Magnetic Resonance Imaging as a Tool for the Study of Mouse Models of Autism

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Abstract

Autism is a heterogeneous disorder, in both its behaviour and genetics. This heterogeneity has led to inconsistencies in the neuroanatomical findings in human autistic patients. The benefit of a model system, such as the mouse, is that there could be a decrease in the heterogeneity of the genetics and standardization of the environment could be done, in order to determine a specific anatomical phenotype, which is representative of a specific genotype. Magnetic Resonance Imaging (MRI) has been used quite extensively to examine morphological changes in the mouse brain; however, examining volume and tissue microstructure changes in mouse models of autism with MRI, is just in its infancy. This review will discuss the current research on anatomical phenotyping in mouse models of autism.

Introduction

In Leo Kanner’s 1943 paper he evaluated 11 children with differing signs and symptoms, describing what have come to be referred to, as Autism. The children in that study were quite heterogeneous in both their symptoms, and the severity of those symptoms. Autism, as currently defined, is still quite heterogeneous. The three hallmark characteristics of autism, social deficits, communication deficits, and repetitive restrictive behavior, have large ranges in severity. For example, the communication deficits range from a delay in the development of spoken language to a total lack of any communication (American Psychiatric Association, 2000). Autism is a genetic disorder, with a 90% concordance rate in identical twins and a 15-20% risk of autism in siblings. Similar heterogeneity is seen in the genetics, with well over 200 genes associated with Autism [2]. However, no single gene accounts for more than 1-2% of autistic cases [3].

Using Magnetic Resonance Imaging (MRI), one can detect subtle volume and tissue microstructure changes in the brain, in both humans and the mouse [4]. Meta-analyses of human brain imaging papers have revealed some overlap across studies, yet autism imaging research is plagued by inconsistencies [5-8]. The authors of these analyses highlight age and IQ as an explanation for these inconsistencies, which is certainly a factor, but it is also the genetic, environmental, and behavioural heterogeneity that is driving this variability in imaging. In an animal model, such as the mouse, almost all of that heterogeneity could be eliminated as the genetics and the environment can be tightly controlled.

This review will focus on MRI in mouse models of autism. Specifically, examination of how MRI is used to assess differences in volume and tissue microstructure in the mouse brain would be done. The current literature will be discussed, followed by a brief synopsis of where to go from here.

The Mouse as a Model System

When the sequencing of the human genome was completed [9,10], researchers started to map the genomes of other mammals. The first mammal examined was the mouse [11]. Knowing the genome of the mouse allows one to gain an understanding of how the genotype relates to the phenotype: the anatomical or behavioural characteristic of the mouse. The genes and pathways in the mouse are very similar to the human; in fact, there is a 99.5% probability that a gene from the mouse is also recognized in the human [11]. Economical reasons also make the mouse an excellent model for research as well. For one, the mouse is quite small in size, limiting housing costs. Secondly, a number of different readily available inbred mouse strains exist, which are, within each strain, genetically identical. Genes can be added, deleted or replaced with relative ease in the mouse, allowing the investigation of the effect of any specific gene. A growing inventory of behavioural tests that show characteristics similar to autism has been reported. Combining all of these factors makes the mouse an easy to use and economical model system, with which the consequences of human disease and behavior could be examined.

Magnetic Resonance Imaging in the Mouse

Where a brain phenotype is unknown, 3D imaging techniques at the mesoscopic scale (which is a range in between microscopic and macroscopic) can detect very subtle differences, which can lead the researcher to a region of interest, for further examination at the microscopic scale [12].

Examples of mesoscopic 3D imaging techniques [12], used in the mouse are Computed Tomography (CT), which is used frequently for investigating high density structures like bone [4], or vascular trees that have been filled with X-ray opaque contrast agents [13,14]. Recently, there has been a growing interest in embryo imaging with microCT, which relies on the use of contrast agents such as iodine, to enhance soft tissue contrast [15-17]. Ultrasound Biomicroscopy (UBM), commonly used for cardiac imaging [18,19] is also useful for studying embryonic development [20]. Positron Emission Tomography (PET) or Single Photon Emission Computed Tomography (SPECT), requires the use of exogenous contrast agents which can be tagged to any molecule, nanoparticle, or cell. Both, however, are difficult to be scaled down from human to mouse [21], and are often combined with other 3D imaging techniques (MRI or CT), to obtain better spatial resolution combined

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with the molecular specificity of the PET/SPECT at low resolution [22]. Optical Projection Tomography (OPT) Imaging, which is basically fluorescence CT, has recently been used to image fixed samples of the brain or embryo of a mouse at quite high resolution [23]. Lastly, the focus of this review, there is Magnetic Resonance Imaging (MRI), which has been used extensively in the brains of both mouse models [24], and human patients.

MRI uses the nuclear magnetic resonance properties of the water molecule to produce an image of the brain, or other organs of interest. MRI has the best soft-tissue contrast of all the 3D imaging techniques. This contrast comes about because water in different regions of the brain interacts differently with the surrounding environment. With MRI, these differences in the water could be harnessed and the MRI sequences could be manipulated, to get differing tissue contrast dependent on our interests. Figure 1 shows the different types of contrast available with MRI imaging.

MRI is not readily scaled from human to mouse due to the decreased signal as the voxels are scaled down, which is caused by less water being within the voxel as they get smaller. The voxel dimensions need to decrease by 10-15 folds in each dimension (human voxel dimensions-1 mm isotropic, mouse voxel dimensions in-vivo-0.125 mm isotropic, fixed brain-0.056 mm isotropic), to achieve comparable images in the mouse as in the human. In order to achieve this increase resolution, several modifications to the MR scanner hardware and imaging protocols are required, including specialized radio-frequency coils, an increase in magnetic field strength, and an increase in the scan duration. The long scan duration causes two additional problems: 1) For in-vivo scanning, there is a time limit due to the anesthesia limits for mice, typically ~3 hrs, and 2) for fixed imaging, when there is no physiological limitation; the problem becomes scanner time, especially on a shared system. This could be overcome by scanning more than one mouse at a time in parallel; a technique coined "Multiple Mouse MRI" by the Henkelman group at the Mouse Imaging Centre in Toronto [25-27].

Currently, the major application of mouse MRI is neuroscience, with much of the work focused on genetic models. Examples include Huntington’s disease [28-31], Alzheimer’s disease [32-34], and other mental health diseases like schizophrenia [35,36], and recently, autism [37-39]. Genetic knockouts are also examined to identify the role of specific genes in development, behaviour and aging. Behaviour links tightly with anatomy, with 90% of gene mutations in mice that show a motor/neurological deficit, featuring an MRI detectable anatomic phenotype [40], and surprisingly even learning and memory can be detected in neuroanatomical changes. Five days of training in the Morris water maze were sufficient to induce changes in mesoscopic neuroanatomy [41], indicating that anatomical phenotyping could be used to assess learning or other behaviours in the mouse.

### Anatomical Imaging with MRI

Anatomical phenotyping with MRI can be used to examine differences between groups of mice, usually a mutant mouse group versus a control mouse group, with the goal being to determine where in the brain they differ. This could be done by measuring the volumes of brain structures, which gives us a quantitative measure that can be compared between groups. In some cases, it may be easy to see a difference in volume; for example, the Engrailed2 Knockout (KO) mouse has a smaller cerebellum, which is clearly visible [42]. However, in other cases, the differences may be quite subtle. Deformation Based Morphometry (DBM) is a commonly used automated technique that can be used to detect anatomical differences between populations. DBM requires no prior hypotheses and produces an unbiased measurement of the volume differences between groups, across the entire brain. DBM is a quantitative image analysis technique which evaluates information contained within the vector field, generated by the nonlinear warping of an individual MRI scan to some sort of reference brain, or to each other [32,43]. DBM has been used previously to examine cross-sectional morphological differences and longitudinal anatomic changes in humans [44], as well as in mouse models [45-47]. Figure 2 is a diagram of the process used for DBM. Using a method such as this, is highly specific and reproducible [48], and with only 10 mice in each group (genetic mutant vs. control), a 5% difference in volume could be detected.

### Diffusion Tensor Imaging (DTI)

DTI is an alternative method used to generate different type of contrast on an MRI image, and can provide quantitative information that can be related to the tissue microstructure. DTI was originally proposed in 1994 by Basser et al. [49], and in that work they estimate, what is called the effective diffusion tensor by measuring the diffusion of water in multiple directions. This diffusion tensor is representative of how water diffuses within a certain voxel, and highlights differences between isotropic (unordered or spherically symmetric) tissues, such as gray matter, and anisotropic (highly ordered) tissues, such as the white matter. The major quantitative measures taken from DTI imaging are Fractional Anisotropy (FA), which measures the degree of anisotropy
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in the cerebral hemispheres, cerebellum, and caudate nucleus, whereas the
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[56] published a Meta analysis determining when exactly the brain
autism with MRI, without identifying much consensus. However,
differences can be seen with the amygdala [53,55]. In the mouse, the
other studies report a decrease or no change at all [54,55]. Similar
structural connectivity in the brain.

As demonstrated from this previous work in the mouse, using
MRI can provide the researcher with an enormous amount of data that
identifies previously unknown regions of interest, and may help answer
questions about both the effect of genetics or behaviour on the brain.

Imaging in Autism

In human autism, the results are often compounded by confounds
such as age, IQ, environment, genetics, etc. These factors increase the
heterogeneity of an already heterogeneous disorder. For example, while
some studies report an increase in the size of the hippocampus [53],
other studies report a decrease or no change at all [54,55]. Similar
differences can be seen with the amygdala [53,55]. In the mouse, the
 genetics of the subjects could be matched, such that a single genetic
mutation could be looked at, and further the environment could be
controlled and most of these confounding factors could be eliminated.

Humans

Several meta-analyses have examined volumetric findings in human
autism with MRI, without identifying much consensus. However,
there are trends worth mentioning. In 2005, Redclay and Courchesne
[56] published a Meta analysis determining when exactly the brain
is enlarged in autism, as there have been conflicting reports. They
conclude that there is an early period of pathological brain overgrowth,
followed by normalization in autism, and this happens during the first 5
years of life. Stanfield et al. [6] performed a meta-analysis on structural
MRI studies, in order to determine the neuroanatomy of Autism. They
also looked at the total brain and found that it increased in size as well as
the cerebral hemispheres, cerebellum, and caudate nucleus, whereas the
corpus callosum was reduced. They also noted that the inconsistencies
in the literature might relate to differences in age and IQ, as well as
different regions showing abnormal growth trajectories. A review that
summarizes the findings from structural MRI studies of human autism
has recently been published by Stigler et al. [57].

While these studies highlight some consistent anatomical findings
in autism, there is no possibility of accurately diagnosing a child with
autism, using structural MRI findings alone. The most consistent,
well replicated finding is the reported decrease in size or thinning of
the corpus callosum, and there are still reports that have not found
differences. Two possible causes lead to this inconsistency: 1) The
noise of the given study is too high to find the subtle changes that are
happening in the brain, and 2) there are multiple causes of autism
(i.e. different genes) that result in different anatomical correlates,
yet produce similar behavioural symptoms. Thus, a model system in
which the heterogeneity of the genetics could be decreased and the
environment could be standardized is needed, which makes the mouse
ideal.

Mouse

As mentioned previously, human autism is defined by three
behavioural characteristics: social deficits, communication deficits,
and repetitive restrictive behaviours. While it may follow that autism
in the mouse should be equivalently behaviourally diagnosed, how to
determine a communication or a social deficit in the mouse? Jacqueline
Crawley’s lab has pioneered behavioural testing in the mouse to help
define autistic behaviour [58-60], and in fact there have been a few
behaviourally autistic mouse model strains that have been discovered.
An example of a mouse that encompasses all 3 of the core behavioural
features of Autism, would be the BTBR mouse [61,62].

For the most part, however, autism in the mouse is defined only
through genetics. Autism related syndromes account for a small
portion of autistic patients. The rest of the autism population is
made up of abnormal Copy Number Variations (CNVs), single gene
mutations, or currently unknown causes [3]. These unknown cases are
thought to be the cause of multiple genetic mutations. Currently, the
SFARI gene database lists 200+ genes that have been associated with
autism [2], with no single gene accounting for more than 1-2% of
autistic cases [3]. Of those 200+ genes, 70+ are listed as having animal
models, with that number increasing every year. Typically, the way a
new mouse model of autism is created is as follows: a genetic study of
models, with that number increasing every year. Typically, the way a
new mouse model of autism is created is as follows: a genetic study of
human autism is performed, and a genetic mutation is
discovered. Then a mouse model, which is representative of that genetic
mutation, is created and analyzed to see how it relates to the human
case. For example, Jamain et al. [63] found an inherited mutation in
the NeuroLigin3 (NL3) gene in a family with two brothers, one with
typical autism and the other with Asperger’s syndrome. This mutation
replaced a highly conserved arginine Residue with Cysteine at amino
acid position 451 (R451C), which caused a decrease in the amount of
NL3. Tabuchi et al. [64] later introduced that same mutation into a
mouse, creating the NL3 R451C Knockin (NL3 KI) mouse model.

Of those 70+ genetic mouse models, less than 10 have published
on volumetric analysis using MRI; most of them recently (Table 1). Therefore, using MRI to detect differences in mouse models of autism is
just in its infancy. However, there is a growing literature on the subject.
Originally, the papers focused on single gene syndromes that were related
to autism, such as Fragile X Syndrome (FXS) and Rett Syndrome (RTT).
Approximately, 15-33% of the patients with FXS are also classified as
having autism, and currently under DSM IV (although this is changing
in DSM V), Rett Syndrome is classified as an Autism Spectrum Disorder
Patients. Furthermore, the thinning of the corpus callosum and motor cortex are also commonly found in RTT. The authors do note, however, that not all the morphologic abnormalities that are found in RTT were seen in the mouse model, as the caudate nucleus and thalamus were not decreased in size in this mouse. In 2008, Ward et al. [69] preformed a longitudinal study on the brains of a RTT null mouse from 21 to 42 days of age. They used MRI to calculate 4 different measures of brain development: total brain volume, cerebellar volume, ventricle volume, and motor cortex thickness. Similar to the 2006 study, total brain volume was decreased in the RTT null mice at all time-points in the study, and the cerebellum volume was also decreased initially, but normalized by 42 days of age; however, the motor cortex thinning reported in the 2006 study was not replicated. The same group later assessed the response to environmental enrichment on these same 4 regions [70]. They determined that the environmental enrichment not only improved the performance of the RTT mouse in locomotor and fear conditioning tasks, but it also showed that the ventricular volume negatively correlated with the improved locomotor activity. In 2011, Ellegood et al. [38] examined the brain of the MeCP2truncation RTT mouse (Society for Neuroscience Annual Meeting). They reported the volumes of 62 different structures. Similar to the previous RTT studies, the total brain volume was decreased, and they report volume changes that are consistent with what has been found previously in human RTT. These four studies highlight the power of using MRI to detect volume differences in the brain. Not only are the changes found often replicated in subsequent studies, but also these changes show similar findings to human RTT patients, showing that anatomical phenotyping in mouse models can replicate volumetric abnormalities found in human patients.

Copy number variations are quite common in the human population, and specific CNVs have been found to be associated with autism susceptibility. The long arm of chromosome 16 is an example. A deletion of the 16p11.2 region is associated with autism, while a duplication of this region is associated with both autism and schizophrenia. Recently, Horev et al. [71] created mouse models of 16p11.2 deletion and duplication. These mice were then anatomically phenotyped to look for differences in the brain between groups. In this study, the authors report a strong dosage effect on the volumetric findings in the brain. Specifically, deletions in the 16p11.2 increased brain size, in comparison to controls. Conversely, 16p11.2 duplications lead to decreases. In fact, these mice had dosage dependent effects in gene expression, brain architecture, and behaviour. Furthermore, they found that the deletion was more severe than the duplication. Strong increases in brain size between the 16p11.2 deletion and the WT were found in a number of midline structures, with the hypothalamus findings being the most intriguing. The hypothalamus finding in this study was a previously unreported finding in mouse or human; however, it did account for the behaviour seen in the mouse. Thus, anatomical phenotyping added a previously unknown region of interest that in fact was responsible for the behavioural phenomenon.

Recently, two additional single gene mutations which are associated with autism, have been examined in the mouse. Many common volumetric findings were found in the two models. The two seemingly unrelated models are the Neurologin3 R451C Knockin (NL3 KI) and the Integrinb3 Knockout (ITGb3 KO) mouse. The Neurologin genes are synaptic adhesion genes located on the postsynaptic membrane, and the ITGb3 gene's role is to control platelet function, cell adhesion and cell signaling, as well as being related to the serotonin system. Both of these genes have been associated with Autism in separate human studies [63,72]. These mouse models were both studied using the same MRI sequence and analysis [38,39]. The NL3 KI mouse model had marked

<table>
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<tr>
<th>Gene/Disorder</th>
<th>Model</th>
<th>Volume Measured</th>
<th>DTI Performed</th>
<th>Paper</th>
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<tr>
<td>Fragile X Syndrome</td>
<td>FMR1 KO</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Rett Syndrome</td>
<td>Mecp2 null</td>
<td>Yes</td>
<td>No</td>
<td>Saywell et al. [67]</td>
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<td>Mecp22truncation</td>
<td>Yes</td>
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<td></td>
<td>Mecp21lox</td>
<td>Yes</td>
<td>No</td>
<td>Nag et al. [69]</td>
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<td></td>
<td>Mecp22lox</td>
<td>Yes</td>
<td>Yes</td>
<td>Ellegood et al.</td>
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<td>No</td>
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<td>NL3 KI</td>
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<tr>
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<td>Yes</td>
<td>No</td>
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<td>BALBC/J</td>
<td>Social</td>
<td>No</td>
<td>Yes</td>
<td>Kumar et al. [72]</td>
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</table>

Table 1: Studies that have examined volume and Diffusion Tensor Imaging (DTI) changes with MRI in mouse models related to autism.
volume differences in many different structures, including the total brain volume, which was decreased by 8%. Specific gray matter regions such as the hippocampus, striatum, and thalamus were significantly decreased; in fact, the total gray matter in the brain was decreased by 8%. Similarly, white matter regions had quite strong decreases in size, the corpus callosum, cerebral peduncle, fornix, and internal capsule were all strongly decreased in volume, and the total white matter volume was also decreased by 10%. The ITGβ3 KO mouse model also had strong volume differences. Similar to the NL3 KI, the ITGβ3 KO mouse’s total brain volume was decreased by 11%, and while not all the volume differences were similar to the NL3 KI mouse, there were some striking similarities. 1) In both the NL3 KI and the ITGβ3 KO mice, the white matter was strongly affected. White matter differences have become common findings in human autism, with the theory that children undergo a period of abnormal white matter development. Furthermore, white matter deficits in Autism have been thought of as atypical or incomplete connectivity; 2) Volume differences in the corpus callosum have a similar pattern in both models. Figure 3 shows an image of the significant decreases in the corpus callosum in both models. As mentioned, a decreased volume or thinning of the corpus callosum has been one of the most consistent findings in human autism; 3) Both the ITGβ3 KO and NL3 KI also had significantly smaller hippocampi, and in both cases, the dentate gyrus and stratum granulosum were much smaller. These similarities between two seemingly unrelated mouse models of Autism highlight a large benefit of the unbiased volumetric measurements performed with Deformation Based Morphometry, using MRI. Anatomical phenotyping can show similarities and differences across the spectrum of autistic models, perhaps grouping some of the genetic causes.

All the white matter volume difference reported in these mouse models, make DTI increasingly necessary to look at the tissue microstructure of the white matter. Only a few studies have looked at mouse models of Autism with DTI. One study examined the FMR1 KO and found no differences in any of the diffusion measures [67]. Another study on the NL3 KI model found only small differences in FA in the globus pallidus of the mouse brain, in spite of the large number of volume differences found in the white matter structures in that model [38]. Given these large volume differences in the white matter in the NL3 KI, the authors were surprised to find a lack of FA differences. They speculated that this could be caused by a loss in the number of axons (a decreased bandwidth), but that the density, size and organization of the axons remained consistent between models. Recently, Kumar et al. [73] used DTI to examine the BALB/CJ mouse. The BALB/CJ mouse is a model of reduced sociability relevant to Autism. In that study, they examined the social behaviour of the BALB/CJ mouse at 3 different time-points and scanned the mice longitudinally with DTI at each of these times. The authors examined 8 manually selected regions of interest (5 gray matter and 3 white matter), in which they reported trends (as noted in that paper, these findings did not hold up when corrected for multiple comparisons) of higher Mean Diffusivity (MD) in the corpus callosum, and a reduced Fractional Anisotropy (FA) in the external capsule. They attribute the change in FA in the external capsule to reduced myelination, although it could also be attributed to a change in the structure or density of the axons in that region.

Conclusions and Future Directions

Anatomical phenotyping at the mesoscopic scale in autism is obviously still in its infancy and no strong conclusions about autism, as a whole can be made from the imaging that has been performed so far. In spite of the findings that the ITGβ3 KO and NL3 KI have similar anatomical characteristics, there is no great overlap across the small number of mouse models of autism that have been examined currently, and perhaps one should not be expected. The anatomical findings of each individual gene or CNV are certainly relatable to the same genetic case in the human population, as illustrated by the RTT findings. With the 70+ mouse models of autism currently existing and <10 models examined, a larger overlap or grouping of models could not be found, until more are investigated. The goal should be to examine as many models of Autism, in as similar a way as possible. Then the findings from all of those mice should be pooled together to cluster the different models based on their neuroanatomical findings. These clusters could then give rise to different Autism subsets allowing for different treatments. This in turn, could lead to better individual treatments of human autism.

References

vasorum in aortas of apoE/-/LDL/- double knockout mice. Atherosclerosis 212: 444-450.


