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The genetic and epigenetic landscape for CNS drug discovery targeting cross-diagnostic behavioral domains

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ABSTRACT

Animal studies play a central role in the identification and testing of novel drugs for CNS disorders. In his longstanding career, Berend Olivier has significantly contributed to CNS drug discovery by applying and supporting novel views and methodologies in the fields of behavioral neuroscience, pharmacology, and (epi-) genetics. Here we review and put forward some of these integrated approaches that have led to a productive collaboration and new insights into the genetic and epigenetic regulation of neurobehavioural traits related to psychiatric disorders.

1. Introduction

The development of etiology-directed treatment for psychiatric disorders requires insights into the neurobiological mechanisms underlying maladaptive behavioral responses to environmental cues. Already in his early days of behavioral pharmacology, Berend Olivier focused on ethological relevant behavioral phenotypes, such as intermale and territorial aggressive behavior (Olivier, 1981). These studies have led to the identification of serotonergic compounds that modulated these type of behaviours in rodent species (Miczek et al., 1989). Following these methods, he coined the term "Ethopharmacology", a creative approach to identification and characterisation of novel psychotropics (Olivier et al., 1990). It was this way of thinking that led to further understanding and pharmacological interventions of animal behavior with the notion to identify novel drugs for psychiatric disorders.

With the emergence of studies that aimed to identify genetic factors underlying psychiatric disorders, the application of genetics for distinct behavioural phenotypes was an interesting next step (de Mooij-van Malsen et al., 2008; de Mooij-van Malsen et al., 2011; Kas et al., 2007). In line with the ethopharmacology concept, novel approaches were developed that focused on ethologically relevant behavioral traits in view of finding determinants of psychiatric disorders. These studies underscored the complexity of psychiatric disorders in view of genetic, environmental, developmental, and phenotypic heterogeneity. Furthermore, they challenged the definitions and assessment methods of behavioral phenotypes that are at the core of brain diseases.

Here, examples of genetic and epigenetic approaches in the field of behavioral neuroscience and psychiatry will be addressed. Some of these methods were applied in close collaboration with Berend Olivier over the past years and have led to novel insights in determinants of behavioral outcome. These studies aim at the translational aspects of behavioral studies across species.

2. Genetics of behavioral domains

2.1. Genetic association studies of psychiatric disorders

Before the era of genome wide association studies (GWAS) commenced, genetic profiling of psychiatric disorders such as major depressive disorder (MDD), schizophrenia (SZ) and bipolar disorder (BD) met very little success. Linkage studies based on affected family, twin and adoptive studies, which had proven to be successful when applied to diseases affected by genetic loci of large size effect, were ineffective for complex genetic disorders. Also, association studies for candidate genes were designed based on the hypothesized etiology of the disease, pharmacological observations or abnormalities recorded in post-mortem human brain tissue from patients. Even though some compelling candidate genes were identified this way, such as BDNF and COMT (Craddock and Sklar, 2009), most results were inconsistent due to

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small sample sizes, as well as poor understanding of the biological, molecular background of the disease.

With the increasing knowledge on the human genome (Venter et al., 2001; Lander et al., 2001; International Human Genome Sequencing, 2004), as well as the progress made in fast and – relatively – inexpensive screening methods for single nucleotide polymorphisms, GWAS rapidly increased our capability to investigate the role of genetic variations involved in psychiatric disorders. However, the successes stayed somewhat behind to the expectations. The main caveat in these studies has proven to be the large patient groups necessary to significantly associate a genetic locus with the disease. Further, due to the large amount of loci being investigated within the same subjects, the level of significance for each locus should reach 1×10^{-8}. Therefore, large consortia have started to be built combining data sets from different patient populations, as well as the use of meta-analysis of data obtained by several research groups. This offers significant progress in identifying loci implicated in these complex disorders. One prominent example of this is association of SNPs in the gene CACNA1C, a pore-forming α_{1c} subunit of the L-type voltage-gated calcium channel with bipolar disorder (for a review see (Bhat et al. (2012)).

However, we are still far from claiming to have found all the different genes involved, let alone our understanding of the actual functionality of these genetic variants in disease development. The results obtained using patient groups with different – but partly phenotypically overlapping – psychiatric disorders show great potential. CACNA1C, for example, has been shown to be associated with several psychiatric disorders, and is not specific for bipolar disorder. A recent schizophrenia genome wide association study showed that by combining datasets from unipolar, bipolar depression and schizophrenic patients, the level of significance of the association of CACNA1C was greatly enhanced (Psychiatric Genetics Consortium, 2011; Craddock and Sklar, 2013). The same has been shown by O'Donovan et al. (2008) for ZNF804A with a combination of bipolar and schizophrenic patients. The apparent premise would be that these genes serve similar (dis-) function in the examined disorders and that these involve common endophenotypes. It still remains to be proven that genetic variations associated with, for example, both schizophrenia and bipolar disorder, but not in major depressive disorder are functional on endophenotypes such as hyperactivity and delusions, whereas variations associated with bipolar disorder and depression, but not schizophrenia, would have a greater role in endophenotypes such as depression and anxiety. As for the case of CACNA1C, which was found to be associated in a combined study for depression, bipolar disorder and schizophrenia would suggest that there is a common ground for the development of these distinct disorders. At this time point we are, however, far from proving these assumptions.

One other way to look at these results is that it proposes that genetic association studies of underlying, common endophenotypes can have a significant meaning for psychiatric genetics. In 2013, the Consortium on the Genetics of Schizophrenia successfully characterized 12 neurophysiological and neurocognitive endophenotypes for schizophrenia and identified a genome-wide significant linkage area for an antisaccade task, a near-genome wide significant region linked to emotion recognition, as well as several other suggestive areas for individual endophenotypes such as spatial processing, prepulse inhibition and face memory (Greenwood et al., 2013). Despite the successes of using endophenotypes for schizophrenia (Greenwood et al., 2013; Knowles et al., 2014; Lencz et al., 2014), as well as shared endophenotypes for autism and schizophrenia (Goodbourn et al., 2014), BP and schizophrenia (Ruderfer et al., 2013), relatively few human genetic studies have actually used this method so far. Future research will require the evaluation of the biological significance of these findings.

2.2. Transgenic models

Once candidate or affected genes have been identified, they may be used as leads to develop novel pharmacological treatments. Transgenic animal models serve an important role in identifying gene function, molecular pathways and pharmacological efficiency. Generally, there are two ways to evaluate the linked genetic regions or genes within an animal model. When using reverse genetics, a knockout/knockdown animal for the desired gene is made and screened for behavioral or neurobiological changes. This strategy has provided important information on the functioning of several human candidate genes, among CACNA1C (Lee et al., 2012a, 2012b; Dao et al., 2010). In forward genetics, a mouse model with endophenotypes corresponding to the disease profile of the disorder is identified and investigated for mutations within the genetic background (for a review, see Tarantino and Bucan (2000)). However, to be able to use forward genetics, sensitive strategies on both behavioral and genetic screening level are essential.

2.3. Translational endophenotypes

As stated before, the translational value of animal behavior-genetics greatly increases when comparing behavioral domains instead of full psychiatric diagnostics (de Mooij-van Malsen et al., 2011). Over the last few years, several powerful mapping strategies have been developed to dissect quantitative traits in rodent models (for a review, see Mott and Flint (2013)). To quickly be able to screen for genetic linkage regions, several mouse genetic reference populations (e.g. BxD strains, chromosome substitution strain panels) have been developed of which the full genome, and with that the SNPs between the strains, is available via online sources (Blake et al., 2014; Karolchik et al., 2014). By testing mice from these sensitive genetic reference populations in behavioral tests with high resolution, homologous human genetic regions of interest can be identified and, subsequently, be investigated in view of functionality. We approached this issue by developing a sensitive, automated home cage environment in which we screened a panel of male and female chromosome substitution mouse strains to efficiently identify novel genetic loci involved in avoidance behavior, independent of locomotor activity (de Mooij-van Malsen et al., 2009). This mouse genetic mapping strategy allowed rapid identification of chromosomal regions linked to specific behavioral endophenotypes. In behavioral testing, we identified different chromosomes regulating either motor activity levels or sheltering preference. Further genetic fine mapping of the F_2 generation revealed a Quantitative Trait Locus (QTL) for baseline avoidance in mice on mouse chromosome 15. As several interesting candidate genes were present within the QTL region, homology screening was performed and the linkage region proved to be syntenic with an established human genetic linkage region for bipolar disorder (de Mooij-van Malsen et al., 2009). The combination of mouse sequence data with human data from the WTCCC GWA study on bipolar disorder (Wellcome Trust Case Control, 2007) identified 3 candidate genes with significant allele frequency differences (2938 healthy controls; 1868 bipolar patients). By mapping a second locus on mouse chromosome 19 that contributed to avoidance behavior in mice we obtained another bipolar disorder linkage region (de Mooij-van Malsen et al., 2013), indicating that (at least) two different mouse loci contributing to the mouse behavior are linked to this human mood disorder. These findings advocate that our methodology identifies genes and functional mechanisms underlying psychiatric endophenotypes.

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