European project on the characterisation of transgenic rat models for neurodegenerative and psychiatric diseases: Automated home cage analyses, live imaging and treatment.

PUBLISHABLE FINAL ACTIVITY REPORT

Objectives

RATstream™ is an ambitious European project that aims to characterize and use three transgenic rat models of neurological diseases which – in humans – present with a wide range of neurological and psychiatric phenotypes: (1) transgenic rat model of HD (von Hörsten et al. 2003, Hum Mol Genet 12:617-624), (2) transgenic rat model of PD overexpressing alpha-Synuclein, and (3) transgenic rat model of SCA17 with 64 expanded CAG repeats in the TATA binding protein. These transgenic rat models are worldwide unique.

The project aims to pursue a completely novel gene-to-function approach resulting from a comprehensive and standardized phenotyping classification which comprises four components: (i) classical phenotyping, (ii) monitoring of behavioural and physiological performance in fully automated physiological and behavioural home cage test systems, which are being developed during the project by two SMEs, (iii) non-invasive imaging technologies which have been adapted to small animals (iv) neuropathology and (v) microarray analysis. All data will be integrated into a specially designed data base which will allow retracting the most stable readouts and defining a minimal set of data which predicts therapeutic efficacy. Finally, proof-of-concept of this comprehensive high-quality characterization of the models will be achieved by first pre-clinical studies.

Two SME members of the consortium, TSE and NewBehavior, respectively, aim to develop automated home cage test systems for rat models, which do not exist yet but are imperative in view of the upcoming large number of transgenic rat models in both, industry and academia. Collaboration with the academic partners FAU and Uni Tuebingen will provide an optimal environment for development and refinement of home cages which are validated via correlation with data from classical read-outs and by cross comparison between two experienced academic partners. Cage systems will be suitable of continuously monitoring of spontaneous, social, cognitive, emotional and physiological measures (drinking, feeding, metabolic performance/calorimetry, telemetry for temperature and biopotentials) in home-cage-like environments for rats.

The phenotyping set up (i-v) will be used to develop a minimized and essential set of biomarkers in order to reliably monitor disease progression in the transgenic rat models of PD, HD, and SCA17, respectively. Phenotype data are also correlated to neuropathological features as protein aggregates, neuronal cell loss, and neurotransmitter alteration at different disease stages. The phenotyping approach will be used to characterize for each disease model a minimized set of markers suited best as read-outs in pre-clinical studies applying novel compounds to delay or prevent neurodegeneration.

The objective of this project will be to provide the proof-of-principle that it is possible in the rat

- to develop, validate, and use standardized automated home cage systems and to adapt in vivo imaging techniques for phenotyping neurological and cognitive function,
- to harvest large data sets on gene functions and corresponding phenotypes thereof,
- to determine and validate a minimised set of predictive parameters and experiments as well as of appropriate time slots for each rat model (low cost approach), and
- to develop and apply specific quality standards applicable for phenotyping tools and models (over the entire project duration).

Furthermore, these rat models will be used to

- to scrutinise novel experimental, pre-clinical treatments chiefly with regard to effectiveness, applicability, and transferability.
Partners:
- Eberhard-Karls-Universität Tübingen, Germany, Prof. Olaf Riess
- Friedrich-Alexander-Universität Erlangen, Germany, Prof. Stephan von Hoersten
- NewBehavior AG, Switzerland, Prof. Hans-Peter Lipp
- TSE Systems GmbH, Germany, Dr. Silvia Brenda
- Commissariat à l'Energie Atomique, France, Prof. Bertrand Tavitian
- Trophos S.A., France, Dr. Rebecca Pruss
- CrossLinks B.V., Netherlands, Dr. Ronald Naninga
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Major achievements of RATstream
- Development, set-up, validation, use and first attempts for commercialization (by SMEs) of new automated home cage systems
- Large scale phenotyping of HD, SCA17 and PD rat models
- Minimised set of parameters and experiments for SCA17 and HD model
- Development of RATstream™ Server for data collection and of RATstream™ PDM, a rat data analyser which uses statistical methods for correlation analyses
- Performance of pilot efficacy studies for Trophos compounds RS1 and RS2
- Performance of preclinical trials in SCA17 and HD rat models

Illustration of the work done
See below in "publishable results of RATstream" chapter

RATstream website www.ratstream.eu
### Objectives, starting points and results achieved

<table>
<thead>
<tr>
<th>Objectives</th>
<th>Starting point</th>
<th>Achievements</th>
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<tbody>
<tr>
<td>To develop, validate, and use standardized automated home cage systems</td>
<td>No such automated home cage systems suitable for rats</td>
<td>New systems for Phenomaster and IntelliCage developed and set up; validation of PhenoMaster system done, Industrial version of IntelliCage, new software tools developed and available; first across-lab comparison, first cage systems marketed and sold</td>
</tr>
<tr>
<td>To harvest large data sets on gene functions and corresponding phenotypes thereof</td>
<td>Data from classical phenotyping available for HD model</td>
<td>Comprehensive phenotyping data for HD, SCA17, and PD model available, longitudinal studies for HD and SCA17 rats applying automated and classical behavioural phenotyping, PET and DT imaging, gene expression profiling and neuropathology done; Phenotyping for novel PD model far advanced; Novel MRI findings</td>
</tr>
<tr>
<td>To determine and validate a minimised set of predictive parameters and experiments as well as of appropriate time slots for each rat model</td>
<td>No such minimised set of predictive parameters and experiments</td>
<td>Minimised set of parameters and experiments has been derived from large scale phenotyping for SCA17 and HD model, Similar minimised set for PD model will be available after completion of the phenotyping effort</td>
</tr>
<tr>
<td>To develop and apply specific quality standards applicable for phenotyping tools and models</td>
<td>No such quality standards</td>
<td>Development of quality standards is still ongoing in particular regarding automated physiological and behaviour testing in home cage environment</td>
</tr>
<tr>
<td>To scrutinise novel experimental, pre-clinical treatments</td>
<td>TRORS1 and TRORS2 in preclinical development, however, not tested in diseased rat models</td>
<td>Pilot efficacy studies using gene expression profiling as read-out for TRORS2 in HD rats and TRORS1 in HD rat done, Long term treatment study of TRORS2 in HD rats done, trial with an antipsychotic drug (clozapine) in SCA17 rats using the results from classical phenotyping that revelaed a schizophrenic-like phenotype</td>
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Publishable results of RATstream

Work package 1: Classical versus novel, automated, and integrated behavioural and physiological phenotyping: Approach, validation, time line, throughput, and reliability

Main partners: Uni Tuebingen, FAU, TSE, New Behavior

Major achievements:

(A) Development of PhenoMaster Systems for rats with various measuring modules, that can be configured according to the necessary test design.

- Measuring modules:
  - Calorimetry (indirect gas calorimetry) – O₂, CO₂, cage temperature, RER (Respiratory Exchange Rate) and further derived calorimetric parameters
  - Telemetry technique based on transponder principle – temperature, ECG, blood pressure
  - Activity (X,Y,Z) measurements via light-beam frames – spontaneous home cage activity (locomotion, ambulatory and fine movements, rearing, active and inactive phases)
  - Drinking and feeding sensors for the measurement of the water and food consumption - amounts, patterns
  - Running wheel – passive (enabled or disabled, number of turns and distance, pattern), active wheel
  - Operant wall consisting of single modules – free configurability of operant functional units
  - PhenoMaster software

- Project-accompanying works
  - Cage racks and control cabinets for space-saving setup of the cage systems
  - Definition of room requirements for the setup of the cage systems
  - Development of a cage concept for weighing urine and feces
  - Animal weighing sensor instead of a liquid weighing sensor

- Relevant deliverables:
  1. Physiological cage
  2. Behavioral cage
  3. PhenoMaster software

Illustrations:
1. Physiological cage setup

Physiological cage setup: cages in racks (A) and control instruments in control cabinets (B), cage cover suspension with a hook system (C)
The physiological cage was equipped with the following modules:

- Indirect calorimetry
- Activity measurement using light-beam frames
- Weighing sensors for registering liquid and food consumption
- Temperature sensor for measuring the temperature in the cage
- Passive running wheel for registering spontaneous wheel activity

2. Behavioral cage - details

**Operant wall design**

1 Home cage type IV
2 Calorimetry cage cover
   2a Connections for air tubing
   2b Suspension hook for cover
3 Running wheel
   in adaptation of cage cover
4 Drilling and feeding sensors
5 Containers
   5a Drinking bottle
   5b Food container
6 Temperature sensor
7 Activity frame
   7a X,Y level
   7b Z (rearing) level

See below
6 Frames to insert the single operant functional units
7 Cable and plug system
3. PhenoMaster software - interface

User interface of the operant wall module (simulation mode)
(B) Development of a home cage system permitting automated assessment of spontaneous behavior and cognitive abilities of socially housed rats. This included development of hard- and software.

- The arrangement chosen consisted of 4 interconnected home cages with conditioning boxes accessible through transponder-reading tubes.

- The software delivered included 3 parts:

(i) A **designer module** permitting to set up the control programs using a graphic interface

![Diagram of designer module](image)

(ii) a **controller module** running the experiments and providing on-line monitoring of the behavioral scores shown by individual rats (or, selectable), monitoring of group means:

![Graph of controller module output](image)

(iii) an **analyzer module** allowing to replay the stored data and corresponding graphs. From these graphs, the user can select a time window of interest, extract the data into a spreadsheet that can be imported in any software package
Illustrations:

C: Validation of the PhenoMaster system

- Validation of technological aspects with regard to preciseness
  a. Drinking and Feeding: The system is measuring the correct amount of consumed food pellets and tap water.
  
  b. Activity: Correlation analysis revealed excellent correlations between these technically different detection systems. Activity parameters derived from the two devices and corresponding to vertical distance and rearing resulted in significant correlations. Overall, these experiments confirmed the validity and reliability of x, y, and z based measures in the PhenoMaster.
  
  c. Temperature: Results showed that the cage temperature measures revealed differences between transgenic and wild-type rats but not the body core temperature in the same way, suggesting that additional factors contribute to the differences observed by measuring indirect cage-“Temp”. Regression analysis provides evidence for this differences as the slope of the corresponding regression-line per wild-type and tgHD rats was different.
  
  d. Calorimetry: As here construct validities were considered, different animals were included into analysis. For the calorimetric parameters RER, VO₂ and VCO₂ regression analysis showed a good correlation between the measurements with these systems.

- Characterization of underlying constructs and their validity
  a. Social Interaction test of anxiety: Obviously, within the automated system no measurements of social behaviors were possible as the rats are housed singly. However, screening for anxiety-related parameters, which could be correlated with the social interaction time as a measure of anxiety, revealed that the total activity in central area of the cage (CenT) significantly correlated with SI-time, suggesting that CenT may represents a marker with construct validity for anxiety-related parameters, similar to those detected by the social interaction test of anxiety.
  
  b. Accelerod test: When testing animals in the PhenoMaster, regression analysis revealed that the rearing parameter Z in the PhenoMaster may represent a surrogate marker for the accelerod performance of the rats on the accelerod. For the parameter Z, data from the first 24hours within the PhenoMaster and monthly accelerod-performance were subjected to regression analysis. Results showed a significant correlation between these different motor parameters.
c. Operant learning behavior (OBS): To validate the operant behavior system in the PhenoMaster with regard to construct validity a comparison with operant learning behavior in the animals in the IntelliCage for rats was used. General feasibility and construct validity were confirmed by comparing side discrimination tasks within the IntelliCage for rats with operant tasks in the PhenoMaster using external control animals and revealing good concordance of genotype-specific differences. The operant tests (progressive ratio schedule) in the automated system detected a phenotype in the transgenic HD animals already at the age of 6 months.

- Empirical description of the sensitivity of the system -Comparison of onsets of a priori non-related spontaneous or triggered behavioral responses
  Overall, the first significant differences between wild-type and tgHD rats became apparent after 1 month using the SI test. However, the PhenoMaster system, which only measures spontaneous, ethologically triggered behaviors, does not directly detect emotional parameters. Trying to draw conclusions on the sensitivity of the system thus suffers from the shortcoming that directly comparable apparatus, context, and stimulus would be necessary. Thus, we here remain on empirical description of the sensitivity of the automated system in detecting an otherwise non-overt phenotype. Following this line of reasoning, we conclude that the system (and the approach) provide a very high sensitivity as the second most sensitive parameter, previously being undetected in the tgHD rat model, was rearing behavior in the familiar environment of the home-cage. In addition, complex statistics provide the chance of defining novel multi-dimensional loadings, which sufficiently might be established as most sensitive read-outs in the future.

- Reliability across different sets of animals
  The reliability of the system was proved by comparing experimental results from two different sets of animals screened in the PhenoMaster at several time points. A second set of animals was tested with the same experimental setup at the age of 4, 7, and 10 months. Results showed a good sensitivity in detecting the same scores across two sets of animals also indicating higher activity in the dark cycle. Corresponding regression analysis revealed for the parameter rearing and for parameter locomotor activity significant correlations.

**Distribution of knowledge**

- PhenoMaster website to inform public about PhenoMaster objectives in the course of the RATstream project.

- NewBehavior presented the IntelliCage for Rat system at various meetings:
  - The Society for Neuroscience Meeting 2008 San Diego
  - The 41st European Brain and Behaviour Society Meeting (EEBS).
  - The Society for Neuroscience Meeting Chicago 2009

**Commercialisation**

- TSE was able to sell 13 cage systems for rats already towards the end of RATstream
- NewBehavior was able to sell, during the reporting period, 2 Rat IntelliCage systems to customers in Germany and Switzerland.
- In 2010, NewBehavior concluded with partner TSE Systems a distribution agreement demonstrating the usefulness of FP6 projects in strengthening European SME industry.
**Publications**
- In preparation:

**Work package 2: Comprehensive characterization of novel rat models**

**Main partners: Uni Tuebingen, FAU, CEA Orsay, Uni Antwerp**

**Major achievements**

A) HD rat model

**FAU:**
- Multiple genotype differences in various behavioral domains were detected by automated as well as classical phenotyping. In automated phenotyping, various specific motor activities were found consistently higher in tgHD animals. Spontaneous free rearing is correlating with individual performance in the accelerod test and indicates therefore the progression of motor function and balance in these rats.

### Minimal essential readouts tgHD

<table>
<thead>
<tr>
<th>Classical tests</th>
<th>Changes</th>
<th>Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotarod</td>
<td>tgHD better performance</td>
<td>Months 3-5</td>
</tr>
<tr>
<td></td>
<td>Decreased motor function</td>
<td>Month 7</td>
</tr>
<tr>
<td>Social Interaction</td>
<td>Reduced anxiety</td>
<td>Month 1</td>
</tr>
</tbody>
</table>

**Automated phenotyping (PhenoMaster)**

<table>
<thead>
<tr>
<th>RER (respiratory exchange rate)</th>
<th>decreased</th>
<th>Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cage temperature</td>
<td>decreased increased</td>
<td>Months 3-6, 11-12 Months 7-10</td>
</tr>
<tr>
<td>Activity: total activity (XT+YT) rearing behavior (Z)</td>
<td>increased increased</td>
<td>Month 6 Months 2-6 Months 7-12</td>
</tr>
<tr>
<td>Heat production</td>
<td>increased</td>
<td>Month 2</td>
</tr>
</tbody>
</table>


Uni Tuebingen:

- In collaboration with FAU, several sets of transgenic HD rats were bred, genotyped and distributed to our partners within the RATstream consortium. With the Phenomaster data obtained in Erlangen, it was proposed that as part of a minimal essential read-out for a treatment study with transgenic HD rats, Phenomaster experiments should be performed at the age of 5 and 8 months. To assess the reproducibility of results across labs we therefore conducted these experiments in Tuebingen and re-analysed raw data from both labs. However, we found considerable differences between the two labs, which could be explained by differences in lab conditions such as settings of cages, dark-light cycles, humidity, light intensity etc. as well as by differences in experimental procedures such as adaptation or placement of animals into cages. For example, in Tuebingen animals were monitored over a period of 70 hours, the dark cycle started at 3 p.m. and the animals were pseudorandomized when put into the cages. The measurements in Erlangen took 72h, their dark cycle started at 6 p.m. and pseudorandomisation into the different cages was not applied, i.e. in one run only transgenic animals or only wildtype animals were measured. While we found some differences between transgenic HD rats and controls in the Erlangen experiments such as increased rearing in HD rats at 5 months of age, these differences were not found in the Tuebingen group. Also values for the different parameters differed between the two labs. For example counts for total ambulation in the dark period were significantly higher in Tuebingen than in Erlangen. We are currently analysing possible causes for these discrepancies. Furthermore, we also tried to establish the Intellicage for rats in our lab. However, due to some technical problems and problems arising from social ranking within the Intellicage system, reasonable data from these experiments were not obtained.

- Moreover, to monitor gene expression profile changes during disease progression we examined HD transgenic rats at 3 months of age and 12 months of age. Already at 3 months when motor symptoms and cognitive deficits were not detectable we found many transcripts differentially regulated in transgenic animals compared to control rats. We could also show that with disease progression there were more widespread changes in gene expression, since at 12 months of age, almost the double amount of transcript were dysregulated as compared to 3 months of age. When looking at the expression level of genes that have been shown to be involved in HD (canonical pathway “Huntington’s Disease signaling”), we also observed the double amount of dysregulated genes in this canonical pathway in old transgenic rats compared to young HD rats. This also indicates that the disease progression correlates well with the gene expression profile in this animal model. Although a previous study showed that transcriptomic blood biomarkers are robust in tracking HD progression (Borovecki et al., PNAS 2005), subsequent studies showed no differential expression between manifest HD and control samples in any of 12 previously reported marker RNAs (Runne et al., PNAS 2007). In our lab we also could not find any statistically significant differences in HD patients samples compared to controls so that we concluded that mRNA readouts from peripheral blood are not reliable indicators of HD progression at the current stage.

- Furthermore, we have started several collaborations to identify more potential biomarkers in HD transgenic rats. For example H NMR-based metabolomics approach has revealed a disease specific profile in blood serum of transgenic HD rats (collaboration with group in Antwerp, submitted manuscript). Additionally, in collaboration with Elena Cattaneo’s lab, we have looked for cholesterol biosynthesis deficits in our rat model. At 3 months of age, we did not detect any significant difference in the levels of cholesterol precursors and cholesterol itself between wt and HD rats However, we found that lathosterol levels are reduced in the whole brain of
HD rats with respect to wt rats at 21 months of age. Other precursors of cholesterol are also reduced in HD rats with respect to wt rats (J Neurosci, in press).

B) SCA17 model

Uni Tübingen

- We established a transgenic SCA17 rat model with 64 CAG repeats under the control of the rat huntingtin promoter and bred these animals to heterozygosity at first. However, since the expression level of the mutant protein was not very high in this animal model, we decided to breed transgenic SCA17 rats to homozygosity to achieve a higher level of mutant TBP expression. We expected to induce a stronger phenotype with this procedure.

- The heterozygous transgenic rats were tested in Skinner boxes at the age of 13 months. Here they showed learning deficits as they had significantly less correct responses during week 4 to 9 of testing. However, in the end they reached the same level of correct responses as the wildtype littermates. Moreover, using electron microscopy we found shrunked Purkinje cells and lipofuscin inclusions in ageing heterozygous SCA17 rats. The controls also displayed these lipofuscin inclusions, but to a smaller degree. The results of the microarray analysis did not show significantly dysregulated genes when strict criteria were used for analysis. Under less stringent criteria 230 transcripts were found to be dysregulated. An interesting pathway that appeared to be affected was the FOS pathway.

- For homozygous animals we established a reliable assay for genotyping via qRT-PCR. We stained paraffin- and cryosections of the brain with the DAB method and did not find neuropathological abnormalities in the paraffin sections of 5, 7 and 10 months old animals by using the antibodies 1C2 and 1TBPA18. Cryo sections of a 10 months old rat with the TUJ-1 antibody revealed a reduced number of basket cells and stellate neurons, as well as Purkinje cells which also exhibit corkscrew-like dendrites. But no aggregates could be found. Further we performed AGERA and filter trap experiments, which are two other methods to detect aggregates. Neither with the specific TBP antibody nor with the poly-Q antibody 1C2 aggregated mutant TBP could be shown. Additionally, a set of 12 wt vs 12 homozygous males was tested monthly in the PM system starting at the age of 1 month until the age of 12 months. The results were compared to those from Erlangen.

FAU:

- Initial phenotyping of tgSCA17 revealed a complex phenotype with an onset at 6 months and being composed of disturbances in anxiety-related behaviors, higher activity in the open field test, cognitive decline in associative memory, loss of prepulse inhibition, reduced rearing behavior and changes in energy metabolism with reduced uptake of O2 as well as production of CO2 in the tgSCA17. At the molecular level, striatal transcripts for DARPP-32 were down- and dopamine receptor D1A was upregulated while cortical neuregulin (NRG1) appeared down- and its receptor ErbB4 upregulated. Immunohistological analysis revealed a 12-fold increase in immunopositive cells in the striatum, nucleus accumbens and cerebellar purkinje cells of homozygous animals and a 4-fold in heterozygous, including classical features such as aggregates and dismorphic purkinje cells. Finally, as most of these differences provided potential relevance to signs, symptoms and molecular markers of schizophrenia, experimental clozapine treatment proved to specifically modulate rearing behavior and reversed the loss of prepulse inhibition.
### Minimal essential readouts SCA17 model (Huntingtin promoter)

<table>
<thead>
<tr>
<th>Classical tests</th>
<th>Changes</th>
<th>Onset</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prepulse Inhibition (PPI)</td>
<td>consistent loss of prepulse inhibition</td>
<td>Month 6</td>
</tr>
<tr>
<td>Social Interaction (SI)</td>
<td>disturbances in anxiety related behavior</td>
<td>Month 6</td>
</tr>
<tr>
<td>Open Field Test (OFT)</td>
<td>increased activity, track length, ambulations, amounts of visits</td>
<td>Month 3</td>
</tr>
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#### Automated phenotyping (PhenoMaster)

| RER (respiratory exchange rate)     | increased                                                               | Month 6        |
| Cage temperature                    | increased, decreased                                                    | Months 1-6 Months 7-12 |
| Activity (total activity, rearing behavior) | decreased                                                               | Month 3 (6)    |

#### Automated phenotyping (IntelliCage)

| Corner visits                      | increased                                                               |                |
| Lick numbers                       | decreased                                                               | only month 5-6 tested |
| Place errors                       | increased                                                               |                |

### Uni Tübingen: new SCA 17 model with stronger phenotype

- As our SCA17 models with the rat huntingtin promoter do not exhibit the strong phenotype that is required for a treatment study with a reasonable number of animals, we generated another SCA17 model where the human TBP cDNA is under the control of the human prion promoter. This construct was expected to lead to a stronger expression of the transgene in the rat brain.
- After injection 10 positive founder animals were received and five of them transmitted the transgene to their offspring. The expression of the mutant TBP is constant in the F1 generation and the length of the CAG repeats is also stable. These five lines were tested for protein expression in different brain regions. For DAB stainings the antibodies 1C2 and 1TBP18 were used on paraffin sections. All lines expressed the mutant TBP protein in various brain regions but the expression levels varied.
- Accelerated RotaRod experiments with heterozygous animals at the age of 5 and 8 months did not show significant differences but the rats exhibit strong ataxia related symptoms such as gait abnormalities, impairment of postural reflexes especially
affecting the hind limbs and kyphosis. Further they are poorly groomed and show a severe weight loss beginning at six months of age. Footprint analysis revealed that tg animals have a reduced stride length and decreased overlap of hind and front paws. In the beam walking tests, transgenic Prp-TBP64Q showed significant difficulties in traversing as measured by their latency to traverse each beam compared to control rats.

- Further experiments to characterize this new SCA17 rat model are ongoing.

C) PD model (Uni Tübingen)

- Lines with expression of BAC-WT-SNCA (construct created by R. Takahashi): Injection of the restricted construct was done. Genotyping revealed 7 potential founders. The highest expressing line (BAC-2) was bred to homozygosity; as homozygous SNCA transgenic rats may develop a faster phenotype due to gene dosage effects.

- Analysis of transgene expression in DA-neurons of the nigro-striatal system of BAC-2 rats: Conventional immunohistochemistry and double-IF staining revealed expression in dopaminergic neurons, which was predominantly detected in the neuropil of the caudate-putamen (CPu) and the glomerular layer of the olfactory bulb (OB) (Figure 1). Additionally cellular staining was detected in the somatodendritic part of the substantia nigra pars compacta (SN). Double-IF staining of this region showed additional colocalization of human SNCA in Tyrosine-Hydroxylase (TH) positive dopaminergic cells.

![Figure 1. Expression pattern of Human SNCA in DA brain regions](image)

A punctuate expression of human SNCA and Tyrosine-Hydroxylase (TH) was detected in the striatum (CPu), whereas additional somatodendritic staining was also prominent in the glomerular layer of the olfactory bulb (OB). Double-IF revealed strong transgenic immunoreaction (SNCA) in dopaminergic (TH) cells of the substantia nigra (merged), which may be a precondition for development of Lewy-body-like inclusions.
We conducted accRotarod to analyze motor performance every week: in this test, transgenic animals did present a higher activity in comparison with controls.

![Graph showing motor performance of transgenic and control rats over time.](image)

**Figure 1.** Accelerated Rotarod Testing in Hetero-/Homozygote aSyn-tg rats
Both, homozygote male and female rats and heterozygote male did show significant better performance (p<0.05, 2-way ANOVA, GraphPad) than control rats. Heterozygote female rats present a lower performance than respective controls (p<0.05, 2-way ANOVA, GraphPad).

We prepared and stained paraffin sections of a 7-month old homozygote rat of line2 to make a first screening for aggregate load:

We detected stronger glia immunoreactivity in cerebellar region of transgenic rats, which might be first sign for cellular degeneration. Stainings with Iba-1 to detect activated microglia and confirm neuroinflammation, will be performed in 12-months-old homozygote rats.

We conducted a TMT-smell test to analyze alterations in PD rats more relevant to pre-motor stages. Single tTest and 2-way ANOVA (post-hoc Bonferroni) did not show significant differences but showed functional olfactorial procession in about 11-months-old homozygote rats. Test will be repeated at later time point and with other odors.

**Figure 2.** TMT smell test in aSyn transgenic rats
11-month old homozygote rats were analyzed for their smell ability and anxiety in a fear-related smell test by usage of TMT, a fox-like odor. Transgenic rats did show a increased activity and stronger avoided the TMT corner, however results were not significant in comparison to behaviour of respective controls.
- We conducted behaviour analysis in automated home cages (Phenomaster), measurement of 3 days; DP= dark phase, LP= light phase; 2-way-ANOVA over all time points; post-hoc Bonferroni and single student’s t tests still need to be performed; p value < 0.05 was considered significant) of heterozygote (n=6/6 male + n= 6/6 female) and homozygote rats (male + female = 6/6 each

- We performed WaterMaze Analyses: Result: Significant weaker performance in cued version (p=0.03, two-way ANOVA) and in spatial learning (p= 0.04, two-way ANOVA) of homozygote female rats

- We performed Ultrasonic Imaging, as Imaging via PET was not performed, due to lack of a motor phenotype in 8-12-months old hetero- and homozygote rats

- We prepared rats for analyses of neurotransmitter content and brain regions (olfactory bulb, striatum, nigra, hippocampus; homozygote: n=3, heterozygote n=3, controls n=3) have been sent to Dr. Teismann (april 2010); analyzes still needs to be done

- 4. We perfused rats and sent brains and eyes (n=2 transgenic, 12-months-old homozygous, respective control n=1) for ultrastructural analyzes (EM; cooperation partner: Dr. Elisabeth Petrasch-Parwez) → First screening of semithin section did substantiate light microscopical finding of immunoreactivity in soma, nucleus and neuropil, e.g. granule cells of dentate gyrus

D) PET imaging

- In a preliminary study, different tracers were evaluated for their use in the phenotyping µPET studies of the transgenic animal models of RATstream. This was done by evaluating not only their specific binding in the region of interest (ROI) but also by taking into account the optimal imaging protocol. We aimed at using: (1) clinically validated ¹⁸F -tracers because of their relative long half life; and (2) short
imaging times to reduce the anaesthesia duration and to increase experimental efficacy. Following PET-tracers were evaluated in control animals: $^{18}$F-FDG for the glucose metabolism, $^{18}$F-LBT999 for the postsynaptic dopamine transporter (DAT) binding ($n=6$), $^{18}$F-F-DOPA for the postsynaptic dopamine metabolism ($n=3$), $[11]$C-Raclopride ($n=1$) and $[18]$F-Fallypride ($n=6$) for the postsynaptic D2 receptor, and $^{18}$F-A85380 for the nicotinic α2β4-receptor ($n=4$). Of these tracers $^{18}$F-F-DOPA and $^{11}$C-Raclopride will not be further used in the phenotyping studies, because of low specific binding of the first tracer and stressful pre-treatment (carbidopa 10mg/kg) required, and because higher specific binding together with longer half life of the $^{18}$F-compound (fallypride) as compared with the $^{11}$C-compound (raclopride) in case of the D2 receptors tracer.

**Transgenic rat model for Huntington disease:**

- Transgenic homozygous HD ($n_{Tg}=12$, $n_{WT}=11$) and wild type animals were subjected to $^{18}$F-FDG and $^{18}$F-Fallypride imaging at 5, 10 and 15 months to measure respectively glucose metabolism and D2 receptor binding. For all tracers a similar imaging protocol was developed: after injection of the tracers under light isoflurane anesthesia (2.5%), animals returned to their cages allowing accumulation of the tracer during one hour. Images of 30 min were taken on a FOCUS 220 camera under isoflurane anesthesia which was induced five minutes before scanning. High resolution MRI data were exchanged with P8 in order to perform a more accurate segmentation of the PET data. $^{18}$F-FDG images and $^{18}$F-Fallypride were respectively automatically and manually coregistered to the individual age-matched MRI images of each animal.

- **ROI analyses:** Segmentation of the striatum and the cerebellum was performed manually on the high resolution MRI images. The measured uptake of the radiotracer was normalized for the injected dose and, in addition, the uptake of $^{18}$F-Fallypride in the striatum was calculated relative to the uptake in the cerebellum. Data were analysed by a 2x3 repeated measures ANOVA. $^{18}$F-FDG and $^{18}$F-Fallypride PET data showed similar time effects for transgenic and wild type animals, but no significant differences could be demonstrated between the phenotypes for any time point.

- **SPM voxel based analysis of MRI-PET data:** A procedure has been set up to analyse the $^{18}$F-Fallypride and $^{18}$F-FDG data using statistical parametric mapping (SPM). Different coregistration approaches have been developed and optimized; and data have been processed per time point, as well as repeated measures to study the effect of time, the interactions between aging and the expressed phenotype. Differences that found between transgenic and control animals (for both $^{18}$F-FDG and $^{18}$F-fallypride) were obtained at very low significance levels and were located at the level of the hippocampus between the two lateral ventricles. A detailed volumetric analysis revealed larger ventricles in the control group as compared to transgenic animals, underlying these partial volume differences.

- An additional autoradiography study has been performed on a small sample of rat brains (3 controls, 3 transgenics) to study D2-receptor binding at a higher resolution. The results of this study were in the line of the longitudinal PET imaging study and did not reveal any significant difference between control and Tg animals.

- **Additional batch of HD animals:** To exclude possible influence of stress induced in the animals of the longitudinal study following the repeated travelling and/or induction of anaesthesia, an additional batch of animals has been measured by $^{18}$F-Fallypride and $^{18}$F-FDG μPET imaging at 15 month of age and MRI.

- The additional batch has been processed in a similar way as the longitudinal study, using SPM software and similar statistics as described before. No differences were shown between transgenic and control animals for any tracer, suggesting that the observations from the longitudinal study are indeed the result from anatomical
differences and not differences in uptake or binding. A detailed volumetric study of the lateral ventricles will follow as well on the additional batch.

**Transgenic rat model for SCA17**

- Glucose metabolism of transgenic heterozygous SCA17 and wild type animals was studied using [18F]-FDG at 8 months \((n_{Tg}=8, n_{WT}=8)\) and subsequently 12 months \((n_{Tg}=6, n_{WT}=6)\). At 15 months of age binding of the nicotinergic \(\alpha_2\beta_4\)-receptor using [18F]-A85380 was evaluated \((n_{Tg}=4, n_{WT}=4)\). For all tracers a similar imaging protocol was developed: after injection of the tracers under light isoflurane anesthesia (2.5%), animals returned to their cages allowing accumulation of the tracer during one hour. Images of 30 min were taken on a FOCUS 220 camera under isoflurane anesthesia which was induced five minutes before scanning.

- Because no anatomical MRI images could be provided by P8, due to the extreme size of the animals, P5 passed all SCA17 animals on a 7T clinical MRI scanner (Neurospin, CEA, Saclay). As the clinical scanner was not optimized for experimental animal research, the images were not of sufficient quality for individual use, and thus a template was created for TG and WT animals which was used to coregister PET data.

- SPM analyses were performed following the analysis performed on the HD model. No differences were observed between transgenic and control animals, for \(^{18}\text{F}-\text{LBT999}, \quad ^{16}\text{F}-\text{A84380}, \quad \text{or} \quad ^{18}\text{F}-\text{FDG}.\)

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**Overview of the RATstream of PET experiments and analysis**

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<th>11C-Raclopride</th>
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**E) DT imaging**

**Phenotyping of the rat model of SCA-17**

- The animals were quite large (± 800 g) and could not be properly fixed in the stereotactic device and exceeded the magnet bore size. In spite the fact that new efforts were made to make a new homemade surface coil, we could not solve the problem, as a result no high resolution DTI – of acceptable quality – could be performed on transgenic heterozygous SCA17 and wild type animals.

**Phenotyping of the rat model of Huntington**

In order to deliver an in vivo imaging read out for disease onset and progression, a complete exploration of the HD model (DT-MRI and PET) was done.

a. Longitudinal study

- During a longitudinal study of one year, TgHD and Wt animals were repetitively scanned with MRI and PET. MRI experiments were performed at the age of 2, 6 and
12 months, and were alternated by PET imaging at the age of 5, 10 and 15 months. In addition to volumetric changes, structural changes in tissue microstructure and connectivity changes were traced using diffusion tensor imaging (DTI). Functional metabolic changes and D2-receptor binding were evaluated with PET imaging.

- As the typical brain structures susceptible for HD pathology are the striatum and the surrounding white matter, we segmented in grey matter, the striatum and the globus pallidus. In white matter, the ROI included the corpus callosum, external and internal capsula.
- To process the HD data of all the time points of the longitudinal study with SPM, different registration scenarios were tested and evaluated. However, in none of these cases we succeeded in a satisfactory registration of the individual data. A plausible cause was the fact that the volume differences (e.g. lateral ventricles, striatum) between the TgHD and Wt animals were too large to correct for it during. Therefore, only Region Of Interest (ROI) analyses can be done on the longitudinal data.
- The relative ventricle volume was smaller but increased faster in TgHD compared to Wt animals ($p < 0.05$). The outcome of this longitudinal study was somewhat surprising as it demonstrated a significant differential ageing pattern in TgHD versus the Wt animals, involving the striatum and the white matter structures surrounding it. This HD related shift in maturation and ageing suggests an early developmental origin of the disease and might suggest that mutant huntingtin interferes with brain development.

b. Additional batch of HD animals
- To exclude possible influence of stress induced in the animals of the longitudinal study following the repeated travelling and/or anaesthesia, an additional batch of animals (n=11 TG and n=10 WT) has been used for DTI (at 12 months) and PET imaging (at 15 months). To increase the sensitivity of the MRI method we included DTI and DKI measures (at 16 months) as a method only introduced very recently for assessing ultrastructural changes in grey and white matter.
- DTI: Differences in individual brain regions were statistically analyzed using ROI analysis as described previously. At the age of 12 months, no significant differences could be observed for any DT parameter between TgHD and Wt animals. Voxel Based analyses (=statistical tests are performed for each voxel separately), is a similar method as SPM but has been developed in cooperation with the Vision Lab, Antwerp. The advantage is that some of the preprocessing has been developed in house (allowing for more flexibility) while SPM uses standard routines. DK images were coregistered (affine and non-rigid), in order to create a population based atlas of controls as well as transgenic animals, which was used for further statistics.
- DKI: Recently, diffusion kurtosis imaging was proposed to quantify the non-Gaussian nature of the diffusion process in biological tissue. Non-Gaussian diffusion results from diffusion barriers, such as cell membranes, water compartmentalization, etc. and is therefore an indicator of microstructural complexity. To examine the detailed distribution of the diffusion parameters obtained from both DT and DK imaging, ROI analyses were performed by a manual segmentation of different grey and white matter structures (striatum, cortex, corpus callosum, external capsule). Voxel Based analyses was used for further exploratory statistics. The first neurodegenerative changes, observed with DKI at the age of 16 months suggest that neurodegenerative processes of the HD rat model slowly develops. Non Gaussian diffusion increases in white matter (external capsula) as indicated by an increase in kurtosis anisotropy and radial kurtosis. This might be related to a decreased orientational coherence or packing of the white matter fibre tracts. In the striatum we observed a similar increase in non Gaussian diffusion, which was related to changes in radial kurtosis. Moreover, an increase in anisotropy was observed in the striatum which might be related to selective loss of some of the striatal connections and which may turn the striatum into a seemingly more organized structure.

c. P15 – P30 study
Huntington’s disease (HD) is a neurodegenerative disorder, caused by a mutation in the Huntington gene and is most often diagnosed in mid-life. The key to its clinical expression however, may be found during brain maturation. Although it is known that HD is a late manifesting disorder, it is reasonable to assume that the pathogenetic mutation can cause progressive, subclinical alterations in the cellular ultrastructure, even in very young TgHD rat pups. And indeed as we only discovered differential age related changes in the brain of wild types and controls we decided to go for very young animals to look for early biomarkers. To that end we studied the brain microstructure of young TgHD rat pups with in vivo DKI, which was performed on TgHD animals and control at the age of postnatal day 15 (n=6TgHD and n=6Wt) and 30 (n=6TgHD and n=5Wt). We have demonstrated that there are some distinct differences in postnatal brain development of TgHD pups. Several brain regions were examined throughout development and some morphological changes in grey and white matter regions were found in tissue microstructure. Already at the age of P15, we found a difference in the diffusion pattern in the cortex of TgHD rat pups. Surprisingly, these differences were no longer detected at the age of P30. However, at this age, we did find a change in the diffusion pattern of the striatum and specific white matter structures, like the external capsula and corpus callosum. These results indicate that neuronal development in young TgHD rat pups occurs differently compared to control animals and that the pathogenetic mutation of the gene causes progressive, subclinical alterations in the cellular ultrastructure. The observed changes in diffusion characteristics provided early evidence of diffuse brain developmental abnormalities in distinct brain regions, association with HD. In this context, DKI not only offers the possibility of detecting developmental changes in young TgHD rat pups, but appears more sensitive to detect regional brain changes, compared to other imaging techniques.

d. Metabolomic study

Although the causative gene in HD has been found, the exact mechanism of the pathogenesis and the time of onset are still unknown. Recent investigations point to a metabolic and energetic dysfunction in HD neurons. In an attempt to look for early biomarkers in body fluid we performed a proton nuclear magnetic resonance spectroscopy (1H NMR) study of serum samples taken from presymptomatic HD transgenic rats and their wild-type littermates at an age of 2 months. Both univariate and multivariate analyses were used to compare these metabolic profiles. N-acetylaspartate (NAA), an indicator of neuronal function, was found to be significantly decreased in HD rats compared to wild-type littermates. In addition, levels of glucose, glutamine and succinic acid are significantly increased in HD rats. There is a 1:1 stoichiometry for coupling glucose utilization and glutamate cycling. The observed increase in glutamine, which indicates a shutdown in the neuronal-glial glutamate-glutamine cycling, will thus result in an increased glucose concentration. The elevated succinic acid concentration is due to an inhibition of succinate dehydrogenase, an enzyme linked to the mitochondrial respiratory chain and TCA cycle. Moreover, reduced levels of NAA may reflect an impairment of mitochondrial energy production. All these findings suggest that even in presymptomatic rats, a defect in energy metabolism is already apparent. Moreover, these results support the hypothesis of a mitochondrial energy dysfunction in HD.

Publications and dissemination


Oral presentation at the 4th European Molecular Imaging Meeting, Barcelona, Spain, May 27-30, 2009

- Van Camp N. et al. Longitudinal glucose metabolism and dopamine receptor binding microPET study of a transgenic rat Huntington model, Poster presentation at WMIC, Nice France 2008


- Verwaest KA, Vu TN, Laukens K, Clemens L, Nguyen HP, Van Der Linden A and Dommisse R. H NMR-based metabolomics reveals a disease specific profile in blood serum of transgenic rats of Huntington's Disease. Submitted


- Urbach YK, Raber KA, Andreasson T, Ponten H, Kullingsjö J, Nguyen HP, Riess O, and von Hörsten S. Automated phenotyping and advanced data mining in rats transgenic for Huntington's disease. Submitted


- Yvonne K. Urbach, Martin A. Paucar, Per Svenningsson, Kerstin A. Raber, Hanna Regus-Leidig, Lothar Haeberle, J. Helmut Brandstätter, Jan Kremers, Jenny Atorf, Alexandra Kelp, Olaf Riess, Peter Bauer, Huu Phuc Nguyen, Stephan von Hörsten
Schizophrenia-like phenotype in a rat model transgenic for the human spinocerebellar ataxia subtype 17 (SCA17) mutation. In preparation


Work package 3: Pre-clinical treatment studies

Main partners: Uni Tuebingen, FAU, Trophos

Major achievements:

Trophos provided two novel compounds coded TRORS1 and TRORS2 to be used in treatment trials. One compound, TRORS1, was discovered to treat motor neuron diseases and is currently in Phase 2/3 clinical trials. TRORS2 is a lead molecule originating from a family of compounds issued from the HD drug discovery program.

- TRORS1: Drug powder was used to prepare food pellets for a one month pilot study in HD rats.

- TRORS2: Synthesis of drug powder at >95% purity was supervised by Trophos’ chemistry department. The drug powder was formulated into food pellets as required for a pilot and long-term treatment studies.

The Trophos analytical chemistry department assayed food pellet drug load and stability and performed bioanalytical measurements of TRORS1 and TRORS2 in rat plasma and brain samples by LC-MS/MS. This confirmed the satisfactory amount and quality of the drugs supplied to rats using the food pellet formulation and that drug levels in brain and plasma were above the expected pharmacologically active concentration based on previous data obtained by Trophos in in vitro or in vivo models.
Pilot studies administering TRORS1 and TRORS2 for one month to HD rats and their wild-type littermates. These studies were performed by partners 1 and 2. Data for TRORS2 was reported in annex to progress report 12/2007. Microarray analysis of brain RNA transcripts at the end of the one month studies showed that both drugs were able to reverse HD-associated transcript changes (62 out of 380 for TRORS1 and 31 out of 90 for TRORS2).

TRORS2 was selected for a long-term study in HD rats. Four groups of male rats (12-16 per group) were treated for 9 months: HD rats treated with TRORS2 or placebo food pellets and wild-type littermate rats treated with TRORS2 or placebo food pellets. The treatment study was performed in Tübingen by partner 1 between April and December 2009. Animal behaviour and physiological data was collected using automated cage systems and classical tests. No PET or MRI studies endpoints were established in the HD model so no imaging was performed on animals in the long-term study. At the end of the study brain RNA transcripts analysis was performed by microarray and brain neurotransmitter levels assayed. TRORS2 levels in rat plasma that was collected periodically (3, 6 and 9 months) and brain samples collected at the end of the study. This confirmed that drug levels were in the expected range at 3 and 6 months. A wash-out period followed by re-administration of drug at the end of the study probably accounts for slightly lower than usual levels of TRORS2 in plasma and brain measured at 9 months and probably did not reflect the level prior to the wash-out period. Therefore, rats were satisfactorily exposed to TRORS2 throughout the treatment period.

Results: After a first rotarod test at the age of 1 month, rats were allocated to the various groups: 1. Wt untreated (n=12), 2. Wt treated (n=12), 3. Tg untreated (n=17), 4. Tg treated (n=16) to avoid „uneven distribution“. Treatment was then started and continued until the age of 9 months, followed by 1 month wash-out and retesting on rotarod at the age of 10 months. Treatment was then restarted and animals were sacrificed for neuropathology and gene expression profiling at the age of 11 months. All animals were subjected to Rotarod tests and social interaction tests each month. Phenomaster experiments were performed at the age of 5 months and 8 months. While we found a decrease of rotarod performance in transgenic HD rats as expected, treatment with TRORS2 showed no improvement of motor coordination capabilities. In the Phenomaster experiments, a few changes were detectable in treated tgHD rats. For example there was a significant increase of rearing activity in treated tgHD rats compared to untreated tgHD rats at the age of 5 months in the dark period. This increase in rearing was also seen at 8 months of age, however this did not reach significance. Similarly, the respiratory exchange ratio and the heat production were significantly increased in treated tgHD rats compared to untreated tgHD rats. In the neuropathological analyses, we found no change in the number of aggregates in treated tgHD rats (antibody used was anti-huntingtin S830). Currently ongoing are DARPP32 stainings. In the microarray analysis at the end of the study we found 72 transcripts that were changed between transgenic HD rats and wildtype rats. Treatment with TRORS2 revealed that 15 out of these 72 transcripts were reversed in tgHD rats.

Study report on trial in SCA17 rats: To confirm the results from classical phenotyping that a schizophrenic-like phenotype could be detected in the transgenic SCA17 rats, a treatment with an antipsychotic drug was conducted. Different doses of clozapine (2.5, 5, 10 and 20 mg/kg BW) were tested in the prepulse inhibition test and 5 mg/kg BW were chosen as the most meaningful dose. Two-factorial ANOVA for repeated measurements with the factors "dose clozapine" and "genotype" revealed no genotype effect but a highly significant effect for the factor "dose clozapine". The
startle response in all treated animals was reduced due to the treatment with clozapine compared to sham-treated controls. Reversal of the rearing phenotype in the PhenoMaster and the loss of PPI at 80dB prepulse intensity in tgSCA17 was achieved by treatment with 5 mg clozapine. No significant differences in PPI at 80dB prepulse intensity were detected after treatment.

Illustration

Figure: Principle component analysis of different groups of TRORS2 trial

Publications and dissemination of knowledge:

- A paper on the effects of TRORS2 in HD rats is planned.
- Various parts of this work were presented at international meetings:
  - Brain Diseases & Molecular Machines on 28 March 2008 (Paris, France): invited talk by Rebecca Pruss: Where’s the target? A phenotypic screening approach to the discovery of potential therapeutics for Huntington’s disease
  - Annual HDF Meeting – (Cambridge, USA) 7-10 August 2008: poster presented by Rebecca Pruss: Effects of TRORS2 on HD-modified gene expression in brains of transgenic HD rats. Authors: Rebecca M. Pruss, Pascale Galea, Corinne Chaimbault, Magali Michaud, Trophos, Marseille, France Hoa Nguyen, Michael Bonin, Olaf Riess, University of Tübingen, Germany, Stephan von Horsten, University of Erlangen, Germany
  - ESMEC-European School of Medicinal Chemistry-Urbino. (XXIX Advanced Course of Medicinal Chemistry and Seminar for PhD students) Prof. Gloria Cristalli, Director, 13-19 September 2009, Urbino, Italy, Lecture by Rebecca Pruss on Use of Cell Based Assays to Define SAR and Select Drug Candidates for Motor Neuron Diseases (and Other Indications)
Work package 4: Data mining and information system

Main project partner: Crosslinks

Crosslinks is a company specialising in complex data mining and the complexity of the data generated during phenotyping and detailed characterization of these models of neurodegenerative diseases necessitates the development of sensitive mining applications. The implementation of preclinical trials will allow the re-use of the phenotyping techniques and results for the evaluation of effectiveness and side-effects of treatments. Strict quality management is used to achieve high levels of validity and reliability which sets for the first time standards for genetically modified rat models suitable also for the pharmaceutical industry. In this respect the development of the RATstream™ Server for data collection as a central data repository for the partners in the Consortium will be used by Crosslinks as a proof of concept for future translational projects.

The development of RATstream™ PDM, a rat data analyser which uses statistical methods for correlation analyses on the rat data sets is a combination of commercial available analysis and visualisation tools and (when needed) specially developed analysis software. Both products, in addition to the parameter optimisation analysis carried out by CrossLinks, will be of value to Crosslinks for future translational projects.

Major achievements:
The following table outlines the major deliverables and achievements throughout the project time span.

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<th>Crosslinks Success Criteria / Deliverables</th>
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<td>WP1: raw Product Data Model (PDM)</td>
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<td>WP4: Design of a common data set: Quality gate</td>
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<td>WP1: Defined Product Data Model (PDM)</td>
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<td>WP1: Correlate automated phenotyping: Data mining</td>
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<td>WP2: Disease related screen: Transcription profiling</td>
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<td>WP4: Design of a common data set: RATstream server</td>
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<td>WP4: Implementation of analysis tools: viz &amp; analyses</td>
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<td>WP4: Data sampling: Filing of all data @ RATstream server</td>
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Publications (in progress)
- Multivariate biomarker discovery - integrated clinical analysis of classical and automated phenotyping with PET and DTI data in a transgenic model of Huntington’s disease.
- The RatSTREAM Database: a comprehensive resource for rat integrated phenotyping and genomics of neurodegenerative disease

Products
- P4: Database and bioinformatics for transgenic rat models
Publications out, submitted and in preparation:

   – in preparation

2. The RATstream Database: a comprehensive resource for integrated phenotyping and genomics of neurodegenerative disease in transgenic rat models
   – in preparation