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Genetic dissection of the mouse CNS using magnetic resonance microscopy

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Abstract

Purpose of review—Advances in MR microscopy (MRM) make it practical to map gene variants responsible for structural variation in brains of many species, including mice and humans. We review results of a systematic genetic analysis of MRM data using as a case study a family of well-characterized lines of mice.

Recent advances—MRM has matured to the point that we can generate high contrast high-resolution images even for species as small as a mouse, with a brain merely 1/3000th the size of humans. We generated 21.5-micron data sets for a diverse panel of BXD mouse strains to gauge the extent of genetic variation, and as a prelude to comprehensive genetic and genomic analyses. Here we review (1) MRM capabilities and image segmentation methods; (2) heritability of brain variation; (3) covariation of the sizes of brain regions; and (4) correlations between MRM and classical histological data sets.

Summary—The combination of high throughput MRM and genomics will improve our understanding of the genetic basis structure-function correlations. Sophisticated mouse models will be critical in converting correlations into mechanisms and in determining genetic and epigenetic causes of differences in disease susceptibility.

Keywords

Magnetic Resonance Microscopy; phenotyping; mouse brain; gene mapping.

Introduction

Magnetic resonance imaging (MRI) has made it practical to generate high-quality brain images for large numbers of humans. MRI has recently been combined with genetic and genomic methods. The combination has been used to study variation in brain structure, heritability of neuroanatomical traits, and associations between differences in DNA, brain structures, and brain diseases (Thompson, Cannon et al. 2001), (Chiang, Barysheva et al. 2009). Experimental mouse models are an ideal complement to these human MRI neurogenetic studies. The barrier to exploiting mouse genetic models has been the difficulty of generating sufficiently high-resolution images of a peanut-sized brain (0.45g for mouse vs. 1350g for human). Fortunately, in the last few years, MRM methods have advanced to the point that it is now practical to generate images with a resolution of two or three cell diameters (Badea, Nicholls et al. 2007)

(Johnson, Ali-Sharief et al. 2007; Petiet, Kaufman et al. 2008). The effective neuroanatomical resolution of MRM now matches or exceeds that of clinical MRIs. MRM methods can finally be applied effectively to study thousands of mouse mutants and the progeny of genetic crosses.

We have begun to explore the genetic basis of variation in brain architecture across a large family of strains of mice—the BXD family—that were generated by crossing two of the oldest and most widely used inbred strains—C57BL/6J and DBA/2J. The BXD families are great resources with which to map genes that control the size and shape of many different parts of the brain. In this review we describe progress in four areas that now allow routine high-throughput 3D evaluation of brain morphology in the context of our analysis of BXD strains. We review MRM advances and genetic approaches to exploiting these methods to understand the genetic control of CNS structure and function.

Methodological advances

MRM exploits contrast mechanisms based on proton stains such as T1, T2, T2*, and diffusion (Johnson, Benveniste et al. 1993), and can cover the whole brain in one 3D acquisition, preserving spatial relationships and shapes in the intact brain. The different contrasts provide new perspectives on brain anatomy and histology, while the 3D, nondestructive nature make MRM an ideal imaging method for morphometry. Most MRM-based studies on brain morphometry rely on information from fixed brains, where image resolutions have approached limits (10 microns) imposed by diffusion (Johnson, Ali-Sharief et al. 2007) (Nouls, Izenson et al. 2008). Multiple barriers must be overcome to obtain and analyze such images to ultimately gain better understanding of the relationships between phenotype and genotype in murine models used in translational research.

The main challenges of imaging brains as small as those of a mouse is achieving enough resolution, signal intensity, and contrast to accurately delineate regions as small as those of thalamic nuclei, such as the dorsal lateral geniculate nucleus. Achieving good contrast between gray matter and the meshwork of smaller fiber tracts is critical in this MRM neuroanatomical delineation process. Special specimen preparation and pulse sequence protocols are essential. Progress over the past few years has been dramatic and is highlighted by the differences of images reproduced in Figure 1. Both have a voxel size of 43 μ m (~80 picoliter) that is 50,000-times smaller than voxels of a typical human clinical scan. Figure 1a was acquired in 2004 as part of a study by Cyr et al. using then state-of-the-art methods to identify morphometric changes in a mouse model of dopaminergic hyperfunction (Cyr, Caron et al. 2005). The scan took nearly 2 hours. In contrast, Figure 1b was acquired in 2008 using the current suite of methods over merely a 10-minute period with the brain specimen still in the skull. Since signal increases with magnetic field strength, most high-resolution fixed brain images are acquired using magnets operating at magnetic fields >7.0T, more than 4-times the lower field (1.5T) magnets used for clinical imaging. High-field MRI is the preferred choice, since it can provide better resolution for the same scan duration at lower field strength. Increased gradient strengths and more sensitive radiofrequency probes are also critical.

Specimen preparation is an essential component of the process. One of the strategies to both shorten acquisition time and enhance contrast between brain compartments is to use paramagnetic contrast agents based on Gadolinium (Gd), Manganese (Mn), or iron (Fe). In particular, active staining (Johnson, Cofer et al. 2002) uses a Gd-based contrast agent (ProHance, Bracco Diagnostic, Princeton, NJ) mixed with a fixative, and delivered transcardially, to preserve the tissue and enhance the signal by reducing the spin lattice relaxation time (T1). Optimized active staining of the mouse brain using Gd, combined with fast acquisition strategies relying on dynamic adjustment of the receiver gain during phase encoding, and the use of partial sampling of the k-space (Johnson, Ali-Sharief et al. 2007) have

led to routine imaging of the fixed whole brain at 21.5 μ m isotropic resolution in less than 2 hours. These MRM images of the brain within the skull, at an isotropic resolution of 21.5 μ m allow the discrimination of cellular layers (Badea, Nicholls et al. 2007). Other strategies to increase throughput have been developed using parallel imaging with multiple specimens at the same time (Bock, Konyer et al. 2003).

These large 3D arrays (1024 $\times$ 512 $\times$ 512 or 512 $\times$ 256 $\times$ 256) must be processed to yield morphometric data, and present computational challenges addressed by developing efficient algorithms and processing pipelines. Figure 2 presents a chart of the processes and bioinformatics tools necessary to perform analysis required for quantitative MRM. While manual segmentation methods are tedious and time consuming, they remain the gold standard and are still used for identifying group differences (Sawiak, Wood et al. 2009). Priors originating from manual gold standards are used to generate reference or average templates, and probabilistic atlases usable in automated segmentation of the brain (Ali, Dale et al. 2005), (Kovacevic, Henderson et al. 2005), (Ma, Hof et al. 2005). Automated segmentation usually involves nonlinear registration into a common space. In addition multiple imaging protocols (Figure 3) and Markov random field modeling have been incorporated into segmentation (Sharief, Badea et al. 2008) (Bae, Pan et al. 2009). From the resulting labels are extracted quantitative parameters such as volumes, areas, surfaces characteristics or texture, which need to be archived. Statistical analyses to identify group differences between mouse models of disease or different strains (Chen, Kovacevic et al. 2006) are done on global morphometric parameters (e.g. volumes and areas), on focal regions (voxel or deformation based morphometry) within the brain or to identify local shape differences (Badea, Nicholls et al. 2007; Lerch, Carroll et al. 2008).

High-resolution images and segmentation methods produced efficiently allow the estimation of normal variability bounds within a mouse strain or across strains, as well as differences among subpopulation groups.

MRM for anatomical phenotyping

Advances in MR imaging have spurred a tremendous increase in the number of studies assessing brain phenotypes in different strains of mice: normals, as well as mutants and knockouts (Cyr, Caron et al. 2005); (Chan, Kovacevic et al. 2007), (Nieman, Flenniken et al. 2006; Badea, Nicholls et al. 2007; Benveniste, Ma et al. 2007) (Lau, Lerch et al. 2008) (Badea, Johnson et al. 2009). MR-based atlases of normal mouse brains have been built to establish a baseline for anatomical variability, mostly for the widely used C57BL/6 strain (Ma, Hof et al. 2005; Dorris, Lerch et al. 2008) (Ali, Dale et al. 2005) (Badea, Ali-Sharief et al. 2007), but also for several other common strains (Kovacevic, Henderson et al. 2005), (Chen, Kovacevic et al. 2006). Atlases are usually based on multiple specimens, and on one optimized imaging protocol, but can also contain multiple MR contrasts (Ali, Dale et al. 2005), (Badea, Ali-Sharief et al. 2007), or even imaging modalities (MacKenzie-Graham, Lee et al. 2004) (Chan, Kovacevic et al. 2007). In addition, atlases contain references to labeled regions (Figure 3d), ranging in number from 20 in the live mouse brain (Ma, Smith et al. 2008) to 33 (Badea, Ali-Sharief et al. 2007), to 62 in the fixed brain (Dorris, Lerch et al. 2008).

More interestingly, MRM has been applied in translational studies demonstrating volumetric differences (Figure 4) ranging from more than 100% in the ventricles of the *Reeler* mutant mouse (Badea, Nicholls et al. 2007), to a few percentage (9%) in the anterior striatum (0.03% of the total brain volume) in a mouse model of dopaminergic hyperfunction (Cyr, Caron et al. 2005). In contrast, differences in the hippocampus of the *Reeler* mouse were not reflected in global volume. However, in the same mice, shape analysis identified significant changes in the dorsal hippocampus, the temporal lobe, and cerebellum. A more sensitive approach is therefore

given by regional/voxel based techniques, such as deformation (DBM) or voxel (VBM) based morphometry (Ashburner and Friston 2000). DBM uses deformation fields to identify differences in the positions of structures. The statistics on the Jacobian of the deformation fields is used to characterize local volume differences relative to a reference. VBM produces statistical maps characterizing differences in local gray matter concentration. These techniques have recently found application in rodent brain imaging (Verma, Mori et al. 2005), (Nieman, Lerch et al. 2007; Lau, Lerch et al. 2008) (Sawiak, Wood et al. 2009). The process of adapting processing techniques devised for human brain images to the mouse has faced challenges related to the larger size of the data-sets (we are now routinely acquiring image matrices of 1024×512^2 , 512^3 , or 512×256^2 voxels), as well as to the lower contrast. In addition to imaging single time point data as in fixed brains studies, the noninvasive nature of MR allows longitudinal studies and correlating morphometric changes with aging or disease progression (Lau, Lerch et al. 2008).

Enhanced MRM throughput has allowed to extend phenotyping studies to larger groups of animals per strain (Dorr, Lerch et al. 2008), to multiple strains (Nieman, Lerch et al. 2007), and even families of strains (Badea, Johnson et al. 2009). These coherent data-sets produced by MRM allow exploring statistical correlations between regions of the CNS, and thus complements the traditional quantitative analysis for comparing mouse populations. Quantitative data on individual regions exist from histology studies, but usually one (Lu, Airey et al. 2001) (Mozhui, Hamre et al. 2007) (Martin, Churchill et al. 2009) or at most couples of structures were segmented in the same brain. In a recent study on BXD RI strains (Badea, Johnson et al. 2009), the variability within and between strains was assessed, and then the patterns of correlations within CNS were explored based on semiautomated segmentation of 33 structures from MR images.

Covariance structure within CNS

The BXDs are a family of 80 strains produced by crossing two of the most common types of mice—C57BL/6J (B or black) and DBA/2J (D or dilute). These strains have been extraordinarily well characterized, especially for neuroanatomical and behavioral traits. For example, Neumann and colleagues discovered large and consistent differences in cerebellar foliation patterns that were highly heritable (Neumann, Garretson et al. 1993). Wehner and colleagues demonstrated that variation in the activation of protein kinase C in the hippocampus of these strains correlated well with differences in learning (Wehner, Sleight et al. 1990). Her work was followed by a series of neurogenetic studies of the BXD hippocampus (Lassalle, Halley et al. 1994), (Lu, Airey et al. 2001; Martin, Dong et al. 2006). Another reason for the popularity of these strains is that they have been well genotyped (Shifman, Bell et al. 2006). This makes it possible to map genes that control variation in neuroanatomical traits without the added chore of genotyping. BXD strains have a level of genetic variation that roughly matches that of human populations, making them a good model for the range of variation one might expect among humans. All of these hard-won phenotypes and genotypes have been integrated into an open web resource called GeneNetwork (www.genenetwork.org).

Neuroanatomical phenotypes on BXD strains have been acquired, usually based on histology, for the hippocampus (Peirce, Chesler et al. 2003), cerebellum (Airey, Lu et al. 2001), olfactory bulb (Williams, Airey et al. 2001) (Williams et al., 1999), neocortex (Jan, Lu et al. 2008), thalamus (Seecharan, Kulkarni et al. 2003), and striatum (Rosen et al., 2009) (Rosen, Pung et al. 2008), and have led to identification of genes (QTL) that modulate variation in CNS architecture. These studies have often been conducted on the same strains, but mostly using different animals. This makes it difficult to accurately estimate the covariance among neuroanatomical traits. MRM provides a perfect solution, since we have been able to acquire morphometric data for 33 regions of the brain (Badea, Ali-Sharief et al. 2007), with an average

heritability of 0.6 ± 0.2 . To compare variation within and among strains when environmental variation is minimized, we have used a subset of 9 BXD RI strains plus B and D, male and females from different litters, age matched individuals. One can think of this as an analysis, with replication, of a family of humans—mother, father, and nine offspring.

The agreement between neuroanatomical estimates based on histology and MRM (Table 1) were excellent for the whole brain (433.7 ± 31.6 g versus 447.9 ± 35.7 mm³) and structures that are easily defined in a consistent fashion such as cerebellum (46.7 ± 3.7 mm³ versus 55.19 ± 3.2 mm³). For structures where boundaries are defined ambiguously these values were lower.

After performing regression analysis to remove variability due to overall brain volume, we searched for statistically significant associations between variation in the volumes of the segmented structures, and produced a neuroanatomical covariance matrix (Figure 5) similar to those computed recently using cohorts of monozygotic and dizygotic human twins (Wright, Sham et al. 2002). A limbic component could be singled out based on strong correlations among subcomponents such as the amygdala and hippocampus ($r = 0.61$, $p < 0.05$).

An exploratory analysis using www.genenetwork.org revealed that behavioral phenotypes also correlate with neuroanatomical phenotypes. Memory related traits, such as the latency in the Morris water maze (Milhaud, Halley et al. 2002), correlated with volumes of structures associated with the limbic system such as the hippocampus (0.68 , $p = 0.06$), while the volume of the amygdala was correlated with phenotypes related to addiction, such as ethanol preference (Rodriguez, Plomin et al. 1994) ($r = 0.91$, $p = 0.0006$).

A principal component analysis (PCA) identified morphological subregional systems associated with the largest proportion of total variance. The first principal component (~40% of the variance) provided contrast between the cerebellum (-0.64) and striatum (-0.29) as one group with negative loadings, and cortex (0.35) and brainstem structures (0.44), which share high positive loadings. The second component (~30% of the variance) provided contrast between cortex (-0.76) and striatum (-0.23) as one group and medulla and midbrain (0.54). We found on the third PCA (~15% of variance) contrast between limbic structures and ventricles (strong negative loadings), and olfactory bulbs and cerebellum (strong positive loadings). Another subsystem could be defined on the third PC, consisting of the thalamus-accumbens-striatum, and amygdala, which are parts of a cortico-striato-thalamo-cortical loop. Components of the same subregional system, defined by strong loadings of the same sign on the same PC, might be under the influence of several common factors, including positively correlated growth patterns, anatomical connectivity, and cooperative functioning (Wright, Sharma et al. 1999).

This approach opens up possibility of discovering gene variants and sets of polymorphism, as well as perhaps environmental mechanisms that generate correlations, and that contribute to networks of covarying phenotypes.

Conclusion

Recent progresses on high-resolution mouse brain imaging and processing techniques have allowed the application of MRM to brain mapping studies, the main advantage being the possibility to quantify morphometric parameters including volume and surface areas and shapes for multiple structures from the same organism the same time. Compared to traditional histology, MR histology provides a setting where the shape integrity and spatial relationships between structures are preserved and allows coherent studies from which the covariance of morphometric parameters can lead to the statistically-based identification of functional submodules. We are only at the beginning of the exploration of gene mapping based on MRI

and existing databases on BXD genetic markers, as well as other complementary phenotypes including weight and cell numbers, or even behavioral phenotypes. The model for analysis has been set by histological studies that have identified genetic intervals linked to variations in morphometric parameters. The high heritability values obtained for MRM-based measurements are encouraging us to believe that it will be possible to map genes responsible for such variations. Integration of various approaches and methodologies for mapping genotype-phenotype is possible now, not only at the level of individual experiments on one population, but also by means of databases (eg. www.genenetwork.org), and the sum of these approaches is likely to advance our knowledge on brain mapping more than any of these modalities alone.

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Abbreviations

CNS, central nervous system; MRM, magnetic resonance microscopy; SOP, standard operating protocol.

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Figure 1.

a) Horizontal image through a mouse brain acquired in 2005 (Cyr et al, 2005), out of the skull, at $43\mu\text{m}$ resolution, required a 109 minute acquisition. While excised mouse brain can use a smaller field of view, within skull images should be favored because they preserve the integrity of tissue, especially in areas like olfactory bulbs and cerebellum; b) Horizontal slice at a corresponding level, acquired in 2009, within the skull, with acquisition time of 10 min, i.e. better images in 1/10th the time.

Figure 2.
The main elements of analysis, and bioinformatics tools for an MR based SOP for neuroanatomical phenotyping.

Figure 3.

The use of active staining (Gd) and multiple imaging protocols allows high resolution MRI (T1 weighted at 21.5 (a), T2 weighted at 43 microns (b)) to emphasize a large number of structures in the mouse brain. Besides the volume and shape for the total brain (c), 33 structures have been segmented and quantified, including olfactory areas (Olf), striatum (CPu), septal nuclei, fimbria, hippocampus (Hc), Superior and inferior colliculi (SupCol, InfCol), and cerebellum (Cblm).

Figure 4.

The extent of morphometric changes compared to controls range from subtle so as not be identifiable in the global volume (but localized to smaller regions) in the hippocampi of the Reeler mice, to a few percent (9%) in the striatum of the DAT-KO (Cyr, Caron et al. 2005) to 300% in the ventricles of the reeler mouse (Badea, Nicholls et al. 2007).

Figure 5.
Covariance structure in CNS and identification of functional subgroups (previously published:
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Table 1

The correlations between volumes as estimated by MRM and classical histology are tighter for well defined structures and even for some partial structures compared to those among structures made up by groups of nuclei such as amygdala, more difficult to define in a consistent fashion. Different boundaries definitions as well as segmentation accuracy account in part for discrepancies.

MRM/histology	GN Trait ID	r	p
Brain	11016 (Strom et al, 1999)	0.86	0.003*
Cortex	10997 (Rosen et al)	0.82	0.002*
Thalamus	10738 (Dong et al, 2007)	0.8681	0.0005*
Olfactory Bulbs	10645 (Williams et al, 2001)	0.82	0.003*
Cerebellum	10004 (Airey et al, 2001)	0.7	0.008*
Hippocampus	10376 (Lu et al, 2001)	0.64	0.03*
Striatum	10710 (Rosen, 2009)	0.51	0.11
Amygdala	10674 (Mozhui et al 2007)	0.14	0.69