In vivo imaging of dentate granule cell layer abnormalities in Schizophrenia

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TARGET AUDIENCE: Radiologists, Schizophrenia researchers

PURPOSE: The hippocampus is central to the pathophysiology of schizophrenia[1]. Histology in patients shows abnormalities in the dentate granule cell layer (DGCL), but its small size (~100 micron thickness) has precluded *in vivo* studies. A recent paper demonstrating that the DGCL can be consistently visualized *in vivo* at 7 T [2], motivated us to compare DGCL morphology in schizophrenic patients to matched controls'.

PATIENTS AND METHODS: 25 schizophrenia patients and 16 age and gender matched controls (8 female, 36.1 ± 9.2 years old) were recruited. An intact *ex vivo* hippocampus from a 68 year old woman who died from pneumonia was obtained from autopsy material in order to compare MRI findings with histopathology.

Experiments were done at 7 T (Magnetom, Siemens AG, Erlangen, Germany) using a volume-transmit 24-coil head receive-array (Nova Medical, Boston, MA). A coronal oblique volume-of-interest was placed onto the medial hippocampus as to cover the entire structure. Imaging was done with heavily T_2^*-weighted 2D gradient-echo sequence: *TR/TE*= 944/25 ms, 35° nutation, 238×238 mm^2 FOV, 1024×1024 matrix, ×1 acceleration. Acquiring 17 1.5 mm thick slices with 20% gap yielded 232×232×1500 μm^3=0.08 μL image voxel resolution, in 14 minutes.

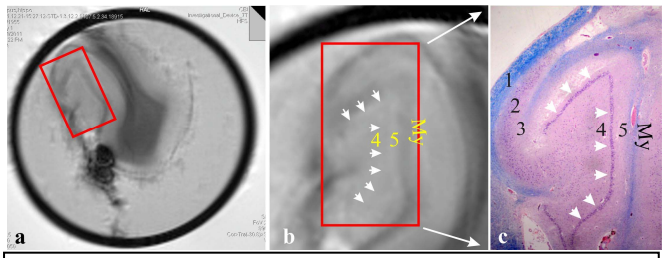

Fig. 1: Postmortem hippocampus, MRI (**a, b**) and histology (**c**). "My": myelinated fibers. "1, 2, 3": *cornu ammonis*' polymorphic (1), pyramidal (2) and molecular (3) layers. "4, 5": dentate gyrus' polymorphic (4) and molecular (5) layers. Arrows: dentate granule cell layer (DGCL). Note correspondence between anatomical detail and T_2^*-weighted MRI.

The same protocol was run on the *ex vivo* hippocampus sample. The specimen was then sectioned in planes matching the coronal MRI as closely as possible and processed for paraffin-embedded tissue block. The histopathology slides were stained with Luxol-Fast Blue combined with hemotoxylin-eosin staining, as shown in **Fig. 1c**.

The *in vivo* T_2^*-weighted images were read by 3 neuroradiologists blinded to the clinical diagnosis and to each other. They rated the bilateral DGCL in every subject on a scale of 1 – 6, described in **Fig. 2**. To determine test-retest reliability, another blinded reading was done by the senior neuroradiologist.

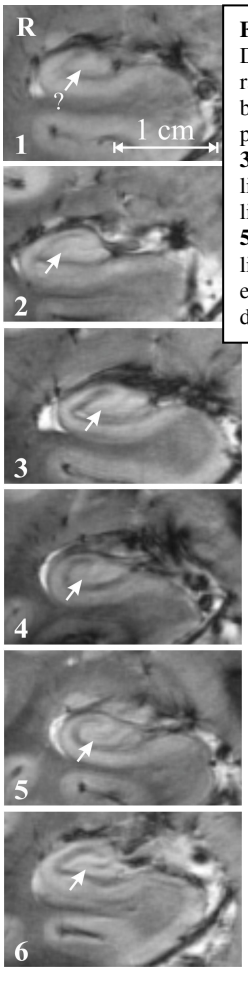

Fig. 2: Examples of the 1 – 6 DGCL (arrows) integrity rating scale used: From top to bottom: **1:** Not discernable; **2:** partially visible but faint; **3:** ‹50% visible and appear light gray; **4:** 50% visible and light gray in appearance; **5:** Entirely visible and appears light gray; **6:** Entire DGCL is easily visualized and appears dark gray or black.

RESULTS: To confirm DGCL identification on the *in vivo* MRI, we compared the *ex vivo* hippocampus T_2^*-weighted images (**Fig. 1a**, **b**) with their approximate corresponding histopathology location, **Fig. 1c**. The DGCL, appearing purple in **Fig. 1c**, is between the other two layers of the dentate gyrus ("4" and "5"). It is reasonable, therefore, to ascribe the thin gray strip (arrows) between "4" and "5" in **Fig. 1b**, to the DGCL.

Nine patients (36%) and 1 control (6%) were excluded for excessive motion, leaving 16 patients (6 female, 40.7±10.6 years old, 20±11 years mean illness duration) and 15 controls (7 female, 35.6±9.4 years old). There were no differences between the two groups in terms of gender (p=0.72) and age (p=0.22). The average right and left ratings of the bilateral DGCL assigned by the 3 neuroradiologists were: 3.2±1.0 and 3.5±1.2 in patients *versus* 3.9±1.1 and 3.8±0.8 in controls. The decreased visibility in patients was statistically significant only for the right DGCL, with and without adjusting for age and gender (p=0.05 and p=0.04, respectively). Receiver Operating Characteristic curve analysis identified a right DGCL rating ≤2 as the optimal predictor of schizophrenia with 48% sensitivity and 80% specificity. Inter-rater concordance was modest with a maximal kappa of 0.13, which did not change when re-examined by the same blinded senior reader. The average left or right DGCL ratings did not correlate with age of patients or controls, or with disease duration (all r‹0.39, p›0.05).

DISCUSSION: The thickness of the DGCL is below the MRI pixel resolution even at 7 T, and the ability to visualize it, therefore, is due to several other factors including partial volume (in 1-2 voxels) and T_2^* field effects[2]. These preclude accurate volumetric analysis of the DGCL with hippocampal segmentation software and thus necessitated a qualitative assessment. The main findings in hippocampal-related dysfunction in schizophrenia are in neuronal morphology, organization and synaptic parameters[1] and the DGCL, as the site of hippocampal neurogenesis, has been extensively implicated. This is the first report showing that abnormalities can be observed in humans *in vivo*. Unfortunately its small sample size precludes examining correlations with disease genotypes and clinical phenotypes. The novelty of 7 T hippocampal MRI limited the usefulness radiologists' experience, as reflected by the small *inter*- and *intra*-rater Kappa.

CONCLUSION: The DGCL was less discernible in schizophrenia patients compared to controls, presumably reflecting morphological abnormalities due to cellular organization. Sensitivity and specificity can be improved by (*i*) studying patients with homogeneous disease subtypes and progression rates and (*ii*) increased radiologists' experience in hippocampal subfield imaging, which will also improve rating concordance.

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