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Invited review

Rats are the smart choice: Rationale for a renewed focus on rats in behavioral genetics

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ABSTRACT

Due in part to their rich behavioral repertoire rats have been widely used in behavioral studies of drug abuse-related traits for decades. However, the mouse became the model of choice for researchers exploring the genetic underpinnings of addiction after the first mouse study was published demonstrating the capability of engineering the mouse genome through embryonic stem cell technology. The sequencing of the mouse genome and more recent re-sequencing of numerous inbred mouse strains have further cemented the status of mice as the premier mammalian organism for genetic studies. As a result, many of the behavioral paradigms initially developed and optimized for rats have been adapted to mice. However, numerous complex and interesting drug abuse-related behaviors that can be studied in rats are very difficult or impossible to adapt for use in mice, impeding the genetic dissection of those traits. Now, technological advances have removed many of the historical limitations of genetic studies in rats. For instance, the rat genome has been sequenced and many inbred rat strains are now being re-sequenced and outbred rat stocks are being used to fine-map QTLs. In addition, it is now possible to create "knockout" rats using zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALENs) and related techniques. Thus, rats can now be used to perform quantitative genetic studies of sophisticated behaviors that have been difficult or impossible to study in mice.

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1. Introduction

For over a century, the rat has been favored for studying neurobiological processes and for providing neuropsychological models for human behavioral disorders (Jacob, 1999; Logan, 2005; Weiss and Feldon, 2001); due in part to its complex behavioral repertoire. Accordingly, many of the behavioral assays used to assess phenotypes of interest and validate pharmacological agents were designed and optimized for use in rats. However, the mouse became the model of choice for mammalian geneticists in the 1990s, after the first mouse study was published demonstrating the capability of engineering the mouse genome through embryonic stem (ES) cell technology (Thomas and Capecchi, 1990). This "knockout" technology allowed mice to be produced that lacked a single, specific gene and was a key factor in choosing the mouse to be the next mammal after humans to have its genome sequenced (Waterston et al., 2002). Thus, the dominance of the mouse as a genetic tool has been long-lasting, despite the abundance of rich behavioral and physiological phenotypes that can be measured in the rat (Jacob, 1999). However, the rat genome was released in 2004 (Gibbs et al., 2004), significantly enhancing efforts at comparative genomics and enabling cross-species data integration. A new build of the rat genome incorporating novel sequence data and mapping technologies was released in March 2012 and is currently being annotated (http://www.ncbi.nlm.nih.gov/assembly/382928/). In

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addition, genetic analyses of experimental rat crosses has allowed for the identification of hundreds of rat quantitative trait loci (QTLs) that were associated with drug-related traits (http://rgd.mcw.edu/rgdweb/search/qtls.html?100). However (as with the QTL studies conducted by mouse geneticists), few genes were identified relative to the number of QTLs. Furthermore, mouse models still possessed a clear advantage for experimental genetics due to the relative ease of obtaining homologous recombination in mouse ES cells, which has allowed the production of 'knockout' and similar genetic models in mice. Thus, two major obstacles have plagued rat geneticists and discouraged the widespread use of rats as a genetic model organism: 1) forward genetic approaches in rats such as QTL mapping failed to identify genes due to difficulties in narrowing QTLs intervals, and 2) the rat genome has not allowed itself to be genetically manipulated in the same way as the mouse, hindering reverse genetic approaches. Now, due to technical advances, these two obstacles have been overcome, allowing for both the localization and functional validation of genes underlying complex traits in rats. Rats and mice have advantages for the understanding of human disease above and beyond what is possible when studying humans directly. Rats possess a rich behavioral repertoire compared to mice; furthermore, their large size makes it easy to carry out detailed physiological measurements not feasible in smaller animal models such as mice (Abbott, 2004). Furthermore, almost all human genes known to be associated with disease have orthologues in the rat genome (Gibbs et al., 2004) and most disease genes identified in rats have also been shown to play a role in human diseases (Aitman et al., 2008). Lastly, because rats and mice are experimental model organisms, researchers can perform potentially stressful, invasive, or even terminal procedures not possible in humans, such as measuring gene expression in key brain regions.

In this review, we discuss how the wealth of new genetic technologies, combined with the phenotypic diversity of the rat make them ideally suited for use in genetic studies of complex behavioral traits. We describe in detail three phenotypes that have been difficult to implement in mouse models, but that have been successfully used in rats. While our review focuses on addiction-related phenotypes, many of these same arguments apply to other complex behavioral and physiological traits. The advantage of using rats as behavioral and neurobiological models is not new. In light of recent technological advances, we argue that complex behavioral tasks that have been difficult or impossible to pursue in mice can now be successfully studied in rats.

2. Genetic approaches in rat models of substance abuse disorders

Genetic techniques used for addiction research in rat models include both "phenotype-to-genotype" and "genotype-to-phenotype" approaches. Phenotype-to-genotype, or forward genetics, begins with the measurement of the trait of interest in order to uncover the underlying genetic architecture in a population. Genotype-to-phenotype, or reverse genetics, is a method to discovering the function of a gene by examining the phenotypic effects that result from a targeted mutation. Both techniques are useful for integrating results from human and rat genetics studies.

2.1. Forward genetics (QTL mapping)

Forward genetic strategies seek to identify the genes and alleles that give rise to variability in a trait of interest. Thus, forward genetics is an unbiased approach that is useful for hypothesis generation. Traditionally, QTL studies have used F_2 crosses between two inbred strains, recombinant inbred (RI) lines or similar populations. Associations between the genetic markers and phenotypes are analyzed to determine the location of the QTLs. Due to limited recombination, these populations are not well suited to fine-mapping the identified loci, which is a necessary pre-requisite to identifying the underlying causative gene(s). This drawback has challenged mouse and rat geneticists for years. However, human genome-wide association studies (GWAS) have been successful precisely because they take advantage of the large number of accumulated recombinations observed among unrelated human subjects. Recombination degrades the non-random associations between adjacent polymorphisms; these associations between nearby markers are known as linkage disequilibrium (LD). Populations that have been intercrossed for multiple generations accumulate many recombinations, which cause a rapid breakdown of LD between adjacent markers. Thus, only markers that are very close and thus in LD with a functional polymorphism will show a significant association with the trait of interest. Populations with more degraded LD allow for more accurate mapping of QTLs, provided that enough markers are genotyped and can ultimately lead to the identification and validation of the causative polymorphism.

Now, as technologies for genotyping have evolved rapidly over the past decade, it is no longer expensive or difficult to perform GWAS in rats. The same improvements in genotyping technology that have been widely used in human genetics and are beginning to be applied to mouse genetics (Parker and Palmer, 2011) also possess enormous but largely unrealized potential to revolutionize rat genetics. Genome technologies such as high-throughput sequencing, RNASeq, or high-density SNP chips would not be useful for standard F_2 crosses, but are extremely helpful when populations with more degraded LD are used. Rat populations such as heterogeneous stocks and commercially available outbred rats have been used for decades for physiological and behavioral analyses and possess very low levels of LD. Now, these highly recombinant rat populations are increasingly attracting the attention of geneticists and their use promises to streamline what has been a very slow and expensive process: definitive identification of the genes that underlie QTLs.

2.1.1. Heterogeneous stocks (HS)

Heterogeneous stocks are created by interbreeding more than two (often eight) inbred strains followed by many generations of randomized outcrossing (Flint and Eskin, 2012; Parker and Palmer, 2011). In mice, the diversity outcross (DO) and the heterogeneous stock (HS) mice have been successfully used for the high-resolution mapping of multiple complex traits (e.g. Svenson et al., 2012; Valdar et al., 2006). The heterogeneous stock rat (N:NIH-HS) was developed by the National Institutes of Health in 1984 (Hansen and Spuhler, 1984) to serve as a source of genetically segregating animals for both experimental and selection studies. Similar to HS and DO mice, the HS rat was originally derived from eight inbred founder strains that are both genetically (Saar et al., 2008) and phenotypically (see Johannesson et al., 2009) distinct: ACI/N, BN/SsN, BUF/N, F344/N, M520/N, MR/N, WKY/N and WN/N. This stock was then outbred for over 50 generations using a rotational outbreeding scheme to minimize the amount of inbreeding, drift, and fixation. The number of generations of outbreeding determines the degree to which LD from the original founder chromosomes is degraded (Mott and Flint, 2002). After 50 generations of outbreeding, the genetic make-up of the resulting progeny represents a random mosaic of the founding animals, with an average distance between recombination events per individual of about one centiMorgan (Mott et al., 2000), enabling the fine-mapping of QTL to only a few Mb (see Solberg Woods et al., 2010a, 2010b, 2012; Johannesson et al., 2009; Rat Genome Sequencing and Mapping Consortium, 2013).