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Chapter 1

General Introduction

Complex genetic traits

With his laws of inheritance presented in 1865, Gregor Mendel explained how biological characteristics, such as pea shape and flower color, were inherited (Mendel, 1866). The presence of only one genetic factor appeared to be necessary and sufficient to determine flower color, and the inheritance of this genetic trait therefore could be explained in simple terms. In the same year Sir Francis Galton, a biometrician devoted to quantifying human characteristics such as reaction time, described that many characteristics do not follow a dichotomous distribution but appear in several intermediate quantifiable flavors (Galton, 1865). He compared biological characteristics of parents and children, and established the degree of correlation between relatives as index of the heritability. The rivalry between the schools of dichotomous Mendelian geneticists and the quantitative biometricians was resolved in the 1918 by Sir Ronald Fisher. He statistically demonstrated that continuous characters as measured by biometricians could be governed by the action of a large number of Mendelian factors (Fisher, 1918), and the 'complex genetic trait' was born. The molecular inheritance of these factors and a functional mechanism by which they were transmitted to the offspring were not defined until 1953, when James Watson and Francis Crick published and discussed the structure of DNA (Watson & Crick, 1953) and suggested a DNA replication mechanism.

Over the years, variation in most human characteristics (even traits such as IQ) has turned out to be, albeit to different extent, genetic in origin and complex in nature. Many genes underlie human behavior and their unique combinatorial inheritance includes the risk to develop various prevalent psychiatric and neurological diseases, such as attention deficit hyperactivity disorder (ADHD), schizophrenia and drug addiction (Faraone & Biederman, 1998; Greenwood *et al.*, 2007; Kreek *et al.*, 2005). By identifying genetic variants in the human population that predispose individuals to disease, quantitative genetics can yield insights into the contribution of multiple genes to complex disease traits. This is of great importance given the fact that the molecular basis of most psychiatric and neurological diseases is at best poorly understood.

Dissecting complex traits into core elements will facilitate gene finding

In genetic linkage studies and genome wide association studies, the aim is to relate the presence of a specific genetic variant to the observation of a specific phenotype. Over the last decade it has become clear that for prevalent human psychiatric and neurologic diseases, with overall substantial heritability, it is difficult to identify the causal genetic variants. To date, association studies have cumulated evidence for a limited number of genes associated with the clinical diagnosis of psychiatric disease, e.g. ADHD (DRD4, DRD5, SLC6A3, SNAP-25 and HTR1B; Faraone & Mick, 2010) and Schizophrenia (NRG1 and DTNBP1; Munafo *et al.*, 2006 and Riley & Kendler, 2006). Together these risk alleles explain only a fraction of total risk contributed by genetic factors to these

diseases despite tremendous advances in SNP genotyping technology and increases in samples sizes owing to large collaborative projects. Two explanations for this 'missing heritability' are being discussed at the moment. First, many genetic variants may act together, each with a small effect (< 1.0 %) that can barely be detected in even the largest collaborative studies. Secondly, each affected person may carry a different variant with a modest effect size that would go unnoticed in current genotyping assays focusing on common genetic variants and would not be picked up by genetic linkage studies due to too low effect size.

Most psychiatric diseases are heterogeneous in nature and patients may have varying degrees of severity of each of the identified symptoms. For instance, in ADHD three subtypes are being recognized, characterized by symptoms of inattention, of hyperactivity/impulsivity, and a combined subtype. In fact, it is under debate whether symptoms of hyperactivity/impulsivity and inattention in ADHD reflect the same or separate disorders (Derefinko *et al.*, 2008; Diamond, 2005; Milich *et al.*, 2001). It is conceivable that quantitative genetic studies are facilitated when the disease phenotype is partitioned better into its core elements, to each of which a limited number of genetic variants contribute (**Fig. 1**; de Geus, 2002; Kas & Van Ree, 2004).

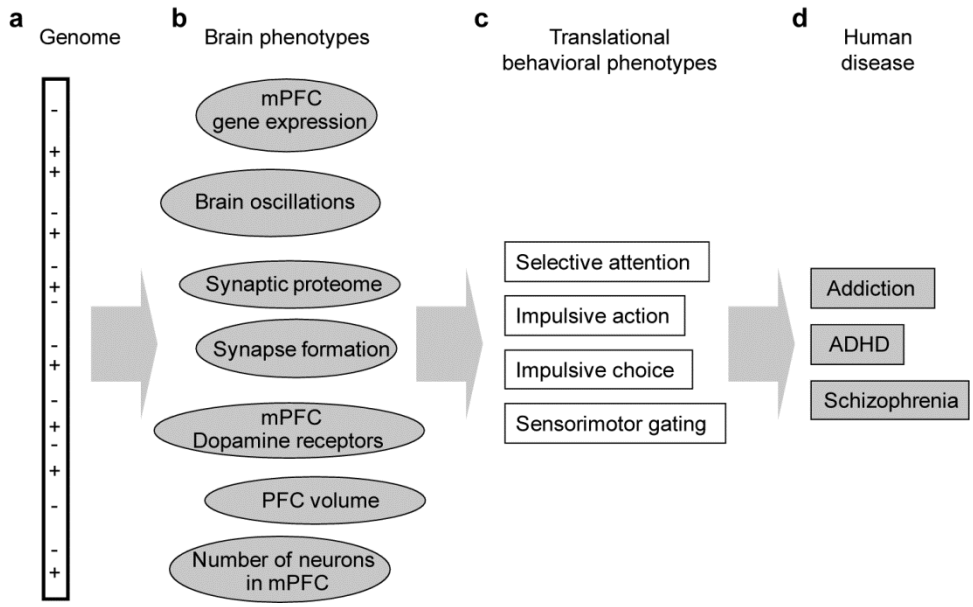

Figure 1 | The genetic architecture of complex traits. (**a**) Many genetic polymorphisms on the genome contribute to higher (+) or lower (-) phenotypic values of brain phenotypes (**b**; grey ovals) and behavioral phenotypes (**c**; white rectangles). These phenotypes are core elements that are intermediate to genome and disease (**d**; grey rectangles). They are genetically less complex than the disease itself and are therefore a suitable starting point for a genetic mapping study to identify disease-contributing genes. Schematic modified after De Geus (2002).

Translation of psychiatric disease symptoms into rodent paradigms

Executive functions are a diverse collection of cognitive processes such as rule acquisition, inhibiting inappropriate actions, planning, cognitive flexibility and selective attention. These executive functions have in common that they require an intact prefrontal cortex. Because these functions are highly demanding, they are sensitive to deterioration by e.g. ageing, chronic stress and occur under certain neurological conditions, such as in Alzheimer's and Parkinson's disease (Perry & Hodges, 1999; Rodriguez-Oroz *et al.*, 2009) and are affected in many psychiatric diseases, such as schizophrenia, ADHD and addiction.

In humans, deficits in executive functions can objectively be quantified in computerized response tasks. Heritability estimates clearly indicate that measures obtained in these tasks are sensitive to genetic effects (Bidwell *et al.*, 2007). Recently, the first linkage studies reported successfully employed neuropsychological measures to identify chromosomal locations contributing to deficits in executive functions that are observed in multiple psychiatric disorders (**Table 1**).

Table 1 | Mapping studies employing executive functions as intermediate phenotypes.

Chr. location	Clinical indication	Neuropsychological measure
12q24.32	Patients with schizophrenia	Sustained attention: undegraded CPT hit rate (Lien *et al.*, 2010)
3q13	Patients with ADHD	Wide range of neurocognitive traits associated with ADHD (Doyle *et al.*, 2008)
2q22.1	Patients with schizophrenia	False alarm rate on the undegraded and degraded CPT (Lien *et al.*, 2011)
2q21.1	Patients with ADHD	Variability in reaction time in a motor timing task (Rommelse *et al.*, 2008)
13q12.11	Patients with ADHD	Verbal working memory: Digit span backwards (Rommelse *et al.*, 2008)

These heritable core elements of complex human disorders play a pivotal role in the translation into genetic animal models of the disorder, for which a large suite of genetic, molecular and physiological techniques is more readily available than for human subjects. Indeed, recently a QTL was reported in a mapping study of a translation of a neuropsychological measure of behavioral flexibility (Laughlin *et al.*, 2011).

Two classes of executive functions are well studied in both humans and rodents, i.e., the capacity to selectively attend to stimuli (attention) and impulse control (i.e. preventing impulsive behavior). Searching for the underlying genetic components of these two aspects of behavior in rodents is the primary focus of this thesis.

Attention

The continuous performance task (CPT) of attentional processing (Beck *et al.*, 1956) has been adapted for rodents into the widely used 5-choice serial reaction time task (5-CSRTT; Robbins, 2002). In this task, rodents are trained to respond to a brief, usually 1 s light stimulus in one of five response holes. During one 5-CSRTT session that typically lasts for 60 to 100 trials during which rodents can obtain a reward by responding into the illuminated stimulus hole. Response accuracy is the primary measure of attention, and is defined by the percentage of correct responses into an activated response hole over the total number of correct and incorrect responses.

In addition, intra-individual variability in correct response latencies (hereafter referred to as response variability) has been recognized as measure of lapses in attention (Castellanos & Tannock, 2002; Leth-Steensen *et al.*, 2000; Loos *et al.*, 2010b; Sabol *et al.*, 2003; Sergeant & van der Meere, 1990). The interest in intra-individual variability in response latencies in human studies has grown over the last decade following the observation of increased intra-individual variability in ADHD (Bidwell *et al.*, 2007; Castellanos *et al.*, 2006; Castellanos & Tannock, 2002; Klein *et al.*, 2006; Leth-Steensen *et al.*, 2000; Spencer *et al.*, 2009). Whereas the peak of a response latency distribution could be viewed as an index of processing speed, larger intra-individual variability in this distribution has been interpreted as lapses in attention (Klein *et al.*, 2006; Sergeant & van der Meere, 1990).

A failure to respond into any stimulus hole after the presentation of the light stimulus, i.e. an error of omission, is a third measure of attention in the 5-CSRTT. This measure may reflect the inability to pay attention to the task in every trial. However, disruptions of locomotor behavior, the motivation to perform or the ability to execute a cognitive task in general can affect this parameter as well. For the interpretation of errors of omission in terms of deficits in attention it is therefore imperative to rule out alternative explanations, for instance by analyzing latency to collect rewards from the magazine in the 5-CSRTT or a test of locomotor function in another apparatus.

Impulsivity

Several operant tasks of impulsivity exist (Evenden, 1999; Winstanley *et al.*, 2006a), broadly separated into those that measure impulsive action and impulsive choice (Winstanley *et al.*, 2006a). Whereas impulsive action involves the loss of inhibitory control over pre-potent response patterns, impulsive choice reflects less beneficial decision-making processes. One frequently employed index to measure impulsive choice is the inability to tolerate delay of reinforcement. In such tasks, ADHD patients show increased preference for immediate small monetary over delayed larger – and therefore more beneficial – rewards (Barkley *et al.*, 2001; Solanto *et al.*, 2001; Sonuga-Barke, 2002; Sonuga-Barke *et al.*, 1992). Methylphenidate (Ritalin), currently the most commonly prescribed drug

for the treatment of ADHD, reduces impulsive choice in a delayed reward task in healthy human volunteers (Pietras *et al.*, 2003) indicating that this task relates to an important clinical symptom, i.e., an intermediate phenotype of ADHD. The rodent analogue of this task measures the preference for immediate small (1 food pellet) over delayed larger reward (4 food pellets).
With respect to impulsive action, currently the 5-CSRTT is the most frequently employed paradigm (Eagle & Baunez, 2010), which is similar to errors of commission in the CPT (Beck *et al.*, 1956). In the 5-CSRTT, a premature response to a food predictive stimulus is a measure of impulsive action. Other tasks of impulsive action require rodents to signal situations in which responding should be inhibited such as in a Go/No-Go task, or in which an already initiated response should be terminated such as in a stop signal reaction time task.

Dissecting complex traits by exploiting genetic and environmental variation
In laboratory animals, such as outbred Wistar rats, individual differences in impulsive action and impulsive choice have been documented that are stable over many days and weeks (Diergaarde *et al.*, 2008). As such, impulsivity is a quantitative trait and should have its molecular and cellular underpinning in frontocortical and striatal brain regions critically involved in impulsivity (for reviews see Cardinal, 2006 and Winstanley *et al.*, 2006a). However, because these strains of rats are bred to maintain a certain degree of genetic variation among individuals, the individual differences can arise from genetic and/or environmental differences among individual rats (Crabbe & Cunningham, 2007). Using such outbred populations of rodents, it is therefore possible to identify molecular correlates of attention and impulsivity, but more difficult to determine the percentage of contribution of genetic variation to a given trait.
In particular, genetically identical (isogenic) inbred strains of mice provide the opportunity to separate genetic and environmental factors (**Fig. 2**). When kept under highly controlled conditions, differences between inbred mouse strains result from additive genetic effects and gene-by-environment interactions (Crabbe *et al.*, 1999). In contrast, behavioral differences among isogenic mice of the same strain result from environmental effects idiosyncratic to each

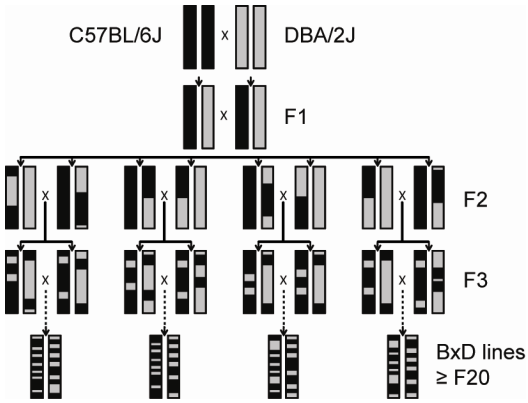

Figure 2 | Generation of BXD recombinant inbred strains of mice. The commonly used laboratory inbred strains C57BL/6J and DBA/2J were crossed (B × D, hence BXD) to obtain an isogenic F1 population. During F1 × F1 matings, genetic recombination occurs randomly at a rate of about 2 events per chromosome, yielding a genetically diverse F2 population. Recombination events continue to accumulate during inbreeding of BXD lines, until fully isogenic recombinant inbred lines are produced.

individual. Comparing within-strain (environmental) with between-strain (genetic) variation therefore indicates the degree of heritability of any given trait. Furthermore, panels of recombinant inbred strains of mice, derived from an intercross of common inbred strains can be used to identify quantitative genetic loci (QTL) underlying phenotypes such as impulsivity (see **Fig. 3**). Currently, the largest and therefore most widely employed panel of recombinant inbred strains was derived from C57BL6/J and DBA2/J mice (**Fig. 2**; Peirce *et al.*, 2004), which are renowned for their difference in alcohol consumption behavior (Crabbe *et al.*, 1983). Furthermore, mice of these two strains show profound differences in their brain dopaminergic system (Cabib *et al.*, 2002; D'Este *et al.*, 2007) that may be relevant for research on dopaminergic dysfunction in relation to psychiatric disease.

Because these panels of inbred mouse strains are retrievable genetic resources, they facilitate the systematic integration of data obtained in different studies. For instance, the available databases of brain gene expression of these strains aid to efficiently identify candidate genes within QTL regions (Chesler *et al.*, 2005).

Finally, genetic reference panels of inbred strains can be used to investigate the relation between any pair of phenotypes measured, from expression levels of proteins in the synapse to behavioral parameters. High correlation between two phenotypes across strains would indicate that these measures are controlled by common genetic or gene-by-environment interaction effects (*genetic correlation*), albeit such genetic correlations inherently contain some residual

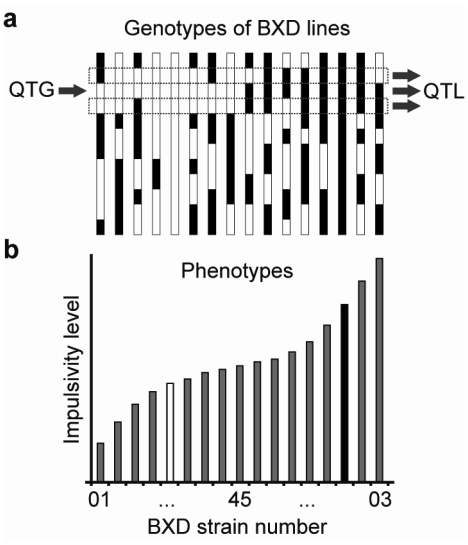

Figure 3 | Mapping a locus on the genome (i.e. QTL) harboring a quantitative trait gene (QTG) affecting the trait. (**a**).The genotype, i.e. whether the locus originates from founder C57BL/6J or DBA/2J, of each strain is determined at regular intervals across the genome (black denotes genotype C57BL/6J, white denotes genotype DBA/2J). The change in color within the genome of a strain reflects the occurrence of recombination between stretches of C57BL/6J and DBA/2J origin during generation of the strain. (**b**) Recombinant inbred strains can quantitatively differ in the expression of a trait, such as the level of impulsivity.

By statistically evaluating the difference in phenotype between carriers of a C57BL/6J versus DBA/2J allele at each locus, the probability can be determined whether a given locus is influencing the trait. The size of the locus is determined by the number and proximity of recombination events surrounding the QTG. The dashed lines indicate upstream and downstream genomic regions surrounding the locus with the highest probability for which – due to linkage – still a significant influence on phenotypes is detected. Due to this effect, a QTL region can become large and can contain up to a few hundred genes.

environmental variation if they are derived from experimentally estimated strain means (Crusio, 2006). In contrast, high within-strain correlations would indicate that these behaviors are controlled by common environmental factors (*environmental correlation*).

Aim and outline of this thesis

The aim of the research projects described in this thesis was to elucidate genetic factors responsible for individual variation in attention and impulsive behavior, in the context of the psychiatric diseases ADHD, drug addiction and schizophrenia. In addition to the symptoms of impulsivity and deficits in attention that these psychiatric diseases have in common, each of them is accompanied by the well-documented disease-specific symptoms of hyperactivity (ADHD), deficits in sensorimotor gating (Schizophrenia) and drug seeking (addiction). To investigate each of these symptoms separately, I therefore employed tailored assays in my thesis.

The aim in **chapter 2** was to understand the role of genes that have been strongly associated with ADHD in impulsive choice behavior. Rats are capable of performing a delayed reward paradigm and show individual variation in impulsive choice behavior (Diergaarde *et al.*, 2008). Correlation analysis between behavior and gene expression suggested a contribution of dopamine D1-like receptors located in the mPFC to impulsive choice behavior. The functional role of these receptors was demonstrated by the disrupting effect of infusing dopamine D_1-like receptor agonists and antagonists into a sub region of the medial prefrontal cortex (mPFC) on choice behavior.

In contrast to inflexible and unstable performance in a delayed reward paradigm, mice were capable of acquiring tasks that rely on sustained attention and inhibitory control, such as the 5-CSRTT and Go/No-Go task (**chapter 3**). Moreover, I observed differences in the expression of dopamine D1, 4 and 5 receptors in the mPFC of these strains, possibly related to the strain differences in behavior.

Differences in inhibitory control between common inbred strains appeared large and reproducible (**chapter 4**). However, both the locomotor response in a novel open field (Piazza *et al.*, 1989) and deficits in inhibitory control (Belin *et al.*, 2008; Dalley *et al.*, 2007) are predictive of addictive behavior, questioning to what extent inhibitory control and the response to novelty are genetically independent. Therefore, I measured the response to novelty in a range of different assays and identified the lack of a genetic relation between this response and inhibitory control in the 5-CSRTT, indicating that QTL studies into both phenotypes were valuable.

In **chapters 5 and 6**, I analyzed the QTL that (i) underlie attentional performance and inhibitory control in the 5-CSRTT, and (ii) sensorimotor gating in a test of prepulse inhibition. The identified QTL did not overlap for the two behavioral phenomena. I also analyzed the genetic correlation among any of these

phenotypes and did not identify a genetic correlation. Using data on single nucleotide polymorphisms (SNPs) and brain gene expression of these strains, as well as the association of QTL genes with diagnosis of ADHD and/or schizophrenia I selected candidate genes potentially involved in the respective behaviors.

In humans and rodents, increased impulsivity scores are predictive of enhanced addictive behavior (Belin *et al.*, 2008; Caspi *et al.*, 1996; Dawes *et al.*, 1997; Diergaarde *et al.*, 2008). Therefore, in **chapter 7** I investigated whether reduced inhibitory control, as observed in the highest impulsive BXD strain, would predict enhanced addictive behavior in a paradigm of alcohol-seeking behavior.

Finally, **chapter 8** summarizes the findings on intermediate phenotypes and discusses these in the context of the psychiatric diseases of ADHD, addiction and schizophrenia. Furthermore, several avenues into new research lines were opened during my thesis, and I discussed the most promising ones in this final chapter.