Using multimodal MRI to investigate alterations in brain structure and function in rats with type 2 diabetes.

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Research

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Abstract

Objectives This is an exploratory study using multimodal magnetic resonance imaging (MRI) to interrogate the brain of rats with type 2 diabetes (T2DM) as compared to controls. It was assumed there would be changes in brain structure and function that reflected the human disorder, thus providing a model system by which to follow disease progression with non-invasive MRI.

Methods The transgenic BBZDR/Wor rat, an animal model of T2MD, and age-matched controls were studied for changes in brain structure using voxel-based morphometry, alteration in white and gray matter microarchitecture using diffusion weighted imaging with indices of anisotropy, and functional coupling using resting state BOLD functional connectivity. Images from each modality were registered to, and analyzed, using a 3D MRI rat atlas providing site-specific data on over 168 different brain areas.

Results There was an overall reduction in brain volume focused primarily on somatosensory cortex, cerebellum and white matter tracts. The putative changes in white and gray matter microarchitecture were pervasive affecting much of the brain and not localized to any region. There was a general increase in connectivity in T2DM rats as compared to controls. The cerebellum presented with strong functional coupling to pons and brainstem in T2DM rats but negative connectivity to hippocampus.

Conclusion Are the neuroradiological measures collected in BBBKZ/Wor rats using multimodal imaging methods common to the clinic, similar to those reported in T2DM patients? In comparison to the clinical findings, the data would suggest the BBBKZ/Wor rat is not an appropriate imaging model for T2DM.

Background

Diabetes is a serious metabolic disorder estimated to affect 30 million people in the US as of 2016, with prevalence expected to reach more than 54.9 million Americans by 2030 [1, 2]. Diabetes is broken down into two main categories, type 1 diabetes (T1DM) and type 2 diabetes (T2DM). Destruction of pancreatic beta cells resulting in insulin deficiency is the hallmark of T1DM while T2DM is typically the result of a combination of peripheral insulin resistance and dysfunctional insulin secretion by pancreatic beta cells [3]. T2DM is much more common in the United States with 90–95% of all diabetes cases being such [1]. The pathology of T2MD is systemic, affecting much of the body and most functions. The brain is not spared as there are severe effects on cognition and behavior with disease progression and aging [4]. Studies with magnetic resonance imaging (MRI) report abnormalities in cerebral macrostructure and microstructure such as cortical atrophy [5], regional reductions in brain volume [6], structural deformities in cerebral gray matter [7], increased white matter lesions [8, 9] and changes in blood brain barrier permeability [10]. Indeed, there is a large body of literature using multimodal MRI e.g. voxel based morphometry (VBM), diffusion weighted imaging (DWI) and resting state BOLD functional MRI (rsFC), to interrogate brain structure and function in T2DM patients to better understand disease progression and prognosis for cognitive decline (for review see [11]).

Animal models using magnetic resonance spectroscopy (MRS) have provided insights into the cellular and molecular mechanisms contributing to the metabolic disorders underpinning the cerebral neuropathy of T2DM [12–15]. For example, a recent study by Duarte and colleagues used ^{13}C MRS at ultrahigh field strength (14.1 T) to follow in vivo disruption in energy metabolism and impairment in the glutamate-glutamine cycle between neurons and astrocytes in the Goto-Kakizaki (GK) rat model of T2DM [15]. While these types of animal studies are critical to understanding the neurodegeneration associated with T2DM, they cannot be performed on humans using clinical scanners. Indeed, there is a paucity of MRI studies in animal models of T2DM using imaging modalities commonly performed in the clinic. We know of only two such studies, one looking at ischemic vascular damage and axonal density following stroke in the high-fat diet, streptozotocin treated Wistar rat (HFD/STZ) [16], and a second in the TALLYHO/JngJ (TH) mouse correlating white matter connectivity using DWI with compulsive behavior [17]. Hence, the major advantage of non-invasive animal imaging – to follow disease progression with the same imaging modalities used in the clinic – has not been exploited. To address this short coming, we performed an exploratory study using VBM, DWI and rsFC to interrogate the brain of the obese Bio-Breeding Zucker diabetic (BBZDR/Wor) rat, a model of T2DM [18]. Our findings are discussed in the context of their clinical relevance and whether this animal model and the imaging modalities used would have translational value in a larger prospective study following evolution of cerebral neuropathy in T2DM.

Methods

Animal Model

Male Bio-Breeding Zucker diabetic rats (BBZDR/Wor rats) ($n = 8$) as well as age-matched non-diabetic BBDR littermates ($n = 8$), were obtained from Biobreeding in Worcester, MA for imaging. The BBZDR/Wor rat is an inbred rat strain of T2DM and is emerging as a model to study the many complications that encompass T2DM in humans. In BBZDR/Wor animals, the recessive *Iddm2* gene responsible for lymphopenia and spontaneous autoimmunity is removed while the *Lepr fa* (*fa1*) mutation is retained. Obese male BBZDR/Wor rat spontaneously develops type 2 diabetes at approximately 10 weeks of age ($\sim 100\%$) when fed standard rat chow [18–21]. The BBZDR/Wor diabetic rat displays the clinical symptoms typically associated with T2DM including dyslipidemia, hyperglycemia, insulin resistance, hypertension, and decreased levels of the beta cell-specific glucose transporter type-2 (GLUT-2) [18, 19, 22].

Rats were maintained on a 12 h:12 h light:dark cycle with a lights on at 07:00 h, allowed access to food and water ad libitum and were treated with IP injections of saline at indications of weight loss. The average age of the animals at time of imaging was about 4 months. All animal experiments were conducted in accordance with the Northeastern University Division of Laboratory Animal Medicine and Institutional Animal Care and Use Committee.

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Neuroimaging

Imaging was conducted using a Bruker Biospec 7.0T/20-cm USR horizontal magnet (Bruker, Billerica, MA, USA) and a 20-G/cm magnetic field gradient insert (ID = 12 cm) capable of a 120- μ s rise time. Radio frequency signals were sent and received with a quadrature volume coil built into the animal restrainer (Animal Imaging Research, Holden, Massachusetts). All rats were restrained using a custom restraint kit and imaged under 1–2% isoflurane while keeping a respiratory rate of 40–50/min.

Voxel-Based Morphometry

Images were acquired using RARE sequence with TR/TE = 3310/36 ms; matrix size $256 \times 256 \times 40$, field of view = 30×30 mm, spatial resolution, $0.117 \times 0.117 \times 0.7$ mm. A 3D MRI Rat Brain Atlas (© 2012 Ekam Solutions LLC, Boston, MA) was used to calculate brain volumes, and registered the standard structural rat template image onto high resolution T2-weighted images for each subject using a non-linear registration method implemented by Unix based software package Deformable Registration via Attribute Matching and Mutual-Saliency Weighting (DRAMMS; <https://www.cbica.upenn.edu/sbia/software/dramms/index.html>). The atlas (image size $256 \times 256 \times 63$) (H x W x D) was then warped from the standard space into the subject image space (image size $256 \times 256 \times 40$) using the deformation obtained from the above step using nearest-neighbor interpolation method. In the volumetric analysis, each brain region was therefore segmented, and the volume values were extracted for all 171 ROIs, calculated by multiplying unit volume of voxel in mm^3 by the number of voxels using an in-house MATLAB script. To account for different brain sizes, all ROI volumes were normalized by dividing each subject's ROI volume by their total brain volume

Diffusion Weighted Imaging – Quantitative Anisotropy

DWI was acquired with a spin-echo echo-planar-imaging (EPI) pulse sequence having the following parameters: TR/TE = 500/20 msec, eight EPI segments, and 10 non-collinear gradient directions with a single b-value shell at 1000s/mm² and one image with a B-value of 0 s/mm² (referred to as B 0). Geometrical parameters were: 48 coronal slices, each 0.313 mm thick (brain volume) and with in-plane resolution of 0.313×0.313 mm² (matrix size 96×96 ; FOV 30 mm³). The imaging protocol was repeated two times for signal averaging. Each DWI acquisition took 35 min and the entire MRI protocol lasted about 70 min. Image analysis included DWI analysis of the DW-3D-EPI images to produce the maps of fractional anisotropy (FA) and apparent diffusion coefficient (ADC). DWI analysis was completed with MATLAB and

MedINRIA (1.9.0; <http://www-sop.inria.fr/asclepios/software/MedINRIA/index.php>) software. Because sporadic excessive breathing during DWI acquisition can lead to significant image motion artifacts that are apparent only in the slices sampled when motion occurred, each image (for each slice and each gradient direction) was screened, prior to DWI analysis. If found, acquisition points with motion artifacts were eliminated from analyses.

For statistical comparisons between rats, each brain volume was registered to the 3D rat atlas allowing voxel- and region-based statistics. All image transformations and statistical analyses were carried out

using the in-house MIVA software (<http://ccni.wpi.edu/>). For each rat, the B 0 image was co-registered with the B 0 template (using a 6-parameter rigid-body transformation). The co-registration parameters were then applied on the DWI indexed maps for the different indices of anisotropy. Normalization was performed on the maps since they provided the most detailed visualization of brain structures and allow for more accurate normalization. The normalization parameters were then applied to all DWI indexed maps that were then smoothed with a 0.3-mm Gaussian kernel. To ensure that FA and ADC values were not affected significantly by the pre-processing steps, the 'nearest neighbor' option was used following registration and normalization.

Statistical differences in measures of DWI between experimental groups were determined using a nonparametric Mann-Whitney U Test (alpha set at 5%). The formula below was used to account for false discovery from multiple comparisons.

$$P_{(i)} \leq \frac{i}{V} \frac{q}{c(V)}$$

P(i) is the p value based on the t test analysis. Each of 171 ROIs (i) within the brain containing (V) ROIs was ranked in order of its probability value (see Table 1). The false-positive filter value q was set to 0.2 and the predetermined c(V) was set to unity¹¹. The corrected probability is noted on each table.

Table 1
Apparent Diffusion Coefficient

Apparent Diffusion Coefficient							
	Control			BBDRZ/Wo			
Brain Area	Ave	SD		Ave	SD	P-val	Ωsq
basal amygdaloid n.	2.13	0.14	>	1.87	0.07	0.002	0.722
lateral amygdaloid n.	2.20	0.14	>	1.83	0.12	0.002	0.722
pontine reticular n. caudal	2.54	0.29	>	2.05	0.19	0.002	0.722
parvicellular reticular n.	2.62	0.25	>	1.83	0.39	0.002	0.720
caudal piriform ctx	2.18	0.12	>	1.61	0.13	0.002	0.720
gigantocellularis reticular n.	2.79	0.28	>	2.03	0.42	0.002	0.719
retrosplenial rostral ctx	2.55	0.22	>	2.09	0.15	0.002	0.719
ventral subiculum	2.42	0.29	>	1.95	0.13	0.002	0.719
cortical amygdaloid n.	2.42	0.22	>	1.84	0.22	0.002	0.688
frontal association ctx	2.59	0.15	>	1.78	0.28	0.003	0.655
habenula n.	3.03	0.21	>	2.37	0.30	0.003	0.653
root of trigeminal nerve	3.12	0.26	>	2.25	0.48	0.003	0.651
anterior lobe pituitary	2.97	0.49	>	1.60	0.72	0.003	0.651
CA3 hippocampus ventral	2.29	0.18	>	1.94	0.11	0.003	0.651
crus 1 of ansiform lobule	2.19	0.34	>	1.48	0.28	0.003	0.651
entorhinal ctx	2.68	0.26	>	1.97	0.20	0.003	0.651
glomerular layer	2.66	0.12	>	1.87	0.44	0.003	0.651
paraflocculus cerebellum	2.29	0.18	>	1.86	0.22	0.003	0.651
solitary tract n.	2.91	0.45	>	1.97	0.36	0.003	0.651
superior colliculus	2.57	0.23	>	2.15	0.12	0.004	0.591
dorsomedial tegmental area	2.57	0.29	>	2.15	0.13	0.004	0.589
medial amygdaloid n.	2.84	0.21	>	2.41	0.18	0.004	0.588
reticular n.	2.06	0.08	>	1.87	0.11	0.005	0.558
CA1 hippocampus ventral	2.14	0.21	>	1.77	0.19	0.006	0.530

Apparent Diffusion Coefficient							
5th cerebellar lobule	2.85	0.29	>	2.16	0.30	0.006	0.529
inferior colliculus	2.87	0.32	>	2.39	0.18	0.006	0.529
visual 1 ctx	2.30	0.31	>	1.92	0.16	0.006	0.529
central amygdaloid n.	2.42	0.23	>	1.99	0.17	0.006	0.527
dentate gyrus ventral	2.67	0.35	>	2.13	0.16	0.006	0.527
vestibular n.	2.86	0.38	>	2.21	0.25	0.006	0.527
medial dorsal thalamic n.	2.18	0.15	>	1.90	0.18	0.007	0.501

Resting state functional connectivity

Scans were collected using a spin-echo triple-shot EPI sequence (imaging parameters: matrix size = $96 \times 96 \times 20$ (H x W x D), TR/TE = 1000/15 msec, voxel size = $0.312 \times 0.312 \times 1.2$ mm, slice thickness = 1.2 mm, with 200 repetitions, time of acquisition 10 min. There are numerous studies detailing the benefits of multi-shot EPI in BOLD imaging [23–27]. We avoided using single shot EPI because of its severe geometrical distortion at high field strengths (≥ 7 T) and loss of effective spatial resolution as the readout period increases [24, 28, 29]. There is also the possibility of signal loss in single shot EPI due to accumulated magnetic susceptibility or field inhomogeneity [27].

Preprocessing in this study was accomplished by combining Analysis of Functional NeuroImages (AFNI_17.1.12, <http://afni.nimh.nih.gov/afni/>), FMRIB Software library (FSL, v5.0.9, <http://fsl.fmrib.ox.ac.uk/fsl/>), Deformable Registration via Attribute Matching and Mutual-Saliency Weighting (DRAMMS 1.4.1, <https://www.cbica.upenn.edu/sbia/software/dramms/index.html>) and MATLAB (Mathworks, Natick, MA). Brain tissue masks for resting-state functional images were manually drawn using 3DSlicer (<https://www.slicer.org/>) and applied for skull-stripping. Motion outliers (i.e., data corrupted by extensive motion) were detected in the dataset and the corresponding time points were recorded so that they could be regressed out in a later step. Functional data were assessed for the presence of motion spikes. Any large motion spikes were identified and removed from the time-course signals. This filtering step was followed by slice timing correction from interleaved slice acquisition order. Head motion correction (six motion parameters) was carried out using the first volume as a reference image. Normalization was completed by registering functional data to the 3D MRI Rat Brain Atlas (© 2012 Ekam Solutions LLC, Boston, MA) using affine registration through DRAMMS. The 3D MRI Rat Brain Atlas containing 171 annotated brain regions was used for segmentation. Data are reported in 166 brain areas, as five regions in the brain atlas were excluded from analysis due to the large size of three brains. These brains fell slightly outside our imaging field of view and thus we did not get any signal from the extreme caudal tip of the cerebellum. Whole brains that contain all regions of interest are needed for analyses so rather than excluding the animals, we removed the brain sites across all animals. After quality assurance, band-pass filtering (0.01 Hz ~ 0.1 Hz) was performed to reduce low-frequency drift

effects and high-frequency physiological noise for each subject. The resulting images were further detrended and spatially smoothed (full width at half maximum = 0.8 mm). Finally, regressors comprised of motion outliers, the six motion parameters, the mean white matter, and cerebrospinal fluid time series were fed into general linear models for nuisance regression to remove unwanted effects.

The region-to-region functional connectivity method was performed in this study to measure the correlations in spontaneous BOLD fluctuations. A network is comprised of nodes and edges; nodes being the brain region of interest (ROI) and edges being the connections between regions. Data are reported in 166 brain areas, as five regions in the 3D MRI Rat Brain Atlas were excluded from analysis due to the large size of three brains that fell slightly outside then field of view excluding signal from the most caudal tip of the cerebellum. Voxel time series data were averaged in each node based on the residual images using the nuisance regression procedure. Pearson's correlation coefficients across all pairs of nodes (14535 pairs) were computed for each subject among all three groups to assess the interregional temporal correlations. The r-values (ranging from - 1 to 1) were z-transformed using the Fisher's Z transform to improve normality. 166×166 symmetric connectivity matrices were constructed with each entry representing the strength of edge. Group-level analysis was performed to look at the functional connectivity in the experimental groups. The resulting Z-score matrices from one-group t-tests were clustered using the K-nearest neighbors clustering method to identify how nodes cluster together and form resting state networks. A Z-score threshold of $|Z|=2.3$ was applied to remove spurious or weak node connections for visualization purposes.

Behavioral Tests

The novel object recognition (NOR) task was attempted to assess episodic learning and memory [30, 31]. The apparatus consisted of a black cube-shaped Plexiglass box (L: 60.9, W: 69.2, H: 70.5 cm) with no lid, indirectly illuminated with two 40 W incandescent bulbs. Animals were placed in the empty box (15 min) for acclimation on day 1. On day 2, for the familiar phase (5 min), animals were placed in the box with two identical objects arranged in diagonal corners, 5 cm from each wall. After a 90 min rest period in their home cage, animals were placed back in the box for the novel phase (3 min) with one of the familiar objects and a novel object.

The cold plate assay was attempted to test for cold allodynia and was taken from Brenner and Golden (2012) [32] and modified for rats. Prior to testing, rats were acclimated to standing on a $\frac{1}{4}$ inch glass plate table, confined to an area of 38 cm $\times$ 14 cm by a transparent plastic container. Beneath the glass table a mirror was positioned to permit a clear view of the rat's hind paws for the precise placement of the cold probe. A hollow probe with a tip dimension of 2 mm was filled with compressed, crushed dry ice. The center of the plantar surface of the hindpaw was targeted for stimulation through the floor of the glass platform. The cold probe was tested on each hindpaw with an interval of 7 min between each paw. Both hindpaws of each rat were tested three times with an interval of 15 min between trials. The latency to move the hindpaw away from the cold probe was timed in seconds. The maximum time allowed for withdrawal was 90 sec. Withdrawal latencies were averaged between the right and left hindpaw and did not differ significantly between paws within either the vehicle or paclitaxel treatments. Consequently, the

right and left hindpaw withdrawal times were averaged for each animal, and a single mean determination from each animal contributed to the groups means and standard deviations for each treatment. These data were statistically compared with a Student's t-Test. Experimenters collecting the data were blinded to the treatment condition.

However, due the obesity of the diabetic rats, their mobility was extremely limited which prevented any accurate or meaningful recordings from either of the tests. Additionally, due to the rapid decline of the diabetic rats' health, subsequent behavioral tests that did not require mobility were not possible.

Results

Voxel-Based Morphometry

Shown in Fig. 1 is a table comparing the average brain volumes that were significantly different between control and BBZDR/Wor rats. The brain areas are ranked in order of their significance and are truncated from a larger list of 171 areas (see Supplementary Table S1). Note, in all cases the BBZDR/Wor brain volumes are smaller than controls. While not statistically significant, this is also true for a majority of brain areas, 136/171, as shown in Table S1. Shown to the left of Fig. 1 is a 3D reconstruction summarizing the brains areas listed in the table. Several areas of the somatosensory cortex (upper lip, hindlimb, forelimb, barrel field, entorhinal, temporal, and retrosplenial cortices) showed a reduced volume as did the white matter tracts, basal ganglia (striatum, substantia nigra, septum), anterior cerebellum (2nd, 3rd, 5th lobules) and olfactory bulbs (granular and external plexiform layers).

Diffusion Weighted Imaging

The significant differences in between control and BBZDR/Wor rats for ADC and FA are presented in Tables 1 and 2, respectively. The brain areas in each table are ranked in order of their significance. In the case of ADC, 90/171 brain areas were significantly different and for FA there were 111/171 significant brain areas. Only those brain areas with an effect size greater than 0.5 are listed. The areas in each case spread out to cover much the brain. BBZDR/Wor rats showed greater FA values and lower ADC values as compared to control. The full tables of brain areas are provided in Supplementary Tables S2 and S3.

Table 2
Fractional Anisotropy

Fractional Anisotropy							
	Control			BBDRZ/Wo			
Brain Area	Ave	SD		Ave	SD	P-val	Ω sq
superior colliculus	0.29	0.02	<	0.39	0.06	0.002	0.745
auditory ctx	0.26	0.02	<	0.41	0.05	0.002	0.740
inferior colliculus	0.30	0.02	<	0.40	0.04	0.002	0.738
dentate gyrus dorsal	0.28	0.02	<	0.38	0.05	0.002	0.736
insular ctx	0.30	0.02	<	0.42	0.05	0.002	0.736
ventral lateral striatum	0.28	0.02	<	0.39	0.05	0.002	0.732
perirhinal ctx	0.33	0.03	<	0.47	0.04	0.002	0.730
ventral posteriolateral thalamic n.	0.42	0.03	<	0.52	0.03	0.002	0.730
reticular n. midbrain	0.34	0.02	<	0.46	0.08	0.002	0.728
rostral piriform ctx	0.32	0.03	<	0.43	0.04	0.002	0.726
primary somatosensory ctx jaw	0.29	0.03	<	0.41	0.06	0.002	0.726
subthalamic n.	0.44	0.02	<	0.53	0.03	0.002	0.726
basal amygdaloid n.	0.32	0.05	<	0.42	0.03	0.002	0.724
secondary motor ctx	0.32	0.05	<	0.44	0.04	0.002	0.724
posterior thalamic n.	0.30	0.02	<	0.42	0.06	0.002	0.724
ventral posteriolmedial thalamic n.	0.34	0.02	<	0.46	0.07	0.002	0.724
accumbens core	0.33	0.03	<	0.44	0.04	0.002	0.722
anterior olfactory n.	0.36	0.03	<	0.47	0.05	0.002	0.722
claustrum	0.32	0.02	<	0.45	0.06	0.002	0.722
dorsal lateral striatum	0.32	0.03	<	0.42	0.05	0.002	0.722
endopiriform n.	0.35	0.03	<	0.46	0.03	0.002	0.722
lateral preoptic area	0.31	0.02	<	0.40	0.05	0.002	0.722
medial orbital ctx	0.28	0.04	<	0.45	0.11	0.002	0.722
secondary somaotsensory ctx	0.25	0.02	<	0.39	0.07	0.002	0.722

Fractional Anisotropy							
ventral orbital ctx	0.27	0.02	<	0.44	0.07	0.002	0.722
CA1 hippocampus ventral	0.28	0.06	<	0.47	0.05	0.002	0.720
lateral orbital ctx	0.28	0.02	<	0.44	0.07	0.002	0.720
granular cell layer	0.36	0.04	<	0.51	0.04	0.002	0.719
dorsal medial striatum	0.33	0.04	<	0.44	0.06	0.002	0.695
parietal ctx	0.32	0.03	<	0.39	0.06	0.002	0.692
3rd cerebellar lobule	0.31	0.04	<	0.44	0.05	0.002	0.690
parafascicular thalamic n.	0.29	0.02	<	0.39	0.08	0.002	0.690
primary somatosensory hindlimb	0.29	0.03	<	0.39	0.04	0.002	0.690
ventral subiculum	0.33	0.04	<	0.46	0.05	0.002	0.690
magnocellular preoptic n.	0.34	0.05	<	0.46	0.07	0.002	0.688
temporal ctx	0.30	0.04	<	0.41	0.05	0.002	0.688
prelimbic ctx	0.31	0.04	<	0.46	0.09	0.002	0.686
lateral geniculate	0.31	0.03	<	0.44	0.05	0.003	0.663
dentate gyrus ventral	0.29	0.04	<	0.46	0.06	0.003	0.662
anterior amygdaloid n.	0.30	0.04	<	0.41	0.04	0.003	0.660
lemniscal n.	0.36	0.02	<	0.44	0.07	0.003	0.660
4th cerebellar lobule	0.33	0.03	<	0.43	0.05	0.003	0.655
entorhinal ctx	0.33	0.03	<	0.43	0.04	0.003	0.655
ventromedial thalamic n.	0.33	0.04	<	0.46	0.06	0.003	0.655
olfactory tubercles	0.34	0.03	<	0.47	0.06	0.003	0.653
medial preoptic area	0.27	0.03	<	0.38	0.07	0.003	0.627
reuniens n.	0.28	0.03	<	0.38	0.06	0.003	0.627
primary somatosensory ctx upper lip	0.26	0.03	<	0.37	0.07	0.003	0.624
tenia tecta ctx	0.28	0.03	<	0.42	0.08	0.003	0.624
medial geniculate	0.34	0.03	<	0.47	0.09	0.003	0.622
Ventricle	0.38	0.03	<	0.45	0.04	0.004	0.600
primary somatosensory ctx trunk	0.30	0.04	<	0.38	0.06	0.004	0.597

Fractional Anisotropy							
accumbens shell	0.34	0.03	<	0.44	0.05	0.004	0.594
lateral posterior thalamic n.	0.28	0.04	<	0.41	0.06	0.004	0.594
infralimbic ctx	0.30	0.04	<	0.43	0.06	0.004	0.592
CA2	0.28	0.04	<	0.40	0.07	0.004	0.591
primary somatosensory barrel field	0.27	0.04	<	0.39	0.07	0.004	0.591
primary somatosensory shoulder	0.27	0.04	<	0.39	0.06	0.004	0.591
trapezoid body	0.38	0.06	<	0.49	0.04	0.004	0.591
5th cerebellar lobule	0.35	0.04	<	0.43	0.05	0.005	0.567
arcuate n.	0.31	0.05	<	0.44	0.09	0.005	0.563
2nd cerebellar lobule	0.35	0.05	<	0.48	0.08	0.005	0.561
CA3 hippocampus ventral	0.32	0.07	<	0.46	0.04	0.005	0.561
external plexiform layer	0.41	0.04	<	0.50	0.04	0.006	0.533
periaqueductal gray thalamus	0.31	0.02	<	0.43	0.09	0.006	0.533
ectorhinal ctx	0.34	0.04	<	0.42	0.06	0.006	0.531
ventral medial striatum	0.33	0.05	<	0.42	0.04	0.006	0.530
lateral amygdaloid n.	0.32	0.05	<	0.44	0.06	0.006	0.527
raphe linear	0.37	0.04	<	0.47	0.07	0.007	0.505
zona incerta	0.40	0.04	<	0.54	0.09	0.007	0.503
8th cerebellar lobule	0.34	0.05	<	0.46	0.07	0.007	0.501
ventrolateral thalamic n.	0.31	0.05	<	0.40	0.05	0.007	0.501

Resting State Functional Connectivity

Figure 2 shows a correlation matrix comparing 166 brain areas for rsFC between the controls and BBZDR/Wor rats. Each colored red/orange pixel represents 1 of 166 brain areas that has a significant positive correlation with other brain areas. Pixels in shades of blue have a significant negative, or anticorrelation with other brain regions. The brain areas with significant correlations appear as clusters because they are contiguous in their neuroanatomy and function. The diagonal line separates the control and BBZDR/Wor groups. Each pixel on one side of the line has a mirror image pixel on the other side. The delineated areas serve to focus attention on similarities and differences in connectivity. Area A shows intra-thalamic connections and favors greater coupling for BBZDR/Wor rats. B is the posterior thalamus and C the midbrain. D shows connections between the habenula/tectal/parafascicular thalamus and the

dorsal hippocampus with clear hypoconnectivity in BBZDR/Wor as compared to control. E highlights the area of the prefrontal cortex, and F the cerebellum/pons. G shows a broad area of connections between the cerebellum/pons and the trigeminal brainstem reticular activating system again favoring higher coupling in BBZDR/Wor rats as compared to controls. Area I is the brainstem reticular activating system and deep cerebellar nuclei. Of particular interest is the anti-correlation or uncoupling in area H (blue pixels) between the hippocampus and the posterior cerebellum. A table is provided showing the positive (yellow) and negative (blue) connections with the dorsal hippocampus (CA1, CA3 and dentate) highlighted in red. The Z score to the right of each brain area is the average score to the three areas of the dorsal hippocampus. For example, the lateral geniculate has significant connections to CA1, CA3 and dorsal dentate (denoted by the 3 shown in parentheses) the average of which is a Z score of 4. In contrast, the crus 2 of the cerebellum only has significant negative connections to two of the three hippocampal areas (denoted by two in parentheses) with an average Z score of negative 2.9. This relationship between hippocampus, thalamus and cerebellum/brainstem is highlighted in 3D reconstructions to the right. Shown is the positive coupling between the dorsal hippocampus and multiple thalamic nuclei and the uncoupling or negative correlation to dorsal striatum, posterior cerebellum and brainstem.

Discussion

There are several rat models that may have translational benefits when using MRI to study T2DM [33]. These include the GK rat [34], Zucker Diabetic Fatty (ZDF) rat [35], HFD/STZ rat [36] and BBBZK/Wor, to list a few. This exploratory study began the process of evaluating the BBBZK/Wor model of T2DM with multimodal imaging with the expectation of finding common neuroradiological measures of cerebral neuropathy as reported in the clinic. If so, the model would have the potential to provide valuable imaging data on disease progression from prediabetic to late stage diabetes and a non-invasive means of assessing the efficacy of therapeutic intervention on brain structure and function.

Voxel-Based Morphometry

A reduction in brain volume is a consistent finding across all imaging studies in 2TDB [11]. Cortical volumes show a decrease in gray [6, 37-40] and white matter [6, 40]. The underlying cause of the global reduction in brain volumes is unknown but is thought to be the consequence of small vessel disease [41]. The general brain atrophy and reduction in gray matter is associated with diminished cognitive function [37, 40, 42, 43]. Hippocampal volumes are smaller in T2DM versus age-matched controls [40, 44, 45] and maybe a contributing factor to the cognitive decline. The BBBZK/Wor rat presented with several brain areas that were significantly smaller as compared to age-matched controls. These areas included the somatosensory, entorhinal and temporal cortices but not CA1, CA3, dentate, and subiculum, the primary components of the hippocampal complex.

Diffusion weighted imaging

DWI is an indirect way of assessing the integrity of white and gray matter microarchitecture. A recently published study by Moghaddam and colleagues [46] provides a comprehensive review of the literature on T2DM and DWI. The literature is very consistent reporting changes in microarchitecture in the areas of the frontal-temporal cortex, hippocampus, cerebellum, thalamus and all of the major white matter tracts. The cognitive decline in T2DM is strongly associated with alterations in DWI in white matter tracts [47-49]. With few exceptions, the general DWI profile is a decrease in FA and increase in ADC. This inverse relationship suggests a loss of microstructural integrity and network organization [46]. The BBBZK/Wor rat also presented with global and pervasive changes in measures of DWI. The FA and ADC values were inversely related with FA being greater than ADC a potential sign of global neuroinflammation and cytotoxic edema. The changes in DWI were extensive affecting much of the brain including the hippocampus, thalamus, amygdala, cerebellum and white matter tracts.

Functional Connectivity

A review by Macpherson and colleagues covers much of the literature on rsFC in T2DM [50]. There is general agreement across multiple studies that T2DM presents with a reduction in connectivity in the default mode network, interconnections between the prefrontal cortex, parietal cortex, and hippocampus. Thalamic coupling to cortical and cerebellar regions is also reduced [51]. The effect on global connectivity in this BBZDR/Wor diabetes model is contrary to that reported in the clinical literature. As shown in the connectivity matrix **Fig 2** there is no clear reduction in connectivity when looking at the clusters highlighted. If anything, there are areas of the brain that show hyperconnectivity with diabetes. Intra-thalamic connections are enhanced (area A), as are thalamic connections to the dorsal hippocampus (area D) and cerebellar/pontine connections to the brainstem reticular activating system (area G). The increase in connectivity is not unprecedented and can occur between some brain areas in human T2DM [52], but that would appear to be the exception. The hyperconnectivity observed in BBBZK/Wor maybe a compensatory response to underlying pathology as reported in traumatic brain injury [53] or in young children with early type 1 diabetes [54].

Limitations and Considerations

As an exploratory study we recognize its many limitations. 1) There were no female rats, an issue of concern given data shows differences in diabetic pathology between females and males [55, 56] 2) There were no measures of cognitive function to correlation with the MRI data as is routine with clinical studies. As noted in the Methods, we were unable to collect behavioral data due to the severity of the obesity in the BBBKZ/Wor rat. 3) We did not image for white matter hyperintensities, lesions associated with microvascular insult. Again, this is routine in clinical studies and would have aided in our analysis of BBBKZ/Wor as a relevant imaging model for T2DM. 4) Our rsFC studies were done under light isoflurane anesthesia. These studies could have been done under awake conditions as we have done so in many other task-related BOLD imaging [57-59] or pHMRI studies [60, 61] in rodents. However, “resting state”

poses a dilemma in awake animal imaging no matter the level of acclimation prior to imaging [62]. Any physical restraint will most likely have some level of stress; hence, the rsFC data were collected under light anesthesia. Nonetheless, numerous studies comparing the anesthetized and conscious states show similar rsFC data [63, 64]

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Conclusion

These limitations aside, the original question stands - are the neuroradiological measures collected in BBBKZ/Wor rats using multimodal imaging methods common to the clinic, similar to those reported in T2DM patients? While the VBM data in BBBKZ/Wor reflected the general findings in T2DM showing a decrease in specific brain areas and a global trend toward a reduction in volume, the hippocampus, a critical area linking changes with cognitive function to disease progression, was unaffected. The DWI changes in FA and ADC were global and pervasive with no specific areas that could be identified as potential biomarkers by which to follow the prediabetic to diabetic stages of cerebral neuropathy. The rsFC data were contrary to most findings in T2DM which report hypoconnectivity with disease progression. The hyperconnectivity in BBBKZ/Wor rat most likely reflects an effort to compensate for the pathology that is not seen in humans. From these observations, we would conclude the BBBKZ/Wor rat is not an appropriate imaging model for T2DM.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Yes

Availability of data and material

All data can be accessed through a link to Mandelley. DOI to follow

Competing interests

CFF has a financial interest in Animal Imaging Research, the company that makes the RF electronics and holders for animal imaging

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Authors' contributions

All of the authors have contributed substantially to the manuscript.

Concept, drafting and interpretation - Ferris, Lawson, Rentrup

Execution and analysis - Cai, Kulkarni, Lawson, Rentrup

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Figures

Figure 1

Voxel-based morphometry The table to the right list the brain areas that have significantly different volumes between experimental conditions. These brains areas are shown in the 3D reconstructions summarizing the differences.

Figure 2

Resting State Functional Connectivity To the left is a correlation data matrix comparing control and BBZDR/Wor rats. To the right are tables of positively (yellow) and negatively (blue) coupled areas to the dorsal hippocampus (red) with 3D reconstruction summarizing the brain areas. A - intra-thalamic connections; B - posterior thalamus; C – midbrain, D connections between the habenula/tectal/parafascicular thalamus and the dorsal hippocampus; E- prefrontal cortex; F - cerebellum/pons; G - connections between the cerebellum/pons and the trigeminal brainstem reticular activating system; H - hippocampus and the posterior cerebellum; I is the brainstem reticular activating system and deep cerebellar nuclei

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [S2FullTable1ADC.xlsx](#)
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