Magnetic resonance virtual histology for embryos: 3D atlases for automated high-throughput phenotyping

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Abstract

Ambitious international efforts are underway to produce gene-knockout mice for each of the 25,000 mouse genes, providing a new platform to study mammalian development and disease. Robust, large-scale methods for morphological assessment of prenatal mice will be essential to this work. Embryo phenotyping currently relies on histological techniques but these are not well suited to large volume screening. The qualitative nature of these approaches also limits the potential for detailed group analysis. Advances in non-invasive imaging techniques such as magnetic resonance imaging (MRI) may surmount these barriers. We present a high-throughput approach to generate detailed virtual histology of the whole embryo, combined with the novel use of a whole-embryo atlas for automated phenotypic assessment. Using individual 3D embryo MRI histology, we identified new pituitary phenotypes in Hex1 mutant mice. Subsequently, we used advanced computational techniques to produce a whole-body embryo atlas from 6 CD-1 embryos, creating an average image with greatly enhanced anatomical detail, particularly in CNS structures. This methodology enabled unsupervised assessment of morphological differences between CD-1 embryos and Chd7 knockout mice (n = 5 Chd7+/− and n = 8 Chd7−/−, C57BL/6 background). Using a new atlas generated from these three groups, quantitative organ volumes were automatically measured. We demonstrated a difference in mean brain volumes between Chd7+/− and Chd7−/− mice (42.0 vs. 39.1 mm3, p<0.05). Differences in whole-body, olfactory and normalised pituitary gland volumes were also found between CD-1 and Chd7−/− mice (C57BL/6 background). Our work demonstrates the feasibility of combining high-throughput embryo MRI with automated analysis techniques to distinguish novel mouse phenotypes.

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mutants, effective methods of identifying novel phenotypes in these embryos will be crucial.

Current phenotyping of embryo morphology is generally achieved by histological examination using microscopy. Specimens are dehydrated, wax embedded and thinly sectioned (2–8 μm) (Kauffman, 1992), providing high resolution 2D data and tissue sections that may also be stained for gene and protein expressions. Episcopic imaging is a development of this process, where autofluorescence of each tissue slice can be photographed and combined to generate high resolution 3D volume datasets (1–2 μm isotropic resolution) (Weninger and Mohun, 2002). However these histological approaches are time-consuming, introduce distortions into the final 3D image due to the sectioning process and do not readily enable rapid screening as only one embryo may be imaged at a time.

MRI is now an established method for non-invasive embryo imaging, beginning with the early work of Smith et al. (1994). High resolution 3D datasets with isotropic resolutions of down to 12 μm are created (Smith et al., 1996) with excellent soft tissue contrast, allowing the visualisation and segmentation of individual organ structures (Dhenain et al., 2001). MRI is also capable of high-throughput screening of multiple ex-vivo embryos (up to 32 in a single overnight scan) with the combination of a large volume imaging coil (Schneider et al., 2004) and fixation in an MR contrast agent (Clery et al., 2009). Diffusion tensor imaging (DTI) — an alternative MRI technique — has also been used to investigate the structure of the embryo CNS by exploring the degree and direction that water is able to diffuse along neuronal axons (Zhang et al., 2003). Although this technique is ideal for investigating white matter, it is impractical for high-throughput imaging, as many hours are needed to generate connectivity maps in a single brain.

Other imaging methods such as optical projection tomography (OPT) and micro-computed tomography (μCT) are also able to non-destructively produce 3D datasets. OPT can create images of embryos that combine both anatomical structure and gene expression with conventional fluorophores, at high resolution (5–10 μm) (Johnson et al., 2006; Sharpe, 2004). However the technique requires embryos which are partially transparent thereby making its use challenging in older subjects (>13.5 days post coitum, dpc) (Schneider and Bhattacharya, 2004). μCT is also capable of acquiring high resolution datasets (typically less than 27 μm) in a short scan time (~2 h) (Johnson et al., 2006). Although conventionally μCT has difficulty in distinguishing soft tissues, which have inherently low contrast due to a narrow range of CT numbers (Holdsworth and Thornton, 2002), the use of CT contrast agents as tissue stains, such as osmium tetroxide (Johnson et al., 2006) and potassium triiodide (Degenhardt et al., 2010), have improved its ability to discriminate tissues. μCT is also particularly suited to skeletal studies, as it can produce excellent images of dense radiopaque structures such as bone (Oest et al., 2008).

Despite the availability of these advanced imaging techniques, any embryo dataset must still be manually assessed through inspection by a trained observer. As high-throughput analysis is increasingly demanded, conventional visual assessment for abnormalities is likely to become labour-intensive and insensitive.

Advanced computational techniques such as segmentation—propagation and voxel-based morphometry (VBM) have been used to investigate populations in both clinical and adult mouse MRI studies (Ashburner and Friston, 2000; Calmon and Roberts, 2000; Lerch et al., 2008; Sawiak et al., 2009). These methods enable anatomical differences between groups to be identified with little manual intervention or visual assessment. Integral to these techniques is the use of an atlas, a spatial average image of the whole population (Ashburner and Friston, 2000; Calmon and Roberts, 2000) created by finely warping individual subject images together to locally align anatomical features. Segmentation—propagation is a quantitative method for making volumetric measurements. After segmenting a volume of interest on the atlas image, such as the brain or heart, differences may then be identified between groups in the population by propagating the segmented volume to all individuals, thus providing the group mean and standard deviation from a single volume of interest on the atlas image. However, while average images of registered wild-type embryos have been reported previously (Zamyadi et al., 2008), there has so far been no application of atlas methods for phenotypic assessment. We envisage that a combination of multiple whole-embryo imaging with image processing techniques would allow the creation of an average embryo atlas from a population and enable automated phenotypic comparisons between transgenic and wild-type littermates.

In this study there were three stages to the investigation of our embryo atlas. We started by addressing the lack of brain tissue contrast on MR images. Initially we developed a contrast enhanced MR technique to produce structural detail in the embryo CNS. This was assessed in Hesx1+/− and Hesx1I26ST/126NT mice, models of septo-optic dysplasia (SOD) (Dattani et al., 1998; Sajedi et al., 2008). Combining our contrast enhanced protocol with computational methods, we generated an MRI atlas for a population of CD-1 embryos and compared this against histology. Finally, this enabled the use of a segmentation—propagation technique to assess brain and cardiac phenotypic differences between CD-1, C57BL/6 strains and Chd7+−/− knockout mice (a model of the condition CHARGE syndrome) (Bosman et al., 2005; Randall et al., 2009) based on a novel population atlas.

Materials and methods

Animal preparation

Pregnant female mice were sacrificed by cervical dislocation. Embryos were then dissected from the mother and transferred to warm Hanks solution. Their umbilical cords were cut and the embryos were allowed to bleed out into the solution. The embryos were then fixed in a solution of 4% formaldehyde and Magnevist MR contrast (Gadolinium-DTPA, Bayer-Schering Pharma, Newbury, UK) and left on a rotator. The embryos were removed and embedded in a 50 ml centrifuge tube using 1% agarose gel doped with an identical concentration of Magnevist to initial fixation. After comparing Gd-DTPA concentrations (2, 4, 8 and 16 mM) in groups of CD-1 embryos over two fixation durations (three days and two weeks), we determined that fixing embryos with 8 mM Gd-DTPA for at least two weeks produced the greatest SNR and brain contrast without major susceptibility artefacts. Three groups of mice were imaged at 15.5 dpc: outbred CD-1 mice from an established colony at UCL, and Chd7−/− wild-type and heterozygous knockout mice (C57BL/6 background). Also Hesx1+/−, Hesx1I26ST/126NT and wild-type embryos at 18.5 dpc.

Imaging

Embryos were imaged on a Varian VNMRS 9.4 Tesla MRI system (Varian Inc., Palo Alto CA, USA) using a 33 mm quadrature birdcage volume coil (RAPID Biomedical GmbH, Würzburg, Germany). Maps of T1 and T2 in tissue were created using a single sagittal slice field of view = 27 × 27 mm2, 256 x matrix size, 0.5 mm thickness. For 3D imaging we used a gradient-echo sequence, TR = 20 ms, 7 averages, and flip angle = 60°. Field of view = 27 × 27 × 27 mm3, matrix size = 512, zero-filled to 1024 in the console to a voxel-size of 26 × 26 × 26 μm3. Echo time (TE) was 9 ms for 15.5 dpc embryos and 5 ms for 18.5 dpc embryos to enable better visualisation of the bone structures of the cranial floor.

Image registration

Embryos were first semi-automatically extracted from the dataset and group-wise registration, using global and local transformations, was performed to create an average image atlas. The obtained deformation parameters from each individual to the template were then used to propagate the different regions of interest to each embryo.
Digital extraction

The embryos were extracted from the acquisition volumes using simple operators such as Gaussian smoothing, thresholding and mathematical morphology (erosion, dilation and selection of connected components).

Registration and atlas average creation

The intensity ranges of the embryos were first normalised prior to registration. One embryo was selected and taken as the reference, with all further processing performed in its image space. The others were first spatially normalised using a rigid registration algorithm consisting of 6 global degrees of freedom (DOF): 3 translation and 3 rotation parameters, in order to correct the differing orientations of each embryo in the acquired images. All resulting images were then averaged into a new blurry reference image. This initial registration step, due to the rigid transformation, did not affect the geometry of the embryos. Thus the average image was not biased toward the selected embryo’s geometry. The embryos were then aligned using affine registration (12 global DOF: 3 translations, 3 rotations, 3 scales and 3 shears) to the previously created average image. Rigid and affine registration was performed using a block-matching technique (Ourselin et al., 2001). We performed 5 iterations using the affine algorithm. After each iteration the resulting average image was updated (an example of the result after affine registration is shown in Fig. 5A). A non-linear registration algorithm, Free-Form Deformation (FFD) (Rueckert et al., 1999) was then used to locally align the embryos to the reference average image. Control point positions were optimised in order to locally deform each image to the target reference image. The degree of alignment was evaluated using Normalised Mutual Information (NMI) (Studholme et al., 1999). We performed 18 iterations of non-rigid registration, using a coarse-to-fine approach where the control point spacing was gradually reduced to 5 voxels width (130 μm) overlaid on the full resolution images. After each non-linear registration step, we ensured the average image was geometrically in the middle of all input images by subtracting the mean total displacement from each deformation field before resampling the embryo images to produce the average atlas. All non-linear registration steps were performed using our parallel implementation (Modat et al., 2010) of the FFD algorithm on a computing cluster, which allowed them to run concurrently within each step. We used a cluster with approximately 1000 CPU cores.

Volume segmentation, rendering and propagation

Regions of interest were semi-automatically segmented and rendered using Amira visualisation software (v5.2.2, Visage Imaging Inc., Andover MA, USA). As the result of the group-wise registration consists of an atlas average image and a set of deformation fields, there is a direct spatial correspondence between every voxel in the average image and their corresponding positions in each single embryo. Using this set of deformation fields, segmented regions of interest were propagated from the atlas into the original image space of each embryo.

Signal-to-noise measurements

Signal-to-noise (SNR) in raw image data was calculated by taking the mean signal in a volume of interest and dividing by the standard deviation of the signal in a region of background noise.

Statistical analysis

Analysis of volume data was performed in Prism v5.00 (GraphPad Software, San Diego CA USA) using one-way ANOVA with Tukey testing for multiple comparisons (propagated volumes) and the Kruskal–Wallis test with Dunn’s test for multiple comparisons (normalised volume data).

Results

Micro-MRI for the investigation of embryo brain anatomy in 3D

15.5 dpc CD-1 embryos were fixed in 4% formaldehyde solution with a gadolinium–chelate contrast agent (Gd-DTPA) and imaged at 52 μm resolution, interpolated to 26 μm by zero-filling. Obtaining tissue contrast, particularly in brain structures is often difficult in embryo imaging. We have evaluated images of the embryo CNS acquired from specimens fixed in four contrast agent concentrations (2 to 16 mM) and two fixation durations (three days and two weeks) to determine optimal preparation. Two weeks fixation resulted in better penetration of the agent into brain structures compared to 3 days, as determined by reduced tissue T1 (e.g. 179 to 80 ms, 4 mM). We determined that embryos fixed in 8 mM Gd-DTPA for two weeks, produced images with the highest brain contrast-to-noise (CNR = 8.0, 8 mM vs. CNR = 5.7, 4 mM, between external capsule and basal ganglia regions), without major artefacts. Images also showed excellent tissue contrast throughout the entire embryo. White-matter tracts and developing layers of the cortex were clearly visualised (Fig. 1), which was due to the selection of MRI parameters; a relatively long 9 ms echo time compared to the 6 ms T2* of the CNS (mean T1 = 38 ms) giving T2*-weighted images. Using this imaging protocol, multiple subjects may then be imaged (up to 40: using a 35 mm diameter volume imaging coil, with a 60 mm z-profile) in a single 10-hour overnight scan with high SNR (~23 over the whole embryo).

Following our preliminary investigation, we applied our imaging protocol to a phenotyping study of 18.5 dpc embryo mice (Hesx1+/−, Hesx1I26T/I26T and wild-type) for CNS and pituitary defects. Hesx1-deficient embryos showed hypoplasia of the left olfactory bulb and telencephalic vesicles (Fig. 2). Eyes were absent with no sign of optic

Fig. 1. Sagittal section through an example 3D embryo dataset showing CNS structures in the brain. Structures of the cortex can be seen, including cortical plate (CP), ventricular (VZ), intermediate (IZ) and marginal zones (MZ) (blue labels). Also thalamus (Th.), hypothalamus (H/t) and ventricular septum (Sep.).
Fig. 2. (A) Sagittal and coronal MR images of 18.5 dpc wild-type, Hesx1−/− and Hesx1I26T/I26T 18.5 dpc mice at the levels showing greatest changes in structure. Similar histology views from the same embryo are also shown. The pituitary gland is also visibly enlarged and penetrates the oropharyngeal cavity (Hesx1−/− sagittal view, middle-left). In the Hesx1I26T/I26T mutant, in addition to an enlarged pituitary gland, structures of the left hypothalamic area can be seen to be upwardly shifted (red arrow). (B) 3D volume rendering of whole-brain and pituitary structures. In the Hesx1−/− mutant, the gland is visibly dysmorphic and enlarged compared to wild-type, with a large amount of associated mucosal soft tissue posterior. The rendering also shows an underdeveloped left olfactory bulb (white arrows). The gland in the Hesx1I26T/I26T mutant (bottom) is highly dysmorphic compared to wild-type.

Fig. 3. Similar axial and sagittal images comparing single embryo, atlas and equivalent histology sections. Even with six embryos, there is a noticeable improvement in signal-to-noise. Structural details common to all embryos appear enhanced in the atlas image and corresponding well to histology, such as internal (blue triangles) and external capsule (red arrows).
nerve development. Close examination revealed an apparent hyperplasia of the pituitary gland with an outgrowth of closely associated mucosal tissue, which has not been previously described (Dattani et al., 1998) (Fig. 2A) in Hesx1−/− mutants. Tissue contrast was readily apparent between both parts of the gland in wild-type embryos, which enabled segmentation of the individual lobes (Fig. 2B). The Hesx1I26T/I26T embryo showed milder forebrain defects with normal corpus callosum and olfactory bulbs, but eyes were also absent. This particular specimen showed a distinct phenotype in which the anterior and posterior pituitary lobes could not be identified, but there was a tissue mass invading the oropharyngeal cavity. Although ectopic oral pituitary tissue has been inferred from histological analysis, it has not been shown so evidently as in Fig. 2B. The midcoronal MRI shows an obvious cranial shift of the left anterior hypothalamic area as a result of the malformation of the pituitary gland (bottom of Fig. 2A, red arrow). These MR findings were all confirmed on subsequent histology (Fig. 2A).

**Generation of an MRI embryo population atlas**

Conventionally, embryo atlases use single subjects at a given developmental time point to illustrate representative anatomy. In this study we have generated an average atlas of a population of midgestation embryos. Using computational methods combining 3-stages
of image registration — rigid, affine and non-linear registration — we warped 6 CD-1 embryos into a single average image.

Fig. 3 demonstrates that the atlas reinforces structures common to all, whilst reducing the effect of imaging or preparation artefacts present in individual embryos. Combining images from multiple subjects also increases image signal-to-noise. Comparing our images to histology, we observed that hypointense regions on the MR images, enhanced in the composite atlas, correlate well to large fibre structures in the developing CNS such as internal (Fig. 3, blue triangles) and external capsule (Fig. 3, red arrows). We note that the anatomical detail has been preserved during atlas formation and SNR is improved and as such, the atlas is ideally suited to a host of image processing techniques such as brain volume segmentation–propagation, which will be explored in the following section.

Unsupervised generation of quantitative tissue volumes in a whole population

Fig. 4 shows the steps in the segmentation–propagation process of an MRI embryo atlas. This allows regional segmentation from a single average image to be mapped onto the original individuals for calculation of tissue volumes. We examined the utility of our atlas segmentation in automatically identifying volume differences between two groups of mice with differing backgrounds: outbred CD-1 (n = 6) and C57BL/6 mice (n = 13). The C57BL/6 mice were further subdivided into wild-types and Chd7 +/- knockout mice (n = 5 and n = 8 respectively).

We first created an MRI atlas of these 19 individuals using our registration techniques. The improvement in the average image after 18 iterations of non-linear, compared to initial affine registration, is shown in Fig. 5. The resulting average atlas was then manually segmented (Fig. 6) into left olfactory bulb (coloured green), pituitary (yellow), mesencephalic vesicle (blue), whole-brain (purple) and whole-body

Fig. 5. Overview of average images produced during the registration process: (A) average image of our 19-embryo dataset after global alignment (affine registration). (B) Final average image after multiple iterations of the local deformation algorithm. The sharpness of the non-linear registration result as seen in image (B) indicates good alignment of structure.

Fig. 6. 3D volume rendering of our atlas of 19 subjects indicating the volumes of interest drawn for segmentation–propagation volume measurement.

Fig. 7. Similar sagittal slices through our 19 embryo atlas (left) and a single CD-1 embryo (right) illustrating the good correspondence of segmented regions propagated from the atlas to individual datasets. (Red: heart ventricles, yellow: pituitary gland, green: olfactory bulb, purple: whole-brain, and blue: mesencephalic vesicle.)
(tan) volumes. Heart ventricles (red) were also segmented for comparison. Volumes from the atlas segmentation were then automatically measured for each individual through propagation. As shown in Fig. 7, there was good anatomical agreement between the areas segmented on the atlas with resulting propagation on the original individual dataset. Furthermore, propagated volumes compared favourably against manually segmentation from 6 CD-1 embryos. Using the established Dice similarity measure of spatial agreement (Dice, 1945) (where a value of 1 represents full spatial overlap and 0 is none) there was a mean correspondence between manual and propagated whole-brain volumes of 0.95±0.01. The propagated volumes measured in the three groups are summarised in Table 1.

<table>
<thead>
<tr>
<th>Region</th>
<th>CD-1 (n=6)</th>
<th>SD (%) mean</th>
<th>Chd7 WT (n=5)</th>
<th>SD (%) mean</th>
<th>Chd7+/− (n=8)</th>
<th>SD (%) mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body</td>
<td>388 6.9</td>
<td>322 10.6</td>
<td>312 4.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole brain</td>
<td>45.7 2.6</td>
<td>42.5 6.2</td>
<td>39.2 4.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>0.3 6.2</td>
<td>0.3 15.4</td>
<td>0.2 15.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vesicle</td>
<td>0.2 11.0</td>
<td>0.2 38.1</td>
<td>0.2 47.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pituitary</td>
<td>0.1 7.3</td>
<td>0.1 13.3</td>
<td>0.1 8.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart ventricles</td>
<td>2.4 12.8</td>
<td>2.2 14.3</td>
<td>2.0 7.4</td>
<td></td>
<td></td>
<td></td>
</tr>
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</table>

Analysing the propagated volumes, we found significant differences in the absolute overall brain volume between the three groups (p<0.05, Fig. 8B), with the smallest mean brain volume (39.2 mm³) observed in the Chd7+/− mice. Significant differences were found between CD-1 and Chd7 wild-type embryos in propagated volumes of the whole embryo and olfactory bulb (0.323 vs. 0.258 mm³, p<0.05, Figs. 8B and C).

In an attempt to account for developmental differences between embryos we also normalised the data to embryo body volume. After normalisation, we observed significant differences between strains in brain and pituitary volumes (both p<0.01, Figs. 8D and E).

**Discussion**

The main findings of this study were: i) development of embryo-specific MR methods and contrast agent protocols enabled high resolution structural imaging with enhanced anatomical detail in the CNS, ii) using these methods, we have identified the structural consequences in *Hesx1*−/− and *Hesx1*I26T/I26T mouse mutants, iii) we have generated a composite whole-embryo atlas using computational methods with excellent anatomical tissue contrast, and finally iv) we presented a novel application of a technique to generate a composite atlas from three groups of embryos, allowing automated high-resolution imaging.

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**Fig. 8.** (A)–(C): Graphs showing propagated volume differences in brain and pituitary regions between groups. (D)–(E): Graphs showing volume differences in brain and pituitary regions between strains after normalisation of data to embryo whole-body volumes.
throughput investigation of phenotype, through volume analysis by segmentation–propagation.

**Phenotyping the developing brain using MRI**

We have demonstrated that through our preparation of 15.5 dpc embryos, which combines fixation in a relatively high concentration of MR contrast agent (8 mM) and sufficient fixation time (>2 weeks) enabling the agent to fully penetrate into the CNS, we are able to visualise small brain structures. Image signal-to-noise is high, as brain T1 is now short and uniform across the brain (reduced to ~38 ms).

Previous embryo MRI phenotyping studies have largely concentrated on the heart (Fischer et al., 2007; Petiet et al., 2008; Schneider et al., 2003b), although some have investigated gross changes in the developing brain and other organs (Schneider et al., 2003a). The images acquired in these cases have generally been optimised to give high overall signal from tissue, resulting in proton density (Jacobs et al., 1999) or predominantly T1-weighted MR images (Petiet et al., 2007; Schneider et al., 2003c). Although the use of the gadolinium–chelate active-stains improves signal-to-noise, the narrow range of tissue T1s (Petiet et al., 2007) after staining limits the scope for T1-weighted contrast, producing a generally uniform appearance of the embryo brain. Given the short T1 of our embryos, the brain contrast observed in our images was unlikely to be due to the chosen MR repetition time (TR). We believe that the short T1 observed in our embryos (~6 ms) enabled CNS tissue contrast to be generated through the choice of echo time (TE), resulting in the well-delineated structures seen in the images acquired.

Typically MRI methods are able to produce images with the greatest degree of intrinsic CNS contrast (white-grey matter) when axons are fully myelinated (Miot-Noirault et al., 1997). In the developing embryo, while distinct myelinated brain structures are beginning to form, contrast on MR images is generally limited, as T1 and T2 differences are small in these young brains (Mori et al., 2001), making visualisation of brain structures difficult. Some of this structure has been seen without contrast enhancement, as part of a wider DTI study (Mori et al., 2001). However our technique allows a reduced scan time together with higher resolution (10 h for up to 40 whole embryos at 52 μm isotropic compared to 13 h for a single extracted brain), indicating that high throughput is possible while still maintaining high brain tissue contrast.

Beyond preparation optimisation, further improvements in SNR could be made with the adoption of multiple solenoid imaging coils as opposed to the single, large volume coil used in this study. A recent investigation showed that a greater than threefold increase in SNR is achievable but this typically requires a large number of receive channels (16), which are currently uncommon on most preclinical MR systems (Zhang et al., 2010).

We used our methods to phenotype Hesx1 embryos, which demonstrated novel phenotypic defects showing hyperplasia of the pituitary gland and closely associated mucosal tissue penetrating into the oropharyngeal cavity. The presence of pituitary tissue in this area may also explain the perinatal lethality observed in these embryos (Dattani et al., 1998), as the pups suffer from respiratory difficulties. Furthermore, we also observed a previously unrecognised cranial shift in the anterior hypothalamus. Symptoms such as obesity and circadian rhythm defects are often associated with human SOD (Rivkees, 2003), indicating that high throughput MRI could now be expanded to visualise and identify more subtle CNS defects through the combination of enhanced MRI contrast and atlasing methods.

**Automatic volume measurements in an embryo study**

We used a mixed average population atlas to make regional volume measurements from a single segmentation in the embryo. Previous work on volumetric measurements in the adult mouse brain has allowed the characterisation of wild-type brains (Dorr et al., 2008; Kovacević et al., 2005) and also volume differences between mouse strains (Chd7+/− mice indicating that Chd7 may be implicated in the process of overall brain development. This would be consistent with recent histological findings of impaired olfactory bulb development (Layman et al., 2009) and previous descriptions of hearing and ear defects (Bosman et al., 2005).


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