IMI1 Final Project Report
Public Summary

Project Acronym: NEWMEDS

Project Title: Novel Methods leading to New Medications in Depression and Schizophrenia

Grant Agreement: 115008
Project Duration: 01/09/2009 - 28/02/2015

Executive summary

1.1. Project rationale and overall objectives of the project

Despite remarkable advances in molecular and imaging technologies and nearly 15,000 articles on schizophrenia and depression (S&D) every year, there have been few truly innovative new chemical entities (NCEs) which have made it to the clinic. We think there are three major bottlenecks that are holding the field back: i) a lack of pathophysiologically-accurate animal models guiding the drug discovery of NCEs; ii) a lack of tools and tests in healthy volunteers that can provide early indication of efficacy; and iii) the reliance of clinical trials on symptom-based DSM-categories which inevitably lead to biologically heterogeneous groups of patients. To overcome these limitations, we have brought together a consortium of six leading European and an Israeli academic institution, two SMEs, and ten EFPIA partners in the NEWMEDS consortium, which ties academic expertise in animal models, genetics, functional MRI and PET imaging, clinical settings and analysis methods together with expertise and incentive in drug discovery and development. The purpose of the project was not to develop new drugs – but to develop new methods. Therefore, the focus was on new "pre-competitive" insights, methodologies and analytical methods that would assist drug development and could be shared transparently with academia and industry. Figure 1 below illustrates the overall layout of the NEWMEDS effort as well as the particular bottlenecks which the project aimed to address.

1.2. Overall deliverables of the project

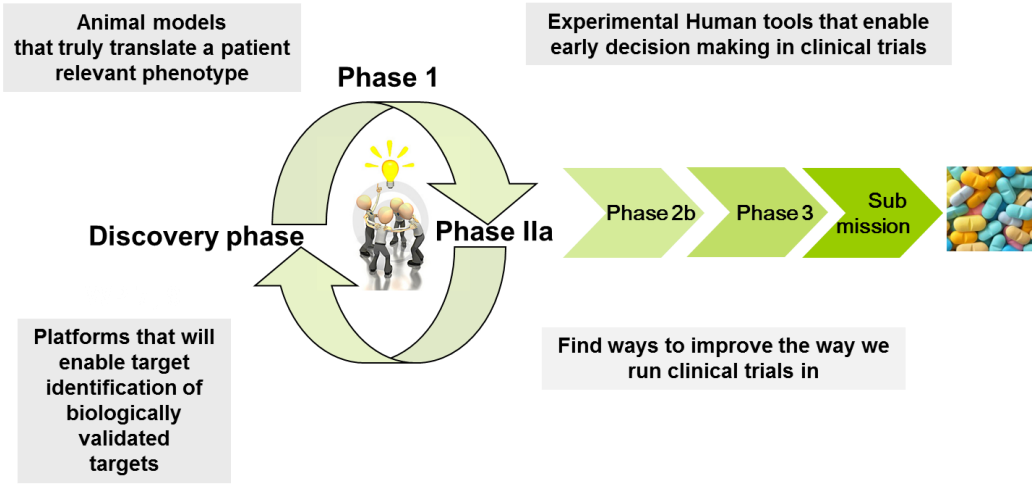

Figure 1. Layout of the strategic intent of the NEWMEDS as related to the stages of drug development.

To specifically target the challenges in psychiatry the NEWMEDS consortium was designed to a) develop standardised animal models that focus on reliable cross-species endophenotypes (e.g. cognitive function) and use cross-species methods (MRI, EEG) to bring animal models closer to clinical endpoints; b) develop fMRI and PET based paradigms which may serve as early or surrogate markers to provide guidance for drug development; c) examine a set of genetic abnormalities (CNV) closely linked with Schizophrenia by studying them in human populations and animal models to achieve one-to-one cross-species correlation of the impact of these mutations on cognition, behaviour and brain physiology and

function; d) identify pharmacogenetic biomarkers that can be used to stratify patients within an umbrella DSM-diagnosis (e.g. Depression) thus allowing for targeted clinical trials and individualized treatment; and e) by bringing together the largest multi-industry clinical database in Schizophrenia and Depression – provide new insights for the intelligent design of clinical trials.

1.3. Summary of progress versus plan since last period

This was the last operating period of the NEWMEDS grant and as authorized included a no-cost extension which accounted for an 18 month period of reporting. The extension has been very helpful, because as reported in Section 2.1 NEWMEDS has managed to deliver on 28 Deliverables that were promised and the extension period also allowed us to complete the additional milestones and deliverables that were agreed as a part of the ENSO addition. Two minor issues relating to a planned delay in publication to coincide with a major Special Issue (WP2), and some delay in recruitment on a sub-project (that is now being completed) is explained in detail (WP3).

1.4. Significant achievements since last report

The detailed achievements since the last report are outlined in Section 2. We highlight here a few particularly notable advances in the last period:

- Comparison of cognitive phenotyping of three CNV knockout mouse lines
- Electrophysiological deficits associated with CNVs in transgenic mice
- fMRI data and neurocognitive data generated for 300 subjects with CNVs
- Publications on the reliability of the imaging battery for drug discovery
- Methodology for PET-measurement of changes in endogenous GABA levels using novel radioligands
- Validation of multivariate linking methods (e.g. genetics-imaging) for stratification of patient groups obtained from biomarker studies
- New classifier algorithm implemented in a distributed toolbox and Proof of principle using novel external data with an experimental compound
- Report of statistical analysis using polygene scores to predict outcome

1.5. Scientific and technical results/foregrounds of the project

Workpakage 1 : Linking animal and clinical models via electrophysiology

The main objective of WP1 was to expand our biological insights and understanding of the brain circuits, neuronal elements and receptors underlying schizophrenia and depression. As a field we need to move beyond animal models based on observed behaviour alone (for example, models such as swim-test for depression, or locomotion for Schizophrenia) and instead focus on neurobiological, molecular and genetic information to develop and test new types of biological assays as models for psychiatric drugs. The altered Prefrontal cortex (PFC) activity as well as the disruption of cortical oscillations induced by pharmacological disruption (PCP and DOI-induced psychosis) used to mimic schizophrenia in animal models have been examined in detail. Early on it was decided to anchor this approach around electrophysiological observations and later compare work with behavioural work (WP2), brain imaging (WP4) and three mice models carrying copy number variants (CNVs) known to be involved in schizophrenia (WP7). A particular emphasis was made on glutamatergic perturbations, since they are implicated in both schizophrenia and depression, and CNVs (as they are the best biologic correlate available for humans carrying a higher risk of developing schizophrenia).

Studies in schizophrenia point to a special role for ventromedial and dorsolateral prefrontal cortex, with an emphasis of abnormal signal-to-noise processing in pyramidal-cell circuits. These cellular elements give rise to a key electrophysiological signal: slow cortical oscillations (SCO). Since both the monoaminergic systems and glutamate systems impact on these pyramidal-cell circuits it provides a key target for influencing prefrontal mediated functions and SCOs may provide a valuable electrophysiological readout for drug discovery. To examine if these clinically-relevant electrophysiological markers could be replicated in animal models, and thus be tools one could use as a screening tool, we recorded selectively from the pyramidal and GABAergic neurons in PFC and investigated their physiological role. We have shown that psychotomimetic drugs such as PCP (phencyclidine, a NMDA receptor antagonist) and DOI (preferential 5-HT2A receptor agonist) markedly disrupted PFC function in rats, increasing pyramidal activity and suppressing SCO. The effects were time-limited and dose-dependent and were observed at doses which produce behavioural disruptions. **The predictive validity of the above model was confirmed by using classical and atypical antipsychotic drugs**, and electrophysiological disturbance was reversed by antipsychotic drugs with different pharmacology, suggesting that **the reversal of drug-induced suppression of SCOs** could be explored as a clinically-relevant, neuroanatomically specific, **potential marker for identifying new medications** (Celada et al. 2013).

Thalamocortical circuits have been validated as a main target for the psychotomimetic action of NMDA-R antagonists, due to the specific blockade of NMDA-R in the reticular nucleus of the thalamus, which provides feed-forward inhibition to the rest of thalamic nuclei, therefore disinhibiting thalamic afferents to the neocortex. We have also characterized the action of other serotonergic hallucinogens, such as 5-MerO-DMT, on cortical networks. All these actions were reversed by classical and atypical antipsychotic drugs, **indicating that the SCO model can be reliably used as a preclinical test for antipsychotic drug development** (Santana et al. and Kiss et al. 2011).

Due to the unique opportunity to collaborate across workpackages and engage similar technologies in rodents and in humans, interactions between the hippocampus and the prefrontal cortex (PFC) were studied using both electrophysiology in animals (WP1), fMRI BOLD in animals (WP4); and fMRI-BOLD and EEG in humans (WP4). A homologous relationship was found in the human hippocampus, with differential functional connectivity between hippocampal locations and the medial prefrontal. These data correlate precisely with the electrophysiological data from WP1, thus these functional connectivity relationships **provide a useful translatable probe of the hippocampal-prefrontal system for the further study of rodent models of disease and potential treatments**.

In an effort to validate a circuit-based approach in depression, we were one of the first to show that interventions that are traditionally used to produce behavioural models of depression (e.g. learned helplessness, acute and chronic stress) dramatically affects the induction of long term potentiation (LTP) in select pathways: plasticity in the HIPP-VMPFC pathway is inhibited, whereas in the orbitofrontal cortex – amygdala (OFC-A) pathway is increased by stress, **once again pointing to that a circuit based approach may provide a useful translatable model of depression** (reviewed in Artigas 2015).

While important observations have emerged in recent years indicating that more rapid and effective treatment paradigm are possible in depression (e.g. deep brain stimulation (DBS), and the non-competitive NDMA receptor antagonist ketamine) the underlying mechanism is still to be uncovered. The role of ketamine has been explored with metabolism, 24 hour and sleep EEG, and also in phosphoproteome studies. The latter studies revealed a major discrepancy in the literature as ketamine did not appear to mediate its effects by mTOR, as proposed by numerous high profile publications by Duman's group stressing the importance of additional work in this area is needed.

Three mice models carrying copy number variants (CNVs) 1q21, 15q13 and 22q11 known to increase the risk in humans of developing schizophrenia were made available to the partners in NEWMEDS. These **animal models are biologically meaningful correlates of the human carriers**. In accordance with

pharmacologic observations reported elsewhere in this report (WP7) the existence of GABAergic abnormalities in the PFC of 15q13 mice, as well as the apparent lack of a dopaminergic phenotype in 22q11 mice point to the biological relevance of these models.

Status of Tasks in WP01:

Tasks	**Status (Please give status: completed, partially completed, deleted, modified, extended)**
Task 1: Study the effects of a.) PCP on slow cortical oscillations in PFC and b.) atypical antipsychotics (clozapine in the first 18 months) on the PCP-induced disruption of slow cortical oscillations. In order to expőlore the neuronal circuits involved pharmacological agents and genetically modified animals (5-HT1A and 5-HT2A KO mice) will be used.	**Completed**
Task 2: Study of the effects of PCP on the neuronal activity and slow cortical oscillations in afferent (intracortical, thalamocortical) areas to PFC.	**Completed**
Task 3: Study of the possible heterodimerization of 5-HT2A and alpha1-adrenergic receptors in PFC - to set up screening methods to assess novel antipsychotic pharmacology.	**Completed**
Task 4: Study of the interactions of glutamate (PCP, NMDA antagonists, AMPA modulators), 5HT (5-HT1A and 5-HT2A agonists and antagonists), noradrenaline (alpha1-adrenoceptor agonist and antagonists) and cannabinoid ligands on synaptic transmission and LTP at hippocampal to prefrontal synapses.	**Completed**
Task 5: Investigate levels of co-ordinated neuronal activity between prefrontal cortex, hippocampus and thalamus in animal models of schizophrenia.	**Completed**

Workpackage 2: Linking animal and clinical models of cognition

The main objective of WP 2 has been **to develop methods to assess cognition which use the same paradigm in rodents as in humans**. Whilst there has been a consensus in the field for decades, that cognitive dysfunction in schizophrenia is a claimable target no drug has successfully been brought to the market to the benefit of patients. Two major hurdles have been holding back the field one being lack of rodent models that translate into human correlates that measure clinically meaningful outcomes and the other being lack of understanding the biological targets that truly have an impact on cognition in schizophrenia (Keelara et al. 2011). WP2 set out to develop animal models/assays that established reliable cross-species intermediate phenotypes, allowing for a more integrated, translational approach to preclinical research.

To initiate this effort we reviewed the literature thoroughly and made a workshop that included external advisors as well as representatives from another IMI project namely PharmaCog. This workshop led to consensus on a cognitive test battery and specific perturbation models that would be tested in NEWMEDS and set the ground rules for sharing of protocol and how we would work together. While the EFPIA partners initiated a cross-laboratory study on assessment of cross-site reliability of behavioural pharmacology with a simple touchscreen task, the UCAM group developed the touchscreen

cognitive battery. Both tasks were major achievements and proved the usefulness of the partnership, where protocols and learning were shared and re-iterated among partners.

For the cross-site touch screen reliability exercise, EFPIA partners compared drug effects in the MAM-17 model in their own laboratories on a common, agreed visual discrimination learning paradigm (Lilly, Janssen, Abbvie, Pfizer, Roche, Orion, Lundbeck) and showed that once standardized there was a very high alignment of results .

Site	PPI	Basal & PCP hyperactivity	Touchscreen VD & Reversal	Attention Learning & Memory	Neuroanatomy
AbbVie	-	↑	-	↓	↓
Lilly	-	↑	-	↓	↓
Orion	-	↑	-	↓	↓
Pfizer	N/A	↑	-	↓	N/A
Lundbeck	N/A	↑	-	N/A	N/A
Reliability	100%	100%	100%	100%	100%

Figure 2: Cross-site reliability at different EFPIA companies

The UCAM team produced a number of tasks that comprised the cognitive test battery- for both mice and rats. These tests included visual discrimination learning and reversal, paired associates learning (PAL), cTUNL- a test of spatial working memory, SWM, a self-ordered spatial working memory task that resembles its human CANTAB equivalent, the touch-screen version of the 5-choice serial reaction time test, and a new continuous performance test (CPT) of sustained attention that resembles paradigms used in human schizophrenia research (Bussey et al 2012). All of these tasks have been made available to the partners in collaborative studies and most of them have been used in partner projects. (Horner et al, Nature Protocols).

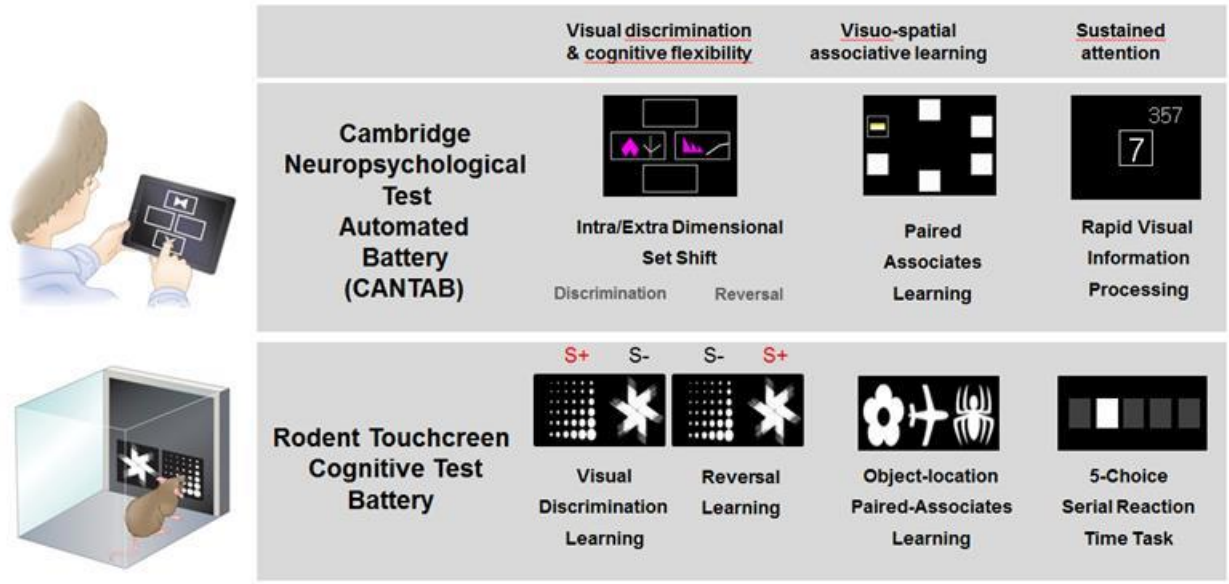

Figure 3: Comparison of CANTAB and the Rodent Touchscreen Test Battery

A second major aspect of the animal modelling work of WP02 focused on behavioural phenotyping of three CNV mouse models made available through the partnership. These three mice models each represent biological correlates of human carriers that share a higher risk of developing schizophrenia and are construct valid rodent models. Data was generated on 1q21, 15q13, and 22q11 mouse lines, and all lines displayed dissociable and discrete cognitive impairments that may have relevance to schizophrenia. Characterisation of the 22q11 model encompassed nearly 20 different behavioural assays, the highlight of which was replicable vigilance impairment in the NEWMEDS-developed continuous performance task (CPT). Although not pharmacological models, these findings suggest that no one rodent model recapitulates the full spectrum of cognitive deficits observed in schizophrenia.

The continuous performance test task was also used to compare effects in rats subjected to MAM E17, chronic PCP or neonatal PCP, and also in NR1 KO and CNV mice. A major finding was that each of these models produced a distinctive pattern of functional effects, with the neonatal PCP model not producing major impairments, while the chronic PCP model mainly affected perceptual aspects of the task. This suggests that each of these perturbations may be relevant to understanding the cognitive phenotype in schizophrenia but that their validity as models recapitulating the spectrum of cognitive deficit may be limited.

Status of Tasks in WP02:

Tasks	Status (Please give status: completed, partially completed, deleted, modified, extended)
Task 1: Assay review and consensus regarding available tests and drugs (M1-18) UCAM and all partners	completed
Task 2: Test modification and innovation (M1-18) UCAM, Lundbeck, Eli Lilly, JP NV, Orion Pharma,	completed
Task 3: Test validation using disrupting agents (i) (M1-18) UCAM, Lundbeck, Eli Lilly	completed
Task 4: Reversal of cognitive dysfunction (ii) (M18-54) UCAM, Lundbeck, Eli Lilly, AbbVie, Orion Pharma, Servier	completed
Task 5: Delivery of test battery (M36) UCAM, Lundbeck, Eli Lilly, AbbVie, GABO:mi, JP NV, Orion Pharma, Servier	completed
Task 6: Test battery mutual investigation and exploitation (M36-54)	completed
Task 7: Analysis and publication of WP2 data plus collation, analysis and publication of data from partners (M48-60) UCAM, Lundbeck, Eli Lilly, AbbVie, GABO:mi, JP NV, Orion Pharma, Servier	completed
Task 8: Cognitive profiling of CNV mice (Month 54 – 66) (HLU, UCAM)	completed

Workpackage 3: Development of cognitive measures ideal for international trials and back-translation to preclinical models

The main objectives of WP3 were to receive and collate the latest data from industry and academia regarding Cognitive Impairment Associated with Schizophrenia (CIAS), and convene a meeting to begin a cross-party discussion about optimal clinical trial designs to study CIAS in Europe, especially on how to combine drug interventions with cognitive remediation. Based on this it was the intention of the WP to evaluate MATRICS [Measurement and Treatment Research to Improve Cognition in Schizophrenia] Consensus Cognitive Battery (MCCB, which was emerging as a standard cognitive test battery in the US in 2009) with some of the emerging computerised options and to use these batteries to undertake a proof of concept trial to test whether the combination of a cognitive training with a cognitive enhancer can lead to better outcomes. The WP held an all-party workshop with Key Opinion Leaders in the field in Madrid in 2009 which advised on potential drug/training trial designs and also decided to focus on the comparison of MCCB vs. CogState and MCCB vs. CANTAB as the two computerised batteries of interest.

A study compared the MATRICS MCCB and CogState Schizophrenia Batteries, looking at the effects of repeated exposure to the tests on consecutive days and following a 4-week break. Data was obtained from the modafinil and cognitive training trial collected for the purposed of NEWMEDS and data provided by Novartis from their ongoing clinical trials. The reliability and validity of these two assessment batteries was compared in 143 participants. While the batteries' total scores correlated strongly (r0.79-0.82), domains of cognition apparently equivalent across both batteries (e.g. psychomotor-attention) correlated between 0.22 and 0.60, **indicating substantial differences between the batteries on some supposed counterparts**. Clinical trials using either battery would benefit from initial practice sessions to ameliorate practice effects (Drake et al. under review).

Accordingly, WP03 carried out a clinical trial investigating the effect of combining modafinil and cognitive training. To prepare for this trial the WP carried out a feasibility study in normal volunteers – recruiting 33 volunteers and randomising them to a modafinil vs. placebo condition, and examining how this affected cognitive training (Gilleen et al. 2014). With this established the WP undertook a Clinical Trial which examined the effect of combining modafinil (200mg/day) vs. placebo with cognitive training in chronic patients with schizophrenia. The trial was successfully completed, randomizing 49 patients, and delivering to each of them 10 episodes of cognitive training (Panayiota et al. In press). Despite a well done trial that met its recruitment goals and cognitive training delivery objectives – there was no interaction between medication and training. Patients improved with training, however, medication did not lead to any synergy. No significant effects of modafinil on symptom severity were found. The study demonstrated that combining pharmacological compounds with **cognitive training is acceptable to patients and can feasibly be implemented** in double-blind randomised controlled trials. However, the lack of differential enhancement of training-induced learning raises questions, such as choice and optimal dose of drug, cognitive domains to be trained, type and intensity of cognitive training, duration of the intervention, chronicity and severity of illness and age of participants that require systematic investigation in future studies.

As a follow-up to the modafinil clinical trial, it was decided to investigate whether the lack of findings could be the result of duration of illness or sensitivity of the test batteries used. A single-dose study investigated the effect of a single-dose of modafinil vs placebo in a cross-over design in both recent onset schizophrenia patients (within first 3 years of onset) and matched healthy volunteers using the Matrics MCCB and CANTAB test batteries. The results of this study are being analysed at the time of this writing. Using these data we will be in a position to assess differential performance of novel Paired-Associates Learning and Continuous Performance Tasks (developed by WP02 to provide direct back-translation to animal models) in patients and healthy volunteers, as well as the effect of modafinil/placebo on the BOLD signal during N-back (as standardised by WP04).

A study being carried out in Madrid, which is due for completion in May 2015, will be combined with in-kind data supplied by Abbvie to compare the MCCB and CANTAB in schizophrenia. This will provide the first such systematic comparison data which is of particular value as several elements of the CANTAB have been standardised by WP2 for direct translational studies.

Status of Tasks in WP03:

Tasks	Status (Please give status: completed, partially completed, deleted, modified, extended)
Task 1: Convening a CIAS state-of-the-science meeting (M3).	completed
Task 2: Calibration and cross-validation of MCCB with Cogstate (M1-18).	completed
Task 3: Cognitive enhancer challenge with Cogstate and CANTAB (M1-12).	completed
Task 4: Confirmatory MCCB calibration with CANTAB (M12-24).	analysis yet to be completed
Task 6: Data from Tasks 1-5 to be used to translate back to preclinical workpackages (M18-30). Data from the results of the experiments in the first 18 months will be translated back into preclinical workpackages, especially WP2. Partner: UCAM.	not done, data not reported in time to be back-translated during NEWMEDS period
Task 7: Proof-of-principle for combining cognitive enhancers with cognitive training.	completed
Task 8: Efficacy of computerised CRT in CIAS (M 18-60).	modified

Workpackage 4: Cross-species and functional imaging models for drug discovery

The main objectives of WP4 were to establish cross-species functional imaging for drug development. In particular, the WP was to establish a standard battery of fMRI tasks for use in experimental human studies, test structural and functional imaging paradigms in subjects with different genetic (CNV) abnormalities relevant to psychiatric disorders and develop closely related animal (rodent) imaging techniques.

An fMRI cognitive-emotional task battery was devised to activate different brain circuits known to be altered in psychiatric disorders. It comprised **reward** (activating the ventral striatum), **working memory** (dorsolateral prefrontal and parietal cortices) and **facial affect** (amygdala) paradigms, along with a task-free "resting state" scan. First, the test-retest within-subject reliability of these tasks was assessed in healthy volunteers [Plichta et al. 2012]. The reward and n-back tasks evidenced good reliability, whereas for the facial affect task, reliable amygdala signal was associated with its habituation to repeated stimuli rather than the mean response over the scan [Plichta et al. 2014]. This novel finding will be important in the use of this task (and hence the full battery) in within-subject study designs, which are generally preferred in early phase, healthy subject pharmacology studies.

The fMRI battery was then applied to study, in a consistent way, the effects of single, acute doses of 8 reference compounds in modulating these brain circuits. The compounds were chosen as marketed psychiatric drugs covering a range of pharmacological and clinical profiles: haloperidol, olanzapine, risperidone, amisulpride, modafinil, lorazepam, ketamine and scopolamine. These data provide an valuable reference data set allowing the effects of novel compounds (acquired using the same standard battery and setting) to be compared with the reference compounds. The NEWMEDs participants can access this data, whereas others can collect data on their new compounds using similar methods and use this dataset for comparison (accessible via Prof. Meyer-Lindenberg's lab).

The tasks in the fMRI battery were adapted for use with EEG (small changes in timing and number of stimuli were needed to yield sufficient power for the EEG analysis). First, the modified task battery was

tested on healthy volunteers using concurrent EEG and fMRI (i.e., EEG in the MRI scanner). This demonstrated that the modified tasks elicited fMRI activation patterns consistent with the original tasks, and allowed the EEG responses, and their relationship to the fMRI signals, to be characterized [Plichta et al. 2013]. Secondly, a test-retest study was performed on subjects outside the scanner. Third, the effects of ketamine on EEG responses to the task battery were tested, using a randomized cross-over design and an identical ketamine dose as used in the fMRI study. The fMRI battery was also transferred to **DeCode (WP7) for application to CNS phenotyping of the CNV carriers**. In addition, two new tasks (dyslexia and dyscalculia) were devised based on the cognitive phenotype of these populations and transferred to create an expanded battery along with structural imaging. Initial analysis of the structural imaging data revealed **a brain atrophy signature similar to that observed in schizophrenic subjects** [Stefansson et al. 2014].

WP4 successfully established rsfMRI in the rat and has been at the forefront of the rapid progress of this technique. We systematically **mapped the functional connectivity signature of the hippocampal-prefrontal network in the rat brain**, demonstrating a high concordance with known anatomical connectivity and increasing confidence in the validity of rsfMRI as a meaningful probe of brain function in the rodent. We were the first to demonstrate the presence of anticorrelated cortical networks (default mode-like and lateral cortical) in the rat, strongly homologous to cardinal networks that are well-established in the human brain [Schwarz et al. 2013]. We were the **first to demonstrate pharmacological modulation of resting state connectivity in the rat by an antipsychotic** (haloperidol), showing that it preferentially acts on the dopaminergic system, consistent with its mechanism of action [Gass et al. 2013]. We also thoroughly characterised the dose- and exposure-related effects of ketamine on connectivity in the rat brain [Gass et al. 2014]. This remains one of the few imaging studies in the literature in which pharmacokinetic-pharmacodynamic (PK/PD) relationships are considered – an aspect of critical relevance for drug discovery.

To parallel the human multi-drug study, we also performed another 6 rsfMRI studies in the rat using the same set of compounds, assessing multiple doses and obtaining peripheral drug concentrations for PK/PD analyses. As for the human study, this will provide an invaluable **reference data set for consortium participants, to elucidate specificity or commonalities across the compounds** and allow novel compounds studied with rsfMRI to be compared and contrasted. Moreover, the drug signatures can be compared with the resting state signatures found in the human study.

Finally, we also established rsfMRI in the mouse, and applied this to **profile functional connectivity along with brain structure in three CNV mouse lines (1q21, 15q13 and 22q11)**. Some differential connectivity in PFC-HC circuit was observed, although the overall effects were quite weak. This is consistent with findings in other WPs using other methods – strong, clear and distinctive phenotypes were not forthcoming despite clear replication of the CNV and standardised methods. The limitations of these models are important findings to inform any potential use as tools for drug discovery.

Status of Tasks in WP04:

Tasks	Status (Please give status: completed, partially completed, deleted, modified, extended)
Task 1 - Establishment of baseline functional imaging battery in humans and rodents	**Completed**
Task 2 - Establishment of human cognitive neuroimaging paradigms for drug discovery	**Completed**
Task 3 - Neural mechanisms of risk genetic variants, SNPs and copy-number variations	**Completed**
Task 4 - Establishment of validity and reliability for drug discovery	**Completed**
Task 5: Pharmacological modulation of Functional Connectivity	**Completed**

Workpackage 5: Cross-Species and Neurochemical Imaging (PET) methods for drug discovery

While PET radioligands are now available for more than forty CNS targets, at the initiation of NEWMEDS in 2009, PET radioligands sensitive to an endogenous neurotransmitter were only validated for dopamine. The objective of WP5 was to develop methods to determine endogenous transmitter alterations in serotonin and norepinephrine systems – and after the successful completion of that the WP focussed on development of a PET methodology for measurement of changes in non-monoaminergic targets, such as GABA and cyclic nucleotides (see Table below).

Neurotransmitter	Target	Radioligand	EFPIA
Serotonin	5-HT1B	AZ10419369	AstraZeneca & Lundebeck
Noradrenaline	α2C-Adrengic	ORM-13070	Rion & Pfizer
GABA	$GABA_A$	RO6899880 RO6900648	Roche & Pfizer
Cyclic nucleotiders	PDE10A	[^{11}C]LuAE92686	Lundeck and Pfizer

Table 1: Neurotransmitters, Radioligands, Targets & EFPIA companies

Within NEWMEDS we validated the use of 5-HT1B receptor radioligand [11C]AZ10419369 for evaluation of changes in extracellular serotonin. The 5-HT1B receptor was considered a promising target as serotonin binds with quite high affinity to this receptor (~1 nM). The serotonin sensitivity of [11C]AZ10419369 binding was initially explored in monkeys using the potent serotonin releaser (±)-fenfluramine. This study showed for the first time a major decrease in radioligand receptor binding (50% by 5.0 mg/kg) measured with PET in the NHP brain after administration of a serotonin releasing agent (Finnema et al. 2010 and 2012). In a consecutive test-retest study in human subjects, [11C]AZ10419369 binding was found to be very reproducible with an absolute mean difference in BPND of less than 3% in serotonergic projection areas. The serotonin sensitivity of [11C]AZ10419369 in humans was therefore

assessed using the SSRI escitalopram which has been shown devoid of significant affinity for the 5-HT1B receptor. In pilot PET studies in monkeys we confirmed that a high dose of escitalopram (2.0 mg/kg, i.v.) decreased [11C]AZ10419369 binding by 11% in serotonin projection areas and by 25% in the raphe nuclei, suggesting, as expected, increase of extracellular 5-HT after escitalopram administration. However, in healthy human subjects (n=9), administration of a single dose of escitalopram (20 mg, p.o.) tended to decrease [11C]AZ10419369 binding in the raphe nuclei, but increased radioligand binding by 5% in serotonergic projection areas ($p<0.05$). These studies suggested that a single clinically relevant dose of escitalopram may decrease extracellular serotonin concentrations in serotonergic projection areas in the human brain (Nord et al. 2013).

At the initiation of NEWMEDS, no methodology for measurement of extracellular noradrenaline in vivo in the human brain was available. Orion Pharma and the Turku University developed together [11C]ORM-13070 as an antagonist radioligand for the α2C-AR. Within NEWMEDS we validated the use of [11C]ORM-13070 for detecting changes in extracellular noradrenaline concentrations. The radiolabeling of [11C]ORM-13070 was achieved readily and the preliminary characterization of the radioligand was successfully performed in rats and α2-AR subtype knock-out mice and paved the way for a metabolism and dosimetry study in healthy human males. The test-retest reliability of [11C]ORM-13070 binding was investigated in 6 healthy male subjects. Absolute test-retest variability in the bound/free ratio of the radioligand was 4.3% in the putamen and also <10% in the caudate nucleus and thalamus. PET data analysis results obtained with a compartmental model fit, the simplified reference tissue model, and a graphical reference tissue analysis method were convergent with the tissue ratio method. The results of this study supported the use of [11C]ORM-13070 in the quantitative assessment of α2C-ARs in the human brain in vivo. The suitability of [11C]ORM-13070 for measurement of amphetamine-evoked changes in extracellular noradrenaline levels was explored ex vivo in rat brain sections and in vivo with PET imaging in monkeys; rat striatal microdialysis experiments. Intravenous administration of amphetamine (0.5 and 1.0 mg/kg) reduced BPND values by 31-50 % in monkeys. Together, these results indicated that [11C]ORM-13070 may be a useful tool for evaluation of synaptic noradrenaline concentrations in vivo (Finnema et al. 2014, Lehto et al. 2015).

In year 4 and 5, we extended our work to non-monoaminergic targets, including the assessments of GABAA receptor benzodiazepine (BZD) site agonist tracers for potentially measuring endogenous GABA levels, and exploring whether the PDE10A PET radioligand [11C]Lu AE9268 could be used to measure changes in endogenous cyclic nucleotide levels.

In vitro receptor binding studies have demonstrated that 3H-labeled agonist radioligands for the BZD site are more sensitive to changes in GABA concentration than weak partial agonists, e.g. [11C]flumazenil, or partial inverse agonists, e.g. [11C]Ro 15-4513. For this reason, two agonist ligands for the BZD site, RO6899880 and RO6900648, were synthesized and characterized in vitro by Roche. Initial PET studies supported that both [11C]RO6899880 and [11C]RO6900648 were suitable for quantification of GABAA receptors in monkeys – however, challenge studies in non-human primates indicated no effect of tiagabine on [11C]RO6900648 binding.

Phosphodiesterase 10A (PDE10A) plays a key role in modulating central 3',5'-cyclic adenosine monophosphate (cAMP) levels, especially in the striatum and substantia nigra (SN). [11C]Lu AE92686 has high affinity for PDE10A and quantification of the radioligand had already been validated in humans. Based on a competition model, alterations in cAMP levels were predicted to induce changes in PDE10A radioligand binding. The quantification of [11C]Lu AE92686 was validated in monkeys and then extended to challenge studies with dopamine agents that are known to alter cAMP. Pretreatment with SCH23390 significantly decreased BPND in PUT (-21±2.0%, $p<0.05$) and CN (-23±1.5%, $p<0.05$). Administration of SCH23390 and apomorphine also decreased BPND in PUT (-13±5.4%, $p<0.05$), CN (-20±5.2%, $p<0.05$) and VS (-15±3.5%, $p<0.05$), but increased BPND in the SN (22±14%, $p<0.05$). Pretreatment with haloperidol with and without amphetamine induced only equivocal changes in BPND across regions. PET imaging with [11C]Lu AE92686 could thus detect dopaminergic modulation induced cAMP changes in the

monkey brain but the mechanisms for the unexpected direction of changes of [11C]Lu AE92686 binding in striatum and the specific role of dopamine D1 and D2 receptors in the regional differences of change in [11C]Lu AE92686 binding warrant further evaluation .

Status of Tasks in WP05:

Tasks	**Status (Please give status: completed, partially completed, deleted, modified, extended)**
Task 1: A molecular imaging approach sensitive to endogenous serotonin concentration	**Completed**
Task 2: A radioligand for the 2c-receptor sensitive to endogenous norepinephrine concentration	**Completed**
Task 3: A functional study linking occupancy measures to transmitter change (Lundbeck).	**Completed**
Task 4: Sensitivity of PET tracers to endogenous GABA levels (Month 54 – 66) (KI, Pfizer, Roche)	**Completed**

Workpackage 6: Image Analysis Methods purpose-made for drug discovery

The main objective of WP6 was to apply machine-learning based classification approaches to imaging for drug development by developing new methods, making them available in an easy to use toolbox and with some exemplar applications so that companies could use the methods for in-house proprietary drug development uses.

The main deliverable for the project was a machine-learning toolbox containing these methods in accessible form so that they could be used by EFPIA partners and by academic researchers. This toolbox called Pharmacological Imaging and Pattern Recognition Toolbox (PIPR) was produced and delivered, along with an explanatory users manual, at a workshop given in Kings College London in October 2013.

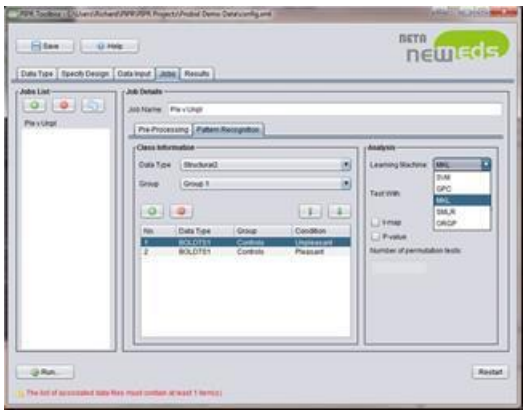

Figure 4: Screenshot of PIPR Toolbox

Toolbox and user manual are publicly available at:
http://www.kcl.ac.uk/ioppn/depts/neuroimaging/research/imaginganalysis/Software/PIPR.aspx

In its release form, the toolbox incorporated machine learning methods (**primarily Support Vector and Gaussian Process based methods** but with other techniques where appropriate) for two-class and multi-class analysis of various type of data. In response to the requirement for later deliverables (multimodal analysis) it has now been further adapted for this purpose.

With the collaboration of our EPFIA partners, the toolbox and the methods it contains have been applied to a variety of pharmacological data sets to address issues of industry interest including:

(1) Increased sensitivity of two-class decisions (e.g. drug-placebo differences or differences between drugs with ostensibly similar actions (atomoxetine/methylphenidate).

(2) Study of the kinetics of drug action in the brain with increased sensitivity.

(3) Multi-class analysis of drug effects (e.g. placebo/ketamine/risperidone vs. placebo/ketamine/lamotrigine) (published as Doyle et al. 2013)

(4) Ordinal regression studies of known ordered effects.

(5) Multimodal analysis (combining imaging, clinical and genetic data).

To further examine the utility of PIPR, in the collaboration with WP04, the **toolbox was applied to comparatively analyse multi-task fMRI data across 8 different drugs in healthy volunteers**. We used a multivariate pattern classification method implemented in the "PIPR"-toolbox to investigate the sensitivity of brain activation to pharmacological modulation in human fMRI data. Specifically, our dataset consisted of three tasks (emotional, motivational and cognitive) that have been applied in fMRI experiments on 8 drugs (ketamine, scopolamine, modafinil, lorazepam, amisulpride, risperidone, olanzapine, haloperidol) in 16-18 healthy volunteers. Following pre-processing and GLM analysis of data with SPM8, the beta values for each task and subjects were fed into a drug-placebo classifier, using Gaussian Process Classification (GPC), resulting in 8*3=24 classifications. This classification approach is now being further optimised.

Status of Tasks in WP06:

Tasks	Status (Please give status: completed, partially completed, deleted, modified, extended)
Task 1: Optimization of algorithms and analysis of datasets (KCL, Pfizer)	**Completed**
Task 2: Development and testing of Matlab Toolbox (KCL, Pfizer, Lilly)	**Completed**
Task 3: Expanding toolbox to additional imaging modalities and approaches	**Completed**
Task 4: Analysis of Pharmacological fMRI data (Month 54 – 66) (KCL, CIMH, Lilly, Roche)	**Completed**

Workpackage 7: Risk pathway via CNV Genetics-Schizophrenia

At the time when NEWMEDS was formed as a consortium one of the most promising genetic links to schizophrenia was published by DeCode. Copy Number Variations (CNVs) with odd-ratios as high as 40 were reported, making these de novo mutations some of the strongest genetic links reported in the field of psychiatry. The main objective of WP 7 was to take advantage of DeCode's access to the Icelandic population and their genetic data and the possibility to follow up with phenotyping profiling on specific carriers. It was clear that NEWMEDS had a unique opportunity to leverage on the large dataset that DeCode had access to and to gain **new insight into disease pathophysiology and possible therapeutic targets**. At the same time Lundbeck had generated the three mouse models (Df(h1q21)/+), (Df(h15q13)/+), and (Df(h22q11)/+) by hemizygous deletion of the orthologous regions and it was decided to share these with partners in the consortium, a unique opportunity to make a translational link between the animal models with construct validity and human carriers.

We examined a large number of subjects with specific genetic alterations (CNVs) linked to schizophrenia and related psychiatric disorders and compared them to relevant normal controls to gain **better understand the disease pathophysiology**. The focus of our human studies was on control subjects carrying CNVs and our studies had two main components, namely to study the effects of CNVs on cognitive traits of the subjects and to study effects of CNVs on brain structure and anthropometric traits. We carried out deep clinical and cognitive phenotyping and carried out fMRI to study brain regions and functions. In collaboration with WP6 we used brain imaging analysis and uncovered brain areas that are affected by CNVs associated with schizophrenia. We demonstrated how cognitive abnormalities and changes in the structure of the brain observed in schizophrenia are also found in control carriers of CNVs that confer high risk of the disease. We have reported that the corpus callosum enlarged in those carrying the 15q11.2 deletion. To our **knowledge this is the largest, systematic, study of healthy volunteers carrying autism/schizophrenia mutations**: 167 controls carrying neuropsychiatric CNVs; 465 controls carrying otherCNVs: 475 controls without large CNVs. In addition, 161 schizophrenia patients were recruited for the neuropsychological phenotyping (**Stefansson et al. Nature, 2014**). In a follow up study, including twice as many scanned subjects, the association with brain structure phenotypes is confirmed.

Animal models with face and construct validity for schizophrenia are scarce, making it difficult to explore the underlying pathology and to develop novel treatments. CNVs in the 1q21.1, 15q13.3 and the 22q11.2 regions constitute some of the strongest known genetic risk factors for schizophrenia. These CNVs are promising starting points for animal models of construct validity. Three mouse models (Df(h1q21)/+), (Df(h15q13)/+), and (Df(h22q11)/+) were characterized in an extensive behavioural test battery with focus on schizophrenia-relevant parameters followed by studies dedicated to clarify the underlying mechanisms and studies of translational relevance.

Although the three models are all based on genetic variants causing high risk of schizophrenia they showed very distinct phenotypes reflecting different aspects of schizophrenia. The 1q21 mice showed a specific presynaptic hyperdopaminergic phenotype revealed by increased neuronal activity and response to amphetamine and apomorphine. Further, the mice expressed increased impulsivity measured as increased premature responses in the 5-choice serial reaction time task. The 22q11 mice showed persistent and antipsychotic-resistant sensorimotor gating deficits and post-pubertal elevations of psychostimulant-induced hyperactivity. The mice also showed increased cortical loudness dependence auditory evoked potentials as well as increased prefrontal and striatal DOPAC levels and elevated striatal GluR1 expression. The phenotype of the 15q13 mice is primarily related to GABA interneuron dysfunction. They showed marked changes in neuronal excitability in acute seizure assays, with increased propensity to develop myoclonic and absence-like seizures, but decreased propensity for clonic and tonic seizures. EEG characterization revealed auditory processing deficits similar to those observed in schizophrenia. Gamma band power was increased during active state, but evoked gamma power following auditory stimulus was dramatically reduced. In addition, the 15q13 mice showed decreases in amplitudes of auditory evoked potentials. Furthermore, 15q13 mice have increased body weight, and a similar increase in bodyweight was subsequently found in human subjects with 15q13.3 deletion underscoring the translational relevance of these CNV mouse models. Although no definite target has evolved from this endeavour, important learning about the disease biology has been obtained and will be the foundation of future research.

Status of Tasks in WP07:

Tasks	Status (Please give status: completed, partially completed, deleted, modified, extended)
Task 1: Identifying and selecting subjects carrying recurrent CNVs under negative selection	**Completed**
Task 2: Phenotyping of individuals carrying recurrent CNVs under negative selection and associating with psychiatric disorders	**Completed**
Task 3: Expression Cohort, Data and Pathway analysis	**Completed**
Task 4: Brain imaging CNV cohort, impact of CNV genotype on brain function	**Completed**
Task 5: Genotyping and expression profiling of clinical trial samples	**Completed**
Task 6: Drug response in CNVs carriers	**Completed**

Workpakage 8: Identifying biomarkers of response and personalized medicine – a focus on depression

The objectives for this WP were set in 2008/2009 a time of great optimism that the candidate genes and GWAS methods would lead to pharmacogenetic predictors and stratification methods. Therefore, the main objectives of the WP were to collate the different pharmacological datasets, standardize the genetic and clinical results, and examine relevant candidate, GWAS (and later CNV and polygenic risk) predictors.

At the start of the project it was determined to be critical that we were looking not for just statistically reliable results, but, for findings that were clinically significant and relevant. A metric and measure had to be developed to determine this. Using the NICE guideline of clinical significance (3 points on the HAMD scale) we developed an algorithm and implemented it in a web-based tool which could be used to assess the clinical significance of biomarkers (Uher 2012). This, **first of its kind, "clinical significance calculator" tool is now publicly available** at: http://www.depressiontools.org/onlinecalculator.html

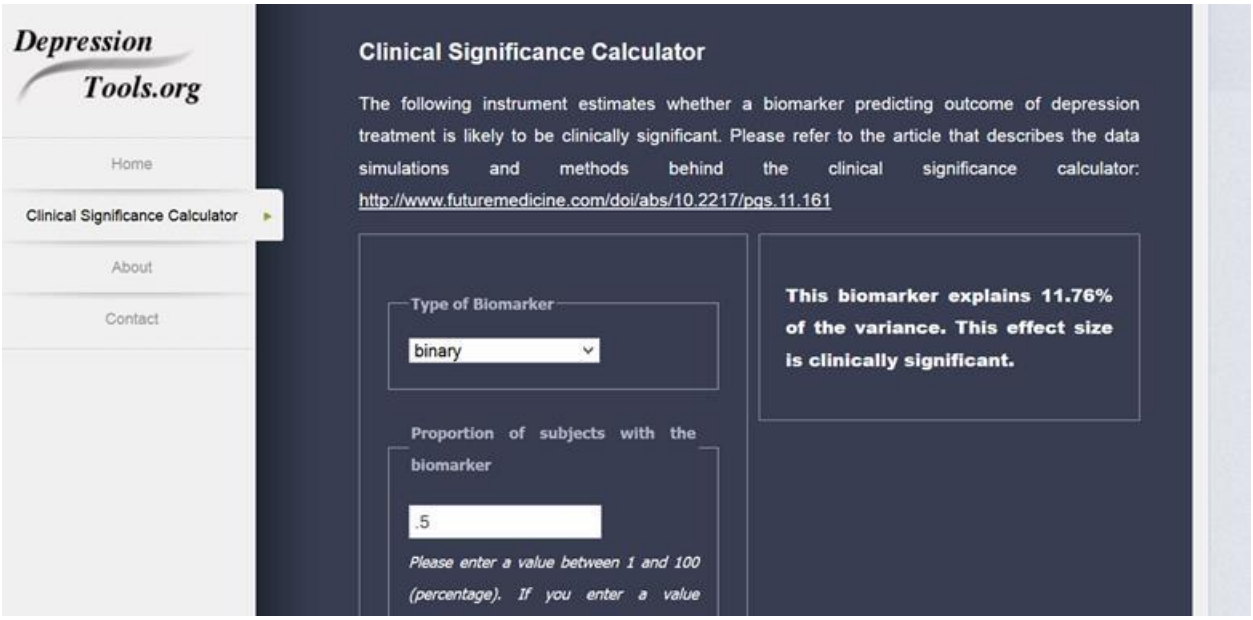

Figure 5: The Clinical Significance Calculator developed by NEWMEDS (http://www.depressiontools.org/)

With this concept of clinical significance as a guiding value we then examined a number of predictors across our datasets. The WP estimated the contribution of common genetic variation to antidepressant response with Genome-Wide Complex Trait Analysis in a combined sample of 2799 antidepressant-treated subjects with major depressive disorder and genome-wide genotype data and found that **common genetic variants could explain 42% (SE = .180, p = .009) of individual differences in antidepressant** response (Tansey 2013). This provides strong basis for looking for genetic predictors of individual antidepressant response – as even a much smaller prediction than the potential of 42% would be clinically significant.

To examine particular predictors using GWAS the WP assembled a database of over 2,000 European-ancestry individuals from five studies (three randomized controlled trials, one part-randomized controlled trial, and one treatment cohort study). **None of the more than half million genetic markers significantly predicted response to antidepressants** overall, serotonin reuptake inhibitors, or noradrenaline reuptake inhibitors, or differential response to the two types of antidepressants (genome-wide significance p<5×10(-8)). No biological pathways were significantly overrepresented in the results. No significant associations (genome-wide significance p<5×10(-8)) were detected in a meta-analysis of NEWMEDS and another large sample (STAR*D), with 2,897 individuals in total. Polygenic scoring found **no convergence among multiple associations in NEWMEDS and STAR*D** (Tansey 2012, Hunter 2013).

While failing to find robust genetic predictors we found very robust clinical and biochemical predictors of response. The interest-activity symptom dimension (reflecting low interest, reduced activity, indecisiveness and lack of enjoyment) at baseline strongly predicted poor treatment outcome in GENDEP (n=811 subjects), irrespective of overall depression severity, antidepressant type and outcome measure used. The prediction **of poor treatment outcome by the interest-activity dimension was robustly replicated** in STAR*D (3637), independent of a comprehensive list of baseline covariates (Uher 2012). The WP also tested the hypothesis that **C-reactive protein (CRP), a commonly available marker**

of systemic inflammation, predicts differential response to escitalopram (a serotonin reuptake inhibitor) and nortriptyline (a norepinephrine reuptake inhibitor) in the GENDEP database. CRP level at baseline differentially predicted treatment outcome with the two antidepressants (CRP-drug interaction: β=3.27, 95% CI=1.65, 4.89). For patients with low levels of CRP (<1 mg/L), improvement on the MADRS score was 3 points higher with escitalopram than with nortriptyline. For patients with higher CRP levels, improvement on the MADRS score was 3 points higher with nortriptyline than with escitalopram. CRP and its interaction with medication **explained more than 10% of individual-level variance in treatment outcome** – a finding that would be clinically significant as per our criteria set out above (Uher 2014).

Despite amassing one of the largest such databases with industry cooperation, and despite several careful analyses **the WP did not find simple predictors/stratifiers**. A significant portion of the prediction was drug-specific suggesting a potential for individualized indications for antidepressant drugs. On the other hand, easily obtained demographic, clinical variables and biomarkers predicted response to antidepressants with clinically meaningful effect size. This suggests a shift is needed towards more integrative methodologies which include clinical as well as demographic data in predictions – and pharmacogenetic based stratified medicine approaches in depression may not be as easy to operationalise.

Status of Tasks in WP08:

Tasks	**Status (Please give status: completed, partially completed, deleted, modified, extended)**
Task 1: Creating a database of antidepressant response with genetic data (low risk; prerequisite to all objectives)	**Completed**
Task 2: candidate gene analysis (low risk)	**Completed**
Task 3: genome-wide analysis (medium risk)	**Completed**

Workpackage 9 : Proteomic Biomarkers for schizophrenia and depression

This work was only funded for a period of 18 months. The objective of WP9 was to compare objectively predefined markers (protein levels in blood or brain) reported to signatures of first onset schizophrenia or depression, in the rodent models used in research. Whereas protein markers are widely used in other areas of drug discovery, psychiatry remains one of the areas where objectively measured markers have yet to be discovered. Modelling neuropsychiatric diseases in animals poses a significant challenge due to the subjective nature of diverse symptoms, and the rudimentary understanding of the pathophysiology. A validated proteomic biomarker panel could potentially provide a platform from which biologically drive hypotheses could be generated and tested. An approximate of 20 different animal models being used as models of schizophrenia or depression were available in the NEWMEDS consortium. When

NEWMEDS was initiated Sabine Bahn and her group had pioneered work in the field of psychiatry and had identified a panel of protein markers that was signature of first onset schizophrenia.

We initiated this work by quantitative western blotting analysis testing seven markers (synapsin II, syntaxin 1a, GAP43, NCAM, aquaporin 4, MARCKS) across frontal cortex tissue from 3 of the models (aPCP, cPCP, MAM). Results showed that there was no significant difference in expression levels observed, and it was decided not to further pursue this route, as the analytical accuracy of the Western blotting technique was insufficiently sensitive, the antibody differences between species were too large and the technique is time consuming and will not satisfy the high throughput criteria to test the 20 models available in the consortium.

We then generated serum proteome profiles using 20 pre-clinical models available in the consortia and compared these with clinical biomarker signatures of first onset schizophrenia (Ernst et al, 2012, Hradetsky et al 2012). Hierarchical cluster analysis showed similarity between the human serum molecular profile and that of 6 animal models. These models (cPCP (CCNR); aPCP (JNJ); MAM E-17 (Lilly), NR1 tg mice (AZ), social defeat and prenatal stress (AZ)) were selected for brain tissue (Frontal cortex and hippocampus) LC-MSE proteome profiling study. aPCP, cPCP and NR1 models seem to mimic schizophrenia peripheral and central molecular phenotypes. Social defeat proteome profile seems to reflect human MDD phenotype. Frontal cortex and hippocampus LC-MSE proteome profiling was conducted on these 6 models and data showed that the proteome profile of aPCP (1mg/kg), cPCP and NR1 TG mice are suitable candidate models.

So, the use of large-scale proteomics assays from animal brain samples to relate to pathophysiologies in psychiatric disorders did not pay off as expected and the methodology for such analyses proved inadequate to the task at least in this consortium. The use of serum markers was explored as a substitute approach, but did not lead to findings that are readily interpretable.

The data assembled in this WP was indeed substantial; however, the consortium decided to focus its work elsewhere and did not pursue this further.

Status of Tasks in WP09:

Tasks	**Status (Please give status: completed, partially completed, deleted, modified, extended)**
Task 1: Quantitative Western blot analysis of frontal cortex biomarker proteins in preclinical models and comparison	**Completed**
Task 2: Serum biomarker profiling of preclinical models.	**Completed**
Task 3: Biomarker and drug target discovery on preclinical models that best recapitulate clinical biomarker features of schizophrenia	**Completed**

Workpackage 10: Advanced data analysis techniques for more efficient trials, biomarker identification and placebo-response differentiation

The purpose of this work package was to examine new ways of analysing trial data to conduct more efficient trials – in particular to examine if trials could be of shorter duration, could use more efficient statistical approaches by using multiple convergent outcomes and if one could differentiate the different trajectories of response.

To address these questions the WP integrated, curated and anonymized databases of patient level efficacy, demographic and treatment data from clinical trials – thus **creating the largest patient level databases in depression and schizophrenia**. One of antipsychotic studies in schizophrenia, with data from Astra Zeneca, Janssen, Lilly, Lundbeck, Pfizer. This included 64 studies (34 placebo controlled studies, 30 active comparator studies) with 25,900 patients (16,105 study drug; 7,119 active comparator; 2676 placebo). The other database of patients from antidepressant studies. This included data from Astra Zeneca, Lundbeck, Pfizer and Lilly from 39 placebo controlled studies of 12,217 patients (8,260 active drug, 3,957 placebo).

Working with industry the WP established that for trials of **antipsychotic medications in schizophrenia: signal detection can be improved and sample size significantly reduced** using enriched populations by including more females and patients with prominent positive and negative symptoms and more early-episode patients (Rabinowitz 2014a). We also found that **trials could be made shorter** and give very similar results (Rabinowitz 2014b). For **antidepressant drugs used to treat depression** we found that signal detection of treatment effects can be enhanced by **increasing proportion of patients on placebo**, excluding centres with high placebo response and by **using both investigator and self-report measures**.

The WP collaborated with WP7 to examine polygenic score for schizophrenia and response to antipsychotic treatment as compared to placebo. We used genetic susceptibility risk scores from mega-analysis cohort of the Psychiatric Genomics Consortium and found preliminary evidence that higher polygenic scores may be associated with less symptom improvement in both placebo and active treatment – this result is being confirmed at present.

A major contribution of the WP was in leading the field by conducting **a consensus building workshop on negative symptom studies that included academics researchers, drug developers and regulators** (Rabinowitz 2012, 2013 and Marder 2013). In addition, our work package members were called upon to serve on the FDA advisory committee in the area of standardized data elements in antidepressant and antipsychotic trials and in committees of the EMA pertaining to the EMA initiative for transparency of data in clinical trials.

Over and above our stated objective of impacting the way trials are conducted in drug discovery for schizophrenia depression, we are having an impact on medicine at large. In the large databases we **discovered the possibility of patients enrolling in the same trial twice – at different sites. Duplicate enrollment harms both patients and studies**. In many therapeutic areas even a small number of duplicate patients can lead to a negative or failed trial. In addition, enrolling duplicate patients can result in a misattributed serious adverse event. This led to developing DupCheck an online tool for preventing duplicate enrollment of subjects across sponsors and therapeutic areas.

Figure 6: DUP Check web page

DupCheck is a web-based tool (https://www.dupcheck.org/) to screen for duplicate patients in clinical trials across studies, sponsors and therapeutic areas. DupCheck provides sites and sponsors with real time information on attempted duplicate enrollment at time of screening. DupCheck is now integrated with a large number of vendor eCRF and trial-management systems [see list here https://www.dupcheck.org/about.html]. **DupCheck is HIPAA and European Data Protection Regulation** compliant. No identifiable patient or sponsor data is transmitted, stored or shared. DupCheck was given a **favourable Qualification Advice review by the European Medicines Agency** and the FDA has stated that using DupCheck or equivalent strategies in trials is advisable.

To facilitate the continuation of this work [beyond the expiry of the NEWMEDS] the industry partners are altering data sharing agreements to allow the continued use of the data under the same terms even once NEWMEDS funding has ended.

Status of Tasks in WP10:

Tasks	Status (Please give status: completed, partially completed, deleted, modified, extended)
Task 1: Hiring personnel, setting up of data-sharing agreements and steering committee	**Completed**
Task 2: Extraction and checking of data at EFPIA sites, transfer and collation of data to IOP/BIU	**Completed**
Task 3: Simultaneous analysis lead by nominated academic, project statistician, with reporting to Steering Committee.	**Completed**
Task 4: Mood Disorders and New Analytic Strategies	**Completed**

1.6. Potential impact and main dissemination activities and exploitation of results

- Gained novel insights into the biology of schizophrenia and depression. In particular, developed better understanding of the thalamocortical and PFC-hippocampal circuits in animal models, and developed EEG based techniques that allow for animal-human translation studies.
- Standardised the application of cognitive models across several industrial partners – leading to a standardised application of touchscreen methodology by several partners.
- Developed translational tools/platforms for drug discovery [animal models, human imaging] – with particular emphasis on pharmaco fMRI, along with the provision of a reference database to which all future new drugs may be compared.
- Standardised a PET methodology for Serotonin release, and evaluated PET methodologies for non-amine transmitters such as GABA.
- Developed new web tool for analysis using machine learning methods [image analysis, biomarker significance], disseminated this via publications and workshop, and have made this tool (PIPR) available publicly. http://www.kcl.ac.uk/ioppn/depts/neuroimaging/research/imaginganalysis/Software/PIPR.aspx
- Have undertaken the largest study of the cognitive and brain effects of autism/schizophrenia related CNVs in healthy populations, have phenotyped three related animal models for precise bedside-bench correlations and have made the transgenic lines accessible to the scientific community.
- Rejected the hypothesis that single genetic mutations, polygenic scores or CNVs can be used to stratify depression. And in turn demonstrated the necessity and value of clinical and demographic predictors for inclusion in any stratification strategy.
- Provided a clinical significance calculator on the Web (http://www.depressiontools.org/) – which is publicly available and can be used to test new biomarkers in psychiatry.

- Provided information for the design of more efficient clinical trials – including suggestions for decreasing sample size and duration in schizophrenia and depression.
- Have highlighted the problems of patients signing up for the same clinical trial twice – and have devised a software solution for it (DupCheck available at https://www.dupcheck.org/) which is now being validated and adopted in different settings.

1.7. Lessons learned and further opportunities for research

NEWMEDS was one of the first projects under the IMI initiative and it brought together expert scientists in the field of psychiatry from industry as well as leading academic institutions around Europe. The field of psychiatry has been in a "drought season" following the successes in the last century introducing SSRIs in the treatment of depression and D2 antagonists and later the D2 partial agonists in the treatment of schizophrenia. The large investments made by the pharma industry, the pharmacogenomic era and thousands of papers being published every year has not yielded the advances one could have hoped for. The launch of the IMI initiative was timely in that respect. Most companies and academic institutions had good experiences in collaborating on a 1 to 1 basis with academia - a collaboration where the industry most often would provide the financial foundation of the collaboration and the academics would contribute with the scientific expertise that was needed to address a certain problem. None of the partners had experience with big public private partnerships, involving several industry partners at the same time. This was a new experience to all partners.

One of the main objectives of IMI was to "Support faster discovery and development of better medicines for patients by addressing pre-competitive bottlenecks in industry". It was thus clear from the very beginning that the foundation had to pre-competitive, rather than the discovery of proprietary compounds.

The EFPIA partners had met prior to launch of the call text to discuss what the consortium objectives should be in order for industry partners to be able to clearly commit resources and expertise to the call. It was decided to have two therapeutic areas of focus – both depression and schizophrenia. As the project evolved it focussed more on schizophrenia – partly to reflect the substantial success of CNV genetics in Schizohprenia rather than Depression. The academic consortium was brought together by the academic lead – each partner representing different expertise, and thus being able to respond to the call text made by EFPIA. From the efforts of the last five years we highlight the lessons we have learnt about academia-industry partnerships.

1) Leadership is critical.

This may seem like a cliché, but, in these large consortia projects – leadership plays an even greater role. Therefore, it is critical to:

a. Ensure joint and strong ownership of the overall project, as well as all the Workpackages, from industry and the academic institutions.

b. To deliver in a timely and accountable fashion, these projects need professional Project Management by a manager who is well versed in EU Grants and their reporting. Academic labs usually do not have this expertise. Industry does, but, there may be limits to how welcome PM from one industrial partner will be. Therefore, ideally a project manager who deals professionally with this and has no scientific or conflicting interest is a critical role.

c. We found that regular, transparent and frank interactions between the Project manager, Coordinator and Academic Lead were critical in delivering the partnership. Thus, special attention should be given to the "chemistry" of these leads as well they must have regular contact and scheduled meetings.

2) Strategic Focus

a. Given the nature of the PPP and the governance mechanisms, the "objectives" of the call need to fit each partner's strategy, so that partners are willing to share and contribute. The agreement also needs to be perceived as fair (financially) and motivate all partners to contribute.

b. Accommodating the diverse interests of different partners [even when focussed on a given question] can dilute the project. Thus, from a maximal impact perspective, the scientific questions need to be as focussed as possible.

c. Balancing the "depth" that science demands and "breadth" that partnerships demand is a constant tension that needs to be balanced. This needs to be particularly managed at two stages: a) it is critical that the call topics be as focussed as scientifically desirable; and b) once funded the leadership team constrain any mission expansion.

3) Communication

a. We found the following mechanisms useful in ensuring active and continuous communications: a) a biweekly teleconference of the Coordinator/Academic/Project Management lead "troika"; b) a quarterly written follow up on milestones and deliverables by all the WPs; c) six-monthly TC between the "troika" and the WP leads based on the quarterly updates; and d) yearly face to face meetings with the whole consortium. In addition, the individual workpackages met on a regular frequency to share data.

4) Resources

a. In-kind contributions from companies should be visible to all partners and publicly available to allow for transparency

b. In the first round of IMI the overheads were lower than that of other EUFP7 and other national programs, that led to substantial challenges for the academics participating in this call. The decision of IMI2 to provide overheads similar to Horizon 2020 is a progressive and welcome step.

5) Sustainability is a challenge

The large IMI projects take a tremendous investment of time, communication and human effort to build the relationships that sustain it. However, as it comes to an end – all of this goodwill and scientific contact becomes vulnerable. There are no easy solutions to ensuring continuity. However, a limited competitive program of funding that would allow elements of a consortium to apply for continuity may be helpful. If such a program is prospectively known – it would be even more effective.