacoMRI with application of the former to two models of depression vulnerability. Although there has been some investigation of glutamate function it has not been possible to extend this to the depression models and studies in humans are limited by uncertainty about whether MRS can detect dynamic changes in glutamate function.

Activity 3: HPA axis gene expression and feedback control

3.2 Influence of CRF1&2, AVP, GC and MR receptors

Site of action of GC and MC on feedback regulation of CRF

Partner 8 (Edinburgh) investigated the role of endogenous CRF receptor ligands (urocortin 3) in urocortin 3 over-expressing (UCN3+) male mice using 2DG autoradiographic imaging.

Widespread attenuation of 8-OH-PDAT-induced reductions in local cerebral metabolic rate were found in UCN3+ mice indicating adaptive changes to the serotonergic system probably at the level of the raphé.

Partner 1 (Manchester) undertook a proof-of-concept pilot study and demonstrated that acute 'fast' effects of steroid receptor activation (believed to be mediated through MR receptors) could be detected in human hippocampus using pharmacoMRI paradigms. This will be applied to the study of the role of corticosteroids in cognitive impairment and poor treatment response in depression in a study commencing in 2010.

Activity 4*: In vivo* studies of vulnerability, depression and recovery in humans

4.1 Neurobiology of reward, emotion, and cognition by risk group and depression

Partners 1 (Manchester) and ***7/14*** (Budapest) undertook community cohorts enriched for depression using questionnaires and taking genetic samples numbering approx 2,800 subjects (Level 1) which providing data for genetic and genetic x environment interactions in relation to depression vulnerability (reported in Workpackage 4)***. Partner 1*** characterised a subgroup of over 250 of these subjects (Level 2) by standardised interviews and computerised tasks to identify groups of never depressed (ND), remitted depressed (RD), and currently depressed (CD) participants in order to investigate affective processing abnormalities related to vulnerability to depression. This fed into a smaller subgroup of nearly 70 participants (Level 3) who underwent pharmacoMRI and fMRI to investigate 5-HT abnormalities and the neural basis for affective processing abnormalities in RDs. ***Partner 1*** also collaborated with a linked MRC study (REMEDi) which studied antidepressant treatment effects in drug-free depressed patients and with SBE at the University of Manchester in a strategic alliance with AstraZeneca plc to study antidepressant drug effects in health volunteers. ***Partner 3*** (Würzburg) studied genetic effects on affective processing in a large healthy volunteer cohort.

Behavioural findings in the Level 2 cohort of relevance to imaging findings

In the Manchester Level 2 cohort (***Partner 1)*** CDs showed abnormalities in affective processing consistent with the literature. RDs did not display the negatively biased memory deficits found in CD but had raised rumination scores and there was a subtle difference in the effect of the reflective component of rumination in RDs which may reflect an impaired ability to problem solve based on previous experience. They had a normalised response to positive social stimuli but still showed the bias for negative situations seen in depression and an increased bias to recognise emotions (driven by negative emotional faces. Medicated subjects had normal face emotion processing which may relate to the known effects of antidepressants on emotional processing.

Regional brain fMRI responses to reward, emotion and cognition

Partner 1. RD participants showed altered affective processing that may be related to vulnerability to depression. RDs showed reduced neuronal activation in a number of areas to negative stimuli, possibly an adaptive response maintaining remission given that this is opposite to the increased responses seen in CD in our findings and the literature. Rumination appears to be a key mediator of vulnerability to depression and we have related this to affective processing at the neuronal level where it appears to work against the 'adaptive suppression' of responses to negative stimuli seen in the RD group as a whole. We also found altered anterior cingulate cortex activation to feedback in RD but the direction of change was task dependent. This is an area that has been associated with error checking and relating performance to external factors and is consistent with vulnerability to depression being associated with altered sensitivity to external events or evaluation of self in relation to life events. Recurrent depression, independent of current symptoms was associated with alterations in grey matter volume in areas associated with functional changes in depression. This includes the hippocampus, which previous studies have shown to be smaller in patients with a long history of depression, possibly due to cortisol hypersecretion.

4.2 Pre and post-synaptic 5-HT function by risk group & depression

Genetic studies

Imaging the effects of 5-HT genotypes may give an insight into the function of this neurotransmitter. ***Partner 1*** demonstrated that homozygous SS carriers of the 5-HT transporter (5-HTT) showed altered BOLD signal responses to citalopram challenge, a presynaptic 5-HT probe, in frontal and parietal cortex and striatum compared to LL carriers suggesting that 5-HT innervation depends on 5-HTT genotype. ***Partner 7/14*** used multimodal magnetic resonance-based imaging (functional, perfusion, and structural), genotyping, and self-reported life stress and rumination. They showed that 5-HTT genotype is associated with differential activation to negative, positive, and neutral stimuli in limbic (including amygdala), striatal, and cortical regions, suggesting a much broader role in modulating brain processes and the default mode of resting brain activity. They also report significant differences in grey matter density in frontal cortical regions, anterior cingulate, and cerebellum as a function of 5-HTT genotype. Their results support for a model by which life stress, rumination and 5-HTT genotype interact to influence amygdala and hippocampal resting activation and functional connectivity. In collaboration with ***Partner 3*** (Würzburg)**,** ***Partner 7*****/14** has also shown that a potentially functional variant of the tryptophan hydroxylase 2 gene (TPH2), involved in the synthesis of 5-HT, modulates amygdala responsiveness to emotional stimuli of both negative and positive valence on its own and in an interaction with 5-HTT. Downstream postsynaptic effects of genotype have also been demonstrated by ***Partner 1*** with two SNPs in the neuroplastic pathway (cAMP, CREB1, BDNF and TrkB) modulating neural responses to negative stimuli and by ***Partner 3*** showing that two SNPs in the regulatory region of the gene coding for stathmin (which regulates microtubules in response to extracellular messengers) modulated anterior cingulate reactivity to emotional stimuli, interacting with gender and life stress.

5-HT neurotransmission studied using 5-HT challenge phMRI

Partner 1 validated mCPP challenge phMRI (a probe of post-synaptic 5-HT_{2C} receptors) in healthy volunteers using the 5-HT_{2C} receptor antagonist, mirtazapine, extending to humans the results found in animals. Using citalopram challenge phMRI (a putative probe of presynaptic 5-HT function) ***Partner 1*** showed that RDs compared to NDs had altered cortical and subcortical activation suggesting altered 5-HT innervation in the drug-free state. In healthy volunteers chronic SSRI treatment modified striatal and cortical responses to citalopram challenge showing for the first time a specific functional effect of treatment on 5-HT neurotransmission in humans.

5-HT modulation phMRI of affective processing

Partner 1 compared RDs with NDs to show altered patterns of neural activation after acute citalopram to positive and negative faces and altered effective connectivity between the amygdala and fusiform gyrus. In healthy volunteers chronic citalopram treatment altered neural responses to emotional faces in limbic and cortical regions and also altered lateral frontal cortical responses in a response inhibition task. In CD patients successful treatment with an SSRI was associated with increased bias towards positive words in limbic and medial prefrontal regions.

Overview

In animal studies considerable success was achieved in collaboration between the Partners to characterise core depression models. The human studies have provided a rich collaboration and source of data providing insight into vulnerability to depression with a focus on 5-HT systems from gene to neuronal function associated with affective processing.

Deviations from the project work programme

MRS studies were not undertaken in the wider depression models or humans as there is doubt that this can be used as a dynamic measure of brain glutamate and further methodological developments are required.

As has been previously mentioned it was not possible to recruit sufficient numbers of depressed individuals into Manchester's NewMood Level 2 and 3 study. The REMEDi study – a complementary research study also based in Manchester – has nearly completed recruiting depressed individuals and the data will feed into the final outcomes from the NewMood study.

Table 15. Deliverables and milestones for Workpackage 7

All the Milestones and Deliverables involved with WP6 have been achieved. For detailed information about the Deliverables, please see separate report ("Deliverables WP 7: "Functional imaging – human and animal")

Below is a list of all Deliverables and Milestones to which WP7 contributed solely or in part.

Deliverable No. (main contributor)	**Deliverable name** **(All Achieved)**	**Date due**	**Actual date**
5	First association study in study participants	18	18
12	MCPP or citalopram pMRI and modulation of reward, aversion and cognition completed.	30	48
13	Initial analysis of data in collaborative community study (Level 1)	30	42
14 & 28a	Two Level III studies (fMRI, pMRI studies in participants) completed	30	48
27a	Initial analysis of gene-environment data in collaborative community study (Level 1)	36	48
27b & 32a	Citalopram pMRI measuring 5-HT sensitivity complete in 15 controls and 15 remitted depressed subjects	42	48
28b	Brain maps of comparative oxidative metabolism in vulnerability to depression	42	60
30b	Completion of the pharmacological profiling of brain function that contributes to the imaging core data set in modified 5-HTT function models	42	48
35	Brain maps of 5-HT receptor function in modified 5-HTT function models	48	48
42	Key results from neuropsychological study (Level 2, 300 subjects) available	54	54
52	Brain maps of 5-HT receptor function in core mouse models	60	60
Deliverable No. (part-contribution)			
4, 15 & 31a, 20, 21, 25a, 33, 37, 51, 56	(various)	18 - 60	18 - 66
Milestone No. (main contributor)			
6a	Data lock on collaborative community study (Level I)	24	48
6b	Completion of motivation-reward and rumination fMRI studies (Level 3).	24	48
11	Completion of sample from community study (Level 1) in Manchester	30	30
12	Evaluation of use of 2-dimensional statistical parametric mapping of group differences applied to autoradiographic brain sections	36	36
19	Completion of motivation-reward and rumination fMRI studies (Level 3).	42	42
26	Completion of direct citalopram phMRI in controls, depressed and remitted patients.	48	54
27	Decision on feasibility of Level 2 study in Budapest	48	54
Milestone No. (part-contribution)			
7, 9, 14, 17, 20, 21, 28, 30, 31, 32, 39.	(various)	30-60	30-66

Section 4 – Plans for Using and Disseminating the Knowledge (summary only)

This section alone is over 100 pages, therefore a summary only is included here. For the full details of all peer-reviewed publications (including book chapters and reviews), posters / verbal presentations at national and international scientific conferences (including published abstracts), conferences workshops attended during the 5 years, and all tables, please see the separate document "Final Plan for Using and Disseminating the Knowledge" (FPUDK 2004-09).

Section 1 – Exploitable knowledge and its use

The knowledge gained through NewMood will lead to a better understanding of the molecular mechanisms in the causation of depression, and ultimately should help the development of new and effective drug-treatments. However, although some "potential commercial applications, products, processes or services" could be eventual goals, these will not occur during the lifetime of the project or fall within the exploitation activities directly linked to the project.

Section 2 – Dissemination of knowledge

Teaching and Training:

All of the NewMood partners are researchers with high-profile international scientific reputations – 9 of the 13 are professors. Ten of the research groups are located at distinguished universities (e.g. Würzburg, Tartu, Oxford), and the remaining three are at internationally acclaimed research institutes (e.g. the Karolinska Institute). Therefore most of the partners are involved with teaching and training undergraduates (including medics), and all have PhD students. Many of these students visit other labs within the consortium to undergo training in specialised scientific techniques. These placements are shown in Table Anx 1 of the PUDK (see separate document).

Many students (29 males and 54 females) have undergone research training within the NewMood study, contributed to the research and successfully defended their PhD thesis or are predicted to submit their thesis in the next year or two; a list of all students is given in Table Anx 2 of the PUDK (see separate document).

The NewMood Annual General Meetings (AGM)

The NewMood AGMs allowed PhD students and young researchers to exchange ideas and information. They were held in a variety of locations:

2005: 1-3 May, Amsterdam, The Netherlands
2006: 24-25 April, Barcelona, Spain
2007: 16-17 April, Budapest, Hungary
2008: 4-5 April, in the Nobel Forum at the Karolinska Institute, Stockholm, Sweden.

Dissemination of Knowledge - Excluding Scientific Publications:

The NewMood public website (http://www.medicine.manchester.ac.uk/psychiatry/newmood/), hosted by the University of Manchester is accessible to the general public. It provides basic details of the NewMood consortium and the research, and also general information about depression, plus links to various sources of further information and help.

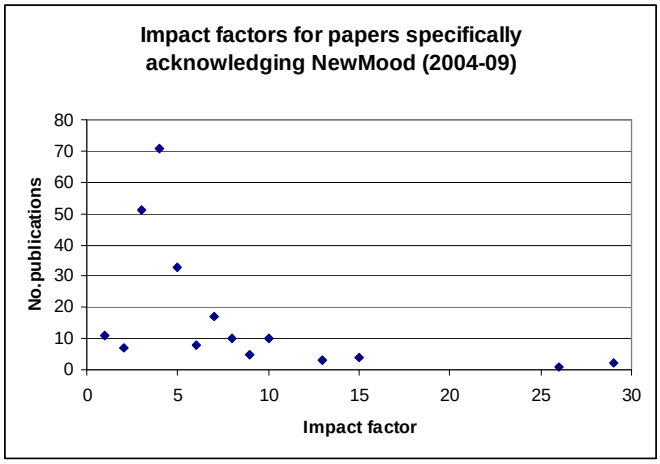
Throughout the project, NewMood results were disseminated primarily at national and international scientific conferences and through publications in peer-reviewed journals. During the five years, the partners attended over 250 different international scientific conferences and workshops in 34 different countries world-wide, predominantly within the EU, but also USA, Canada, Japan, China, Brazil, Mexico, Argentina, Australia. A list of conferences attended is

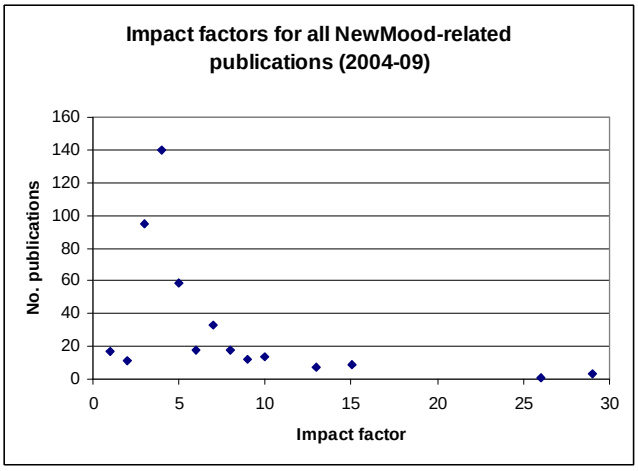
given in Table Anx 3 of the PUDK (see separate document). Many partners were invited to give presentations, or plenary lectures, or chair sessions.

Full publications in peer-reviewed journals

NewMood collaborative effort between two of more groups has resulted in 40 joint full publications to date; more will follow. In addition, the consortium partners individually published over 400 papers relevant to NewMood. Most papers were published in journals with an impact factor around 5 (ISI Journal Citation Report 2008), although there were several with significantly higher impact factors, e.g. Partner 3 (Murphy & Lesch, 2008) published in *Nature Rev Neurosci* (impact factor 25.94) specifically acknowledging NewMood; while Partners 2 (Conti et al. 2006), 9 (Sharp 2006) and 11 (Audero et al. 2008) published in *Science* (impact factor 28.10); Partners 9 and 11 specifically acknowledge NewMood in those publications.

In addition, the Eur J Neuropsychopharmacology is planning a special issue devoted to NewMood. This is expected to be published in summer 2010.





A full list of book chapters, full publications in peer-reviewed journals, conference presentations / abstracts can be found in the separate PUDK document.

Section 3 – Consortium Management (2004-09)

Overview

NewMood has been a great success. The consortium partners worked well together in a spirit of openness and trust, which engendered constructive exchanges of material and information, to the benefit of the programme. Several of the partners had collaborated previously in a programme funded under Framework 5, and some had been involved in an FP4 project; this gave NewMood a positive foundation from which to grow and develop. There was good contact and honesty within the consortium; formal progress meetings for all the consortium members (or their representatives) were held every 6-months in various EU locations, and this fostered both professional relationships and also social friendships. In addition, partners frequently met, and exchanged ideas, information and materials.

Changes to the NewMood contractors / consortium members:

1. In the original proposal, Vernalis (an SME in Wokingham, UK) was expected to collaborate. Due to a company takeover and change in key personnel before the start of the project, the planned collaboration with this company did not take place; however, this was not a major difficulty for the NewMood programme. With full agreement from the consortium, the funding initially allocated for Vernalis, was transferred to the co-ordinator (Manchester) to cover some shared costs.
2. In the original proposal, Partner 13 (Swiergiel, Poland) working at the Institute of Genetics and Animal Breeding (IGAG) in Jastrzebiec, expected that some of the work would be performed by co-workers at Institute of Experimental Biology, Polish Academy of Sciences (NENKI) in Warsaw. However, at the start of the project, NENKI withdrew from the consortium without claiming any costs. This did not affect the progress of the work – all the planned tasks have been performed using resources at IGAB.
3. In 2007, due to the crisis in health services in Hungary, Prof Gyorgy Bagdy was forced to relocate his research group. Prof. Bagdy was originally located at the National Institute of Psychiatry and Neurology (NIPN), which was situated in a large building in extensive grounds on the outskirts of Budapest. As part of the reorganization of the healthcare system, the Hungarian Government decided to close this institute, and Prof Bagdy and his team relocated to Semmelweis University (SE), Faculty of Medicine, Department of Pharmacology and Pharmacotherapy. Thus Partner 7 (Bagdy at NIPN) withdrew from the consortium, to be replaced by Partner 14 (Bagdy at SE). While this caused considerable disruption and some loss to his team, it did not result in any significant delay in the research programme.

Problems encountered and how they have been addressed:

Most problems have been relatively minor and generally of an administrative nature. These included (a) strict control of funds and very cumbersome purchasing procedures encountered by Partner 13 (Dr Artur Swiergiel) resulting to a delay in the purchase of equipment agreed in the contract (this was resolved by year 2); (b) bureaucratic problems delayed the recruitment of new staff members, and there was particularly high turn-over of staff (postdoctoral and temporary research fellows) at the University of Florence (Partner 11, Renato Corradetti) in year 1, although this resulted in minimal disruption because the work was conducted by staff funded from other sources; and (c) the co-ordinator (Manchester) changing banks (from NatWest to Barclays) which incurred a loss of 8.73 euro.

One major problem was encountered during the course of the project: in October 2006, without prior warning, the SME Genescore was declared bankrupt. Genescore had contributed significant

intellectual input into the design of the NewMood oligonucleotides (for the planned "NewMood-1000 gene chip), and many of the gene expression studies had been completed to the satisfaction of the consortium. However, further studies had been planned and these could not now be carried out by Genescore. This bankruptcy forced a radical re-think of the strategy for the gene expression studies and provided an opportunity for greater harmonisation – central facilities, for example, would minimise the variability between labs and improve reliability. Thus the consortium agreed that, to ensure maximum uniformity between the partners, the array studies should be done using a single platform by a core service laboratory, and that all the raw data from each array would be analysed centrally by an expert team.

For several months, a number of different alternative strategies were investigated, (with input from the teams in Manchester, Maastricht, Wurzburg and Paris) and discussed within the consortium. Price quotations were obtained from central service laboratories at the Universities of Würzburg, Maastricht and Manchester (all for the Affymetrix platform), from ServiceXS (for both Affymetrix and Illumina platforms), and from Montpellier (for their own array chips). The price quotations were to provide a complete analysis service, including labelling and hybridisation, for approximately 240 mouse and 200 rat samples.

After careful consideration of various factors, including costs, the consortium decided to use Illumina's MouseRef-8 and RatRef-12 Expression BeadChips, and all lab work to be undertaken by ServiceXS – an SME located in Leiden, with extensive technical expertise in gene expression profiling. Raw data was transferred to the University of Manchester, where it was normalised and put through an analysis pipeline under the direction of Prof Andy Brass who has considerable experience of interpreting array data. The total costs (lab and initial analyses) was shared across the consortium by all partners. Results from the individual models were then sent to the relevant partner(s) for validation (e.g. qRT-PCR) and further analysis.

The data generated by whole genome chips are significantly more extensive than that produced from custom-built chips, and thus the analysis has taken a considerably longer time than initially envisaged. However, the advantage is that no gene, however unlikely its effect on the depressive state, would be discounted from the analysis

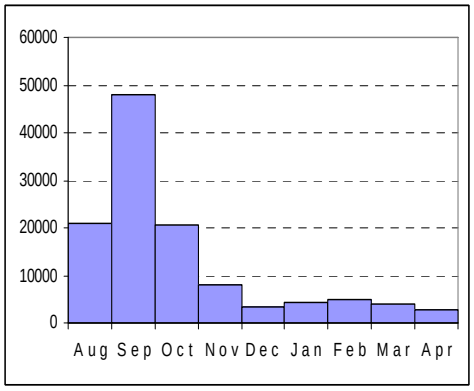
Management websites – ProjectPlace and LiveLink

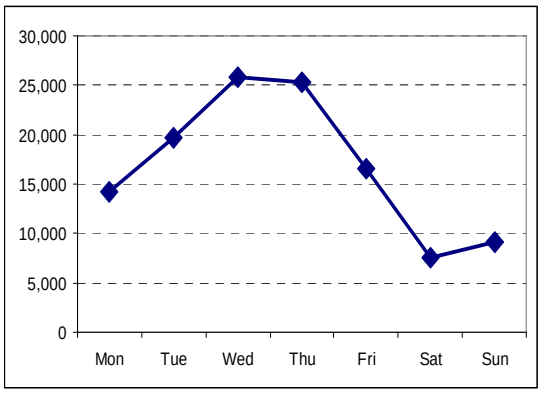
To aid the management tasks and to enhance the likelihood of achieving the targets and goals of the research, initially a NewMood website was created within the web-based Projectplace management system. However, due to cost considerations, various alternatives were investigated and the consortium decided to use LiveLink – an equivalent resource hosted by the University of Manchester and available to the consortium at zero cost. All the files were migrated to LiveLink in October 2006. As with ProjectPlace, this system provided a safe and secure tool both for the management of this research study and for effective communication between partners. The document archive – accessible to all partners – provided a central point of storage for all project documents; any changes and updates, could be flagged in the system. Presentations given at the biannual Partners' Meetings were also uploaded on to LiveLink where they were freely available for later reference.

The NewMood website

This public website (http://www.medicine.manchester.ac.uk/psychiatry/newmood/) is hosted by The University of Manchester. The primary aim initially was to interest members of the UK population in the community study and to give them the opportunity to participate. This website also gives information about the study as a whole, including the locations of the thirteen research centres, with links to the institutes' websites and personal web pages (if appropriate).

The website was launched 9th August 2005 and by the end of April 2006 the website had received a total of 118,172 impressions ('hits') the majority being in the first few months after the launch, the distribution being predominantly on weekdays rather than weekends.





During the first three months, there was an average of 2,394 hits per day, 29% and 27% of these were from the UK and the USA respectively, the remainder being from a total of 97 other countries around the world, including Mexico, Canada, Israel, the Russian Federation, Australia and New Zealand. This demonstrates that the site generated a large amount of interest among the general public world wide.

The website is updated regularly.

Gender of NewMood researchers

During the five years, the researchers involved with NewMood showed a gender distribution shown in the following table (this is an estimate; some discrepancies may occur if individuals were promoted during the five years, or gained a PhD):

Table 16. Gender distribution of NewMood researchers

	PI	Senior Researcher	Researcher	PhD student	Total by gender	% of total
Male	12*	64	24	29	117	38 %
Female	2*	65	71	55	191	36 %
Total	14*	129	95	84	308	100 %

* In close collaboration with Prof. Del Rio (Partner 12, Pamplona), Rosa Tordera took responsibility for the project in the two final years.

Although there are only two female PIs (for the final years of NewMood), female scientists are equally represented at senior research levels (post-docs. etc.) and are over-represented at the more junior levels (including research assistants and technicians) and as PhD students. Overall there are more females than males involved in the NewMood research study.

Harmonisation of methodologies between consortium members

- *Gene expression studies:* as described above, the use of a central facility (ServiceXS), a single platform (Illumina) and a unified method of normalisation and initial analysis, allowed greater harmonisation between consortium members.
- *Brain dissection:* In order for the gene expression data to be valid, it is important that the same brain regions are isolated and compared. To enable the harmonisation of dissection methods, a film crew from Maastricht travelled to Paris to record Michel Hammon dissecting

a rat brain and a mouse brain. The film was transferred to DVD format and made available to each member of the consortium.

- *Animal imaging studies:* the four groups conducting imaging studies of regional brain function in animals (Tartu, Manchester, Oxford and Edinburgh) agreed protocols for cytochrome oxidase and 2-deoxyglucose metabolic mapping and drug administration.
- *Animal microdialysis studies:* the consortium agreed to follow a set protocol recommended by Partner 7 / 14 (Budapest)
- *Human community studies:* Manchester and Budapest collect data using a parallel questionnaire and database – both were devised in Manchester and translated into Hungarian – enabling a direct comparison of the data. Likewise, these two research centres collect buccal swabs and send them to Manchester for genetic analysis at the Centre for Integrated Genomic Medical Research (CIGMR); the results were analysed separately providing a robust study, comparing data from two different European populations.

NewMood provides 'Added Value'

The partners are experts in their own field of research. The consortium therefore provides opportunities for students and post-doctoral fellows to be trained in a variety of techniques by spending time in different laboratories across the EU; this is actively encouraged by the partners. These training placements, which also enhanced harmonisation of methodologies, are summarised in Table 17. Although training in other labs would have occurred, the extent of this inter-group training has certainly been greater because of to the existence of NewMood.

Table 17. Training placements of researchers for the full duration of the project.

From	To	Student	Dates and duration
Paris	Barcelona	Mr Thibault Renoir	January - March 2005 (2 months)
Budapest	Edinburgh	Ms Brigitta Balogh	January – July 2005 (6 months)
Maastricht	Oxford	Mr Yasin Temel	June 2005 (5 days)
Maastricht	Oxford	Mr Yasin Temel	January 2006 (5 days)
Budapest	Manchester	Ms Kriztina Mekli	March – April 2006 (5 weeks)
Maastricht	Oxford	Mr Yasin Temel	April / May 2006 (5 days)
Warsaw	Manchester	Mr Grzegorz Juszczak	March – Sept. 2006 (6 months) (PhD student)
Maastricht	Edinburgh	Ms Eva van Donkelaar	March – November 2006 (9 months)
Oxford	Paris	Dr. Laura Boothman	March 2006-March 2007
Budapest	Manchester	Mrs Krisztina Mekli	June – September 2006 (14 weeks)
Barcelona	Paris	Ms Ester Aso	August 2006 (3 days)
Warsaw	Edinburgh	Mr Grzegorz Juszczak	December 2006 (1 week) (PhD student)
Maastricht	Stockholm	Mr. Rob Vermeulen	January – June 2007 (6 months)
Barcelona	Paris	Ms Ester Aso	April-July 2007 (PhD student)
Pamplona	Oxford	Ms Natalia Elizalde	14 -19 May 2007 (one week) (PhD student)
Oxford	Maastricht	Mr Philip Queree	July-Sept. 2007 (3 months) (PhD student)
Maastricht	Würzburg	Dr. Daniël van den Hove	July 2007- Dec. 2007 (6 months) (Post Doc)
Florence	Maastricht	Dr. Gilda Baccini	September 9-15 2007 (1 week) (Post Doc)
Barcelona	Paris	Ms Ester Aso	September –November 2007 (PhD student)
Paris	Florence	Mr Thibault Renoir	Sept.-Dec. 2007 (3 months) (PhD student)
Budapest	Maastricht	Dr. R. Csaba	Sept. 2007 – Mar. 2008 (6 months) (Post Doc)

From	To	Student	Dates and duration
Maastricht	Oxford	Mr Sonny Tan	January 2008 (one week) (PhD student)
Tartu	Oxford	Ms Kadri Kõiv	January 2008 (one week) (PhD student)
Maastricht	Edinburgh	Ms Eva van Donkelaar	Feb. 2008 – Apr. 2008 (3 weeks total) (PhD student)
Tartu	Oxford	Ms Kadri Kõiv	April – June 2008 (PhD student)
Tartu	Stockholm	Mr Denis Matrov	April – June 2008 (PhD student)
Edinburgh	Maastricht	Dr. Linda Ferrington	May – June 2007 (Post-doc)
Maastricht	Würzburg	Dr Daniel van den Hove	2008 – present: (25% of the time) (post-doc)
Würzburg	Paris	Dr Lise Gutknecht	2008 – 1 month; 2009 1 month (post-doc)
Maastricht	Oxford	Sonny Tan	Dec 2008 – Dec 2009 (PhD student)
Tartu	Stockholm	Denis Matrov	2009 – 3 months (PhD student)
Pamplona	Paris	Alvaro Garcia Garcia	2009 – 3 months; 2010 – 3 months (PhD student)
Maastricht	Stockholm	Ali Jahanshahianvar	May-July 2009 – 3 months (PhD student)
Warsaw	Tartu	Dr Grzegorz Juzsczak	2009 – 2010 (6 months) (post-doc)

Training of PhD students

Over 80 PhD students, listed in Table 18, have been involved with NewMood, including one split-site PhD studentships between Budapest and Manchester, established in October 2006; a second split-site PhD studentship was set-up in 2009 between Maastricht and Paris.

Meetings between the consortium members

Many meetings have taken place between members of the consortium; these are listed in Table 19. These meetings include bi-annual meetings (for all partners and / or their representatives), which allowed an opportunity to discuss progress, and also enabled a social networking (partners met the evening before for a joint meal). This engendered a very friendly, open and harmonious relationship between members of the consortium.

Transfers and exchanges between consortium members

Samples, data and protocols: Transfers and exchanges of data within the consortium, are summarised in Table 20.

Table 18. PhD students (research related to NewMood) in the consortium (2004-2009)

Student name	Male / female	Date of successful defence / predicted date of completion	Title of thesis / proposed title
Partner 1 (Manchester)			
Karen Lythe	female	Awarded 2006	The role of glutamate and NMDA receptors in schizophrenia
Jason Dunham	male	Awarded 2007	Laboratory studies in schizophrenia
Marisha Palm	female	Awarded July 2008	The neurochemistry, neuropsychiatry and functional neuroanatomy of anxiety disorders
Neal Hinvest	male	Awarded August 2008	Neuropsychological structural and biochemical differences in highly impulsive individuals compared to healthy controls and the distribution of 5HT neurons in the human brain
Darragh Downey	male	Awarded August 2008	The neural operations of depression utilising functional magnetic resonance imaging (fMRI)
Emma Pegg	female	Awarded May 2009	Interactions between rumination, depression and cognition
Nia Golden	female	Successfully defended November 2009	Dynamic causal modelling of psychopharmacological effects on effective connectivity between brain regions
Kriztina Mekli	female	Predicted spring 2010	Molecular mechanisms of genetic vulnerability and resilience in depression
Paula Trotter	female	Predicted spring 2010	Investigating the correlation between attitudes and experiences of pleasant touch and depression
Partner 2 (Paris)			
Sabah Kelaï	female	Awarded June 2006	Involvement of 5-HT and CB systems in alcohol consumption in the mouse
Chloé Alexandre	female	Awarded November 2007	Sérotonine et états de vigilance: Implications du transporteur et des récepteurs 5-HT1A et 5-HT1B de la sérotonine dans la régulation du sommeil chez la souris.
Thibault Renoir	male	Awarded June 2008	Neurobiological consequences of MDMA treatment in the mouse
Eleni Païzanis	female	Awarded July 2008	Neuroplastic and behavioural consequences of both genetic and epigenetic modifications in the mouse
Patricia Bonvallon	female	Awarded November 2008	Neuronal pathways of the brainstem involved in the regulation of sleep and wakefulness: distribution and role of 5-HT1A receptors
Adeline Rachalski	female	Planned September 2010	Hypocretin-serotonin interactions in sleep regulation: animal models and pharmacology
Jenny Molet	female	Planned September 2010	Effects of early exposure to ethanol and/or cannabis on addiction at adult age
Cedric Martin	male	Planned Autumn 2011	Relevance of the 5-HT2C receptor desensitization to the anxiolytic effect of chronic antidepressant drugs and mutation in mice

Student name	Male / female	Date of successful defence / predicted date of completion	Title of thesis / proposed title
Fabien Boullé	male	Planned Autumn 2012	Epigenetic modulations of bdnf gene: implications in adult pathology
Cécile Limousin	female	Planned Autumn 2012	Modulation of cerebral focal ischemia-induced neurogenesis by antidepressants and functional correlates
Partner 3 (Würzburg)			
Claudia Kriegebaum	female	Successfully defended November 2009	Spatio-temporal expression patterns of the serotonin synthesis enzymes TPH1 and TPH2 and effects of acute stress
Sarah Nietzer	female	Planned January 2010	Stress reactions and their morphological correlates in genetically modified mice
Sabrina Fritzen	female	Planned July 2010	The influence of NO signalling on adult neurogenesis and its consequences for psychiatric disorders
Jonas Waider	male	Planned November 2010	GABAergic neuron plasticity in the hippocampus of Tph2 modified mice
Naozumi Araragi	male	Planned November 2010	Electrophysiological correlates odf 5-HT dysfunction in 5-HTT deficient mice
Sissi Jacob	female	Planned June 2011	Molecular mechanisms of epigenetic programming in 5-HTT deficient mice
Antonia Post	female	Planned June 2011	Anxiety- and depression-related behavior in Tph2 modified mice
Partner 4 (Barcelona)			
Ester Aso	female	Awarded December 2008	Participation of the endogen cannabinoid system in the control of the emotional responses related to affective disorders and addiction
Lola Galeote	female	Awarded December 2008	Role of the endogen opioid system in nicotine addiction
Simona Andreea Bura	female	Planned March 2010	Interaction between cannabinoids and nicotine in addictive processes
M. Juliana Orejarena	female	Planned July 2010	Participation of the monoaminergic system in mice exposure to MDMA (3,4-methylendioxymetanphetamine) and THC (Δ9-tetrahydrocannabinol): behavioural and neurochemical aspects
Xavier Nadal	male	Planned September 2010	Involvement of the endogenoud opioid system in neuropathic pain
Arnau Busquets-Garcia	male	Planned December 2012	Interaction of the opioid and cannabinoid systems with other cellular signalling pathways in addictive disorders
Clara Touriño	female	Awarded February 2009	Participation of the endogen cannabinoid system in the dependence phenomena to ecstasys
Partner 5 (Tartu)			
Margus Tõnissaar	male	Awarded June 2006	Stress and sociability: individual differences and their neurochemical substrate
Tanel Mällo	male	Awarded June 2008	Exploratory behaviour and 50-kHz ultrasonic vocalizations in rats: behavioural and neurochemical

Student name	Male / female	Date of successful defence / predicted date of completion	Title of thesis / proposed title
			profiles of persistent inter-individual differences
Aet Alttoa	female	Awarded September 2008	Neurochemical regulation of rat exploratory behaviour: focus on dopaminergic and noradrenergic neurotransmission
Argo Vonk	male	Awarded June 2009	Determination of adenosine A2A-and dopamine D1 receptor-specific modulation of adenylate cyclase activity in rat striatum
Denis Matrov	male	Planned June 2010	Monoamine neurochemistry and regional oxidative metabolism in rat models of depression
Kadri Kõiv	female	Planned August 2010	Dopamin- and serotonergic mechanisms in inter-individual differences
Laura Herm	female	Planned August 2010	Dopamine receptors and behaviour: varieties of motivation
Margus Kanarik	male	Predicted June 2011	Search for brain regions involved in social behaviour and stress response: mapping of oxidative metabolism
Karita Raudkivi	female	Predicted June 2011	Role of glutamate and monoamine neurotransmission in inter-individual differences
Partner 6 (Maastricht)			
Daniël van den Hove	male	Awarded April 2006	Prenatal stress and adult psychopathology
Eva van Donkelaar	female	Awarded September 2009	Challenging the serotonergic system
Marisela Martinez	female	Planned Winter 2010	Role of brain derived neurotrophic factor (BDNF) in hippocampal plasticity in relation to stress-cognition interaction
Jochen De Vry	male	Planned Spring 2011	Role of brain-derived neurotrophic factor (BDNF) in neuronal and synaptic plasticity in affective disorder
Annerieke Sierksma	female	Planned Autumn 2011	Depression as a risk factor for Alzheimer's disease
Partner 7 / 14 (Budapest)			
Xenia Gonda	female	Awarded April 2008	Association of thr 5HTTLPR S allele, anxiety, depression and affective temperaments
Brigitta Balogh	female	Awarded October 2008	Effects of MDMA on vigilance and pharmacological effects of neural damage
Tamas Telek	male	Awarded May 2009	Association of premenstrual symptoms with personality dimensions and and mood changes
Eszter Kirilly	female	Successfully defended November 2009	Time course of neuronal damage and recovery induced by MDMA: Expression and distribution of serotonin transporter in rat brain
Judit Lazary	female	Predicted February 2010	Gene x gene and gene x environment interactions in depressive and anxiety phenotypes
Anita Benko	femele	Predicted September 2010	Association of impulsivity, depression and the HT1AR gene

Student name	Male / female	Date of successful defence / predicted date of completion	Title of thesis / proposed title
Eszter Molnar	female	Predicted Sept. 2011	Seasonal affective disorder: association with the serotonergic system
Partner 8 (Edinburgh)			
Neil Dawson	male	Awarded January 2008	Affective-related endophenotypes in serotonin transporter over-expressing mice
Emma Perkins	female	Awarded July 2007	Investigating the modulation of neonatal rat facial motoneurone excitability by monoamine neurotransmitters : Postsynaptic mechanisms and presynaptic modulation of glutamate release
Partner 9 (Oxford)			
Michael Newson	male	Awarded March 2005	Investigation of the effects of combined 5-HT transporter/5-HT autoreceptor blockade on central 5-HT function
Katie Jennings	female	Awarded March 2005	Analysis of brain 5-HT function in transgenic mice overexpressing the 5-HT transporter
Josephine Raley	female	Awarded August 2006	A pharmacological and neuroanatomical study of 5-HT and GABA interactions in the brain
Philip Queree	male	Awarded July 2008	Feedback control in the central 5-HT system: evidence for a role of 5-HT2C receptors
Samantha Line	female	Awarded June 2008	Investigation of the behavioural phenotype of mice overexpressing the 5-HT transporter
Kate Burnham	female	Awarded June 2009	Investigating the role of 5-HT6 receptors in prefrontal cortical function
Sep Hafizi	male	Awarded January 2009	Brain neurokinin-1 receptors in depression and interactions with the serotonin system
Matthew Taylor	male	Predicted Autumn 2009	A pharmacological and neuroimaging study of 5-HT and glutamate interactions in the brain
Christopher Barkus	male	Predicted Summer 2010	Brain glutamate systems in depression and interactions with the 5-HT system
Qin Xie	female	Predicted Spring 2010	Behavioural and molecular effects of immune system activation: the role of 5-HT mechanisms
Partner 10 (Stockholm)			
Susanne Hilke	female	Awarded Oct 2005 at Linköping University	Galanin and Neuropeptide Y in the Rodent Brain: Rapid Effects of 17□-Estradiol and Possible Role in Hippocampal Plasticity.
Eugenia Kuteeva	female	Awarded March 2007	Brain galanin systems and their role in depression-like behaviour
Ming Zhao	female	Successfully defended 29 October 2009	Neurorestorative strategies involving neurogenesis, neuronal precursors and stem cells in animal models of Parkinson's disease
Tara Wardi	female	Planned February 2010	Galanin receptor subtypes in rodent models of mood disorders
Kang Zheng	male	Planned autumn 2010	The brain galanin system: Histochemical and electrophysiological studies
Partner 11 (Florence)			

Student name	Male / female	Date of successful defence / predicted date of completion	Title of thesis / proposed title
Raffaella Morini	female	Awarded March 2007	N-methyl-3,4-methylenedioxyamphetamine (MDMA, ecstasy) effects on hippocampal neurotransmission and synaptic plasticity.
Simona Mascalchi	female	Awarded March 2007	Local regulation of dorsal raphe serotonergic neurone firing activity by serotonin in rat braistem slices.
Gilda Baccini	female	Planned mid 2010	Role of 5-HT_{1A} receptor-mediated autoinhibiton in raphe serotonergic neurone activity in animal models of human psychiatric pathology.
Partner 12 (Pamplona)			
Bárbara Aisa	female	Awarded 2008, "Excellent Cum Laude"	Maternal separation: an experimental model of depression based on an altered response to stress
Natalia Elizalde	female	Planned February 2010	Molecular, cellular and behavioural characterization of long–term chronic mild stress
Alvaro García García	male	Planned September 2010	Molecular, cellular and behavioural characterization of the VGLUT1+/- mice: interaction with environment.
Eva Martisova	female	Planned December 2011	Effect of antidepressant drug therapy on altered response to stress induced by maternal separation.
Elisabet Venzala	female	Planned October 2012	Social versus environmental stress in experimental models of major depression
Partner 13 (Warsaw)			
Grzegorz Juszczak	male	Awarded November 2007	Role of opioid system in emotional behaviors in mice selected for high and low stress-induced analgesia
Adam Sliwa	male	Awarded November 2007	Effects of stress on appetitive behaviors in mice selected for high and low stress-induced analgesia
Patrycja Wolak	female	PhD student 2005-2006 (24 months), resigned from doctoral studies for family and carer reasons	Registered title: Role of cytokines in depressive behaviors in mice
Pawel Lisowski	male	PhD student in 2005 (12 months doing research related to NEWMOOD, for administrative reasons transferred to different laboratory). Planned February 2010	Tentative title while doing research related to NEWMOOD: "Changes in gene expression in brains of mice responding or resistant to citalopram and desimipramine". Current title to be defended: "Gene expression profiling in the liver of dairy and beef cattle breeds"

Table 19. Meetings between Partner groups for the full duration of the project

Meeting between (PIs and / or their representatives)	Location	Date	Purpose of the meeting
All partners / representatives	Paris	01/Oct/04	Biannual progress meeting
All partners / representatives	Amsterdam	1-3/May/05	NewMood Annual General Meeting
Oxford, Tartu	Tartu	15-18/May/05	Discussions on harmonisation of microdialysis protocol
Oxford, Maastricht	Maastricht	03-04/May/05	Discussions on collaborative experiments and future plans
Budapest, Florence, Pamplona, Edinburgh	Budapest	08/Oct/05	Harmonization of methods for collaborative work – WP1, 3, 6, 7
All partners / representatives	Paris	3-4/Nov/05	Biannual progress meeting
Paris, Barcelona	Paris	6-7/Dec/2005	Discussions on collaborative experiments and future plans
Budapest and Stockholm (Bagdy and Hökfelt)	Budapest	24/Jan/06	Discuss experiments with olfactory bulbectomy
Stockholm, Budapest, with Prof. Miklos Palkovits	Budapest	26/Jan/06	WP5 immunohistochemistry and ish – to harmonise procedures
Manchester, Oxford, Barcelona with Geoffroy Golfier (GeneScore) and Prof. Jonathan Flint et al.	Oxford	21/Mar/06	Bioinformatics - to discuss potential databases
Manchester, Tartu, Oxford, Edinburgh, Warsaw, with Prof. Steve Williams et al.	Manchester	27/Mar/06	WP7 Imaging – to harmonise procedures
All partners / representatives	Barcelona	24-25/Apr/06	NewMood Annual General Meeting
Tartu and Budapest (Harro and Bagdy)	Tartu	29/Jun/06	Discussion of experiments and data with partial serotonergic denervation and depression phenotype
Maastricht, Würzburg	Würzburg	01/Aug/06	Discussions on future plans about prenatal stress/5-HTT knock-out mice
Maastricht, Edinburgh	Edinburgh	24/Aug/06	Evaluation of ongoing collaboration on tryptophan depletion and 2DG/blood flow
Paris and Budapest (Veronique Fabre, Michel Hamon, Gyorgy Bagdy)	Paris	19/Sep/06	Discussion of data and further studies related to sleep regulation
All partners / representatives	Paris	25/Oct/06	Biannual progress meeting
Manchester and Budapest (Gyorgy Bagdy et al, Ian Anderson et al.)	Budapest	17/Nov/06	WP7 / Activity 4 – harmonisation of human studies (including genotyping studies and planned joint publications)
Maastricht, Edinburgh	Maastricht	13/Dec/06	Discussions on future plans on 2DG/blood flow

Meeting between (PIs and / or their representatives)	Location	Date	Purpose of the meeting
Oxford, Maastricht	Maastricht	01/Mar/07	Contribution to neuroscience training course
All partners / representatives	Budapest	16-17/Apr/07	NewMood Annual General Meeting
Würzburg and Tartu	Würzburg	5 June 2007	To discuss future plans on LE/HE-model and psychogenetics
All partners / representatives	Brussels	July 2007	To discuss the harmonization of the Illumina gene expression array data
Manchester & Budapest	Budapest	August 2007	To discuss genotyping data results and joint publications
Manchester & Budapest	Budapest	Sept. 2007	To discuss possible modification in handling of databases for further analyses
All partners / representatives.	Florence	October 2007	Biannual Meeting for the Partners
Manchester & Budapest	Budapest	Dec. 2007	To discuss genotyping data and addition of possible further SNPs in the human cohorts
Manchester, Paris, Würzburg ,Tartu, Maastricht, Oxford	Manchester	January 2008	Bioinformatics workshop
All partners / representatives	Stockholm	4-5/Apr/08	NewMood Annual General Meeting
Paris, Würzburg, Budapest, Oxford, Manchester	Oxford	17-21/Jul/08	Serotonin Club meeting
All partners / representatives	Brussels	10/Oct/08	Biannual progress meeting
Tartu / Oxford	Washington, DC	17/Nov/08	To discuss the social defeat model
Paris / Barcelona	Barcelona	19/Dec/08	PhD Defense
Tartu / Würzburg	Würzburg	January-February 2009	Jaanus Harro was a Guest Professor at the University of Würzburg Department of Psychiatry. Meetings with Klaus-Peter Lesch to discuss expression profiling data
All partners / representatives	Manchester	03/Apr/09	Biannual progress meeting
Würzburg / Maastricht	Maastricht	April 2009	To discuss genetic programming studies
All partners / representatives	Brussels	04/Jun/09	To discuss progress of NewMood
Manchester, Paris, Barcelona, Maastricht, Tartu, Oxford, Warsaw	London	03/Jul/09	To discuss results / analysis from Illumina genechip array data
Würzburg / Maastricht	Würzburg	July 2009	To discuss progress in genetic programming studies
All partners / representatives	Istanbul	12–16/Sep/09	ECNP conference / NewMood symposium / NewMood final meeting
Warsaw / Tartu	Tartu	October 2009	To discuss experimental work

Table 20. Material transferred between Partners for the full duration of the project

From	To	Nature	Date
Tartu	Stockholm	Sera from patients with eating disorder	Summer 2004
Paris	Barcelona	Mice (5-HTT mice)	Feb, June, Sept, Nov 2005 & Jan, Feb '06
Stockholm	Budapest	Data (galanin immunohistochemistry) from rat brains sent earlier to Stockholm from Budapest	Paris AGM 2005
Edinburgh	Budapest	Data (LCMRglu) of a parallel collaborative study (see joint publications)	1st September 2005
Paris	Budapest	Data (GTP-gammaS) from rat brains sent earlier to Paris from Budapest	15th September 2005
Budapest	Edinburgh	Data (5-HTT immunohistochemistry) of a parallel, collaborative study (see joint publications)	25th November 2005
Budapest	Manchester	Approx. 300 mouth swab samples for DNA extraction and genetic polymorphism analysis	January & March 2006
Edinburgh	Warsaw	Experimental protocols for 2-DG experiments in mice; 14C standards for preparation of autoradiographic images; access to image analysis equipment in Edinburgh.	January – April 2006 and continuing
Tartu	Edinburgh	Protocols for cytochrome oxidase methodology	March 2006
Maastricht	Oxford	Brain tissue samples for HPLC analysis	April 2006
Wurzburg	Paris	Double knockout (5-HTT/BDNF) mice	July 2006
Wurzburg	Warsaw	Transgenic mice	Summer 2006
Barcelona	Paris	RNA samples for microarrays	August and November 2006
Pamplona	Oxford	Heterozygous VGLUT1 mice for electrophysiological studies	October 2006
Wurzburg	Oxford	5-HT transporter k.o. mice	October 2006
Edinburgh	Budapest	Data (LCMRglu) of a parallel collaborative study with SSRIs	3 October 2006
Würzburg	Budapest	Antibody for tryptophan hydroxylase staining	9 October 2006
Budapest	Stockholm	Brain tissue samples for immunohistochemistry and *in situ* hybridization	10 November 2006
Würzburg	Oxford	Transfer of 5-HTT KO mice	November 2006
Budapest	Maastricht	Experimental protocols for immuno-staining of 5-HT transporter and tryptophan hydroxylase	15 December 2006
Budapest	Manchester	Buccal samples for genotyping	Feb. & Oct. 2006, Feb. 2007
Manchester	Budapest	SNP and length polymorphism data	continuously
Edinburgh	Tartu	Frozen brains harvested from 5-HTT over-expressing male mice, for cytochrome oxidase staining	April 2007
Pamplona	Tartu	Brain tissue from maternal separated and control rats for cytochrome cytochrome oxidase activity	April 2007
Barcelona	Paris	CB1 knockout mice	April 2007

From	To	Nature	Date
Maastricht	Florence	Protocols for Acute Tryptophan Depletion and Diet	May 2007
Würzburg	Florence	Protocols for Tryptophan Hydroxylase IHC and Antibodies	May 2007
Paris	Stockholm	Brain tissue (5-HTT-/- and 5-HTT+/+) for ISH analysis	May 2007
Pamplona	Paris	VGLUT1 mouse brains for GTPγS	May 2007
Pamplona	Paris	Brain tissue from VGLUT1+/- and WT mice (control and CMS) for 5-HT1A activity in DRN	May 2007
Würzburg	Florence	Protocols for Tryptophan Hydroxylase IHC and Antibodies	May 2007
Maastricht	Florence	Protocols for Acute Tryptophan Depletion and Diet	May 2007
Paris	Stockholm	Brain tissue (5-HTT-/- and 5-HTT+/+) for immunohistochemistry / ISH analysis	May 2007
Pamplona	Paris	VGLUT1 mouse brains for GTPgS	May 2007
Pamplona	Paris	Brain tissue from VGLUT1+/- and WT mice (control and CMS) for 5-HT1A activity in DRN	May 2007
Oxford	Paris	5-HTT over-expressing mice for GTP□S, sleep study and microarray studies	May 2007 and Jan. 2008
Budapest	Manchester	Buccal samples for genotyping	May 2007 and April 2008
Edinburgh	Warsaw	Protocols and supplies for 2DG method to determine metabolic activity in mice brain	June 2007
Paris	Florence	Transfer of 5-HTT k.o. mice	August 2007
Maastricht	Florence	Protocols for Acute Tryptophan Depletion and Diet	September 2007
Tartu	Pamplona	Data on cytochrome c oxydase activity for brain tissue from maternal separated and control rats	October 2007
Pamplona	Tartu	Brain tissue from VGLUT1+/- and WT mice for cytochrome c oxydase activity	November 2007
Oxford	Maastricht	Plasma and brain tissue samples for analysis of BDNF protein	November 2007
Tartu	Edinburgh	Data on cytochrome c oxydase activity for brain tissue from 5-HTT o.e. male mice	April 2008
Oxford	Warsaw	Protocol for measurement of 5-HT1A autoreceptor function (hypothermia model)	April 2008
Tartu	Pamplona	Data on cytochrome c oxydase activity for brain tissue from VGLUT1+/- and WT mice	April 2008
Tartu	Stockholm	Brain tissue from the rat social defeat model for ISH analysis	April 2008
Barcelona	Tartu	Frozen brain tissue from CB1 knockout and WT mice for cytochrome c oxidase activity	April 2008
Barcelona	Maastricht, Pamplona & Tartu	Illumina microarray data	April 2008
Pamplona	Paris	Transfer of frozen brains from VGLUT1+/- mice and WT littermates to perform *in situ* hybridisation studies (n= 10 brains [May] and 11 brains [July])	May and July 2008
Paris	Tartu	GR-i mice (frozen brain)	July 2008

From	To	Nature	Date
Oxford	Paris	Transfer of frozen brains from 5-HTT over-expressing mice and WT littermates to perform RNA extraction for microarray analysis.	July 2008
Barcelona	Paris	CB1 KO mice (frozen brains)	September 2008
Pamplona	Oxford	Transfer of live VGLUT1+/- mice and WT littermates to perform behavioural studies.	September 2008
Budapest	Stockholm	Human brain tissue (frozen)	October 2008
Pamplona	Tartu	CB1-KO mice (frozen brains)	October 2008
Barcelona	Tartu, Maastricht, Pamplona	Inter-exchange of data from microarrays experiments (several models)	October 2008
Pamplona	Paris	Transfer of live VGLUT1+/- mice and WT littermates to perform electrophysiology studies (n= 16 animals)	January 2009
Paris	Barcelona	5-HT2A mice	February 2009
Barcelona	Pamplona	Validation data from VGLUT1+/- mice microarrays experiments	Feb 2009
Pamplona	Paris	Transfer of frozen brains (n= 11) from VGLUT1+/- mice and WT littermates to perform autoradiography studies	March 2009
Paris	Stockholm	5-HTT-KO mice (frozen brain)	May 2009
Pamplona	Stockholm	VGLUT +/- mice (frozen brains)	May 2009
Maastricht	Würzburg	5-HTT KO mouse brains following prenatal stress	June 2009
Tartu	Paris	COX data on GR-i mice	July 2009
Tartu	Pamplona	COX data on CB1-KO mice	July 2009
Pamplona	Stockholm	CB1-KO mice (frozen brains)	October 2009
Warsaw	Tartu	LA/HA mice (frozen brains to assess cytochrome oxydase activity)	October 2009