Genetic architecture and behavioral analysis of attention and impulsivity

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Chapter 8

General discussion

1. Discussion

1.1. Background

Of most psychiatric diseases the etiology is at best poorly understood, which hampers the development of adequate treatments. Psychiatric diseases are clinically diagnosed based on the presentation of a collection of behavioral symptoms. Among these symptoms, deficits in inhibitory control and attention are prominent features and are observed in multiple psychiatric diseases, such as schizophrenia, ADHD and drug addiction. The aim of this thesis was to identify genes and molecular processes that contribute to inhibitory control and attention, to provide entry points for understanding and treatment of the frequently observed deficits they underlie. Because inhibitory control and attention are complex cognitive functions, they rely on various brain systems and circuitries therein. They contain a substantial genetic component, as is clear from human genetic studies (Bidwell *et al.*, 2007). Due to this overall complexity they are considered 'complex traits'. In order to identify genes underlying these traits, adequate measures of these functions need to be available. As described in more detail in **chapter 1**, to study inhibitory control and attention in humans, specific computerized quantitative response tasks have been developed. These well-defined behavioral measures of brain function represent phenotypes that are intermediate to genome and the individual's behavior, and may therefore be influenced by only a limited number of genetic factors. As such, they facilitate gene hunting (de Geus, 2002). Importantly, several of these response tasks are readily adaptable for use with rodents, providing an entry into molecular (genetic) dissection, as described in this thesis.

1.2 Exploiting individual variation to specify the role of dopamine-related genes in impulsive choice behavior

Human genetic studies have identified a number dopamine-related genes associated with psychiatric disorders, most notably ADHD (Kreek *et al.*, 2005; Waldman & Gizer, 2006). Nonetheless, genetic studies in humans do not easily provide insight into the temporal and spatial aspects of aberrant gene function; this requires studies in model organisms.

Genetically modified mice are powerful tools to investigate the involvement of specific genes in a behavioral phenotype, such as impulsivity or attention, based on a priori knowledge on the function of the respective gene. By testing mutant mice in the 5-CSRTT, several genes have been implicated in impulsivity and/or attention (Bailey *et al.*, 2010; Davies *et al.*, 2007; Guillem *et al.*, 2011; Hoyle *et al.*, 2006; Lambourne *et al.*, 2007; Relkovic *et al.*, 2010; Romberg *et al.*, 2011; Young *et al.*, 2007; Young *et al.*, 2004). However, finding novel genes, not previously implicated in impulsivity or attention requires other strategies than targeted mutagenesis. In this thesis individual variation in cohorts of outbred rats

(**chapter 2**) and variation among (recombinant) inbred mouse strains was exploited for gene hunting (**chapters 4–6**).
In **chapter 2**, we aimed to further specify the role of dopamine related genes in impulsive choice, an important intermediate phenotype in ADHD (Barkley *et al.*, 2001; Solanto *et al.*, 2001; Sonuga-Barke, 2002; Sonuga-Barke *et al.*, 1992). Individual differences in levels of impulsive choice among rats were shown to correlate with variation in gene expression of Drd1, Drd5 and Calcyon, specifically in the mPFC. Subsequent pharmacological intervention showed for the first time that signaling through dopamine D1/D5 receptors was functionally involved in impulsive choice behavior.
In conclusion, this rodent study identified Drd1, Drd5 in the mPFC to be involved in impulsive choice, and in addition it specified the brain region in which these genes may lead to impulsive choice deficits in ADHD. However, implicating novel genes that have not been associated with impulsivity and/or attention before requires another approach, such as the mapping of QTL in this study.

1.3 Evaluating mouse tasks of impulsivity and attention for QTL analyses

QTL mapping studies require large cohorts of subjects - in our case BXD strains - that are typically tested in serial batches across multiple months. In some behavioral assays, differences between strains of mice are poorly reproducible, especially in cases where strain differences are subtle (i.e., when the genetic effect size is low; Crabbe *et al.*, 1999). For the purpose of a QTL study that requires multiple months of experiments, it is important to select a robust task that leads to reproducible results.
Operant tasks have the advantage that training can be performed systematically according to objective criteria programmed into an automatic analysis of training results, which enhances the consistency of training across multiple months. Furthermore, handling of animals, which is thought to contribute to idiosyncratic results, is limited to introduction and removal of animals in and from operant boxes thereby increasing reproducibility. Two operant tasks measuring aspects of impulsivity and attention analogous to computerized response tasks for humans were reported for use with mice, i.e., a Go/No-Go task (Logue *et al.*, 1998; McDonald *et al.*, 1998) and 5-CSRTT (Humby *et al.*, 1999). We therefore compared the 5-CSRTT with Go/No-Go task in the context of a QTL study.
We detected promising strain differences between the founder strains of the BXD lines (**chapter 3**) in both 5-CSRTT and Go/No-Go tasks. In the 5-CSRTT, we described behavioral effects of amphetamine in mice similar to those observed in rats (Cole & Robbins, 1987; Harrison *et al.*, 1997; van Gaalen *et al.*, 2006a) indicating that dopamine modulates inhibitory response control in this task as expected. Other neurotransmitter systems involved in inhibitory response control in this paradigm are those using GABAA, NMDA and serotonin 5-HT_2 receptor ligands (Fletcher et al. 2007, Oliver et al. 2009). In contrast, although

psychostimulants are known to affect inhibitory control in the Go/No-Go task in human studies (Trommer *et al.*, 1991; Vaidya *et al.*, 1998), we did not find such an effect in the Go/No-Go task, meaning that this murine task may be less well-suited as translational assay.
In further support of the 5-CSRTT, several studies genetically validated this task using mouse models of neurological disease that are characterized by deficits in attention and/or inhibitory control (Davies *et al.*, 2007; Lambourne *et al.*, 2007; Relkovic *et al.*, 2010; Romberg *et al.*, 2011). Furthermore, the nicotinergic system is critically involved in attentional processes (Mansvelder *et al.*, 2006) and mice lacking subunits of nicotinic receptors show deficits in attention (Bailey *et al.*, 2010) (Guillem *et al.*, 2011; Hoyle *et al.*, 2006; Young *et al.*, 2007; Young *et al.*, 2004).
The differences in impulsivity between C57BL/6J and DBA/2J mice in the 5-CSRTT detected in **chapter 3** could be replicated in **chapter 4**. In addition, we detected substantial heritability for 5-CSRTT parameters, suggesting that alleles segregate in the panel of BXD recombinant inbred lines that affect 5-CSRTT performance. Taken together, the 5-CSRTT appeared to be a good starting point for a QTL study for impulsivity and attention phenotypes in the BXD RI resource.

1.4 Discovering genes for impulsivity and attention using the BXD resource
In **chapters 5 and 6**, we observed a strong transgression in terms of impulsivity and attention phenotypes among BXD lines outside the behavioral range covered by the two parental lines. Together with the gradual distribution of these phenotype, this suggested the action of multiple genetic loci to these phenotypes, indicating that even these well-dissected behavioral phenotypes are genetically complex. Nonetheless, we detected QTL for attention (response variability) and impulsivity (premature responses), indicating that dissection of complex traits into translational behavioral phenotypes (**chapter 1, Fig. 1**) was a successful approach. These QTL showed striking specificity. Impulsivity and attention, of which deficits co-occur in ADHD, mapped to different loci. This is underscoring the relevance of intermediate phenotypes in genetic studies, in which a combined score of impulsivity and poor attention may have obscured the detection of these distinct QTL. The advantage of intermediate phenotypes is once more illustrated by the fragmentation of the response latency measure into its mode (also interpreted as sensorimotor processing speed; Sabol *et al.*, 2003) and standard deviation (i.e., response variability). Respective traits mapped to QTL at different locations on chromosome 16, showing that even relatively simple measures, such as response latency, are complex traits that can be further dissected in order to identify underlying genetic loci.
Prepulse inhibition is a presumed pre-attentive phenomenon that is affected in Schizophrenia (for review see Braff *et al.*, 2001), and hence was of interest in the context of studies on attentional performance in this thesis. Nonetheless,

measures of PPI were genetically independent from measures of attention in the 5-CSRTT, as shown by lack of genetic correlation and separate QTL, indicating that lower levels of attentional performance are not caused by deficits in PPI in the BXD panel. The QTL for PPI therefore harbors genetic variants specifically influencing PPI and not attentional performance in general, and for that reason is of relevance to Schizophrenia but not to ADHD and addiction.
Promisingly, none of these QTL harbored genes previously implicated in attention and impulsivity, suggesting that these QTL contain one or more novel genes that may help us to better understand these phenotypes. The power of previous human genome wide association studies (GWAS) on ADHD and Schizophrenia was insufficient to detect significant SNPs. However, the synergism between human GWAS and mouse QTL analyses pinpointed a limited set of genes in the QTL that are the most promising ones for follow up studies (i.e., Nrg3, Ppyr1, Mmrn2, Wapal and 4930474N05Rik).
Each of the detected QTL only explained a fraction of the variation observed among strains. This indicates there were multiple genetic loci with presumable lower effect size that we did not detect, hence confirming the idea that impulsivity and attention phenotypes are complex traits. At least two factors explain why we did not detect those QTL. First, the lower the additive effect of a QTL, the larger the sample size required to detect a significant QTL. For reasons of logistics, we were able to test approximately 40 BXD lines in this study. According to simulation studies this results in a power (power = 0.80, $p < 0.0001$) to reliably detect QTLs accounting for ~40% of the strain variance (Belknap *et al.*, 1996; Peirce *et al.*, 2004). Second, another complicating issue is the effect of genetic interactions, or epistasis. Testing for genetic interactions requires testing of epistatic interactions between all pairs of loci in the genome. This inflates the number of statistical tests and therefore requires even higher numbers of strains than used for single QTL. Obviously, the number of strains in the current studies was insufficient to detect these interactions.

2. Perspectives

2.1. Novel behavioral assays to measure mouse cognition at higher throughput

The power to detect QTL, let alone epistatic interaction between QTL, was limited in the current study. For reasons of limited recombination rates, it is unlikely that all genes contributing to a trait (quantitative trait genes; QTG) can be detected in the BXD panel. The current panel of BXD strains contains approximately 4100 recombinations (Peirce *et al.*, 2004). If these recombination events would be distributed evenly across the 2.5 Gb mouse genome (Waterston *et al.*, 2002), then every recombination would occur approximately every 600 Kb. In case a region in between two recombination events contains two QTG

with an opposite additive effect, those QTG would not be detected. Recombinant inbred strains from an eight-way cross between genetically distant founders (the collaborative cross; CC) will yield higher mapping precision, enabling the detection of these neighboring QTG (Chesler *et al.*, 2008). However, assessing well-dissected phenotypes, such as impulsivity and attention in operant tasks, requires a vast amount of labor as compared to for instance anxiety and certain learning and memory phenotypes. Therefore, increasing power using more BXD strains or extending to CC lines is a challenging project unless novel less-labor intensive assays will be developed.

Another challenge would be to delineate the underlying genes related to the decision-making counterpart of impulsivity, namely impulsive choice. One frequently employed index to measure impulsive choice is the inability to tolerate delay of reinforcement. In contrast to available assays to study this aspect of impulsivity in rats, to date only one laboratory succeeded in setting up a paradigm for impulsive choice in mice using a within-session increase in delay to reinforcement (Isles *et al.*, 2004; Isles *et al.*, 2003) similar to our paradigm in rats (Loos *et al.*, 2010a). Despite effort, we did not succeed in setting up a within session protocol in mice, as well as three different other labs (personal communication with A. Holmes, T. Kalenscher and F.S. van den Bergh). In our hands, mice were not able to flexibly shift choice preference between large or small reward within one session, but habitually chose one of the two throughout a session. Numerous studies reported delayed reward tasks in mice that use an across-session increase in delay (Adriani & Laviola, 2003; Brunner & Hen, 1997; Oberlin & Grahame, 2009; Wilhelm & Mitchell, 2008). It is possible that mice solve these across session tasks by slowly shifting from one habit (responding for large reward) to another (responding for small reward), which not necessarily reflects the same flexibility displayed by rats, when shifting preference within one session. This may suggest that a task requiring this type of flexibility is cognitively challenging for mice. Yet, this type of executive function, cognitive flexibility, is essential for adequate behavior and is impaired in many psychiatric and neurological diseases. It may take a more potent reward (e.g. sweet condensed milk, Isles *et al.*, 2003) to successfully implement delayed reward tasks, or other inventive assays to assess cognitive flexibility, tailored to the species under investigation.

One direction to improve throughput and develop novel tasks of impulsivity, attention and cognitive flexibility is by exploiting the home cage environment of mice. The training intensity can be increased from 25 min in classical operant tasks to theoretically all awake hours in automated home cages to intensify the training. Automatic control over food and water availability would enable the experimenter to administer the tasks at the time of day when animals are most willing to earn food or liquid reward to enhance the motivation to acquire even the most cognitively demanding tasks. Initial steps in this direction have been taken by administering a simple reaction time task to group-housed female mice

in an automated home cage (Vannoni *et al.*, 2010). This task measured aspects of impulsivity and attention and was acquired by mice in less than 2 weeks time, exemplifying the improvement in phenotyping efficiency by applying tasks in a home cage environment.

2.2. Towards a better molecular and cellular understanding of impulsivity and attention using new mouse lines

Drawing parallels between behavioral phenotypes and brain phenotypes within one individual or strain is a powerful and frequently employed method to understand behavior and the etiology of associated disease. In this thesis, cohorts of individual outbred rats with extreme phenotypes (**chapter 2**) and panels of (recombinant) inbred strains of mice (**chapters 3–7**) have been instrumental in this respect. A third method, exploiting two strains of rodents with extreme phenotypes provides a convenient alternative. In **chapter 7**, we identified a BXD strain (BXD16) that was more impulsive in both the 5-CSRTT and a simple choice reaction time task, which also showed enhanced alcohol-seeking behavior in terms of higher motivation to lever press for alcohol and an enhanced cue-induced reinstatement of lever pressing after extinction. However, in terms of genetic interpretation, the coexistence of two phenotypes within these lines not necessarily depends on the same genetic factor, since these lines and their respective controls are polymorphic at multiple loci. It is imperative to identify and isolate the causative genetic variant to unequivocally relate two phenotypes to the same genetic factor. So, the ultimate aim of the QTL studies in **chapters 5 and 6** was to end with a mouse line that, in comparison with its wild type counterpart, possesses one single genetic polymorphism causally affecting the trait. This can be achieved by, rigorous backcrossing, identifying the QTG and creating a genetically modified line and by viral over-expression to rescue the behavioral trait. Together, the QTL and genes identified in this thesis may generate insight into molecular mechanisms contributing to deficits in inhibitory control and attention, which may provide entry points for further research into treatment of these deficits in ADHD, schizophrenia and drug addiction.