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# Table of contents

1. Executive summary ............................................................................................................. 1

2. Summary description of project context and objectives .......................................................... 2

3. Description of the main S&T results / foregrounds ............................................................... 4
   WP01 Animal models, neurochemistry & pharmacology .......................................................... 4
   WP02 Animal neuroimaging ................................................................................................... 7
   WP03 Human neurochemistry ............................................................................................... 9
   WP04 Human neuroimaging .................................................................................................. 13
   WP05 Genotyping and phenotyping ...................................................................................... 16
   WP06 Pilot testing of medication in clinical paediatric populations (GOAT) ....................... 19
   WP07 Machine learning ....................................................................................................... 21
   WP08 Business development and dissemination ................................................................. 22
   WP09 Ethics and Training .................................................................................................... 23
   WP10 Project Management .................................................................................................. 24

4. Potential impact, main dissemination activities and exploitation of results .......................... 26
   4.1 Socio-economic impact and wider societal implications of TACTICS ............................ 26
   4.2 Main dissemination activities of TACTICS .................................................................... 27
   4.3 Exploitation of TACTICS results ................................................................................ 29

5. Address of the public website of TACTICS and relevant contact details .......................... 30
Final publishable summary report

1 Executive summary

TACTICS is a multidisciplinary project that includes preclinical and clinical research and involves experts from academia and small and medium-sized enterprises (SMEs) that has been developed to identify the neural, genetic and molecular factors involved in the pathogenesis of compulsivity. Compulsivity is defined as the repetitive, irresistible urge to perform a behaviour, the experience of loss of voluntary control over this intense urge, the diminished ability to delay or inhibit thoughts or behaviours, and the tendency to perform repetitive acts in a habitual or stereotyped manner. Compulsivity is a cross-disorder trait underlying phenomenologically distinct psychiatric disorders that emerge at early age (autism spectrum disorder, ASD), in late childhood (obsessive-compulsive disorder, OCD) or during adolescence (substance use disorders, SUD, and behavioural addictions such as gambling, gaming and internet addiction). Compulsivity is closely linked to two other concepts, namely impulsivity and addictive behaviour. In particular Attention-deficit Hyperactivity Disorder (ADHD) is a disorder characterized by high impulsivity and known for an increased risk for later SUD.

The preclinical studies provide no evidence for changes in frontostriatal glutamate tone in animal models of compulsive behaviour. Both the NMDA antagonist memantine and the glutamate release inhibitor riluzole do not have effects on compulsive behaviour. The absence of treatment effects of memantine may be at least partly be explained by interactions between the dopaminergic (quinpirole) and the glutamatergic (memantine) systems. Our results further suggest that anti-glutamatergic drugs may not be effective in individuals with altered dopaminergic neurotransmission.

In the human MRI studies low grey matter volumes of the (pre)frontal, central and cerebellar rather than from the striatal regions were related to persistence versus remission of ADHD. Polygenic risk scores for ADHD did not differentiate between persistence and remission. ASD, OCD and ADHD share common alterations of the subcortical structures, but also each have unique alterations of particular cortical areas. Exposure to stress influences ADHD severity, but only in individuals with an s-allele of the serotonin transporter gene. Stimulant treatment may lower the risk for later SUD, and affect white matter infrastructure and brain regions involved in cortical control.

MR spectroscopy learned that levels of glutamate were higher in the anterior cingulate cortex (ACC) of children with compulsivity disorders compared with controls. There were no differences in glutamate levels between ASD and OCD. This increase in glutamate in the ACC is consistent with several prior studies.

Genetics work in TACTICS identified and confirmed brain-based insulin signalling as an important biological process disturbed by OCD and compulsivity. This led to the generation of a new animal model that was highly compulsive and provided leads for future studies to develop novel therapeutic for OCD and the compulsivity trait. TACTICS further confirmed an important additional concept that there is genetic continuity between psychiatric disorders (ASD, ADHD, as well as OCD) and related population behavioural traits.

The planned randomized, double-blind, placebo-controlled add-on treatment study with a glutamatergic compound (memantine) in ASD and OCD faced several severe challenges and recruitment problems. As a consequence, few participants have been enrolled and only descriptive reporting will be done.

TACTICS has built a multi-task and multi-source learning tool for causal discovery that extends previously existing algorithms by relaxing several assumptions these methods typically rely on, namely data obeys a Gaussian distribution or is discrete, and data has no missing values. This algorithm and software has been valorised by formation of the spinout company Machine2Learn.

By now, TACTICS has published more than 80 peer-reviewed publications, more than 10 of which are in high-impact journals.
2 Summary description of project context and objectives

The "compulsivity disorders" ASD, OCD, ADHD and substance use disorders (SUD)/addictions carry an enormous burden for the patients and their family members and society in general. This burden is due to the overall high prevalence in the population (ASD 1-2%; OCD 3%; ADHD 5-7%; SUD/addiction 6-10%), their most often chronic lifetime persistent course, limited knowledge of the underlying pathophysiology and the lack of curative treatments. Further negative consequences are manifold, and include tremendous human, social and economic costs, including individual suffering, break-up of families, suicide, crime, violence, homelessness, physical illness, impaired performance at work and total disability, and this has serious public health consequences. The clinical burden is further amplified by the frequent comorbidity between these disorders. For example, between 25% and 50% of subjects with ASD have clinically impairing symptoms of impulsivity, inattention and hyperactivity that pass diagnostic thresholds for ADHD. Subjects with ADHD and ASD have a 3-5-fold increased risk to develop addictive behaviours in adolescence, such as excessive use of alcohol, marijuana, and other substances.

The frontostriatal circuits are neural pathways that connect frontal lobe regions with the basal ganglia (striatum) and that mediate motor, cognitive, and behavioural functions within the brain. They receive input from dopaminergic, serotonergic, noradrenergic and cholinergic cell groups that modulate information processing. The frontostriatal circuits are assumed to underlie impulsive, compulsive and addictive behaviours. In the impulsive circuit, a striatal component (ventral striatum/nucleus accumbens shell) may drive impulsive behaviours and a prefrontal component (anterior cingulate/ventromedial prefrontal cortex, VMPFC) may exert inhibitory control. Similarly, in the compulsive circuit, a striatal component (caudate nucleus) may drive compulsive behaviours and a prefrontal component (orbitofrontal cortex, OFC) may exert inhibitory control over them. Increased activity within the striatal components or decreased activity in the prefrontal components may thus result in an increased automatic tendency for executing impulsive or compulsive behaviours, depending on the sub-component afflicted. Overlap between these functional systems may contribute toward the impulsive–compulsive addictive behaviours cycle model. Other possible abnormalities within frontostriatal circuits related to altered striatal activation to rewards may also contribute to the occurrence of impulsive or compulsive behaviours during engagement in reward-related behaviours.

In addition to the neurotransmitter systems mentioned above, also glutamate plays a role in these frontostriatal circuits. Glutamate-dopamine-serotonin interactions are critical to the understanding of the top-down control from prefrontal sub-regions to the dorsal and ventral striatum. In fact, glutamate is the major excitatory neurotransmitter in the brain, including the cortical–subcortical projections and cortico-cortical interconnections. The frontostriatal circuits implicated in compulsivity and impulsivity are notable for their relatively rich glutamatergic receptor density. Moreover, glutamate modulates the metabolism and function of the frontostriatal circuits, as is reflected, for example, in modulatory effects on synapse induction and elimination, as well as cell migration, and “fast” (ionotropic) and “slow” (metabotropic) synaptic transmission.

The objectives of TACTICS were:

1. Identifying determinants and mechanisms
   a. To identify predictive neural, genetic and cognitive markers of compulsivity
   b. To develop and validate novel animal models for pharmaceutical screening and proof-of-concept studies, and test the effects of early intervention therapies in animal models
   c. To examine the interactive (moderating) effects of disorder, age, gender, ethnicity, socioeconomic status, and environmental factors on the relationship between markers and compulsivity in human populations

2. Testing promising medication and developing translational and clinical tools
   a. To provide evidence of disorder-modifying pharmacologic strategies as a therapeutic approach, and provide data on safety and efficacy of promising medication in paediatric populations
   b. To develop a new integrative framework and software for probabilistic multi-source and multi-task learning, which facilitates the robust determination of markers from a collection of different tasks (animal and human) and different sources (neurochemistry, behaviour, MRI, fMRI, genotypes)
   c. To develop translational tools for the early prediction of diagnosis and course of compulsivity and addictive behaviours focusing on Risk Assessment Charts
   d. To develop a new framework for understanding the relationships between the cross-disorder traits impulsivity, compulsivity and addictive behaviours, and for how these traits explain the clinical overlap between different disorders (ADHD, ASD, OCD, ICD, substance use disorder (SUD))
3. **Management, impact and dissemination**
   
a. To liaise with SME’s to support future large-scale clinical trials according to these disorder-modifying strategies to obtain Paediatric Use Marketing Authorisation (PUMA)
   
b. To create value for money by making available data collected in TACTICS in order to facilitate unencumbered global data sharing between researchers
   
c. To disseminate results to a critical mass of stakeholders for policy counselling, integrating the expertise and views of the European Commission and all relevant stakeholder groups to develop policy recommendations and guidelines
3 Description of the main S&T results / foregrounds

WP01 Animal models, neurochemistry & pharmacology

Background

Compulsivity is characterized by a repetitive, irresistible urge to perform a behaviour, the experience of loss of voluntary control over this intense urge, the diminished ability to delay or inhibit thoughts or behaviours, and the tendency to perform repetitive acts in a habitual or stereotyped manner. These behaviours form part of the phenotype of obsessive compulsive disorder (OCD) and autism spectrum disorders (ASD). Increased cortico-striatal glutamate tone has been postulated to underlie in part the compulsive phenotype. In a series of ‘proof-of-concept’ studies, the neurochemistry, behaviour, and proteomics has been examined in behavioural animal models in an effort to better understand underlying glutamate related mechanisms in frontostriatal circuits and its remediation / prevention by early intervention studies with glutamate-based (riluzole and memantine) clinically used drugs. In addition, we sought to validate in animal models the novel mechanism of insulin-related signalling underlying compulsive behaviour identified by the TACTICS project (Van de Vondervoort et al. 2016).

Overall Objectives

1. Test the ability of two glutamate based drugs (riluzole and memantine) to alter compulsive behaviour in animal models of compulsive behaviour.
2. Assess glutamate related neurochemical changes in an animal model of compulsivity.
3. Select a candidate target mechanism related to insulin signalling based on genetic data in OCD, and assess compulsive behaviour in constitutive knockout mice of this target as a putative novel animal model of compulsivity.
4. Assess frontostriatal glutamate and glutamine in a selected animal model which shows deficits in impulse control.
5. Analyse protein levels of the novel insulin-related mechanisms in both brain (frontal cortex, striatum and cerebellum) and blood samples.

Results

Glutamate-related interventions and measures in compulsivity

The rat signal attenuation model is a gold standard animal model of OCD which has been well validated in the Joel lab and others as being responsive to the primary pharmacotherapy of OCD, namely Prozac (fluoxetine) and other selective serotonin reuptake inhibitors (Goltseker et al. 2015). It was the starting point for translational preclinical studies based in the Joel lab. The glutamate based drugs memantine but not riluzole reversed the increase in compulsive lever pressing seen in vehicle treated animals in this task. This was observed in male but not female animals. However, further investigations showed that memantine had no effect in those animals where the vehicle treatment did not elicit increased compulsive lever pressing. Preliminary genetic analyses suggested a role for the MTOR signalling pathway in OCD which was not subsequently highlighted by more extensive analyses. Early stage testing of the MTOR antagonist rapamycin in the rat signal attenuation model confirmed no effect of this mechanism on compulsive lever pressing. It was considered it was worth performing this early exploratory investigation as rapamycin is a drug in clinical use for other indications.

Compulsivity is not confined to OCD and ASD but is also part of drug seeking behaviours which impulsive individuals (such as those with attention deficit hyperactivity disorder; ADHD) are more prone to. This was investigated by the Cryan lab. It was anticipated that early life stress would increase this drug seeking susceptibility. Contrary to expectations, in male rats, early life stress induced by removing the presence of the mother for a short period during early life appears to decrease the amount of compulsive cocaine seeking. This may be in line with literature with suggests that early life stress may also have positive effects on individuals related to their coping strategies. Paradoxically, this anti-drug seeking effect was diminished by the glutamate based drugs riluzole and memantine (O'Connor et al. 2015). These effects did not appear to be related to changes in salience.

The dopamine D_{2/3/4} receptor agonist quinpirole when given sub-chronically (10 days) induces a well-validated, robust compulsive checking phenotype in rats as an OCD model and was performed both in the Glennon and Dijkhuizen labs. Initial experiments with riluzole and memantine in adult quinpirole-treated animals were inconclusive. Follow-up experiments in collaboration with the Dijkhuizen group (WP2 lead) concluded that memantine fails to alter compulsive checking induced by sub-chronic quinpirole in rats.

The phenotype of the SLITRK5 -/- mice was extensively tested by the Petryshen lab and effects on compulsive behaviour reported in the literature were not reproduced despite repeated attempts. Efforts to reproduce the phenotype extended to back-
crossing and breeding new mutant mice lines but unfortunately no compulsive phenotype was seen. On this basis, work on the SLITRK5 /– mouse model was stopped in favour of characterising another relevant gene (ANK3) in terms of compulsive behaviour. These ANK3 knockout mice have been demonstrated to display increased reward and stress-sensitive phenotypes which may impact on impulsive-compulsive behaviours (Gottschalk et al. 2017). Knockout of ANK3 in mice is associated with enhancement of microtubule dynamics via glycogen synthase kinase 3 (GSK3)-related mechanisms (Garza et al. 2018). Whether this underlies this phenotype or not remains to be clarified.

In a final effort to assess glutamate related mechanisms in a mouse model showing compulsive behaviour — the Glennon lab utilised the TALLYHO/JngJ model (outlined below). Proton spectroscopy was used to determine metabolic / chemical changes in this model in frontostriatal brain regions. The Gtx signal is a composite signal (also employed in WP3/4) of glutamate turnover and was measured in the TALLYHO mice. No effect was observed in either orbitofrontal, anterior cingulate cortex or dorsomedial striatal glutamate or glutamine levels in the compulsive TALLYHO mice versus their controls (SWR/J mice; Van de Vondervoort et al. 2018; accepted). Thus 1H-MRS glutamate hyperactivity in frontostriatal circuits could not be confirmed in the TALLYHO mouse model.

Validation of insulin-signalling mechanisms in compulsivity

The Glennon lab further validated the link between insulin-signalling raised in WP5 in three ways — a) examining compulsivity in Type I and Type II diabetes models (Alloxan and TALLYHO/JngJ mice) and a third model (DMSXL mice) showing altered insulin signalling, b) acutely testing the anti-diabetic drug metformin in quinpirole-treated rats, c) examining insulin related gene (mRNA) and microRNA expression.

Phenotypic and MRI characterisation of dysregulated insulin signalling in a Type II diabetes mouse model (the TALLYHO/JngJ mouse) has been performed. TALLYHO mice show compulsive behaviour (versus Swiss SWR/J mice and C57/Bi6 mice) controls in three independent tasks; (i) decreased spontaneous alternation and increased repeated arm entries (in the Y-maze), (ii) increased number of correction errors, correction trials and preservation index during touchscreen operant reversal learning and (iii) enhanced suppression of a non-rewarded stimulus during appetitive extinction (Van de Vondervoort et al. 2018; accepted; Van de Vondervoort et al. (submitted)). Furthermore, increased anxiety-like behaviour is observed in the elevated plus maze (as indicated by reduced open arm entries) in the TALLYHO mice versus controls. To compare the effect of depleting peripheral insulin generation, we utilized treatment with alloxan in adult mice to selectively destroy pancreatic beta cells (as a model of Type I diabetes) and tested these alloxan treated mice versus C57/Bi6 vehicle treated controls. Alloxan treatment had no effect on spontaneous alternation or repeated arm entries in the Y-maze unlike the TALLYHO Type II diabetes mouse model (Van de Vondervoort et al. (unpublished)). Taken together, this suggests that either a) the neurodevelopmental exposure at the juvenile phase to altered insulin signalling (present in the TALLYHO but not alloxan-treated mice) or b) central but not peripheral insulin signalling is important in inducing compulsive-like behaviour. Furthermore, an animal model (the DMSXL mouse) with a splice variant of IGF1 (one of the targets in the OCD genetic analyses) which shows insulin resistance was tested in both reversal learning and appetitive extinction operant tasks. The DMSXL mice also exhibit a compulsive phenotype as demonstrated by habitual responding in the late phase of reversal learning and enhanced suppression of a non-rewarded stimulus during appetitive extinction (Soldt et al., manuscript in preparation).

Initial studies involving acute testing of the anti-diabetic drug metformin in quinpirole-treated rats demonstrated that it decreases quinpirole-induced compulsive checking (Kapusta et al., unpublished). Further dose-response studies and sub-chronic testing of metformin will need to be performed in the future in this and other models of compulsivity to confirm the utility of metformin as an anti-compulsive agent.

Neurochemical / molecular profiling, microarrays and micro-RNA sequencing has been performed on the orbitofrontal cortex and dorsomedial striatum of the brains from the signal attenuation task which confirmed the genetic analyses (see WP5) of the role of insulin signalling but also identified microRNAs targeting insulin mechanisms as well as novel microRNA targets related to compulsive behaviour. The same 20 top-ranked OCD GWAS landscape associated mRNAs expression has been performed in the TALLYHO mice which show a compulsive phenotype.

The top-ranked gene candidate (confidential but here named „K”) from the OCD genetic analyses in WP5 is involved in insulin secretion while insulin growth factor 1 (IGF1) plays a prominent role in the growth / differentiation of brain cells. Validation of „K” and IGF1 as a target for compulsive mechanisms was performed in two ways. Firstly protein expression studies of „K” and IGF1 in blood serum and brain was performed in the compulsive TALLYHO mouse model in the Bahn lab. Within brain, the prefrontal cortex, dorsomedial striatum and cerebellum from the TALLYHO mouse model were utilised for proteomics. The data suggests that there is an increase in serum „K” and IGF1 gene expression while in the brain, a decrease with cerebellar IGF1 expression is found in TALLYHO mice versus controls. Moreover, IGF1 protein expression in the cerebellum correlated with the percentage spontaneous alternation in the TALLYHO mice (Van de Vondervoort et al. 2018; accepted). In addition, gene (mRNA)
expression of \(^K\) was decreased in the cerebellum of TALLYHO mice. To confirm the role of \(^K\) further, novel transgenic mice targeting \(^K\) were created at GenOway. Transgenic conditional heterozygous KO mice were bred with mutant embryos cryopreserved in order to secure the line. GenOway finalised the development of constitutional (whole body) homozygous knockout and Cre-synapsin \(^K\) line which is brain specific and can be optogenetically switched on/off. The constitutive (brain-only) knockout has been tested in a mouse battery of compulsive and anxiety related tasks. \(^K\) knockout is associated with decreased anxiety but increased compulsive-like behaviours – such as increased object checking, decreased spontaneous alternation and increased stereotypy (circling and head shakes). All conditional and constitutive \(^K\) knockout brains have been prepared for ex-vivo MRI and immunohistochemistry of insulin markers which is currently ongoing.

**White-matter markers**

The rat quinpirole model has been selected for further MR imaging profiling and has been reported in WP2. In short, no effect of the glutamate based drug memantine was seen either on compulsive behaviour or on MRI metrics (Straathof et al. submitted). In addition, MR imaging of the compulsive TALLYHO/JngJ type II diabetes model (to examine functional and structural changes) related to insulin signalling in OCD show changes in white matter microstructural integrity. MRI-DTI studies showed clear increases in the fractional anisotropy in the corpus callosum and superior cerebellar peduncle similar to that seen in the juvenile quinpirole model and clinical OCD (Van de Vondervoort et al. 2018; accepted). With regards to the MRI connectivity metrics, their relationship to the glutamate and insulin signalling aspects studied in WP1 remains an area of study.

**Conclusions**

- No evidence for changes in frontostriatal glutamate tone in animal models of compulsive behaviour.
- Both the NMDA antagonist memantine and the glutamate release inhibitor riluzole do not have effects on compulsive behaviour (cocaine seeking, quinpirole induced checking, and signal attenuation task). The evidence for glutamate tone modifying agents in compulsive behaviour is not supported by either WP1 or WP2.
- Phenotype of SLITRK5 knockout mice reported in literature (repetitive grooming) was not reproducible.
- ANK3 knockout mice demonstrate an impulsive-compulsive phenotype. Separate studies show that this mechanism enhances microtubule dynamics via GSK3 signalling. Whether this underlies this phenotype remains to be clarified.
- Together with WP5 colleagues, insulin signalling was identified as a key mechanism in obsessive compulsive disorder. Validation of this mechanism was undertaken in WP1. The type II diabetes animal model, the TALLYHO mouse shows an anxious and compulsive phenotype (spontaneous alternation and reversal learning) which is not observed in the type I diabetes Alloxan-treated mouse model.
- IGF1 signalling in TALLYHO mice (particularly in cerebellum) may contribute to compulsive behaviour.
- The top hit from the OCD GWAS analysis, \(^K\) was observed to be down-regulated in the cerebellum.
- Constitutive knockout of \(^K\) resulted in repetitive behaviour (circling and head shakes), compulsive object checking and decreased spontaneous alternation independent of anxiety (increased time in centre of open field and open arms of elevated plus maze). This validates \(^K\) as a target involved in compulsive behaviour.
- Cre-synapsin \(^K\) conditional knockout mice have been created to validate in the future whether the effect of \(^K\) is centrally mediated. This line is currently cryopreserved.
- Preclinical efforts in TACTICS provide evidence of insulin signalling but not glutamate related mechanisms as being involved in compulsivity.

**References**


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**WP02 Animal neuroimaging**

**Background**

Compulsivity is the repetitive, irresistible urge to perform certain behaviours without voluntary control, and can be considered to be a core symptom of different psychiatric disorders like obsessive compulsive disorder (OCD) and autism spectrum disorder (ASD) (Jacob et al, 2009). Current treatment strategies for these disorders typically focus on diminishing symptoms associated with a specific diagnosis, and are often not fully effective (Accordino et al, 2016; Franklin and Foa, 2011). This stresses the need for alternative treatment approaches, for example by focusing on symptoms, e.g. compulsive behaviour, so treatment can be tailored to specific symptom domains.

Development of treatment approaches that focus on compulsive behaviour requires knowledge of the underlying neural circuits. Compulsive behaviour has been associated with abnormalities within the frontal cortico-striatal-thalamo-cortical circuits in humans, as demonstrated with magnetic resonance imaging (MRI) (Figeer et al, 2016; Montigny et al, 2013). Within these circuits, compulsive behaviour may either be caused by hyperactivity within the striatal component or by a failure of top-down control of the frontal cortical regions over the striatal component (Fineberg et al, 2010). It has been theorized that this top-down cortical control is mediated by the neurotransmitter glutamate (Sesack et al, 2003). This points towards a relationship between compulsive behaviour and altered glutamate concentrations, which is further supported by the high density of glutamate receptors in the frontostriatal circuits (Monaghan et al, 1985) and dysregulation of glutamatergic signaling in individuals with ASD and OCD (Naajen et al, 2015; Pittenger et al, 2011).

The role of glutamate in patients with OCD and ASD may imply that anti-glutamatergic drugs could be effective as medication against compulsivity (Mechler et al, 2017). One potential drug is the N-methyl-D-aspartate (NMDA) receptor antagonist memantine, an FDA approved drug used in the clinic for the symptomatic treatment of Alzheimer’s disease (see for a systematic review and meta-analysis: Matsunaga et al, 2015). Memantine has shown some first promise as a successful treatment against OCD in an animal model and in human adults with OCD (Egashira et al, 2008; Ghaleiha et al, 2013; Haghighi et al, 2013). Since three-quarters of people with OCD experience their first symptoms in mid childhood, OCD is suggested to be a neurodevelopmental disorder (Boileau, 2011). Therefore, it is important to assess the treatment potential of anti-glutamatergic drugs, like memantine, in this developmental period (Mechler et al, 2017). In addition, mechanistically it remains unclear how these anti-glutamatergic drugs exert their therapeutic effects on neural circuits involved in OCD. In recent years a variety of translational animal models has been developed to study the pathophysiology of OCD and to test therapeutics (Albelda and Joel, 2012a, 2012b: Alonso et al, 2015). Therefore, in this work package, we determined the neural underpinnings of compulsive behaviour in an appropriate adolescent rat model of compulsive behaviour, as determined in WP1. In addition, we investigated the therapeutic potential and working mechanisms of the most potent therapeutic drug, as determined in WP1, to reduce this compulsive behaviour.
Overall Objectives

1. To clarify the neural underpinnings of compulsive behaviour, and to reveal potential targets for therapy, we assessed the development of functional and structural brain connectivity in the frontostriatal circuit by means of multi-parametric MRI in appropriate rodent models, in combination with specific treatment strategies. Our objectives were: To characterize developmental changes in a) morphometry, b) white matter integrity, and c) functional connectivity in the frontostriatal circuit from the juvenile to adolescent phase in an appropriate rodent model of compulsivity, as determined in WP1, with similar neuroimaging protocols as in WP4, and analysis tools as in WP7 (Task 1).

2. To identify effects of promising therapeutic drugs, as selected in WP1, on functional activation of the frontostriatal system in rats with compulsive behaviour (Task 2).

3. To determine the neural correlates of behavioural improvement after pharmacological treatment with the most potent drug, as identified in WP1, in a rodent model of compulsivity (Task 3).

Results

In close agreement with WP1 (RUNMC: Glennon), we have chosen the quinpirole-induced compulsive checking behaviour model in rats to measure, with MRI, morphometric, structural integrity and functional connectivity changes in the frontostriatal circuit. In this model (Szechtman et al, 1998), compulsive checking behaviour is induced by repetitive injections with the dopamine D2/D3 receptor agonist quinpirole in combination with placement on the open field. Similar to this OCD model in adult rats, which has been used in WP1, we showed that adolescent rats developed a compulsive checking behaviour phenotype after repeated injections with quinpirole. The OCD phenotype was not associated with large-scale volumetric changes in frontostriatal circuit structures. Analyses of the fractional anisotropy values (a measure of structural integrity) of relevant white matter tracks showed that these were higher for the internal capsule and corpus callosum in animals with OCD as compared to controls. Analyses of functional connectivity data showed that the developmental change in functional connectivity between prefrontal cortical areas and striatal areas was reduced in OCD animals.

In close collaboration with WP1 (RUNMC: Glennon), memantine was chosen as the promising therapeutic drug of which we investigated the effects on functional activation in the frontostriatal system in the adolescent rat model of compulsive behaviour developed in Task 1. Memantine is an anti-glutamatergic drug that is also used in the clinical trial (WP6). We demonstrated that memantine acutely induced a 7-16% increase in BOLD signal in frontal cortical areas in adolescent control animals, lasting for about 1 h after the memantine challenge. This memantine-induced brain activation response was absent in adolescent OCD animals. Memantine did not induce a measurable activation response in striatal regions of the frontostriatal system in both adolescent OCD and control rats. In additional experiments we found that the memantine-induced activation in frontal cortical areas was also absent in rats that received a single quinpirole injection.

Memantine was sub-chronically administered for seven consecutive days between the 10th and 12th quinpirole injection. We measured compulsive behaviour before and after the sub-chronic memantine treatment, and demonstrated that this treatment did not significantly reduce compulsive behaviour in the quinpirole-induced compulsive checking behaviour model in adolescent rats. Correspondingly, MRI-based measures of structural and functional connectivity within the frontostriatal circuit were not altered after sub-chronic memantine treatment. The sub-chronic memantine treatment did influence the body weight of rats, by inhibiting the developmental body weight increase in both control and quinpirole-injected rats.

Conclusions

We have successfully adapted an adult rat model for compulsive behaviour for studies in adolescent animals. This model offers opportunities to further unravel underlying mechanisms of compulsive behaviour in adolescents and to study novel treatment strategies. Compulsive checking behaviour in this adolescent rat model is associated with altered development of structural and functional connectivity within the frontostriatal circuit. An acute memantine challenge induced brain activation in frontal cortical areas in control rats, but not in rats that received repetitive or a single quinpirole injection. Subchronic memantine did not reduce compulsive behaviour in this adolescent rat model and correspondingly did not influence structural and functional connectivity in the frontostriatal system. Results from task 2 and 3 together suggest that the absence of treatment effects of memantine may be at least partly explained by interactions between the dopaminergic (quinpirole) and the glutamatergic (memantine) systems. In addition, our results may suggest that anti-glutamatergic drugs may not be effective in individuals with altered dopaminergic neurotransmission. Future studies that apply this model should carefully consider possible interactions between quinpirole and pharmacological treatments, which may be verified with in parallel pharmacological MRI experiments.
Compulsivity is defined as the repetitive urge to perform certain behaviours, loss of voluntary control over this urge, the diminished ability to delay or inhibit thoughts and behaviours, and the tendency to perform repetitive acts in a habitual or stereotyped manner1. It is a trait that occurs in a variety of different disorders, including obsessive compulsive disorder (OCD) and autism spectrum disorders (ASD) and has been linked to behavioural disinhibition (a component of executive functioning) in both2. This involved control of the prefrontal cortex over the striatum, brain regions in the frontostriatal circuit. Changes in this circuit have been implicated in compulsive behaviours in both ASD & OCD34. In neuroanatomical models the dorsal striatum drives compulivity, while the prefrontal cortex exerts control over it5. The overlap between ASD and OCD is likely related to repetitive behaviours6 that have been linked to deficits in functioning of the frontostriatal circuit7.
Glutamate is the most abundant excitatory neurotransmitter and is involved in synaptic transmission, plasticity and long-term potentiation\(^9\). It can be measured non-invasively, along with several other cerebral metabolites, using proton magnetic resonance spectroscopy (\(^1\)H-MRS). Unfortunately, it is not possible to distinguish between the metabolic pool of glutamate from glutamate acting as a neurotransmitter. There is evidence, though, that glutamate/glutamine cycling accounts for 80 – 100 % of glutamate trafficking in the brain\(^9,10\).

**Overall Objectives**

1. To develop a set of standardised MRS acquisition strategies, quality control measures and standard operating procedures (SOP) for each of the centres in the study.

2. To investigate whether glutamatergic concentrations in the frontal-striatal circuit were different in children with ASD and OCD compared with healthy controls, and in conjunction with WP4, to investigate whether glutamate concentration in the frontostriatal circuit predict compulsivity, impulsivity and addictive behaviours, and to assess structural and functional characteristics of brain maturation to the development of compulsive behaviours. As part of this study, we also investigated the relationship between striatal volume and shape and concentrations of N-acetyl aspartate and glutamate.

3. To study whether there were any neuroimaging or neurochemical differences between patient subject groups following medication (with WP6).

**Results**

**Glutamate levels between groups for the first wave of the longitudinal study:**

A group difference was found for glutamate levels corrected for voxel composition in the ACC, including gender, medication and scanner site as covariates. Since age didn't affect the ANCOVA model, it was excluded. There were group differences in gender, but these did not affect glutamate concentrations. Post-hoc between group tests showed increased glutamate in the ACC in both ASD and OCD groups compared with controls but not between the ASD and OCD groups. In a restricted set of ASC and OCD subjects with no previous or current use of medication, however, glutamate levels did differ between groups. A dimensional analysis showed a positive correlation between glutamate levels in the ACC and compulsivity (measured with the Repetitive Behaviour Scale\(^13\)). However, there was no significant correlation between glutamate concentration and total RBS score. No differences in water scaled glutamate levels were found in the striatum.

**Glutamate levels between groups for the second wave of the longitudinal study:**

Data for the second wave has been collected at all centres. Image segmentations to determine voxel composition and LC model measurements to determine glutamate concentrations have been performed. Currently, the second wave results are awaiting statistical analysis to determine whether there are any differences compared with the first wave results.

**GOAT – Effects of Medical Intervention**

The GOAT study (into the effects of medication) has been delayed due to a number of issues and is scheduled to complete its last scan in the first week of October 2018, by which time seven participants will have been scanned. The first issue was that Lundbeck were initially involved with the project which would have brought all their expertise in the conduct of drug studies in patients, but unfortunately, they withdrew their commitment and it was not possible to find any other personnel from the commercial sector to engage with the project. This left a large knowledge and funding gap. In addition to this there were also several logistical issues: there were severe delays in setting up the clinical monitor, especially as the first clinical monitor involved closed after seven months on the project. It then took another month to get a new clinical trial monitor up and running.

Other issues involved the more complicated ethics process for partner CIMH (Zentralinstitut für Seelische Gesundheit), which ideally required the involvement of a commercial team, which would have been straightforward if Lundbeck had remained involved. Getting the required approval appeared to be much easier at the other German or Dutch sites.

**Striatal Structure and its Association with NAA and Glutamate**

A group difference was found in NAA in the left striatum while including scanner as a covariate. Medication use, sex and age were excluded from the model since they had no effect. Post-hoc analysis revealed lower NAA in ASD compared with controls. There were no statistically significant differences for other group comparisons.

Associations between metabolite concentrations and striatal volume were investigated separately for each group. There was a significant relationship for both NAA and glutamate with striatal volume for children with ASD.
There was no main effect of diagnosis on striatal volume and no effects of IQ, sex, age, medication, or scanner site. A hemisphere by diagnosis interaction suggested differences in left-right asymmetry in striatal volume across the three groups (right > left). Post-hoc tests comparing the groups with each other using 2×2 designs showed the same diagnosis by hemisphere interaction when comparing ASD with controls and OCD with controls but not ASD with OCD. After removal of subjects using medication and those with comorbidities, the asymmetry result remained significant and respectively for ASD and OCD. There were no differences in striatal shape between groups.

Conclusions
Levels of glutamate were higher in the ACC of children with compulsivity disorders compared with controls. There were no differences in glutamate levels between ASD and OCD subjects for either voxel. This increase in glutamate in the ACC is consistent with several other studies that have found increased glutamate or Glx (the sum of glutamate and glutamine concentrations) in the ACC of children with ASD. Conversely, adults with ASD show decreased levels of glutamatergic compounds in prefrontal brain regions. It seems that glutamatergic overactivity in the ACC may be specific for children with ASD. However, this overactivity may not be limited to just the ACC, since previous studies have reported increased glutamate within the amygdala-hippocampal complex and the cerebellum and striatum. This study did not confirm increased glutamate within the striatum. The higher than normal glutamate levels in the early stages of development (8 – 13 years) compared with lower levels in adulthood suggests excitotoxicity in youth attenuates glutamate signalling in adults. However, metabolite levels are known to change with age in normal controls.

Previous findings in OCD have been less robust, with studies showing increased Glx in the caudate while others have shown decreased levels across the brain, including in the ACC. However, most previous studies have been performed in adults with most not finding any differences between patients and controls. In this study, we specifically focused on an important age-range within neurodevelopment. As such, our data fill an important void in current research, especially given the knowledge about age-specific effects in the overlapping disorder ASD.

The most important result of this study was our finding there was that while both disorders showed higher levels of glutamate in the ACC compared with controls, there was no significant difference in glutamate levels between the two disorder groups ASD and OCD. This similarity may be due to similar mechanisms for compulsivity in the two disorders. Our finding that increased compulsivity was associated with increased levels of glutamate in the ACC supports this suggestion. Since this correlation was not found for the total RBS score, it suggests that ACC glutamate specifically underlies compulsivity, but not other measures of repetitive behaviour such as self-harm, resistance to change and restricted interests.

In children with ASD, left striatal volume was associated with both NAA and glutamate concentrations. Volume and shape analysis didn’t show any structural changes in the striatum that were associated with ASC, OCD, compulsivity or metabolite concentrations. We did, however, find a hemispheric asymmetry (right > left) in striatal volume in ASD & OCD compared with controls, but this asymmetry did not exist between disorder groups. Additionally, the asymmetry was neither associated with phenotypic measures nor with total brain volume.

Total NAA was reduced in children with ASD compared with controls, in line with previous studies. In the same group, striatal volume has been shown to positively correlate with NAA concentration. Other studies on the relationship between cortical volume and NAA have suggested NAA is related to neuronal integrity, since NAA is mostly found in neurons in the brain. This suggests children with ASD may have reduced neuronal integrity within the striatum. It is, however, possible that reduced NAA may reflect reduced neuronal functioning rather than a reduction in the number of neurons.

In ASD, there was only a trend-level association between glutamate and striatal volume after correcting for total brain volume. Previous studies in other disorders have suggested excitotoxicity causes neuronal loss in the striatum. Our result that there was a positive correlation between glutamate and striatal volume in ASD contradicts this excitotoxicity hypothesis. It should be noted, however, that MRS measures the total metabolic pool of glutamate, not just glutamate acting as a neurotransmitter. Children with ASD thus show impaired neuronal metabolism in the striatum, paired with alterations in volume.

Both disorders have been associated with alterations in the striatum, but with inconsistent findings. In ASD and OCD, we did not replicate increased volumes for the caudate and putamen. In contrast to our results, a previous study has found striatal asymmetry controls but not in ASD. Other previous studies have shown inconsistent findings, with some showing increased, decreased or no change in striatal volumes. Ours is, however, the first study to show regional volumetric asymmetry. The striatal asymmetry in both ASD and OCD could point to similar alterations in striatal structure in both disorders. This cross-order finding warrants replication in an independent sample but may reflect similarities in the underlying neurobiology of both disorders.
References


WP04  Human neuroimaging

Background

Autism Spectrum Disorder (ASD), Attention-Deficit Hyperactivity Disorder (ADHD) and Obsessive-Compulsive Disorder (OCD) are rather common neurodevelopmental disorders with onset in very early childhood (ASD), early and middle childhood (ADHD) or late childhood-early adolescence (OCD). These disorders have both common and distinct clinical features, and are characterized by various combinations of impulsive and compulsive behaviours. The neural underpinnings of these disorders are the frontostriatal-thalamic and fronto-cerebellar circuits. Current neuroanatomical models posit the existence of separate but intercommunicating impulsive and compulsive loops in the frontostriatal circuits, differentially modulated by neurotransmitters. In the impulsive circuit, a striatal component (ventral striatum/nucleus accumbens shell) may drive impulsive behaviours and a prefrontal component (anterior cingulate/ventromedial prefrontal cortex) may exert inhibitory control. Similarly, in the compulsive circuit, a striatal component (caudate nucleus) may drive compulsive behaviours and a prefrontal component (orbitofrontal cortex, OFC) may exert inhibitory control over them. Overlap between these functional systems may contribute toward the impulsive–compulsive addictive behaviours cycle model. Other possible abnormalities within frontostriatal circuits related to altered striatal activation to rewards may also contribute to the occurrence of impulsive or compulsive behaviours during engagement in reward-related behaviours.
Overall Objectives

1. Identify the neural, neurocognitive, genetic, and biomarker mechanisms underlying impulsive and compulsive behaviours in high-risk subjects (those with ADHD) and controls, and examine the developmental links between early impulsive and compulsive behaviours and later outcome including addiction.

2. Examine the common and distinct neural and neurocognitive mechanisms underlying compulsivity and identify disease-modifying factors (OCD, ASD) of these neural, neurocognitive and genetic mechanisms.

3. Examine effects of a glutamatergic intervention on frontostriatal MRI measures in individuals with OCD and ASD.

Results

We found that persistence versus remittance of ADHD from childhood into adulthood was associated with low volumes of prefrontal, frontal, precentral and cerebellar regions. Conversely, low volume in visual/auditory cortices was related to ADHD remission. ADHD full-persisters had significantly larger left amygdala volumes relative to controls. These findings suggest a possible earlier maturation in visual/auditory cortices in remitters and further support previous theories that over-reliance on sensory processing might compensate for frontal control deficits in ADHD (Adamo et al. submitted; PhD thesis 2018). Polygenic risk scores differentiated ADHD (both persistent and remittent) from controls, but did not differentiate between persistent and remittent ADHD, but this may be due to limited statistical power (Adamo, PhD Thesis 2018). Those with late, adolescent-onset ADHD displayed more impairment on all cognitive measures (motor speed and variability, memory span, response inhibition, timing) in childhood than stable unaffected individuals (Ilbegi et al. submitted-b).

The prediction of later substance use disorder (SUD) and nicotine dependence (ND) was related to a complex set of variables. Risk factors for later SUD and ND were persistent ADHD, severe ADHD, late-adolescent onset ADHD, and positive family history for SUD. The ADHD effect was in part mediated by Conduct Disorder problems. A protective factor in individuals with ADHD was early initiated and high-dose stimulant treatment for ADHD. Severity of ADHD inattention symptoms was linked to later gaming (Groenman et al., 2018; Ilbegi et al, submitted-a; Paraskevopoulo et al. submitted a, b; Schoenmacker et al. 2018).

ASD was associated with smaller subcortical volumes of the pallidum, putamen, amygdala, and nucleus accumbens (all small effect sizes), as well as increased cortical thickness in the frontal cortex and decreased thickness in the temporal cortex in the ENIGMA ASD case-control mega-analysis. Analyses of age effects indicate that the development of cortical thickness is altered in ASD, with the largest differences occurring around adolescence. No age-by-ASD interactions were observed in the subcortical partitions (van Rooij, Anagnostou et al. 2018). In the ENIGMA ADHD case-control mega-analysis, ADHD was associated with smaller volumes of the accumbens, amygdala, caudate, hippocampus, putamen, and intracranial volume (all small effect sizes). Effect sizes were highest in most subgroups of children (<15 years) versus adults (>21 years), and case-control differences in adults were non-significant (Hoogman, Bralten et al. 2017). ADHD was further associated with subtly reduced surface area in childhood, mainly in frontal but also in parietal cortices; temporal cortices had both smaller surface area and thickness in patients during childhood.

When findings in ASD and autism are cross-compared and are compared to findings in OCD (Boedhoe, Schmaal et al. 2017, Boedhoe, Schmaal et al. 2018), there are no differences in subcortical structures between ASD and ADHD but different patterns of cortical abnormalities; adult OCD patients had a larger pallidum compared to ADHD patients, adolescent OCD patients showed larger thalamus, putamen and pallidum volumes compared to ASD patients, and paediatric OCD patients had larger hippocampal and amygdala volumes compared to ADHD patients.

In conclusion, ASD, OCD and ADHD share common alterations of the subcortical structures, but also each have a unique alteration of particular cortical areas.

In terms of functional activations and connectivity, the cross-disorder analysis of ASD and OCD showed similar abnormalities in reward processing, and resting-state connectivity changes (Akkermans et al submitted-a,b; Boecker-Schlier et al, submitted). There were no alterations in response inhibition in ASD and OCD, compared to controls, but abnormalities were associated with comorbid ADHD (Gooskens et al., submitted).

Stress exposure was associated with more severe ADHD symptoms in carriers of the s-allele compared with individuals homozygous for the l-allele of the serotonin transporter gene (van der Meer, Hartman et al. 2014). This gene-environment interaction was mediated by grey matter volume in the frontal pole and anterior cingulate gyrus (van der Meer, Hoekstra et al. 2015). We further found that s-allele carriers showed a more negative relation between stress exposure and connectivity of the executive control network than l-allele homozygotes, specifically in the pre/postcentral gyrus, striatum, and frontal pole. In the default mode network, we found a positive association between the GxE and supra-marginal gyrus connectivity (van der Meer,
Hartman et al. 2017). In summary, this shows that the serotonin transporter genotype moderates the effect of stress on brain regions involved in social cognitive processing and cognitive control.

We studied the long-term functional and structural brain changes after stimulant treatment for ADHD. Individuals with ADHD showed thinner bilateral medial temporal cortex throughout adolescence and young adulthood compared to healthy controls, there was no association between cortical thickness and stimulant treatment (Schweren, Hartman et al. 2015). However, stimulant treatment was associated with the integrity of the orbitofrontal-striatal white matter tract (Schweren, Hartman et al. 2016). A history of intensive stimulant treatment did affect activation of brain regions for cognitive control and/or decision-making to reward stimuli, whereas no effects on striatal activations were found (Schweren, Groenman et al. 2017).

Conclusions
Low grey matter volumes of the (pre)frontal, central and cerebellar rather than from the striatal regions were related to persistence versus remission of ADHD. Polygenic risk scores for ADHD did not differentiate between persistence and remission. ASD, OCD and ADHD share common alterations of the subcortical structures, but also each have unique alterations of particular cortical areas. Exposure to stress influences ADHD severity, but only in individuals with an s-allele of the serotonin transporter gene. Stimulant treatment may lower the risk for later SUD, and affect white matter infrastructure and brain regions involved in cortical control.

References
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WP05 Genotyping and phenotyping

Background

When we started the TACTICS project, little was known about the molecular basis of OCD and related traits. In WP5, we aimed to identify novel candidate genes and biological pathways for compulsivity through a battery of complementary approaches, involving patient and population-based samples, exploring also the overlap between different psychiatric disorders showing comorbidity with compulsivity/OCD. In addition, we aimed at finding new clues about the biological underpinnings of compulsivity/OCD by integrating data across different levels of organismal complexity, especially with neuroimaging genetics studies. We aimed to use the largest data sets available for our studies. Through our leading role in different international consortia on neuroimaging, genetics, and neuroimaging genetics, this could be realized in an even more powerful way than we had anticipated when writing the project.

Overall Objectives

1. Identification of new candidate genes for compulsive behaviour from the results of genome-wide association studies (GWAS) through endophenotype approaches in patient and population-based samples.

2. Integration of the findings across own data and those from affiliated consortia to pick the most interesting candidate for animal studies (WP1) and promising new treatment strategies (for WP6).

3. Characterization of samples, pre- and post-medication, from patients with OCD, ASD, ADHD, and healthy controls analysed in WP4 and treated in WP6 through genetics and proteomics analysis of candidate genes/biological pathways (in collaboration with UCAM) to select biomarkers.

These objectives were translated into tasks, according to which the work was structured

Task 1: Genome-wide association studies of compulsivity-related traits / endophenotypes in patient samples of OCD and related disorders.

Task 2: Genome-wide association studies of brain morphology in the population-based samples.

Task 3: Integration of findings from various GWAS of OCD- and compulsivity-related phenotypes to identify novel candidate genes. Those data were analysed with bioinformatics approaches and manual integration as well as using Bayesian causal modelling (with WP7). From this work, a candidate gene for the WP1 animal model was extracted and a promising new treatment strategy defined.

Task 4: Genetics analyses of samples from WP4 (and WP6).

Task 5: Proteomics analyses of samples from WP4 (and WP6).

Results

In the lifetime of the TACTICS project, we took on leading roles in the ENIGMA Consortium on neuroimaging genetics, the ENIGMA-ADHD Working Group on neuroimaging of ADHD and its comparability with other disorders, and the Psychiatric Genomics Consortium ADHD Working Group on the genetics of ADHD. Each of those consortia, which work in a largely unfunded manner, works with the world-wide largest number of samples and provided us with the opportunity to perform statistically highly powerful gene-finding studies. We combined this with novel data integration approaches, which we had developed ourselves.

For Task 1 (Genome-wide association studies of compulsivity-related traits / endophenotypes in patient samples of OCD and related disorders), highlights of our work include the following studies:

- We performed studies of impulsivity, inattention, and compulsivity, autism symptom severity, and inhibition, in which we investigated the role of specific biological pathways for their role in such behaviours. Through gene-set analyses, we
observed associations with monoaminergic, glutamatergic, and GABAergic genes, as well as a set of genes involved in neurite outgrowth (Bralten et al., J Am Acad Child Adolesc Psychiatry 2013; Naaijen et al., Transl Psychiatry 2017).

- In terms of finding new genes for compulsivity, we recently published the largest GWAS of ADHD as part of the Psychiatric Genomics Consortium ADHD Working Group. Next to identifying the first 12 genome-wide significant loci for ADHD, we also established the genetic overlap with other psychiatric disorders and related traits (Demontis et al., Nature Genetics, in press), which confirm the biological link between ADHD and OCD-relevant behaviour.

- For ADHD, we also performed brain-wide studies of neuroimaging phenotypes. Those were the world-wide largest studies so far (3200 participants in the first, over 4000 individuals in the second study) and observed novel as well as confirming previous findings on the involvement of striatal and limbic structures in the disorder. Importantly, our study was the first to take a lifetime approach, and report that the structural alterations in the disorder were more strongly observed to childhood, suggesting a prolonged timeframe needed for maturation of the brain in ADHD (Hoogman et al., Lancet Psychiatry 2017). The second paper, describing widespread differences between children (but not adults) with ADHD and healthy controls in cortical surface area, is currently in preparation. In addition, we are working on a meta-analysis of the neuroimaging findings for ADHD, ASD, and OCD, based on the samples from the ENIGMA Working Groups on these disorders, in which we aim to identify specificity and overlap among them.

For Task 2 (Genome-wide association studies of brain morphology in the population-based samples), highlights of our work include the following studies:

- We identified genes involved in different brain phenotypes relevant for compulsivity. In a first study, we performed genome-wide association studies of hippocampus and total brain volumes in over 10,000 individual samples (Stein et al., Nature Genetics 2012). Subsequently, we extended these studies to include more subcortical structures as well as more individuals (over 20,000 individuals) (Hibar et al., Nature 2015). These studies were performed in the context of the ENIGMA Consortium. Through collaboration with another large imaging genetics consortium (CHARGE), we were able to further enlarge sample sizes with the result of even more identified loci influencing the volume of the different structures, in particular for hippocampus (Hibar et al., Nature Commun 2017), intracranial volume (Adams et al., Nature Neurosci 2016), and also the other subcortical volumes (Satizabal et al., in revision). A first study of cortical brain phenotypes, being regional and global surface area and thickness, was also recently concluded, and the manuscript on data from over 40,000 individuals is currently in preparation (Medland et al., in preparation).

In Task 3, we have worked on the integration of findings across different GWAS of OCD and compulsivity-related phenotypes to identify novel candidate genes. Through bioinformatics approaches and manual data integration as well as using Bayesian causal modelling (with WP7). From this work, a candidate gene for the WP1 animal model was extracted and a promising new treatment strategy defined.

- We used our data integration approach of different genetic approaches in ASD, in which compulsivity and rigidity are prominent symptoms. Through this, we were able to develop a molecular landscape of ASD, in which we identified steroidogenesis, neurite outgrowth, and (glutamatergic) synaptic function to be enriched in the genetic data. Importantly, A-kinase anchor proteins (AKAPs) were found to functionally integrate signaling cascades within and between these networks (Poelmans et al., Transl Psychiatry 2013).

- We subsequently found that the genetic variants for ASD overlap with those for autistic traits in the general population. In this, using factor analysis, we could identify trait rigidity/compulsivity as the trait on which the ASD genetic risk loaded most strongly (Bralten et al., Mol Psychiatry 2018).

- Through our data integration approach, we also brought together all hitherto available genetic data on OCD and compulsivity, and developed a molecular landscape for such behaviour. The resulting landscape was enriched for proteins involved in regulating postsynaptic dendritic spine formation - and hence synaptic plasticity - through insulin-dependent molecular signalling cascades (van de Vondervoort et al., J Psychiatry Neurosci 2016).

- To validate the findings we performed a genome-wide association study on OCD traits in a population-based sample (CHOP) that we compared with clinical OCD data from the Psychiatric Genomics Consortium. Our results showed shared genetic aetiology between clinical OCD and three OCD traits as well as a significant association of the insulin gene-set to one of the OCD traits. The manuscript reporting these findings is currently being finalized (Bralten, Widomska et al., in preparation). Insulin-based signaling may thus provide an interesting lead for the development of new therapeutic approaches for OCD.
• We also used integration of different data sources to understand more of the links between disease, brain volume, and genetics in ADHD. With the information from the analyses in ENIGMA-ADHD (Hoogman et al., Lancet Psychiatry 2017) and our earlier GWAS analyses of brain volume (Hibar et al., Nature 2015 and Nature Commun 2017; Adams et al., Nature Neurosci 2016), we asked the question whether the genetic variants for ADHD identified in our recent GWAS (Demontis et al., Nature Genetics, in press), overlap with those influencing brain volume in regions known to be involved in compulsivity. Using a comprehensive battery of analyses, in which we investigated overlap both at the global genetics level and at the single variant/gene level, we showed a significant negative genetic correlation between clinical ADHD and brain volume especially for total brain volume and identified neurite outgrowth as contributing to the overlap (Klein et al., in revision). Performing such an analysis for OCD awaits the publication of a larger GWAS for this disorder, as we currently lack power.

• Through Bayesian causal modelling performed in collaboration with WP7, we could finally explain the existence of the literature of inconsistent findings from association studies of genetics, neuroimaging, cognitive performance in reward processing, and symptom severity of ADHD based on insight into dependencies of different factors in the analysis (Sokolova et al., Am J Med Genet Part B 2016).

For Task 4 (Genetics analyses of samples from WP4 (and WP6)), we were severely hampered by the limited sample size of the COMPULS cohort. However, we did generate polygenic risk scores for ASD, OCD, and ADHD for all individuals in the COMPULS study - based on the publicly available Psychiatric Genomics Consortium data. A polygenic risk score provides a single number representing an estimate of the burden of genetic risk (based on common genetic variants) of an individual. Integration of those scores into the COMPULS database provides researchers using the e.g. neuroimaging and cognitive data the ability to take into account genetics as a single variable.

In Task 5 (Proteomics analyses of samples from WP4 (and WP6)), we performed several studies, the highlights including the following:

• We have performed a proteomics analysis in patients with ASD (in which compulsivity/rigidity is an important symptom) and controls. This analysis identified several biological pathways involved in disease severity (Ramsey et al., Mol Autism 2013).

• Being hampered by the limited sample size of the COMPULS cohort, we found an additional way to investigate biomarkers for OCD and related traits and disorders. For this, we used a genetic integration analysis, in which we combined genome-wide genetic data on OCD with those on blood/serum/plasma levels of multiple metabolites (available from literature). This allowed us to e.g. confirm the role of insulin-based signaling in OCD and its constituent traits and identify several additional pathways of interest (Widomska et al., in preparation).

Conclusions

TACTICS allowed us to produce a large translational data set, and also got us in touch with several other international consortia in which TACTICS members often took on a leading role. Through those, we were able to make substantial progress in the understanding of the molecular underpinnings of OCD, its constituent traits, as well as related disorders and (endophenotypic, brain) features. Most importantly, we identified and confirmed brain-based insulin signalling as an important biological process disturbed by OCD and compulsivity. This finding provided input for the generation of a new animal model, which was shown to be highly compulsive. Furthermore, it provides an interesting lead for future studies aiming at the generation of a novel therapeutic approach for OCD and the cross-disorder symptom of compulsivity. An important additional concept tested and confirmed as part of TACTICS, is the continuum between psychiatric disorders and related population behavioural traits. We were convincingly able to show such continuity for ASD, ADHD, as well as OCD.

References


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**WP06  Pilot testing of medication in clinical paediatric populations (GOAT)**

**Background**

Compulsivity is a cross-disorder trait underlying phenotypically distinct psychiatric disorders that emerge in (early) childhood (ASD, OCD), or adolescence (addiction to substance use). Compulsivity is defined as the repetitive, irresistible urge to perform a behaviour, the experience of loss of voluntary control over this intense urge, the diminished ability to delay or inhibit thoughts or behaviours, and the tendency to perform repetitive acts in a habitual or stereotyped manner (Chamberlain et al. 2006). In this study, the focus was set on compulsive behaviours across the different clinical phenotypes and the developmental links between compulsivity and impulsivity.

Obsessive Compulsive Disorders (OCDs) are characterized by repetitive thoughts, impulses or images (obsessions) and repetitive behaviours or mental acts (compulsions). Autism Spectrum Disorders (ASDs) are characterized by deficits in (i) reciprocal social interaction and (ii) communication, and by (iii) restricted, repetitive and stereotyped patterns of behaviour, interests and activities. Compulsivity and the closely associated impulsivity trait are characterized by behavioural disinhibition maintained by maladaptive frontostriatal circuits (Fineberg et al. 2010). Compulsivity has also been considered an overarching concept that includes both failure to resist an impulse or an urge (impulsivity), maladaptive habitual behaviours (addiction), repetitive motor behaviours, ritualistic and stereotyped behaviours, and feelings of loss of control (ECNP, 2010). Glutamatergic projections to and from frontal subregions to the striatum play a key role in the regulation of various compulsive behaviours in humans including: 1) a maladaptive habitual pattern (addiction), 2) repetitive motor behaviours (stereotypy in ASD) and 3) the feeling of loss of control (OCD). Glutamatergic imbalance in frontostriatal regions has been observed, e.g., in childhood OCD (MacMaster et al. 2008).

This suggests that impaired functioning of both frontal and striatal areas may be due to a common underlying pathophysiology, such as dysregulation of glutamatergic mechanisms and glutamatergic genes. Preclinical animal models of compulsivity support glutamatergic interventions, e.g., excessive stimulation of the orbitofrontal cortex and the anterior cingulate may result in the generation of excessive, erroneous glutamatergic signals to the basal ganglia, leading to compulsive behaviour. Rodent studies of the TACTICS project will add to pre-existing knowledge by, e.g., providing proof-of-concept predictive data for glutamatergic medication strategies in both male and female models, with high relevance for the exploratory clinical studies in paediatric populations with OCD and ASD outlined in the (GOAT) study protocol.

**Objectives**

1. To investigate clinical effectiveness of the glutamatergic compound memantine in paediatric patients with OCD and ASD
2. To explore tolerability and safety (based on laboratory measures, adverse events) of the compound in these clinical indications
3. To explore the effects of glutamatergic interventions at the level of the structure, function and biochemistry of the frontostriatal circuits

**Summary of Study Design**

This 12-week study had a randomized, double-blind, placebo-controlled add-on design of treatment with a glutamatergic compound (mantine), including an up-titration phase (forced flexible dose design), in paediatric patients with OCD or ASD. Participants were assessed at various points in time according to the schedule of events.

Psychiatric and behavioural data were collected, including measures of (a) rigid and compulsive patterns of behaviour that are appropriate for these disorders; and (b) MRI and MRS data were collected for subgroups, meeting inclusion/exclusion criteria of the COMPULS study protocol from the TACTICS project. (c) Blood was collected for genetic analyses and for biomarkers. Sample size: 50 patients with OCD and 50 patients with ASD centre were planned to be enrolled.

**Results**

Since there were a number of unexpected and unforeseen difficulties and challenges to implement and run this study, the planned milestones and above objectives could be achieved only to a limited degree. These obstacles included, e.g., choice of study compound had to be based on an extra literature review instead - as planned - on animal study findings from the TACTICS project; ERB and regulatory requirements/requests; study medication supply; management of SAE reporting; and, patient recruitment in particular.

At the time of this reporting (end of June 2018), the achievements for this study comprised:

- All the organizational preparatory and implementation tasks for the GOAT study were finished for all 4 sites
- ERB approvals as well as regulatory and research agency approvals had been achieved for all sites (including a new satellite site to Nijmegen, i.e. Groningen)
- Intensive pre-screening and recruitment activities had been implemented.
- A literature review (extra task) and the study protocol were published in international journals.
- N=7 patients were enrolled (only; out of > N = 200 pre-screened), 2 are still ongoing; 3 finished the study according to protocol, 2 dropped out before the end of the study (discontinuation); there were no serious adverse events reported from the study, and no early drop-outs due to adverse events.

**Conclusions**

- In general; this study could be implemented and performed with the given design according to the protocol at all 4 sites/in all 3 countries, but unfortunately with substantial delays.
- Analyses as to, e.g., ‘clinical effectiveness and tolerability/safety’ will be limited to descriptive statistics from this case series and single case findings; the study has stopped recruitment (due to: official end of TACTICS project, expiration of study drug), but is currently still ongoing/treating single patients to the end of the observation period.
- A thorough and comprehensive analysis and review of the complexities, circumstances, challenges and delays - with respect to planning, implementing and managing a paediatric psychopharmacology clinical trial in the set-up of a publicly funded project - for an international group of university departments is strongly recommended and intended, and should lead to an international publication and, thus, potentially to further discussions/solutions in the fields of paediatric psychopharmacology and child and adolescent psychiatry, and beyond.

**References**


WP07  Machine learning

Background: An increase of gathered data and a proper analysis of this data can potentially improve the treatment of patients, find unknown risk factors of diseases, or detect co-morbid disorders if the causal relationships are understood. To be able to infer valid conclusions from observed patterns in the data, however, advanced machine learning techniques are needed. A major problem in causal discovery is the uncertainty about the interpretation of inferred correlations illustrated by the well-known statement in statistics: ‘correlation does not imply causation’. For example, yellow teeth and having lung cancer are highly correlated, but neither one is a cause for the other. The correlation can be explained by another factor, smoking, which influences both yellow teeth and lung cancer. With a causal model one can explain which interventions may have an effect on the outcome and which may not. In the example, quitting smoking will reduce the risk of lung cancer whereas whitening your teeth will not. Whereas in this example how to interpret the causal nature of the factors involved is clear, this is often not the case for disease mechanisms.

Overall Objectives

1. The first goal was to build an integrative framework for multi-task and multi-source learning that can handle heterogeneous medical data.

2. The second goal was the application of the developed methods to real-world data sets gathered within the TACTICS project to provide more insight into the underlying mechanisms.

Results

We have built a multi-task and multi-source learning tool for causal discovery that extends previously existing algorithms by relaxing several assumptions these methods typically rely on, namely data obeys a Gaussian distribution or is discrete, and data has no missing values. These assumptions are typically unrealistic for data from the medical domain. We developed a method that can infer the structure of a causal graph when the data set contains both discrete and continuous data, where continuous data is not necessarily Gaussian distributed. In addition the method can deal with data missing at random, which is less restrictive then data missing completely at random often assumed by alternative methods [1, 2, 3]. In [4] we have proposed a new method for estimating the reliability of causal statements which has been incorporated in our own tool, but can in principle also be used in other causal discovery techniques.

The causal discovery method that we developed has been used on several real-world data sets, in particular data about ADHD.

Our aim was to use causal discovery to provide a better understanding of the aetiology of ADHD, to develop a large model that can include different potential risk factors, and understand the interactions of ADHD with other disorders that often co-occur with ADHD, such as aggression and ASD. We used our algorithm to determine whether there is a direct or indirect link between a candidate gene for ADHD and brain activation during a monetary incentive delay task [5]. The causal model inferred from the data showed that there is no direct link between these two variables; however, there is an indirect path between them. This finding might explain existing discrepancies in the current literature, but to make solid conclusions further experiments should be performed.

In [6] we applied causal modelling to answer the question whether inattention drives hyperactivity/impulsivity or hyperactivity/impulsivity drives inattention in ADHD. Based on the results of the causal modelling the most probable model explaining the conditional independencies observed in the data is the model where inattention causes hyperactivity/impulsivity. We discuss our results providing a historical context and relate our results with other studies that showed different aetiology of the hyperactivity/impulsivity for subjects that have a high level of inattention from subjects with a low level of inattention.

In [7] we studied the interactions between ADHD and its co-morbid disorder ASD. The causal modelling builds a more complete model than standard statistical techniques, distinguishes between direct and indirect associations, and allows us to make preliminary predictions about causation. Applying causal modelling to a large phenotypic data set suggested three distinct pathways between the disorders where the strongest link was found between social communication difficulties, inattention, and impulsivity. These findings may help future studies on understanding the (pathophysiological) mechanisms behind the overlap between ASD and ADHD.

Conclusions

In conclusion, the methods that we developed provide a means to infer causal relationships from observational data and provide several examples of its application to real-world medical data. Based on the inferred models new hypothesis for experimental studies are proposed that can help scientists to understand the mechanisms of ADHD.
**WP08 Business development and dissemination**

**Background**

This work package has dealt with the dissemination and valorisation efforts within the TACTICS consortium. As such it is tasked with increasing the visibility of TACTICS by reaching out to the scientific community, industry, patient organisations and other interested or potential stakeholders. In addition, it seeks to identify areas of TACTICS output which may serve as starting points for valorisation, commercialisation and future efforts requiring industry support.

**Overall Objectives**

- Make TACTICS known to the scientific community and the public.
- Disseminate the results to the scientific community in the academic, healthcare and pharmaceutical sectors and foster interaction and exchange with the scientific community and the public.
- Identify and valorise the intellectual property rights (IPR) on biomarkers of compulsivity generated within WP1-7.
- Initiate next steps for full scale clinical trials of the most promising candidate drug identified in WP6 to treat childhood compulsivity in OCD, ASD and ADHD – high risk of substance abuse populations.

**Results**

The TACTICS Consortium was highly active in dissemination and resulted in a number of out-reach initiatives including the consortium website at www.tactics-project.eu. At the end of the project, we have now produced 84 peer-reviewed publications, 10 papers as conference proceedings, 9 theses / dissertations, have submitted many more manuscripts, anticipate to submit at least 10 more manuscripts to high-impact journals within the next year, have given multiple lectures on TACTICS-related topics, organized a TACTICS symposium at the ECNP congress in Amsterdam 2015 and at the Dutch Neuroscience meeting 2018, presented TACTICS results at international meetings, and participated in over 170 dissemination activities for the general public, patient organisations, the scientific community and other stakeholders.

WP1 and WP5 have generated strong evidence that insulin signalling markers could be used as a biomarker of compulsivity but this requires additional validation in independent cohorts prior to any valorisation steps. Equally, while white matter marker changes have been documented across both animal and human cohorts demonstrating compulsive behaviour, this affects white matter tracts that have also been implicated in other conditions. As such, the selectivity of such markers needs to be confirmed. The utility of the causal modelling platform developed within WP7, namely an algorithm and software for causal modelling, has...
resulted in concrete valorisation steps: A spin-out company has been formed (Machine2Learn), and their services have already been applied in independent FP7 and Horizon 2020 projects.

The GOAT study in WP6 did not provide a conclusion on the clinical efficacy of memantine in compulsive syndromes (WP6), the preclinical data in WP1/2 did also not support the utility of memantine because it did not show robust anti-compulsive action across preclinical models. No recommendation can be made for the initiation of full scale clinical trials with glutamate modifying agents. Further follow-up studies with agents modifying insulin-related signalling are warranted, but will require preclinical hit-to-lead, lead optimisation, and drug repurposing efforts prior to considering clinical studies.

Conclusions

- Good project visibility to the scientific and medical community, patient organisations, and the public was accomplished. The project website (www.tactics-project.eu) serves as the main public platform for TACTICS news and information.
- 84 peer-reviewed publications and about 10 more manuscripts currently in preparation
- Successful organisation of two TACTICS symposia (at ECNP 2015 and at Dutch Neuroscience 2018)
- 170 dissemination activities for the general public, patient organisations, the scientific community and other stakeholders.
- The data did not justify efforts to initiate future clinical trials related to glutamate modifying agents in compulsivity.
- Insulin-related and white matter-related markers of compulsivity were established in preliminary phase studies, but require additional validation for selectivity.

WP09 Ethics and Training

Background

The ethics and training work package was a key work package, as the studies in the TACTICS consortium involved both animals and children. It was therefore essential that the work conducted in the consortium was held to the highest scientific and medical ethical standards. This was addressed in several ways.

Overall Objectives

- Ensuring that the highest standards for the scientific and medical ethical aspects of our studies are set and met.
- Monitoring the adherence of sites involved in data collection to standard operating protocols (SOPs), quality standards and implement relevant training strategies regarding inter-laboratory and inter-rater reliability, assessment measures, instruments and procedures including their precise application, evaluation, documentation and transfer of results
- The studies were conducted in compliance with
  - European Directive 86/609/EEC
  - Recommendation 2007/526/65/EC
  - Legal European Union & national regulations
  - Local animal use and medical ethical committees
- Studies started only after
  - protocols were approved by the responsible ethics committees and competent authorities
  - the relevant approvals were sent to the commission

Preclinical studies (work packages 1 and 2)

Task 1 Ethical use of animals
  - Minimise animal use & suffering
  - High quality animal husbandry

Task 6 Standardise preclinical cross-laboratory training & common SOPs
  - Overlapping preclinical behavioural readouts and microdialysis / HPLC procedures will use a shared SOP
  - Standardisation of behavioural assessment
  - Standardisation of microdialysis / HPLC procedures
Getting approvals by the responsible ethics committees

Clinical studies (work packages 3, 4, 5 and 6):

Task 2  
*Informed consent & assent*
- Follow GCP standards

Task 3  
*Protection of research participants’ confidentiality*
- Follow European Guidance for Healthcare for Professionals on Confidentiality and Privacy in Healthcare

Task 4  
*Data Monitoring and Safety Board*
- Was integrated with Scientific, Clinical and Ethical Advisory Board
- Analysed criteria for discontinuation single subjects & study

Task 5  
*Care and protection of research participants*
- Linked to hospitals with emergency services
- Burden was minimized, incl. min invasive procedures

Task 7  
*MRS, MRI, blood sampling protocols*
- Shared training across sites for imaging (break-out session & site visits – IoP & UMCU)
- SOP for blood sampling – RUNMC-Gen & UCAM

Task 8  
*Pilot clinical pharmacological studies training (WP 6)*
- GCP guidelines for training procedures: working group to appraise assessment tools & their availability, estimations of required training procedures & need for interrater agreements, GCP certification of research staff, and providing a standard of rating via meetings

Results

Task 1 and Task 6 in WP 1 and 2
- SOPs for ethical use of animals were developed in the first year of TACTICS and training for preclinical work packages 1&2 took place at the GA meeting in Munich, March 2013.
- SOPs for standardisation of biological and behavioural assessments were also developed in this period and were part of the training in Munich.
- Medical ethical approval was gained for all preclinical studies in the period 2012-2014 (approval for one study predated the consortium and was already available in 2010). The approval letters were filed with the third periodic report.

Tasks 2, 3, 4, 5, 7 and 6 in WP 3, 4, 5 and 6
- All studies involving human subjects were conducted following GCP standards and the European standards for confidentiality. Study procedures were monitored locally at individual sites, in line with the monitoring plan, and a monitor from RUNMC visited some sites to ensure standards were being met (Tasks 2, 3 and 5).
- A report on compliance with informed consent procedures was drafted and filed (MS123).
- The DSMB was established on March 31st 2012, integrated with the SCEAB. One of the members, Dr. Ulrike Schulze acted as an independent ethics consultant for the duration of the consortium (task 4).
- SOPs were developed for human data collection, and training was completed in the course of 2013 and 2014 (task 7). A report was filed in April 2014.
- There were two independent training sessions for clinical pharmacological studies, on January 29th 2015 and on October 13th and 14th, 2016 (task 8). Instructions were developed and included for training for CRF and query, drug handling, informed consent, monitoring, plasma level, SAE, pregnancy report, pseudonymisation, randomization, and unblinding.
- Medical ethical approval was acquired at each site before data collection commenced. The letters of approval were submitted with the third Periodic Report, except for two that were still pending. These have been submitted with this final report.
- Site initiation visits were conducted at each site before commencing the studies.
- Reliability of experimental measures across sites was assessed and a report filed (MS120).

Conclusions

All studies were conducted in compliance with the high scientific and medical ethical standards put forth in the grant proposal.
WP10 Project Management

Effective project management is a central element of successful research. This is because large research projects often entail a lot of administrative work which needs to be dealt in an efficient and timely manner. In view of this, the purpose of WP10 was project management for the TACTICS project. This WP took care of all administrative and coordinating tasks.

To ensure compliance of the beneficiaries with their obligations under the grant agreement, the project management office at concentris routinely supported the Coordinator in monitoring the partners’ performance, for example in form of monthly telephone conferences of the steering committee (SC). Specific management responsibilities were to ensure the following:

- That tasks assigned to each partner were performed correctly and in a timely manner.
- That all reports were submitted according to the guidelines and on time.
- That all funds were used and claimed according to the EU guidelines and rules.
- That all partners followed the rules for dissemination, funding acknowledgements and intellectual property rights.
- That any changes to the work plan were communicated to the European Commission (EC) efficiently.
- That all project work and activities were compliant with the ethical guidelines.

The project management office acted as a helpdesk for all participants. It was the central node of communication on a day-to-day basis and communicated with the European Commission on behalf of the Coordinator regarding administrative and managerial issues (i.e. initial contract negotiations, amendments, reportings).
4 Potential impact, main dissemination activities and exploitation of results

4.1 Socio-economic impact and wider societal implications of TACTICS

Social and economic impact

Compulsivity and compulsivity-related disorders affect altogether more than 10% of the paediatric population worldwide. In more than 50% of the cases, the disorders have a chronic persistent course far into adulthood. It is precisely this combination of high prevalence and strong persistence which poses a major medical and socioeconomic problem in our society, and leads to substantial economic costs. For example, the cost of illness of autism is very similar to that of schizophrenia, and in the USA and Australia is estimated at between 3.5 and 5.0 million US dollars per patient lifetime! The direct and indirect costs of ADHD are estimated at 15,000 US dollars per patient per year (Pelham et al., 2006). Reports of the European Brain Council in 2005 indicate that the annual costs of addiction are around 10,000 € per patient per year, and of OCD around 800 € per patient per year. Current treatments have limited efficacy or only benefit a subsample of the clinical population. Designing new and more effective interventions requires a much better understanding of the neural, genetic, cognitive, and biomarker mechanisms involved in compulsivity. Despite protracted public health campaigns, addictive behaviour, especially adolescent smoking and alcohol abuse is not declining, particularly among females and lower income groups.

Impact on health care

The original plan was for TACTICS to provide efficacy and safety data of more effective medication for OCD, ASD and high-impulsive subjects who are at increased risk to develop addictive behaviours. These data then would have been an important step to develop Paediatric Investigational Plans and submit requests for Paediatric Use Marketing Authorisations to EMA. However, although the clinical studies performed in TACTICS provide evidence for the involvement of the glutamate system in the functioning of the frontostriatal system and in compulsive behaviour, data from the literature review, more recently published clinical studies as well as the results from our preclinical studies significantly temper enthusiasm about the clinical relevance of glutamatergic interventions for compulsivity disorders in general. Though on itself a negative result, an important message for clinicians is that more general glutamatergic interventions for compulsivity cannot be supported. This does not preclude that in the context of personalized medicine approach, patients with compulsivity disorders and identified alterations of the glutamate system may benefit from glutamatergic interventions. The feasibility for such an approach has recently been demonstrated (Elia et al., Nat Commun 2018 Jan 16; 9(1):4). This study showed that fasoracetam, a metabotropic glutamate receptor (mGluR) activator, was very effective in patients with ADHD with alterations in core glutamatergic genes, but was not effective in patients with ADHD with alterations in genes that were more remote from the core glutamatergic genes.

TACTICS has also provided new information on risk factors for the development of compulsivity and the link with addictive behaviour. The prediction of later substance use disorder (SUD) and nicotine dependence (ND) was related to a complex set of variables. Risk factors for later SUD and ND were persistent ADHD, severe ADHD, late-onset ADHD, and positive family history for SUD. The ADHD effect was in part mediated by Conduct Disorder problems. A protective factor in individuals with ADHD was early initiated and high-dose stimulant treatment for ADHD. Severity of ADHD inattention symptoms was linked to later gaming. Our progress in understanding this set of risk factors will lead to the development of specific preventive interventions targeting compulsivity-related behaviour in childhood. The further identification of neural, cognitive, genetic and biomarkers from our neuroimaging, cognitive and genetics work will further impact on strategies for stratification and early intervention in at risk groups.

Impact on psychopathology and neuroscience research

TACTICS has brought several conceptual innovations to the field of psychiatry and neuroscience. In fact, the studies in TACTICS started within a cross-disorder perspective, aiming to dissect “conventional” categorical disorders and examine shared behavioural traits and shared cognitive and neural mechanisms across ASD, OCD and ADHD. This was even before the NIH launched the Research Domain Criteria Initiative (RDoC) (https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml). RDoC is a research framework for new approaches to investigating psychiatric disorders. It integrates many levels of information (from genomics and circuits to behaviour and self-reports) in order to explore basic dimensions of functioning that span the full range of human behaviour from normal to abnormal. The goal is to understand the nature of mental health and illness in terms of varying degrees of dysfunctions in general psychological/biological systems. TACTICS is among the first research programmes to demonstrate the feasibility and relevance of this approach by outlining cross-disorder shared cognitive, neuroimaging and genetic mechanisms of ASD and OCD, and of ASD and ADHD.
Opening up new research avenues and identifying new drug targets

While the studies on glutamate did not lead to further large scale clinical trials with glutamatergic interventions and planned biomarker research was hampered by the difficulties with GOAT, the genetic studies in TACTICS were highly successful. Integration of information across different types of existing genetic studies led to the identification of an entirely new biological process involved in compulsivity and OCD: insulin-related signalling. The involvement of insulin-related signaling in compulsivity was successfully validated through the generation of a mouse model and by additional confirmation in two mouse models with altered insulin-related signalling. Importantly, insulin-related signalling is a "druggable" biological process, which could make valorisation possible after further validation research has been successfully conducted. A new grant proposal for further evaluation of the relationship between insulin-related signaling/disorders and compulsivity is currently in process, led by RUNMC and DTID.

Promoting international collaborations

The data bases collected by the TACTICS team and the created synergy between the research teams has also led to successful applications for and links with other funded EU projects such as Aggressotype, MATRICS, IMAGEMEND, CoCA and Eat2beNICE. TACTICS has further facilitated the further set-up and development of the ADHD and the ASD working groups within NIH sponsored ENIGMA programme (http://enigma.ini.usc.edu/) (Enhancing Neuroimaging Genetics through Meta-analysis). TACTICS was further able to build links with two successful IMI programmes EU-AIMS and PRISM.

New tools

The TACTICS team also developed an algorithm to allow pattern recognition of preclinical data sets. Preliminary discussions have started with Noldus B.V., a software development company marketing software solutions for animal research, about whether further valorisation can be enabled within their infrastructure and existing products, e.g. EthoVision.

Training and supervising your scientists

An important spin-off of TACTICS has been the opening up of career opportunities of numerous young scientists. They have been supervised and coached by local and international experts of different disciplines, been facilitated to start publishing first-authorship peer-reviewed papers, and finalized their PhD thesis to obtained their doctorate degree.

Dissemination and communication

TACTICS has had a major impact on the field of psychiatry and neuroscience research by its many scientific peer-reviewed and high-impact publications, multiple poster and oral presentations at scientific meetings of prestigious international societies such as ECNP, FENS, ADHD World Federation, Eunethydis, and the International Society for Psychiatric Genetics meetings (for full details, see section 4.2 below).

4.2 Main dissemination activities of TACTICS

Flyers, newsletters, interviews & articles published in the popular press

In the beginning of the project (November 2012), the official TACTICS flyer (info brochure) was designed, printed and distributed to all partner institutions. The flyer was used to inform study participants, parents and relatives, the general public, as well as the scientific community about the main goals of the project, the preclinical background studies, who the consortium members and the coordinator were, and what groups of patients should participate in and benefit from the project.

In January 2018, Science Magazine published a popular press article about the ENIGMA project, a higher-order international project that brought together 900 researchers across 39 countries to analyse and contribute data from magnetic resonance imaging (MRI) brain scans and genetic data of more than 30,000 people to reveal the underlying mechanisms behind brain disorders. The TACTICS project has also contributed data to this large undertaking, and was interviewed for this article: http://www.sciencemag.org/news/2018/01/world-s-largest-set-brain-scans-are-helping-reveal-workings-mind-and-how-diseases

On a biannual basis (Spring and Fall), the TACTICS newsletter was disseminated within the consortium, announcing the most recent scientific developments of the project, new colleagues within the research teams, important deadlines, and reminders to register for upcoming conferences and meetings.
Organisation of workshops & symposia

The TACTICS consortium was keen on emphasising international collaboration as well as the educational mission associated with academic research. Within this realm, we organised the following 4 workshops and 2 symposia:

- The endophenotype concept: developmental issues, August 2014, Nijmegen, The Netherlands
- Translation from neuroscience to clinical practice, June 2015, Stockholm, Sweden
- How to conduct longitudinal studies, March 2016, Antalya, Turkey
- Emotion processing in autism, June 2016, Cologne, Germany
- Traits in the general population: A solution for genetic studies of psychiatric disorders, symposium at the 25th World Congress of Psychiatric Genetics (WCPG), October 2017, Glasgow, Scotland (UK)
- Translational approaches to compulsivity, symposium at the Dutch Neuroscience Meeting, June 2018, Lunteren, The Netherlands

Oral presentations, posters & exhibitions at scientific events

A lot of basic, preclinical and clinical research efforts went into the TACTICS project. This resulted in 84 peer-reviewed publications, 10 papers in form of conference proceedings, and 9 theses / dissertations. Given the large amount of data, it is not surprising that the consortium was most active within this dissemination category. In total, more than 170 dissemination activities occurred since the beginning of the project. Amongst those, 128 oral presentations were given and 35 posters were presented at scientific events all over Europe, in the United States of America (USA), and even in Canada, Turkey, Lebanon, Israel, Russia, Australia, Singapore and India. This included major international gatherings of experts, such as annual meetings of the Society for Neuroscience (SfN), the Society for Biological Psychiatry (SOBP), the International College of Psychopharmacology (CINP), the European College of Neuropsychopharmacology (ECNP), the American Professional Society of ADHD and Related Disorders (APSARD), the Organisation for Human Brain Mapping (OHBM), the International Society for Magnetic Resonance in Medicine (ISMRM), the European Network for Hyperkinetic Disorders (Eunethydis), the International Society for Research on Impulsivity (InSRI), the International Scientific Board of Experts on ADHD (ISBEA), and the annual World Congress of Psychiatric Genetics (WCPG). The scientific results and core messages of the TACTICS study, thus, were received by a widespread audience. The events that received the most attention (with an estimated 200-600 number of attendees) were the following talks and presentations:

- The effect of smoking on the development of frontal cortical thickness in participants with a history of attention-deficit/hyperactivity disorder and healthy controls: a longitudinal study, October 2016, Berlin, Germany (600 attendees)
- Causal discovery for dopamine transporter haplotype and reward-related brain activation for adult ADHD, September 2013, Nijmegen, The Netherlands (500 attendees)
- Effect of smoking on frontal cortical thickness: a longitudinal study in participants with a history of attention-deficit/hyperactivity disorder and healthy controls, September 2016, Austria (about 500 attendees)
- What is the relation between ADHD and other neurodevelopmental disorders such as autism and schizophrenia?, April 2017, Vancouver, Canada (500 attendees)
- Functional connectivity patterns between putamen and anterior cingulate cortex during response inhibition in smokers and non-smokers, August 2015, Amsterdam, The Netherlands (400 attendees)
- Deficit hyperactivity disorder and autism spectrum disorders: two manifestations of one overarching disorder?, January 2017, Washington DC (400 attendees)
- Frontostriatal glutamate in compulsive and impulsive syndromes, October 2014, Berlin, Germany (300 attendees)
- The genetic and neural landscape of ADHD, February 2016, Beirut, Lebanon (about 300 attendees)
- Basal ganglia response to reward anticipation and receipt in Tourette Syndrome and ADHD, June 2017, Vancouver, Canada (300 attendees)
- The EU TACTICS Study: Translational adolescent & childhood therapeutic interventions in compulsive syndromes, May 2016, Singapore (200 attendees)
- The neurobiology of ADHD, September 2017, Apeldoorn, The Netherlands (about 200 attendees)
Presentations of 200-500 attendees that addressed scientists as well as the general public, policy makers and the media, were:

- What is ADHD?, August 2015, Netherlands, Amsterdam
- Research on ADHD in European consortia and the contribution of ADHD Europe, February 2016, Brussels, Belgium
- Autism as a complex and heterogeneous disorder, April 2016, Moscow, Russia
- ECNP Congress 2017 (booth, presentation, flyer, personal conversations), September 2017, Paris, France
- Psychopharmacology of autism, April 2018, Gothenburg, Sweden

**Website & Intranet**

In March 2012, 3 month after the funding of the project started, the official TACTICS website went online ([http://www.tactics-project.eu/](http://www.tactics-project.eu/), see screenshot below). It was updated on a regular basis (most recently in August 2018), and serves not only as a showcase of the project, but mainly to inform the general public, patients and relatives of the project’s key goals, the scientific background, the main objectives, news, events, and most recent results. It aims to convey complex scientific information in a comprehensive manner, and makes the project transparent by presenting all partner institutions, individual researcher profiles and profile pictures, as well as the tasks of each work package.

**4.3 Exploitation of TACTICS results**

Two major exploitable foregrounds have resulted from research performed over the course of the TACTICS project.

- Identification and both genetic and in vivo validation of insulin signalling mechanisms (specifically „K”) as a target for compulsive syndromes. This paves the way for the identification of „K” ligands as possible treatment opportunities of compulsive syndromes. The foreseen embargo date for commercial exploitation of the R&D results of the TACTICS project is January 2023.

- The consortium has built a multi-task and multi-source learning tool for causal discovery of biological drug targets that extends previously existing algorithms by relaxing several assumptions these methods typically rely on. This algorithm and software has been developed and valorised by WP07 members, and resulted in the formation of the spinout company “Machine2Learn” on 29 August 2017. The RUNMC team designed the business plan for this company in collaboration with the valorisation department of Radboud University and science management students as part of their Master’s thesis or internship.
## Address of the public website of TACTICS and relevant contact details

The aim of TACTICS is to identify, over a 5-year period, the neural, genetic and molecular factors involved in the pathogenesis of compulsivity, in order to develop a knowledge base and clinical trial methodologies that will work together to further understanding and treatment of obsessive compulsive disorders.

**Screenshot from the official TACTICS website: [www.tactics-project.eu](http://www.tactics-project.eu)**

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<td>Jan</td>
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<td>Jeffrey</td>
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<td>Sabine</td>
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<td><a href="mailto:sb209@cam.ac.uk">sb209@cam.ac.uk</a></td>
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<td>Florea</td>
<td><a href="mailto:IOFL@lundbeck.com">IOFL@lundbeck.com</a></td>
</tr>
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