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TITLE: Neurovascular Changes Associated with Olfactory Deficits in Early Parkinson's Disease

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14. ABSTRACT The purpose of this study is to investigate neurovascular changes related to the olfactory deficits in early PD patients to develop novel biomarkers for PD. We plan to focus on these Specific Aims: 1) neurovascular abnormalities associated with olfactory deficits in early PD patients; 2) dynamic changes in neurovascular and metabolic parameters during olfactory stimuli in early PD patients; and 3) the progression of such neurovascular abnormalities across different stages of PD patients. We have completed all proposed recruitment and data acquisition and analysis. Scientifically, we found significant neurovascular changes (Aims 1 and 2), impaired dynamic responses to olfactory stimulation (Aims 2 and 3), and structural abnormalities (additional exploratory analysis) in primary and secondary olfactory regions in the brains of PD patients compared to controls. We have published our results in 2 journal papers and 9 conference abstracts, and are working on 2 additional journal papers.					
15. SUBJECT TERMS Parkinson's disease, olfaction, neurovascular, imaging, non-invasive, MRI, ultra-high field, arteriole, olfactory cortex, BOLD, functional MRI, biomarker					
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1. INTRODUCTION:

A major hurdle for treatment development in Parkinson's Disease (PD) has been the lack of reliable measures to evaluate the efficacy of experimental therapeutic strategies. The investigation of functional deficits and associated physiological abnormalities in early PD may advance our understanding of the neuropathology, and facilitate the development of in vivo biomarkers for assessing disease progression and evaluating efficacy in therapeutic trials. Olfactory deficits in PD are prevalent, can precede motor symptoms, and are associated with alpha-synuclein pathology, which is a major pathological hallmark of PD. Therefore, non-invasive and repeatable in vivo measures (such as neurovascular measures) associated with olfactory deficits may be excellent candidates as quantitative in vivo biomarkers in early PD for tracking disease progression and evaluating treatment outcomes. Such measures may provide more specific and sensitive information, and may change earlier and faster with disease progression compared to behavioral measures of olfactory functions. Neurovascular abnormalities have been linked to the neuropathology and functional deficits in PD. In this study, we will use advanced MRI technologies developed at ultra-high field (7T) to investigate neurovascular changes related to the olfactory system in the brain in early PD patients. The enhanced sensitivity at 7T will allow us to detect subtle changes with unprecedented spatial specificity, and the advanced MRI methods developed by us will enable us to study physiological changes in specific types of blood vessels (arteriolar, capillary, and venous), and to detect robust fMRI signals in brain regions close to air cavities (resulting in large artifacts with conventional methods) such as the olfactory cortex. We will focus on the following Specific Aims: 1) To investigate neurovascular abnormalities associated with olfactory deficits in early PD patients; 2) To measure dynamic changes in neurovascular and metabolic parameters during olfactory stimuli in early PD patients; and 3) To conduct a cross-sectional study on the progression of such neurovascular abnormalities across different stages of PD patients.

2. KEYWORDS:

Parkinson's disease, olfaction, neurovascular, imaging, non-invasive, MRI, ultra-high field, arteriole, olfactory cortex, BOLD, functional MRI, biomarker

3. ACCOMPLISHMENTS:

What were the major goals of the project?

- a) Local IRB and HRPO approval, Month 3, completed.
- b) Participant recruitment, clinical Assessment, MRI and analysis: We plan to recruit 60 subjects in the proposed study. Target enrollment for Q1-Q4 Year 1: 7+6+6+6, Q1-Q4 Year 2: 7+6+6+6, Q1-Q2 Year 3: 5+5.

Specific Aims of the proposed study:

Aim 1: To investigate neurovascular abnormalities associated with olfactory deficits in early PD patients.

Aim 2: To investigate dynamic changes in neurovascular and metabolic parameters during olfactory stimuli in early PD patients.

Aim 3: To investigate the progression of neurovascular abnormalities associated with olfactory deficits across different stages of PD patients.

▪ **What was accomplished under these goals?**

We have completed all proposed recruitment and data acquisition and analysis. **Table 1** summarizes the number of participants we recruited in this projects.

Table 1: Summary of recruitment.	Recruitment	Completed data acquisition
Year 4 (NCE) total:	13	21
Year 3 total:	20	7
Year 2 total:	28	16
Year 1 total:	18	16
All Years total:	79	60

In this project, we have recruited 79 participants, and have completed MRI and clinical data acquisition in 60 subjects. Some of the recruited participants did not complete all of the research procedures.

COVID-19: In Year 3 and Year 4, participant recruitment and visits for data acquisition were significantly impacted by the COVID-19 restrictions from the institute. Recruitment and data acquisition were still on-going during this period but were slower than expected.

Scientific findings:

a. Neurovascular abnormalities (Specific Aims 1 and 3):

Our key finding is that in PD patients with normal cognition, the volume of small pial arteries and arterioles (CBVa) was significantly decreased in the substantia nigra, caudate and putamen when compared to controls. In PD patients with impaired cognition, CbVa showed a decreasing trend in the substantia nigra, caudate and putamen, but was significantly increased in the presupplementary motor area and intracalcarine gyrus compared to controls. More details are described in our journal publication #1 listed in Section 6.

b. Dynamic changes in neurovascular and metabolic parameters during olfactory stimuli (Specific Aims 2 and 3):

First, we devoted much time to establish and optimize the functional paradigm, functional MRI experiments and data analysis strategies for the measurement of dynamic neurovascular changes during olfactory stimulation. Such experiments are not trivial to conduct in human subjects. We published our results in journal publication #2 listed in Section 6.

Second, we used the established methodology to measure dynamic neurovascular changes during olfactory stimulation in PD patients. We found significantly reduced responses in the primary olfactory cortex and

associated olfactory areas in the brains of PD patients compared to controls. Such MRI measures correlated with behavioral olfactory performance in the same cohort. We are currently working to publish these results in PD patients.

c. Additional results: structural changes in the olfactory regions in PD patients

Besides the studies planned in the original research proposal, we also conducted exploratory analysis of the acquired data in this project. One unique advantage of the current study is that it was conducted on a ultra-high magnetic field (7T) human MRI system, which allowed us to acquire whole-brain high resolution MRI images in all participants. Using these high resolution images, we characterized the volumes of small regions in the olfactory pathway in the brain which are typically very challenging to measure. We found significant structural changes in the olfactory bulb, primary olfactory cortex and secondary olfactory regions in PD patients compared to controls. We have published these results in 3 conference abstracts (see conference publication #7-9 listed in Section 6), and we are currently working to publish these results in a journal paper.

Stated Goals Not Met

The stated target recruitment in the SOW was met.

▪ **What opportunities for training and professional development has the project provided?**

Nothing to report.

▪ **How were the results disseminated to communities of interest?**

Nothing to report.

▪ **What do you plan to do during the next reporting period to accomplish the goals?**

Nothing to report.

4. IMPACT:

▪ **What was the impact on the development of the principal discipline(s) of the project?**

Nothing to report.

▪ **What was the impact on other disciplines?**

Nothing to report.

▪ **What was the impact on technology transfer?**

Nothing to report.

▪ **What was the impact on society beyond science and technology?**

Nothing to report.

5. CHANGES/PROBLEMS:

- **Changes in approach and reasons for change**

Nothing to report.

- **Actual or anticipated problems or delays and actions or plans to resolve them**

Nothing to report.

- **Changes that had a significant impact on expenditures**

Nothing to report.

- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

Nothing to report.

- **Significant changes in use or care of human subjects**

Nothing to report.

- **Significant changes in use or care of vertebrate animals.**

Nothing to report.

- **Significant changes in use of biohazards and/or select agents**

Nothing to report.

6. PRODUCTS:

- **Publications, conference papers, and presentations**

- **Journal publications.**

1. Paez AG, Gu C, Rajan S, Miao X, Cao D, Kamath V, Bakker A, Unschuld PG, Pantelyat A, Rosenthal LS, **Hua J***. Differential changes in arteriolar cerebral blood volume between Parkinson's disease patients with normal and impaired cognition and mild cognitive impairment (MCI) patients without movement disorder – an exploratory study. *Tomography*. 2020 Dec;6(4):333-342. PMC7744190.
2. Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J***. Functional Activities Detected in the Olfactory Bulb and Associated Olfactory Regions in the Human Brain Using T2-Prepared BOLD Functional MRI at 7T. *Front Neurosci*. 2021;15:723441. doi: 10.3389/fnins.2021.723441. eCollection 2021. PubMed PMID: 34588949; PubMed Central PMCID: PMC8476065.

- **Books or other non-periodical, one-time publications.**

Nothing to report.

- **Other publications, conference papers, and presentations.**

Conference publications:

- 1) Paez AG, Rajan S, Chaney G, Miao X, Cao D, Gu C, Liu D, Pantelyat AY, Rosenthal LI, Mari ZK, Mills K, Dawson TM, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Olfactory functional MRI (fMRI) using T2-prepared BOLD fMRI at ultra-high field (7T). *Proceedings International Society for Magnetic Resonance in Medicine*, May 2019. **Montreal, Canada**.
- 2) Paez AG, Rajan S, Chaney G, Miao X, Cao D, Gu C, Liu D, Pantelyat AY, Rosenthal LI, Mari ZK, Mills K, Dawson TM, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Olfactory Functional MRI (fMRI) Using T2-prepared BOLD fMRI at Ultra-high Field (7T). *Military Health System Research Symposium (MHSRS)*, August 2019. **Orlando, FL, USA**.
- 3) Paez AG, Rajan S, Miao X, Pantelyat AY, Rosenthal LI, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. Olfactory functional MRI (fMRI) using T2-prepared BOLD fMRI at ultra-high field (7T). *ICP Network Symposium-Physiological and Functional MRI of the Brain: Emerging Techniques and Clinical Applications*, September 2019. **Baltimore, MD, USA**.

- 4) Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Functional MRI in the olfactory bulb and associated olfactory regions in the human brain on 7T. *Proceedings International Society for Magnetic Resonance in Medicine Workshop on Ultra-High Field MR*, March 2022. **Lisbon, Portugal**.
- 5) Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Functional activities detected in the olfactory bulb and associated olfactory regions in the human brain using T2-prepared BOLD fMRI at 7T. *Proceedings International Society for Magnetic Resonance in Medicine*, May 2022. **London, England, UK**.
- 6) Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. High-resolution functional MRI in the human olfactory bulb at 7T. *Proceedings 28th Organization for Human Brain Mapping (OHBM) Annual Meeting*, June 2022. **Glasgow, Scotland, UK**.
- 7) Paez AG, Rajan S, Fang Z, Pantelyat AY, Rosenthal LI, Faria A, Miao X, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. High resolution structural MRI reveals abnormalities in olfactory brain regions in early Parkinson's disease (PD) patients. *Proceedings International Society for Magnetic Resonance in Medicine Workshop on Ultra-High Field MR*, March 2022. **Lisbon, Portugal**.
- 8) Paez AG, Rajan S, Pantelyat AY, Rosenthal LI, Faria A, Miao X, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. Structural abnormalities in olfactory brain regions in early Parkinson's disease (PD) patients measured by high resolution MRI at 7T. *Proceedings International Society for Magnetic Resonance in Medicine*, May 2022. **London, England, UK**.
- 9) Paez AG, Rajan S, Pantelyat AY, Rosenthal LI, Faria A, Miao X, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. Volumetric changes in olfactory regions in Parkinson's disease measured by high-resolution 7T MRI. *Proceedings 28th Organization for Human Brain Mapping (OHBM) Annual Meeting*, June 2022. **Glasgow, Scotland, UK**.

Note that some of the conference publications may have similar or same titles. The main purpose of the conference publications is to disseminate the scientific results to researchers from different fields. The content of each presentation was tailored to the specific audience at each conference.

- **Website(s) or other Internet site(s)**

Nothing to report.

- **Technologies or techniques**

Nothing to report.

- **Inventions, patent applications, and/or licenses**

Nothing to report.

- **Other Products**

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

▪ What individuals have worked on the project?

Name:	<i>Jun Hua</i>
Project Role:	<i>PI</i>
Researcher Identifier (e.g. ORCID ID):	<i>JUNHUA</i>
Nearest person month worked:	<i>2</i>
Contribution to Project:	<i>Dr. Hua prepared the IRB protocol for this study, which was approved by the JHU IRB and the HRPO. He interviewed several candidates for the postdoc/research associate position and recruited a highly qualified person for this important position. He implemented and optimized the MRI pulse sequences on the 7T MRI system as proposed in the study. He developed and implemented robust technology to achieve ideal B0/B1 shim, which is critical for the optimization of the MRI pulse sequences needed in this study on the new 8-channel 7T MRI system. He supervised the data acquisition and analysis. He organized regular meetings to coordinate and speed up the efforts for patient recruitment.</i>
Funding Support:	<i>N/A</i>

Name:	<i>Adrian Paez</i>
Project Role:	<i>Research coordinator</i>
Researcher Identifier (e.g. ORCID ID):	<i>N/A</i>
Nearest person month worked:	<i>12</i>
Contribution to Project:	<i>Mr. Paez assisted in the preparation of the IRB protocol. He has completed all trainings needed for recruitment and cognitive evaluation. He implemented the olfactometer setup for the olfactory fMRI experiments in the study. He implemented and optimized the MRI pulse sequences needed for this study on the new 8-channel 7T system. He has been working with the clinics to screen and recruit study participants. He conducted all data acquisition and analysis.</i>
Funding Support:	<i>N/A</i>

▪ Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

▪ What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

▪ COLLABORATIVE AWARDS:

Nothing to report.

▪ QUAD CHARTS:

Neurovascular Changes Associated with Olfactory Deficits in Early Parkinson's Disease

PD160104, W81XWH-17-1-0667

PI: Jun Hua Org: HUGO W. MOSER RESEARCH INSTITUTE AT KENNEDY KRIEGER

Award Amount: \$531,188.00

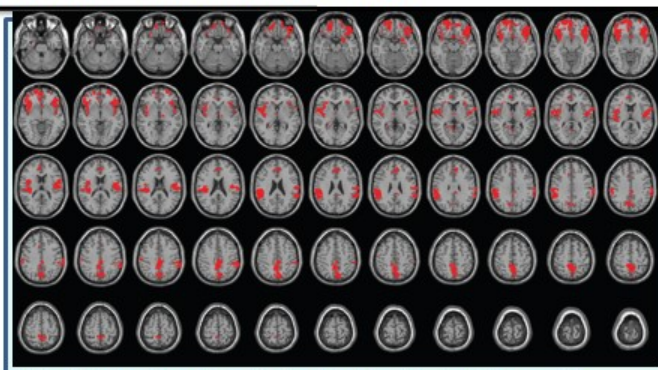


Study/Product Aim(s)

- Aim 1: Neurovascular abnormalities associated with olfactory deficits in early PD patients.
- Aim 2: Dynamic changes in neurovascular and metabolic parameters during olfactory stimuli in early PD patients.
- Aim 3: Cross-sectional study on the progression of neurovascular abnormalities associated with olfactory deficits across different stages of PD patients.

Approach

Advanced MRI methods developed at 7T will be used to measure neurovascular changes in related brain regions in early PD patients and controls (Aim 1). We will also measure dynamic responses of neurovascular parameters during olfactory stimulation in related brain regions (Aim 2). These MRI measures will be correlated with olfactory test scores, and compared to brain atrophy in the same regions.



Activated brain regions during olfactory stimulation detected by the recently developed T2prep BOLD fMRI approach. The olfactory cortex is notoriously difficult to image with conventional fMRI methods due to larger susceptibility artifacts. With our T2prep BOLD fMRI, robust activation can be detected in regions related to olfaction.

Timeline and Cost

Activities	CY	17	18	19	20
IRB, personnel and other prep					
Aim 1					
Aim 2					
Aim 3					
Estimated Budget (\$K)		\$000	\$000	\$000	\$000

Updated: 09/29/2021

Goals/Milestones

CY17 Goal – IRB approval, personnel recruitment and other preparation

☑ IRB approval and personnel recruitment.

CY18 Goals – Aim 1

☑ Experimental setup and optimization.

☑ Patient recruitment, MRI and analysis.

CY19 Goal – Aim 2

☑ Patient recruitment, MRI and analysis.

CY20 Goal – Aim 3

☑ Patient recruitment, MRI and analysis.

Comments/Challenges/Issues/Concerns

- If timelines change, comment here.
- If off by more than one quarter in spending, comment here.

Budget Expenditure to Date

Projected Expenditure:

Actual Expenditure:

9. APPENDICES:

The publication listed in Section 6 are attached in the following order.

Journal publication:

- 1) Paez AG, Gu C, Rajan S, Miao X, Cao D, Kamath V, Bakker A, Unschuld PG, Pantelyat A, Rosenthal LS, **Hua J***. Differential changes in arteriolar cerebral blood volume between Parkinson's disease patients with normal and impaired cognition and mild cognitive impairment (MCI) patients without movement disorder – an exploratory study. *Tomography*. 2020 Dec;6(4):333-342. PMC7744190.
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Conference publication:

- 1) Paez AG, Rajan S, Chaney G, Miao X, Cao D, Gu C, Liu D, Pantelyat AY, Rosenthal LI, Mari ZK, Mills K, Dawson TM, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Olfactory functional MRI (fMRI) using T2-prepared BOLD fMRI at ultra-high field (7T). *Proceedings International Society for Magnetic Resonance in Medicine*, May 2019. **Montreal, Canada**.
- 2) Paez AG, Rajan S, Chaney G, Miao X, Cao D, Gu C, Liu D, Pantelyat AY, Rosenthal LI, Mari ZK, Mills K, Dawson TM, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Olfactory Functional MRI (fMRI) Using T2-prepared BOLD fMRI at Ultra-high Field (7T). *Military Health System Research Symposium (MHSRS)*, August 2019. **Orlando, FL, USA**.
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- 4) Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Functional MRI in the olfactory bulb and associated olfactory regions in the human brain on 7T. *Proceedings International Society for Magnetic Resonance in Medicine Workshop on Ultra-High Field MR*, March 2022. **Lisbon, Portugal**.
- 5) Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. Functional activities detected in the olfactory bulb and associated olfactory regions in the human brain using T2-prepared BOLD fMRI at 7T. *Proceedings International Society for Magnetic Resonance in Medicine*, May 2022. **London, England, UK**.
- 6) Miao X, Paez AG, Rajan S, Cao D, Liu D, Pantelyat AY, Rosenthal LI, van Zijl PCM, Bassett SS, Yousem DM, Kamath V, **Hua J**. High-resolution functional MRI in the human olfactory bulb at 7T. *Proceedings 28th Organization for Human Brain Mapping (OHBM) Annual Meeting*, June 2022. **Glasgow, Scotland, UK**.
- 7) Paez AG, Rajan S, Fang Z, Pantelyat AY, Rosenthal LI, Faria A, Miao X, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. High resolution structural MRI reveals abnormalities in olfactory brain regions in early Parkinson's disease (PD) patients. *Proceedings International Society for Magnetic Resonance in Medicine Workshop on Ultra-High Field MR*, March 2022. **Lisbon, Portugal**.
- 8) Paez AG, Rajan S, Pantelyat AY, Rosenthal LI, Faria A, Miao X, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. Structural abnormalities in olfactory brain regions in early Parkinson's disease (PD) patients measured by high resolution MRI at 7T. *Proceedings International Society for Magnetic Resonance in Medicine*, May 2022. **London, England, UK**.
- 9) Paez AG, Rajan S, Pantelyat AY, Rosenthal LI, Faria A, Miao X, Dawson TM, van Zijl PCM, Kamath V, **Hua J**. Volumetric changes in olfactory regions in Parkinson's disease measured by high-resolution 7T MRI. *Proceedings 28th Organization for Human Brain Mapping (OHBM) Annual Meeting*, June 2022. **Glasgow, Scotland, UK**.

Differential Changes in Arteriolar Cerebral Blood Volume between Parkinson's Disease Patients with Normal and Impaired Cognition and Mild Cognitive Impairment (MCI) Patients without Movement Disorder – An Exploratory Study

Adrian G. Paez^{1,2}, Chunming Gu^{1,2,3}, Suraj Rajan^{4,5}, Xinyuan Miao^{1,2}, Di Cao^{1,2,3}, Vidyulata Kamath⁵, Arnold Bakker⁵, Paul G. Unschuld⁶, Alexander Y. Pantelyat⁴, Liana S. Rosenthal⁴, and Jun Hua^{1,2}

¹F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD; ²Neurosection, Division of MR Research, Department of Radiology, ³Department of Biomedical Engineering; ⁴Department of Neurology; and ⁵Department of Psychiatry and Behavioral Sciences, Johns Hopkins University School of Medicine, Baltimore, MD; and ⁶Department of Psychogeriatric Medicine, Psychiatric University Hospital Zurich, Zurich, Switzerland

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Key Words: Dementia, blood vessel, perfusion, iVASO, MRI

Abbreviations: Parkinson's disease (PD), mild cognitive impairment (MCI), arteriolar cerebral blood volume (CBVa), PD dementia (PDD), cerebral blood flow (CBF), presupplementary motor area (preSMA), inflow-based vascular-space-occupancy (iVASO), gray matter (GM), time of repetition (TR), time of inversion (TI), statistical parametric mapping (SPM), signal-to-noise ratio (SNR), magnetic resonance imaging (MRI), Unified Parkinson's Disease Rating Scale (UPRDS)

ABSTRACT

Cognitive impairment amongst Parkinson's disease (PD) patients is highly prevalent and associated with an increased risk of dementia. There is growing evidence that altered cerebrovascular functions contribute to cognitive impairment. Few studies have compared cerebrovascular changes in PD patients with normal and impaired cognition and those with mild-cognitive-impairment (MCI) without movement disorder. Here, we investigated arteriolar-cerebral-blood-volume (CBVa), an index reflecting the homeostasis of the most actively regulated segment in the microvasculature, using advanced MRI in various brain regions in PD and MCI patients and matched controls. Our goal is to find brain regions with altered CBVa that are specific to PD with normal and impaired cognition, and MCI-without-movement-disorder, respectively. In PD patients with normal cognition (n=10), CBVa was significantly decreased in the substantia nigra, caudate and putamen when compared to controls. In PD patients with impaired cognition (n=6), CBVa showed a decreasing trend in the substantia nigra, caudate and putamen, but was significantly increased in the presupplementary motor area and intracalcarine gyrus compared to controls. In MCI-patients-without-movement-disorder (n=18), CBVa was significantly increased in the caudate, putamen, hippocampus and lingual gyrus compared to controls. These findings provide important information for efforts towards developing biomarkers for the evaluation of potential risk of PD dementia (PDD) in PD patients. The current study is limited in sample size and therefore is exploratory in nature. The data from this pilot study will serve as the basis for power analysis for subsequent studies to further investigate and validate the current findings.

INTRODUCTION

Parkinson's disease (PD) is defined by its characteristic motor symptoms of bradykinesia, rigidity, and tremors. However, non-motor symptoms such as cognitive impairment are frequently reported in PD, with more than one-third of patients showing signs of impairment in at least one cognitive domain at the time of diagnosis with PD (1). Gaining a better understanding of the mechanistic underpinnings of cognitive impairment is important, as cognitive impairment is associated with accelerated functional decline and neuropsychiatric symptoms including anxiety and

depression, and the risk of progression to dementia is over four times greater in PD patients with cognitive impairment than in PD patients with normal cognition (2, 3). Despite significant efforts, currently there is no robust measure to predict which patients with PD are at the greatest risk of developing PD dementia (PDD).

Cognitive impairment in PD is likely due to the presence of pathologic alpha-synuclein in the cortex, although in ~30% of individuals, there is additional amyloid and tau pathology (4–6). There is also growing evidence that cerebrovascular disease is an

important contributor to cognitive impairment. Cerebrovascular abnormalities including altered cerebral blood flow (CBF), cerebral blood volume (CBV), and blood-brain barrier permeability (7–10) have been linked with pathophysiology in various dementias (11, 12). Following those reports (13), we previously used advanced neuroimaging methods to show increased volumes of small pial arteries and arterioles (arteriolar cerebral blood volume, CBVa) in several brain regions such as the orbitofrontal cortex and the hippocampus in elderly adults with mild cognitive impairment (MCI) compared with the volumes of those in age-matched elderly controls. Cerebral vascular risk factors have also been associated with PDD. Within PDD, there is growing recognition regarding the importance of vascular pathology. Among individuals in the Parkinson's Progression Markers Initiative cohort, we found that the rate of change in measures of global cognition was greater among those with white matter hyperintensities on magnetic resonance imaging (MRI) (14). Altered CBF, CBV, and microvasculature have also been shown in patients with PD (15–23). However, to date, few studies have examined and compared cerebrovascular changes in PD patients with normal cognition, PD patients with impaired cognition, and MCI due to Alzheimer's disease.

PD-related cognitive impairment and AD-related cognitive impairment manifest differently (24), with the former being a subcortical dementia and the latter a cortical dementia (25–28). The type and regional distribution of pathology differ between PD and AD. The pathognomonic changes in PD include loss of pigmented dopaminergic cells and presence of Lewy bodies in the substantia nigra (29, 30). Dopamine loss changes the relationship within the basal ganglia pathways and subsequently changes the signaling between the basal ganglia and the cortex, leading to motor and some executive dysfunctions observed in individuals with PD (31). Indeed, multiple studies have shown small blood vessel damage in patients with PD, mainly in the substantia nigra, caudate, and putamen (15–23). The presupplementary motor area (preSMA) receives significant inputs from the basal ganglia and, in individuals with PD, significant atrophy (32), metabolic changes (33), and hypoactivation (34) have been observed in their preSMA. These are considered to be markers of changes in motor planning and not cognitive change per se. Although hypoactivation and atrophy in the entorhinal cortex, hippocampus, parahippocampus, and posterior cingulate gyrus have been identified in individuals with AD (26–28, 35) and in those with PD-related cognitive changes (36–38), other brain regions including the intracalcarine gyrus (39), thalamus (40), and lingual gyrus (41) appear to subserve PD-related cognitive impairment than AD-related amnesic MCI. Still other areas such as the nucleus accumbens (26–28, 42, 43) are affected primarily in AD-type dementia than in PD-related cognitive impairment. Different patterns of neuronal loss were reported in the nucleus basalis of Meynert in AD, PD, and PDD (44).

In this study, we used the inflow-based vascular-space-occupancy (iVASO) MRI approach (45–50) to determine potential arteriolar abnormalities (CBVa) in the brain in a cohort of patients with PD and matched controls and in a cohort of MCI patients without movement disorders and matched controls. Pial arteries and arterioles are the most actively regulated blood

vessels (51–55) and are affected by aging before venous vessels (56). Therefore, the measurement of changes in CBVa may provide a more sensitive marker than measurement of changes in total CBV and CBF, which include both arteriolar and venous vessels. Hua et al. (13) have previously reported on the MCI without movement disorder cohort and their data has been reanalyzed here with a different approach (see Methods). Based on the literature discussed previously, CBVa in preselected brain regions was calculated and compared in patients and matched controls in each cohort, with the goal of finding brain regions with altered CBVa that are specific to PD with normal cognition, PD with impaired cognition, and MCI without movement disorder.

METHODS

Study Participants

In total, 2 cohorts of participants were recruited for this study. The first cohort includes 10 PD patients with normal cognition, 6 PD patients with impaired cognition, and 7 healthy controls matched in age, sex, and education level. All patients with PD had a clinically established or clinically probable PD diagnosis according to the criteria described in the study by Postuma et al. (57). The Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (58, 59) was used as a key part to evaluate clinical symptoms. All participants were recruited through the Johns Hopkins Parkinson's Disease and Movement Disorders Center. This study has been approved by the Johns Hopkins Institutional Review Boards. Demographic data for this PD cohort are summarized in Table 1.

The second cohort consists of 18 MCI patients without movement disorder and 22 age-, sex-, and education-matched cognitively normal controls. This second cohort was recruited at the University of Zurich, Switzerland. The current study uses recently published MRI and clinical data of this cohort (13). The published MRI data was reanalyzed using a different method in the current study (see Data Analysis). As reported earlier, the study procedures were in accordance with guidelines issued by the local ethics committee (Kantonale Ethikkommission Zürich), as well as with the Declaration of Helsinki (60). Demographic data for this MCI without movement disorder cohort are summarized in Table 2 [data indicated in Table 2 has been published recently in (13)].

In both cohorts, each participant gave written informed consent for their participation. Each participant completed an MRI session on a 7T human MRI system and received a cognitive assessment (see Cognitive Assessment). None of the participants had other neurologic disorders or met *Diagnostic and Statistical Manual-5* criteria for psychiatric disorders.

Cognitive Assessment

All participants completed a cognitive assessment. All cognitive tests were administered and scored according to standardized procedures. The cognitive battery for the PD cohort consists of the following tests:

(1) The Logical Memory Subset of the Wechsler Memory Scale (WMS-III) (62)

Table 1. Demographic Data and Clinical and Cognitive Assessment of the Parkinson's Disease (PD) Cohorts

				<i>p</i> ^a			
	Controls (Con)	PD Cognitive Normal (PDcn)	PD Cognitive Impaired (PDci)	Overall	PDcn vs Con	PDci vs Con	PDci vs PDcn
Demographics							
N	7	10	6	N/A	N/A	N/A	N/A
Sex (female)	4	5	3	.95	.77	.80	1
Age (years)	59.86 ± 6.09 ^b	64.90 ± 7.85	66.33 ± 10.37	.32	.16	.22	.78
Education (years)	15.71 ± 2.69	17.20 ± 1.40	16.50 ± 3.21	.46	.22	.65	.63
Disease Duration (years)	N/A	2.80 ± 1.34	3.36 ± 1.41	N/A	N/A	N/A	.45
Unified Parkinson’s Disease Rating Scale							
Motor	N/A	25.11 ± 8.09	27.02 ± 6.33	N/A	N/A	N/A	.55
Neuropsychological Assessment							
Logical Memory Subset of the Wechsler Memory Scale	26.75 ± 6.80	27.30 ± 4.19	19.00 ± 5.57	.02	.80	.05	.02
Logical Memory Subset of the Wechsler Memory Scale Recall	25.00 ± 5.29	25.20 ± 4.47	11.60 ± 7.06	.001	.95	.01	.009
Controlled Oral Word Association Test	88.50 ± 16.20	97.30 ± 12.92	70.00 ± 15.76	.007	.108	.04	.01
HVLT-R Learning Trials	22.00 ± 6.32	23.90 ± 5.00	14.80 ± 4.32	.02	.62	.05	.005
HVLT-R Recall	6.75 ± 2.63	7.40 ± 3.66	3.60 ± 2.88	.14	.72	.13	.05
60-Item Boston Naming Test	51.67 ± 3.51	52.30 ± 1.34	53.80 ± 6.22	.06	.08	.06	.08
Longest Digit Span Forward	6.75 ± 9.00	6.70 ± 1.83	7.00 ± 2.00	.95	.95	.81	.79
Longest Digit Span Backward	5.00 ± 7.00	5.70 ± 1.34	4.60 ± 1.34	.25	.13	.54	.17

Please see Methods on details regarding the test applied.

^a *P* values were derived with the ANOVA, chi-square test, or *t* test, as appropriate.

^b mean ± standard deviation.

- (2) Controlled Oral Word Association Test (COWAT) (62)
- (3) Hopkins Verbal Learning Test–Revised (HVLT-R) (62)
- (4) The 60-item Boston Naming Test–2nd Edition (BNT-60) (62)
- (5) Digit Span Forward and Backward (63).

Individuals were classified as either PD with normal cognition or PD with impaired cognition according to the Level 1 classification outlined by Litvan et al. (64). In particular, individuals with impairment on at least 2 tests were stratified to the PD with impaired cognition group.

In the MCI without movement disorder cohort, the following cognitive tests were performed:

- (1) The Mini-Mental State Exam (MMSE)
- (2) The Revised Boston Naming Test (BNT-15) (65)
- (3) Digit Span Backward (63)
- (4) Trail Making Test (TMT) (66)
- (5) Verbal Learning and Memory Test (VLMT) (67).

Participants were categorized as cognitively normal or cognitively impaired according to established criteria (61).

MRI

Participants in both cohorts underwent a 7T MRI scan (Philips MRI scanner; Philips Healthcare, Best, The Netherlands). The hardware and software on the 7T MRI systems at both sites were identical. A 32-channel phased-array head coil (Nova Medical,

Wilmington, MA) was used for radiofrequency reception and a head-only quadrature coil for transmittal. High-resolution anatomical images were acquired with a 3D Magnetization Prepared Rapid Gradient Echo (MPRAGE) sequence (voxel = 0.75 mm isotropic) (68, 69). A 3D iVASO MRI scan covering the entire brain (13, 70, 71) was performed to measure regional gray matter (GM) CBVa using the following parameters: time of repetition (TR)/time of inversion (TI) = 10 000/1383, 5000/1093, 3800/884, 3100/714, 2500/533, and 2000/356 millisecond; voxel = 3.5 × 3.5 × 5 mm³, slices = 20; and parallel imaging acceleration (SENSE) = 2 × 2. A reference scan (TR = 20 seconds, other parameters identical) was obtained so that the scaling factor M0 in iVASO images can be determined to calculate absolute CBVa values.

Data Analysis

The statistical parametric mapping (SPM) software package (Version 8, Wellcome Trust Centre for Neuroimaging, London, United Kingdom; <http://www.fil.ion.ucl.ac.uk/spm/>) and other in-house code programmed in Matlab (MathWorks, Natick, MA) were used for image analyses. Motion correction in iVASO images, coregistration between anatomical and iVASO images, and normalization to the Montreal Neurological Institute space were performed using SPM. Regional GM CBVa maps in the whole brain were calculated from the iVASO signals after

Table 2. Demographic Data and Clinical and Cognitive Assessment of the Age-Related MCI Patients Without Movement Disorder

	Controls	Age-Related MCI Patients Without Movement Disorder	<i>p</i> ^a
Demographics			
N	22	18	N/A
Sex (female)	8	6	1
Age (year)	72 ± 5 ^b	75 ± 7	.08
Education (year)	13.64 ± 2.56	15.06 ± 3.28	.13
Number of APOE4 Alleles	7	9	N/A
Neuropsychological Assessment			
Mini-Mental State Exam	29.45 ± 0.86	28.44 ± 1.42	.01
15-Item Boston Naming Test	14.41 ± 0.73	14.17 ± 1.25	.47
Digit Span Backward	6.27 ± 1.39	5.94 ± 1.76	.52
Verbal Learning and Memory	11.05 ± 2.57	5.39 ± 2.93	<0.001
Trail Making B/A	2.98 ± 1.37	2.75 ± 1.40	.62

Please see Methods on details regarding the test applied; Data indicated in Table 2 have been published recently in (13).

^a*P* values from 2-sample *t* tests between the 2 groups, or from chi-square test for categorical variables.

^bmean ± standard deviation.

surround subtraction (72) based on the iVASO equations [73]. GM, white matter, and cerebrospinal fluid maps generated from the anatomical images using the SPM segmentation algorithm were applied to correct for the partial volume effects of white matter and cerebrospinal fluid on the iVASO difference signal in GM (74). A signal-to-noise ratio (SNR) threshold of one standard deviation below the mean SNR was applied to exclude low SNR voxels from further analysis (73).

The IBASPM116 atlas (75–79) (PickAtlas software, Wake Forest University, NC) was used to identify the preselected anatomical regions based on the literature reviewed in the Introduction, from which average CBVa values were calculated. Group differences in GM CBVa in each region were examined using analysis of covariance with age, sex, education, regional GM volume from anatomical scans, and motion parameters estimated from the motion correction routine in SPM as covariates in the analysis. Effect size was estimated with Cohen's *d*. All statistical tests were corrected for multiple comparisons by controlling the false discovery rate (adjusted *P* < .05) (80). Note that data from all patients and their corresponding control participants were acquired at the same site and no statistical comparison between the data acquired from different sites was performed in this study.

Note that the current study adopted a region of interest-based analysis strategy to test our hypotheses in preselected brain regions based on literature. CBVa in each brain region identified on magnetic resonance images using the IBASPM116 atlas was averaged and compared. This result is different from that of our previous study on the MCI without movement disorder cohort (13), in which CBVa was compared across the brain on a voxel basis and significant clusters of altered CBVa were identified. CBVa in each cluster within each brain region (which may not cover the entire region) was averaged and compared.

RESULTS

Demographic data for the PD cohorts are summarized in Table 1. Age, sex, and education levels were matched among PD patients with normal or impaired cognition and controls (*P* > .1). Disease duration and UPDRS motor score were matched among PD patients with normal or impaired cognition (*P* = .45, .55). Significant deficits were observed in PD patients with impaired cognition compared with the other 2 groups in the following tests: Logical Memory Subset of the Wechsler Memory Scale (*P* = .02), Controlled Oral Word Association Test (*P* = .007), and HVLT-R (*P* = .02); and trending significant in BNT-60 (*P* = .06).

Demographic data for the MCI without movement disorder cohort are summarized in Table 2. Individuals with MCI and controls in this cohort had matched age, sex, and education levels (*P* > .1). Patients with MCI showed significantly lower scores compared with controls on the Verbal Learning And Memory Test (*P* < .001) and Mini-Mental State Exam (*P* = .01).

The main findings in CBVa changes are summarized in Tables 3–5, and in Figure 1. The CBVa values in controls in all brain regions investigated were in the normal range of CBVa reported for healthy human subjects in the literature (81).

In PD patients with normal cognition (*n* = 10), CBVa was significantly decreased in the substantia nigra (*P* = .04), caudate (*P* = .04), and putamen (*P* = .01) compared with controls (*n* = 7), but comparable with controls in all the other regions investigated.

In PD patients with impaired cognition (*n* = 6), CBVa showed a trend toward decrease in the substantia nigra (*P* = .06), caudate (*P* = .09), and putamen (*P* = .06) compared with controls (*n* = 7). CBVa was significantly increased in the preSMA (*P* = .01) and intracalcarine gyrus (*P* = .03) compared with controls, and it also showed a trend toward increase in the hippocampus (*P* = .07), entorhinal cortex (*P* = .09), and parahippocampus (*P* = .07).

Table 3. Altered Gray Matter CBVa in PD Patients Compared With Controls—CBVa Values in Each Brain Region

Regions	Control (n = 7)		PD Cognitive Normal (n = 10)		PD Cognitive Impaired (n = 6)	
	Mean	SD	Mean	SD	Mean	SD
Substantia Nigra	0.90	0.15	0.63	0.22	0.63	0.31
Caudate	0.90	0.05	0.76	0.26	0.71	0.26
Putamen	0.90	0.09	0.63	0.20	0.63	0.32
Nucleus Accumbens	0.89	0.06	0.85	0.19	0.92	0.07
Posterior Cingulate Cortex	0.93	0.04	0.87	0.20	0.96	0.07
Hippocampus	0.91	0.08	0.90	0.13	1.01	0.12
Entorhinal Cortex	1.00	0.06	1.01	0.05	1.33	0.46
Parahippocampus	0.99	0.10	1.01	0.07	1.35	0.46
Presupplementary Motor Area	1.10	0.08	1.06	0.12	1.34	0.13
Thalamus	0.99	0.09	0.97	0.04	1.09	0.15
Intracalcarine Gyrus	1.08	0.09	1.14	0.45	1.50	0.37
Lingual Gyrus	1.04	0.03	1.04	0.16	1.20	0.24
Nucleus Basalis of Meynert	1.00	0.03	1.02	0.07	1.03	0.09
Cerebellum	1.03	0.01	1.01	0.08	1.04	0.07

In MCI patients without movement disorder (n = 18), CBVa was significantly increased in the caudate ($P = .04$), putamen ($P = .05$), hippocampus ($P = .02$), and lingual gyrus ($P = .05$) compared with controls (n = 22). CBVa showed a trend toward increase in the nucleus accumbens ($P = .08$), posterior cingulate

cortex ($P = .06$), entorhinal cortex ($P = .06$), and parahippocampus ($P = .06$).

In all patients with PD and MCI, the CBVa values in the cerebellum were not significantly different from those in controls in the respective cohorts.

Table 4. Altered Gray Matter CBVa in PD Patients Compared With Controls—Statistical Results

Regions	PD Cognitive Normal vs Control					PD Cognitive Impaired vs Control					PD Cognitive Impaired vs PD Cognitive Normal				
	Relative Change (%) ^a	Effect Size ^b	P	t	df	Relative Change (%)	Effect Size	P	t	df	Relative Change (%)	Effect Size	P	t	df
Substantia Nigra	-29.56%	-1.31	.04	-4.32	12	-29.63%	-1.01	.06	-3.04	11	-0.11%	0.01	.99	-0.01	11
Caudate	-14.86%	-0.57	.04	-2.90	15	-20.30%	-0.84	.09	-2.86	10	-6.39%	-0.19	.55	-0.65	12
Putamen	-29.78%	-1.45	.01	-5.96	14	-29.63%	-1.00	.06	-3.29	11	0.21%	0.01	.99	0.02	11
Nucleus Accumbens	-4.04%	-0.21	.38	-0.96	15	3.75%	0.50	.31	1.28	10	8.12%	0.44	.10	1.94	14
Posterior Cingulate Cortex	-6.38%	-0.32	.17	-1.60	15	3.34%	0.48	.27	1.37	11	10.38%	0.54	.06	2.38	14
Hippocampus	-0.99%	-0.08	.80	-0.28	13	11.97%	1.01	.07	2.86	11	13.10%	0.94	.03	3.31	12
Entorhinal Cortex	1.24%	0.23	.65	0.57	11	32.67%	0.88	.09	2.99	10	31.04%	1.14	.10	2.92	10
Parahippocampus	1.96%	0.24	.68	0.53	11	35.36%	0.93	.07	3.10	10	32.75%	1.19	.09	3.06	10
Presupplementary Motor Area	-3.45%	-0.33	.34	-1.18	12	22.37%	2.14	.01	6.21	11	26.75%	2.28	.01	7.73	12
Thalamus	-1.58%	-0.28	.69	-0.51	11	10.48%	0.79	.11	2.27	11	12.25%	1.25	.07	3.35	10
Intracalcarine Gyrus	5.67%	0.15	.48	0.76	15	38.86%	1.36	.03	4.51	10	31.40%	0.85	.03	3.11	13
Lingual Gyrus	0.05%	0.00	.99	0.02	15	15.38%	0.80	.11	2.73	10	15.33%	0.82	.09	2.51	11
Nucleus Basalis of Meynert	1.60%	0.25	.36	-1.33	14	3.33%	0.46	.24	0.81	10	1.71%	0.23	.51	0.97	13
Cerebellum	-1.70%	-0.24	.27	-1.26	14	1.37%	0.26	.47	0.88	10	3.13%	0.41	.19	1.51	13

^a Relative change was defined as $100 \times (\text{mean CBVa in patients} - \text{mean CBVa in controls}) / (\text{mean CBVa in controls}) \%$.

^b Effect size was estimated with Cohen's $d = (\text{mean CBVa in patients} - \text{mean CBVa in controls}) / s$, where s is the pooled standard deviation of the 2 groups.

^c Degree of freedom.

Table 5. Altered Gray Matter CBVa in Age-Related MCI Patients Without Movement Disorder Compared With Matching Controls

Regions	MCI (n = 18)		Control (n = 22)		Relative Change (%) ^a	Effect Size ^b	P	t	df ^c
	Mean	SD	Mean	SD					
Substantia Nigra	1.15	0.83	1.09	0.88	5.50%	0.07	.59	0.55	31
Caudate	2.31	1.65	1.17	1.31	97.44%	0.77	.04	3.22	35
Putamen	2.15	1.38	1.29	1.01	66.67%	0.72	.05	3.15	35
Nucleus Accumbens	1.52	1.00	1.02	0.99	49.02%	0.50	.08	3.01	32
Posterior Cingulate Cortex	1.55	0.78	1.11	0.82	39.64%	0.55	.06	3.06	32
Hippocampus	1.77	0.93	1.07	0.65	65.42%	0.89	.02	3.33	34
Entorhinal Cortex	1.89	0.78	1.08	0.52	75.00%	1.25	.06	3.05	32
Parahippocampus	1.81	0.53	1.05	0.70	72.38%	1.21	.06	3.05	32
Presupplementary Motor Area	1.76	0.47	1.32	0.52	33.33%	0.88	.12	1.97	36
Thalamus	1.63	0.71	1.17	0.66	39.32%	0.67	.15	1.90	36
Intracalcarine Gyrus	1.82	0.68	1.50	0.77	21.33%	0.44	.13	1.95	36
Lingual Gyrus	1.85	0.88	1.45	0.69	27.59%	0.51	.05	3.12	35
Nucleus Basalis of Meynert	1.21	0.98	1.17	1.00	3.42%	0.04	.60	0.57	35
Cerebellum	1.29	1.01	1.19	0.88	8.40%	0.11	.50	0.69	35

^a Relative change was defined as $100 \times (\text{mean CBVa in patients} - \text{mean CBVa in controls}) / (\text{mean CBVa in controls}) \%$.

^b Effect size was estimated with Cohen's $d = (\text{mean CBVa in patients} - \text{mean CBVa in controls}) / s$, where s is the pooled standard deviation of the 2 groups.

^c Degree of freedom.

DISCUSSION

In this study, microvascular abnormalities as reflected by volume changes in small pial arteries and arterioles (CBVa) were investigated using iVASO MRI in PD patients, MCI patients without movement disorder, and matched controls. As pial arteries and arterioles are the primary regulators of regional perfusion in brain tissues (82–84), CBVa is considered as an indicator of the homeostasis of microvasculature that may provide additional information regarding the underlying neurophysiological changes than behavioral measures and conventional structural MRI. The same MRI scans and analyses were performed in 2 separate cohorts of PD patients and MCI patients without movement disorder and corresponding control groups recruited at 2 sites. This allowed us to better match the controls in each cohort, as the age and many other factors can differ substantially among PD patients and MCI patients without movement disorder. The MRI scans were acquired on a 7T human MRI system with identical hardware and software at both sites. Only data acquired on the same MRI system were compared to minimize the confounding effects from the potential differences between the 2 sites.

Patients with PD who enrolled for this study had an average disease duration of ~3 years and an average UPDRS score of 20–30, which is generally considered to be early stage (but not prodromal) PD (85). The degree of cognitive decline in PD patients with impaired cognition group was mild, as reflected by their performance on the cognitive assessments, and was comparable to that in the MCI patients without movement disorder group as reported in our previous study (13).

The main finding in this study is that PD patients showed significant CBVa *decreases* in the substantia nigra, caudate, and putamen compared with controls, whereas MCI patients without movement disorder and PD patients with impaired cognition showed significant CBVa *increases* in several brain regions closely related to cognition, compared with controls. We interpret the decreased CBVa as an indicator for microvascular damage, especially in the substantia nigra in PD patients, as evidenced in several studies in the literature (15–23). In contrast, similar to our previous studies in MCI patients without movement disorder (13) and Huntington's Disease patients (86) in which the same MRI methods were used, one possible explanation for the elevated CBVa observed in several brain regions may be a *compensatory* mechanism in the earlier stages of the diseases, in which the number of blood vessels increases to normalize the restricted blood flow owing to the reduction of diameter in individual vessels. The exact mechanism is unclear and warrants further investigation that integrates MRI and other imaging and histological techniques.

The substantia nigra is one of the first brain regions that accumulates Lewy bodies in postmortem pathological studies in PD. In our data, CBVa decreased in the substantia nigra in both PD patients with normal cognition and PD patients with impaired cognition. CBVa in the substantia nigra in MCI patients without movement disorder did not show significant changes compared with controls. The dorsal striatum, which consists of the caudate and the putamen, is another region that is known to be affected early in PD. Interestingly, our data showed decreased CBVa in

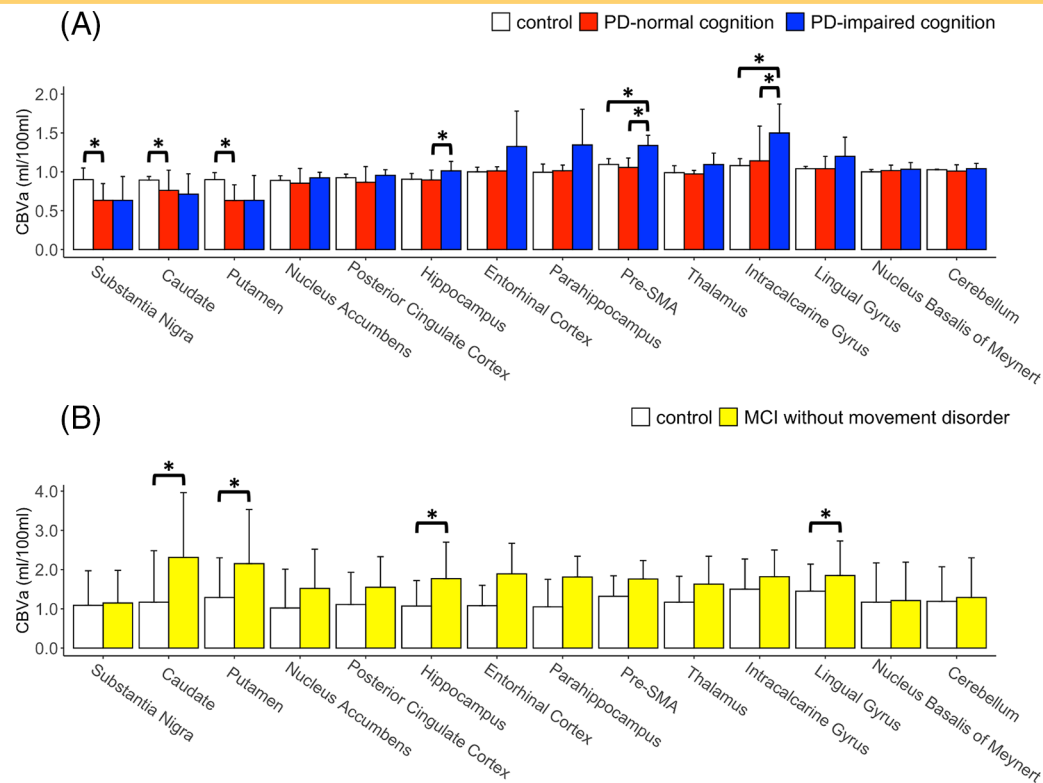


Figure 1. Comparisons of arteriolar cerebral blood volume (CBVa) values in chosen brain regions between Parkinson's Disease patients with normal cognition, PD patients with impaired cognition (A), and mild cognitive impairment (MCI) patients without movement disorder with matching controls (B). * $P < .05$.

the caudate and putamen in PD patients but increased CBVa in these regions in MCI patients without movement disorder compared with their respective controls.

In this exploratory study, our data seem to suggest that CBVa increase in the preSMA and intracalcarine gyrus, and possibly the hippocampus, entorhinal cortex, and parahippocampus, may be differentiating between PD patients with normal cognition and patients with impaired cognition. The hippocampus, entorhinal cortex, and parahippocampus are closely related to overall cognition and to episodic memory and are known to be affected in dementia (87–91). Our data showed relatively large effect sizes (close to 1) in CBVa increase in these three regions in both PD patients with impaired cognition and MCI patients without movement disorder compared with matching controls. In PD patients with normal cognition, CBVa values in these regions did not show significant changes. The preSMA and intracalcarine gyrus are two regions that are considered to be primarily affected in PDD but not in AD-MCI. In our data, PD patients with impaired cognition showed significantly increased CBVa in these two regions compared with controls, with the largest effect sizes among all regions investigated. No significant changes in CBVa in PD patients with normal cognition and MCI patients without movement disorder were detected in these two regions. In contrast, the opposite CBVa changes in the caudate and putamen, along with CBVa changes in the substantia nigra, nucleus accumbens in the ventral striatum, and the posterior cingulate

cortex, seem to suggest that measuring CBVa in these regions may be key in differentiating between PD patients with impaired cognition and MCI patients without movement disorder. In addition, the lingual gyrus is another region that showed increased CBVa only in the MCI patients without movement disorder cohort. To the best of our knowledge, there are very few studies currently on the potential differential neurovascular changes in different brain regions among PD, PD-MCI, and AD-MCI. The preliminary findings in this study require further investigation and validation in subsequent studies.

The cerebellum is known to be largely spared in the early stages of both PD and AD-MCI. In our data, all PD and MCI patients showed comparable CBVa values in the cerebellum as in controls in respective cohorts. Besides, the CBVa values in all regions in the control subjects were in the normal range of CBVa in human subjects reported in the literature (81). These results provide validation for the CBVa values measured in this study.

No comparison between the MCI patients without movement disorder and PD cohorts were conducted in the analysis described in Results, as the data were acquired on the same type of MRI system but at two different sites. Nevertheless, the CBVa values in the MCI without movement disorder cohort seemed to be slightly greater overall than those in the PD cohort. As CBVa values tend to increase with age (81), one possible explanation is the ~10-year age difference between the patients in MCI and PD cohorts.

There are several limitations in this exploratory study. First, although significant effects were detected in our data, the sample size is small, especially for the PD cohort. Subsequent studies will continue to recruit PD patients with normal and impaired cognition and matched controls at the Johns Hopkins site to validate the current findings. Second, the cross-sectional design is also a fundamental limitation. Future studies with longitudinal measures at different stages of the disease are required to evaluate whether regional CBVa changes can be a predictor for the risk of developing PDD in PD patients. Using the smallest effect size (~ 0.5) for significant between-group CBVa differences detected in this study, we were able to conduct a power analysis that determined we would need ~ 30 participants per group in subsequent studies to achieve a power of 0.8 with $\alpha = 0.05$.

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In summary, CBVa abnormalities in different brain regions were detected in PD patients with normal cognition, in PD patients with impaired cognition, and in MCI patients without movement disorder compared with matched controls by use of iVASO MRI. Our data implies that CBVa changes in several key brain regions may be specific to each condition and thus may provide clues to differentiate one condition from the others. These findings provide further details regarding microvascular abnormalities in different brain regions in PD patients and in MCI patients without movement disorder who have not been reported in existing literature. This may help advance our understanding of the pathophysiology of PDD and may aid the development of imaging biomarkers in PDD. The data from this study will serve as the basis for power analysis for subsequent studies to further investigate and validate the current findings.

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Functional Activities Detected in the Olfactory Bulb and Associated Olfactory Regions in the Human Brain Using T2-Prepared BOLD Functional MRI at 7T

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Olfaction is a fundamental sense that plays a vital role in daily life in humans, and can be altered in neuropsychiatric and neurodegenerative diseases. Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) using conventional echo-planar-imaging (EPI) based sequences can be challenging in brain regions important for olfactory processing, such as the olfactory bulb (OB) and orbitofrontal cortex, mainly due to the signal dropout and distortion artifacts caused by large susceptibility effects from the sinonasal cavity and temporal bone. To date, few studies have demonstrated successful fMRI in the OB in humans. T2-prepared (T2prep) BOLD fMRI is an alternative approach developed especially for performing fMRI in regions affected by large susceptibility artifacts. The purpose of this technical study is to evaluate T2prep BOLD fMRI for olfactory functional experiments in humans. Olfactory fMRI scans were performed on 7T in 14 healthy participants. T2prep BOLD showed greater sensitivity than GRE EPI BOLD in the OB, orbitofrontal cortex and the temporal pole. Functional activation was detected using T2prep BOLD in the OB and associated olfactory regions. Habituation effects and a bi-phasic pattern of fMRI signal changes during olfactory stimulation were observed in all regions. Both positively and negatively activated regions were observed during olfactory stimulation. These signal characteristics are generally consistent with literature and showed a good intra-subject reproducibility comparable to previous human BOLD fMRI studies. In conclusion, the methodology demonstrated in this study holds promise for future olfactory fMRI studies in the OB and other brain regions that suffer from large susceptibility artifacts.

Keywords: olfaction, habituation, MRI artifacts, T2prep, high-field

INTRODUCTION

The sense of smell has a profound yet underappreciated influence on physical and mental wellbeing. Olfactory information aids the detection of spoiled food and environmental hazards and influences personal hygiene and feeding behavior. Odorants are received by olfactory sensory neurons embedded in the nasal epithelium (Buck and Axel, 1991), which project first to glomeruli within the olfactory bulbs (OBs) and then to subcortical and cortical targets. Olfactory deficits have emerged as an early risk factor in many neurodegenerative and neurodevelopmental conditions (Moberg et al., 2014; Fullard et al., 2017; Marin et al., 2018), as well as in healthy older adults (Doty and Kamath, 2014). Olfactory disturbance is also a common symptom in coronavirus disease (COVID) infection (Moein et al., 2020).

Blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) has been widely used to investigate functional activities in brain regions associated with olfaction. Functional activations during olfactory stimulation have been detected by BOLD fMRI in the primary olfactory (piriform) cortex and many secondary olfactory regions in the human brain (Yang et al., 1997; Sobel et al., 1998, 2000; Poellinger et al., 2001; Gottfried et al., 2002). However, several olfactory related regions such as the OB and orbitofrontal cortex are difficult to image with the conventional gradient echo (GRE) echo-planar-imaging (EPI) based BOLD fMRI methods, mainly due to the well-known signal dropout and distortion artifacts caused by large susceptibility effects from the nearby sinonasal cavity and temporal bone especially the aerated petrous apex (Yang et al., 1997; Sobel et al., 1998, 2000; Poellinger et al., 2001; Wang et al., 2010; Zong et al., 2014; Lu et al., 2018). Such susceptibility artifacts in olfactory regions have been reported at 3.0 Tesla (3T), which is currently the most commonly used field strength for clinical MRI, and lower fields (Yang et al., 1997; Sobel et al., 1998, 2000; Poellinger et al., 2001; Wang et al., 2010; Lu et al., 2018); and are exacerbated at higher magnetic fields such as 7.0 Tesla (7T). It is especially challenging to do fMRI in the OB because of significant susceptibility artifacts and the small size of the bulb. Functional activities in the OB have been reported in rodents using fMRI (Xu et al., 2000, 2003, 2005; Schafer et al., 2005; Martin et al., 2007; Li et al., 2014; Poplawsky and Kim, 2014; Murphy et al., 2016; Zhao et al., 2016, 2017; Muir et al., 2019) and manganese enhanced MRI (Cross et al., 2004), in dogs (Jia et al., 2014; Berns et al., 2015) using fMRI, and in non-human primates using fMRI (Boyett-Anderson et al., 2003; Zhao et al., 2015). A recent human study used a surface coil covering most of the primary olfactory regions to do fMRI in the OB (Fournel et al., 2020). The electrobulbogram (EBG) technique has been developed recently for non-invasive recording of functional signals in the human OB (Iravani et al., 2020). To date, however, few studies have reported successful fMRI in the OB in humans with whole brain coverage. As olfactory stimulation can elicit functional activities in a variety of brain regions along the olfactory pathways (Doty, 2015), a whole brain coverage will be ideal for many olfactory fMRI studies in humans.

Recently, a whole-brain T2-prepared (T2prep) BOLD fMRI (Hua et al., 2014) approach showed minimal susceptibility

artifacts across the entire brain in healthy subjects. In the T2prep BOLD approach (**Supplementary Figure S1**), the BOLD contrast is induced using driven equilibrium (Becker, and Farrar, 1969), also referred to as a T2 preparation or T2-prep (Haase, 1990; Parrish and Hu, 1994) module, followed by a single-shot 3D fast GRE readout, which is commonly used in high-resolution anatomical scans with much reduced susceptibility artifacts compared to EPI. The T2-prep BOLD method minimized the susceptibility artifacts and enhanced functional sensitivity in the brains of individuals with metallic head implants (Miao et al., 2020), and in brain regions close to blood products and/or calcifications in patients undergoing presurgical brain mapping (Hua et al., 2017; Miao et al., 2020). In the current study, we tested the performance of T2prep BOLD fMRI in olfactory functional experiments in healthy subjects conducted on a 7T human MRI system. The contrast-to-noise ratio (CNR) was compared between T2prep BOLD fMRI and conventional GRE EPI BOLD fMRI in the same subjects. Functional activation in olfactory-eloquent brain regions, especially the OB, was assessed using T2prep BOLD fMRI. The reproducibility of the T2prep BOLD fMRI results was also evaluated. The primary goal of this technical study is to evaluate the feasibility and reproducibility of performing olfactory fMRI using T2prep BOLD fMRI in healthy human subjects. A comprehensive assessment and validation of the underlying neuronal and physiological origins of the fMRI signal changes is beyond the scope of the current study and is being pursued in subsequent studies.

MATERIALS AND METHODS

Participants

Fourteen healthy participants (47 ± 11 yo, 8 females) were recruited in the study. Some experiments such as GRE EPI BOLD fMRI scans during olfactory stimulation (see section “MRI Scans”) was performed in a subset of participants ($n = 5$, see power analysis in section “Statistical Analysis”) for comparison. We declare that all experiments on human subjects were conducted in accordance with the Declaration of Helsinki. This study was approved by the Johns Hopkins Institutional Review Board, and written informed consent was obtained from each participant. Participants had no history of neurologic or psychiatric disorders, or history of sinus surgery, craniofacial abnormalities, or nasal trauma or surgery. All participants were right-handed, non-smokers, and were not on any medications. Individuals experiencing respiratory infection, sinus allergies or symptoms of a common cold within a month before the study visit were excluded. The University of Pennsylvania Smell Identification Test (UPSIT; Doty et al., 1984, 1989) was administered to all participants, and the results indicated that all participants had normal olfactory functions.

Magnetic Resonance Imaging Scans

All scans were performed on a 7.0 Tesla (7T) Philips MRI scanner (Philips Healthcare, Best, Netherlands). An 8-channel transmit head coil was used for signal transmission and a 32-channel phased array head coil was used for signal reception. An advanced

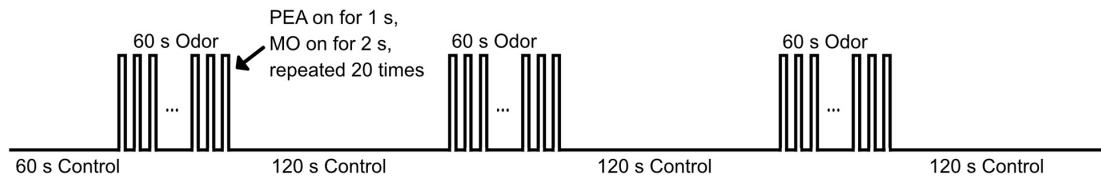


FIGURE 1 | Illustration of the olfactory paradigm used during fMRI. The odorant during the 60 s stimulus-on block was delivered in a pulsed pattern, with 1 s odorant of PEA and 2 s of odorless mineral oil (MO) repeated for 20 times. During the 120 s stimulus-off block, only MO was delivered continuously.

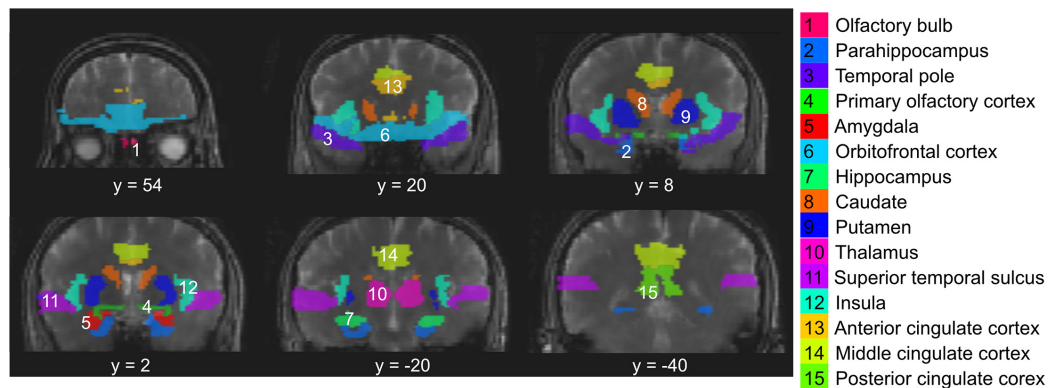


FIGURE 2 | Regions-of-interest (ROI) evaluated in the study overlaid on T2prep BOLD fMRI images from one subject. These include the olfactory bulb (#1), primary olfactory cortex (#4, piriform cortex), and other secondary olfactory regions known from the literature. Note that only six coronal slices were provided to illustrate the locations of the ROIs, but many ROIs can cover more slices. Most olfactory regions are best viewed in the coronal plane.

B0 shim algorithm was applied using the MRCodeTool software (v1.5.9, TeslaDC, Zaltbommel, Netherlands) installed on the scanner to improve B0 field homogeneity over the entire brain. In order to improve B1 field homogeneity, rectangular pads filled with high dielectric constant materials (Teeuwisse et al., 2012) were placed on the side of the subjects' head. The breathing pattern was recorded for each participant using a respiratory belt placed around the participant's abdomen so that it can be regressed out for fMRI analysis.

The following scans were performed for each participant:

- (1) 3D T1-weighted Magnetization Prepared Rapid Gradient Echo (MPRAGE): repetition time (TR)/inversion time (TI)/echo time (TE) = 4500/563/1.81 ms; field of view (FOV) = 220 mm × 220 mm; voxel = 1 mm isotropic; 180 sagittal slices;
- (2) T2prep BOLD fMRI during the olfactory paradigm described next: TR = 2000 ms; flip angle = 4°; T2prep effective TE = 50 ms; FOV = 180 (RL) × 222 (AP) mm²; voxel = 1.5 mm isotropic; 84 axial slices covering the entire brain; parallel imaging with SENSE factor = 3 × 3 (AP × FH), single-shot 3D turbo field echo (3D TFE, also known as 3D fast GRE) readout, centric phase encoding profile starting from the center of k-space, TR_{GRE}/TE_{GRE} = 2.90/1.32 ms. A first-order volume shim was performed on the imaging volume, which is usually sufficient for improving field homogeneity as shown in previous studies (Hua et al., 2014; Miao et al., 2020).

The following scan was performed in a subset of participants ($n = 5$, see power analysis in section "Statistical Analysis") on a different day (in order to avoid olfactory habituation effects) to compare the CNR between GRE EPI BOLD and T2prep BOLD fMRI:

- (3) GRE EPI BOLD fMRI during the same olfactory paradigm: TR = 2000 ms; flip angle = 70° (approximately the Ernst angle); TE = 22 ms; FOV = 180 (RL) × 222 (AP) mm²; voxel = 1.5 mm isotropic; 33 axial slices; SENSE = 3 (AP), single-shot 2D GRE EPI readout. Note that the coverage of conventional 2D GRE EPI scan was reduced as our current settings on 7T cannot achieve whole brain coverage with the chosen resolution, but the spatial and temporal resolutions were kept identical for the comparison. An optimal high-order shim method was performed on the imaging volume of the GRE EPI scan to improve field homogeneity and reduce dropout and distortion, and a field map based method was employed for distortion correction in GRE EPI (Schar et al., 2004).

Olfactory Paradigm

An enhanced model of multi-channel computer-controlled olfactometer (Lundström et al., 2010) (Whiff LLC, Swarthmore, PA, United States) was used to deliver the odorants in precisely timed pulses. The olfactometer was placed outside of the scanning room and was connected to pressured air tanks. Phenyl ethyl alcohol (PEA, Sigma-Aldrich) diluted in odorless mineral oil (50% v/v, 60 ml) was embedded in a constantly

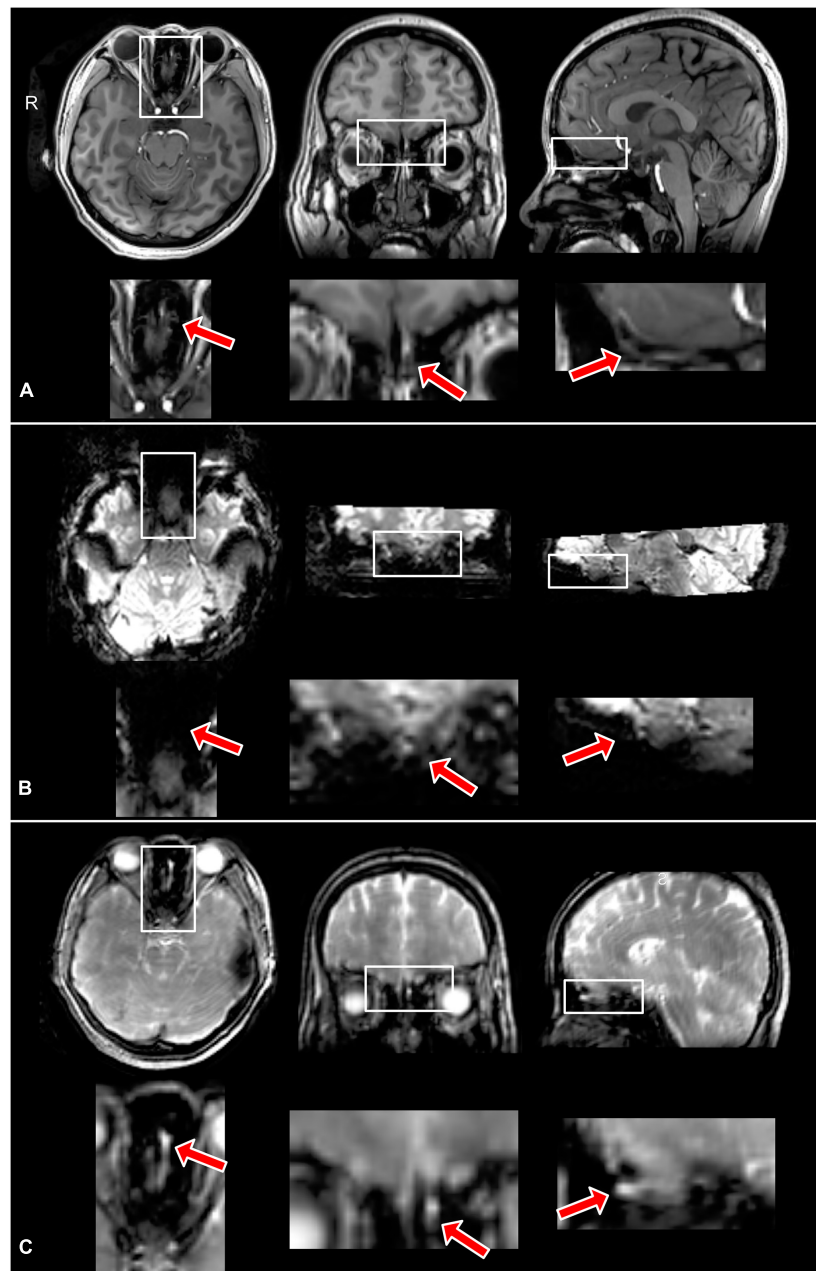


FIGURE 3 | Typical MPRAGE (A), GRE EPI BOLD (B), and T2prep BOLD (C) images from one subject. As the size of the olfactory bulb is small, the regions in the white boxes covering the areas around the olfactory bulb are magnified in the panel below each image for better visualization. The red arrows indicate the location of the olfactory bulb. The olfactory bulb can be clearly identified in the MPRAGE and T2prep images, but not in the GRE EPI images due to substantial susceptibility artifacts. All images are from the same location. Note that the eye ball region in the GRE EPI (B) images also showed high distortion and dropout [the outline of the eye balls can be seen at the approximately same location as images shown in panels (A,C)].

flowing humidified air stream (1.5 L per min/nostril) at body temperature. PEA is a relatively pure olfactory nerve stimulant with relatively low trigeminal stimulation properties (Doty et al., 1978, 1984, 1986). The odorants was presented to both nostrils using a nasal cannula (Teleflex Medical) connected to the olfactometer via Everbilt vinyl tubing (inner diameter: 0.25 inch). The olfactory paradigm (Figure 1) started with a stimulus-off

period of 60 s with odorless mineral oil, followed by three blocks of a stimulus-on period of 60 s with PEA and a stimulus-off period of 120 s with odorless mineral oil (total duration 10 min). The relatively long stimulation period was chosen in this study to evaluate habituation effects in the olfactory system. During the stimulus-on periods, the PEA was delivered in a pulsed pattern with 20 repetitions of 1 s PEA and 2 s odorless

TABLE 1 | Comparison of contrast-to-noise ratio (CNR) between T2prep BOLD and GRE EPI BOLD fMRI ($n = 5$).

ROI	ROI size (# voxel)	T2prep BOLD	GRE EPI BOLD	<i>P</i>	Relative difference (%)	Effect size
Olfactory bulb	15.4 ± 5.1	1.3 ± 0.3	0.5 ± 0.1	0.02*	183.3	4.7
Parahippocampus	268.1 ± 83.4	0.9 ± 0.2	0.8 ± 0.1	0.57	8.5	0.4
Temporal pole	785.0 ± 245.3	1.7 ± 0.3	0.4 ± 0.1	0.01*	296.5	5.3
Primary olfactory	67.0 ± 21.2	1.1 ± 0.3	1.1 ± 0.2	0.68	6.1	0.3
Amygdala	57.0 ± 16.0	0.5 ± 0.1	1.7 ± 0.7	0.15	-68.0	-2.4
Orbitofrontal	263.0 ± 93.0	0.9 ± 0.1	0.2 ± 0.1	<0.01*	417.7	7.5
Hippocampus	212.1 ± 71.7	0.9 ± 0.2	2.1 ± 0.5	0.16	-58.4	-3.4
Caudate	185.0 ± 58.5	1.1 ± 0.2	1.6 ± 0.4	0.44	-27.0	-1.4
Putamen	259.1 ± 91.4	0.7 ± 0.1	0.7 ± 0.2	0.62	6.9	0.3
Thalamus	244.9 ± 73.9	0.9 ± 0.2	1.5 ± 0.3	0.23	-39.4	-2.0
Superior temporal	498.8 ± 163.8	0.7 ± 0.2	0.3 ± 0.1	0.07	125.4	3.2
Insula	458.2 ± 149.4	1.0 ± 0.2	0.9 ± 0.1	0.71	6.8	0.4
Anterior cingulate	283.2 ± 95.0	0.9 ± 0.2	1.2 ± 0.2	0.24	-24.6	-1.4
Posterior cingulate	289.5 ± 97.0	0.7 ± 0.1	1.6 ± 0.4	0.12	-58.7	-3.0
Middle cingulate	413.3 ± 130.5	0.9 ± 0.1	2.3 ± 0.4	0.02*	-62.3	-4.9

Mean ± standard error. * $P < 0.05$.

The same voxels were used in both fMRI methods. The number of voxels in the olfactory bulb is small as it is a very small region.

Relative difference was defined as $100 \times (\text{mean CNR in T2prep} - \text{mean CNR in EPI}) / (\text{mean CNR in EPI}) \%$.

Effect size was calculated using Cohen's $d = (\text{mean CNR in T2prep} - \text{mean CNR in EPI}) / s$, where s is the pooled standard deviation of the two groups.

mineral oil, similar to previous studies (Welge-Lussen et al., 2009; Georgiopoulos et al., 2018). All participants were instructed to breathe passively through the nose and avoid sniffing. In addition, all participants were instructed to press a button box when they start to smell the PEA odor at the beginning of the 60 s stimulus-on period. If the button press is delayed for more than 3 s from the actual onset of the stimulus, the data is discarded and the participant is scheduled for another experiment on a different day (to avoid any potential habituation effects).

Data Analysis

Data analysis was performed mainly using the statistical parametric mapping (SPM) software package (Version 12, Wellcome Trust Centre for Neuroimaging, London, United Kingdom) and in-house routines coded in MATLAB R2019a (MathWorks, Natick, MA, United States). Realignment was performed for all fMRI images to correct for subject motion during the scans. Spatial smoothing was performed for fMRI images using an isotropic Gaussian kernel of 4 mm. The baseline drift of fMRI time series was removed by applying a high-pass filter with a cut-off frequency of 1/180 Hz (as the duration of one block in the olfactory paradigm is 180s) using the FMRIB Software Library (FSL6.0.1; FMRIB, Oxford, United Kingdom). An independent component analysis (ICA) based denoising approach (Salimi-Khorshidi et al., 2014) was performed on the fMRI data using FSL6.0.1, from which components related to motion and physiological noise were removed. This includes, in particular, removing components that showed significant correlation with the recorded breathing and cardiac patterns. Temporal filtering was performed using a low-pass filter with a cut-off frequency of 0.03 Hz. No normalization was performed during preprocessing, and all subsequent fMRI analysis was performed in the subject space. The MPRAGE structural images were co-registered to the fMRI images for each participant. The

Automated Anatomical Labeling (AAL) atlas (Rolls et al., 2020) was used to identify primary and secondary olfactory regions in the brain according to the literature (Poellinger et al., 2001). The inverse deformation field was obtained to transform the regions-of-interest (ROI) identified in the AAL atlas from the MNI (Montreal Imaging Institute) space to the subject space. Since the OB is not included in the AAL atlas, it was manually delineated on the MPRAGE images for each participant. The manual segmentation of the OB was performed on all subjects by two researchers (XM and AGP) independently, who have been trained by senior neuroradiologists and have been performing OB segmentation in various studies in the group for over 3 years. After segmentation was completed, discrepancies between the two researchers were assessed and final measurements agreed upon. **Figure 2** shows the 15 ROIs investigated in the current study overlaid on T2prep BOLD fMRI images from one subject. Note that only six coronal slices were provided in **Figure 2** to illustrate the locations of the ROIs, but many ROIs can cover more slices. Most olfactory regions are best viewed in the coronal plane. To compare the quality of fMRI images in the OB, the OB was also identified on individual T2prep BOLD and GRE EPI images. To do that, the OB ROI from MPRAGE was overlaid on each fMRI image. A threshold of two standard deviations below the average signal intensity of the entire slice (not just the OB) was used, and voxels with intensities above this threshold within the MPRAGE OB ROI were counted in each fMRI image. Note that only the ROIs from MPRAGE were used for subsequent functional analysis.

Functional activation during olfactory stimulation in the whole brain was detected using the non-parametric Kolmogorov-Smirnov (KS) two-sample test (Siegel and Castellan, 1988) (adjusted $P < 0.01$). Compared to the commonly used general linear model (GLM), previous

olfactory fMRI studies (Sobel et al., 2000; Poellinger et al., 2001) have shown that the KS statistic may be more suitable for detecting functional activations with strong habituation effects. Note that the KS statistic is also suitable for detecting functional activations without habituation effects (Sobel et al., 2000; Poellinger et al., 2001). Nuisance parameters such as motion and breathing and cardiac pattern that are usually controlled in GLM have been removed using the ICA based denoising approach (Salimi-Khorshidi et al., 2014) described above. Relative signal changes ($\Delta S/S$) between the stimulus-on and stimulus-off periods were calculated for each voxel. Note that due to the transition period following the cessation of stimulus that usually lasts for 50–100% of the stimulus-on period (Hua et al., 2011; van Zijl et al., 2012), only signals from the second half of the stimulus-off period were included when calculating $\Delta S/S$. Because the two-sample KS statistic detects both positive and negative activation during stimulation (Georgiopoulos et al., 2018), the activated voxels were separated into positively ($\Delta S/S > 0$) and negatively ($\Delta S/S < 0$) activated voxels subsequently. Temporal signal-to-noise ratio (tSNR) was calculated as the signal divided by standard deviation along the time course in each voxel. CNR was defined as the product of tSNR and $\Delta S/S$ from the 1st block. The CNR comparison was conducted in voxels that were positively activated in either GRE EPI or T2prep fMRI scans in each participant. We chose to use the combined activated voxels from both scans because in some ROIs with significant susceptibility artifacts such as the OB, GRE EPI scans showed little activation. The same voxels were used in both fMRI methods and results in each ROI are shown.

Statistical Analysis

The comparison between GRE EPI and T2prep BOLD fMRI was performed in a subset of participants ($n = 5$). Power analysis was performed using the approach described by Cohen et al. (Cohen, 1988) based on the average effect size (approximately 1.3) reported in previous studies (Hua et al., 2017; Miao et al., 2020) to ensure that this sample size can provide a power of 0.8 with significance set at $\alpha = 0.05$ (type I error, two tailed) for the CNR comparison between these two methods. This is consistent with similar technical studies using the same MRI methods performed at 3T (Hua et al., 2017; Miao et al., 2020). As the CNR difference between the two fMRI methods in the OB is mainly caused by the well-known susceptibility artifacts from the nearby nasal cavity (Yang et al., 1997; Sobel et al., 1998, 2000; Poellinger et al., 2001; Wang et al., 2010; Zong et al., 2014; Lu et al., 2018), the effect sizes in the current study on 7T are expected to be greater than previous 3T studies (Hua et al., 2017; Miao et al., 2020). We therefore believe that this sample size is sufficient for this technical comparison.

Paired t -tests were performed to compare CNR from GRE EPI BOLD and T2prep BOLD fMRI. Effect size was estimated with Cohen's d . One-way repeated-measures analysis of variance (ANOVA) was conducted to examine differences of $\Delta S/S$ among the three blocks of the olfactory paradigm. All statistical tests were corrected for multiple comparisons by controlling the false-discovery rate (adjusted $P < 0.05$).

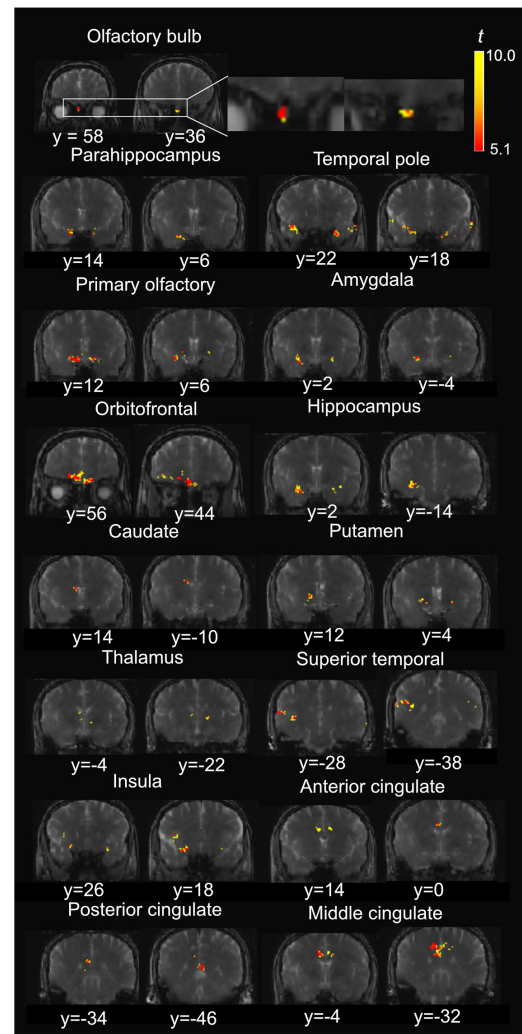
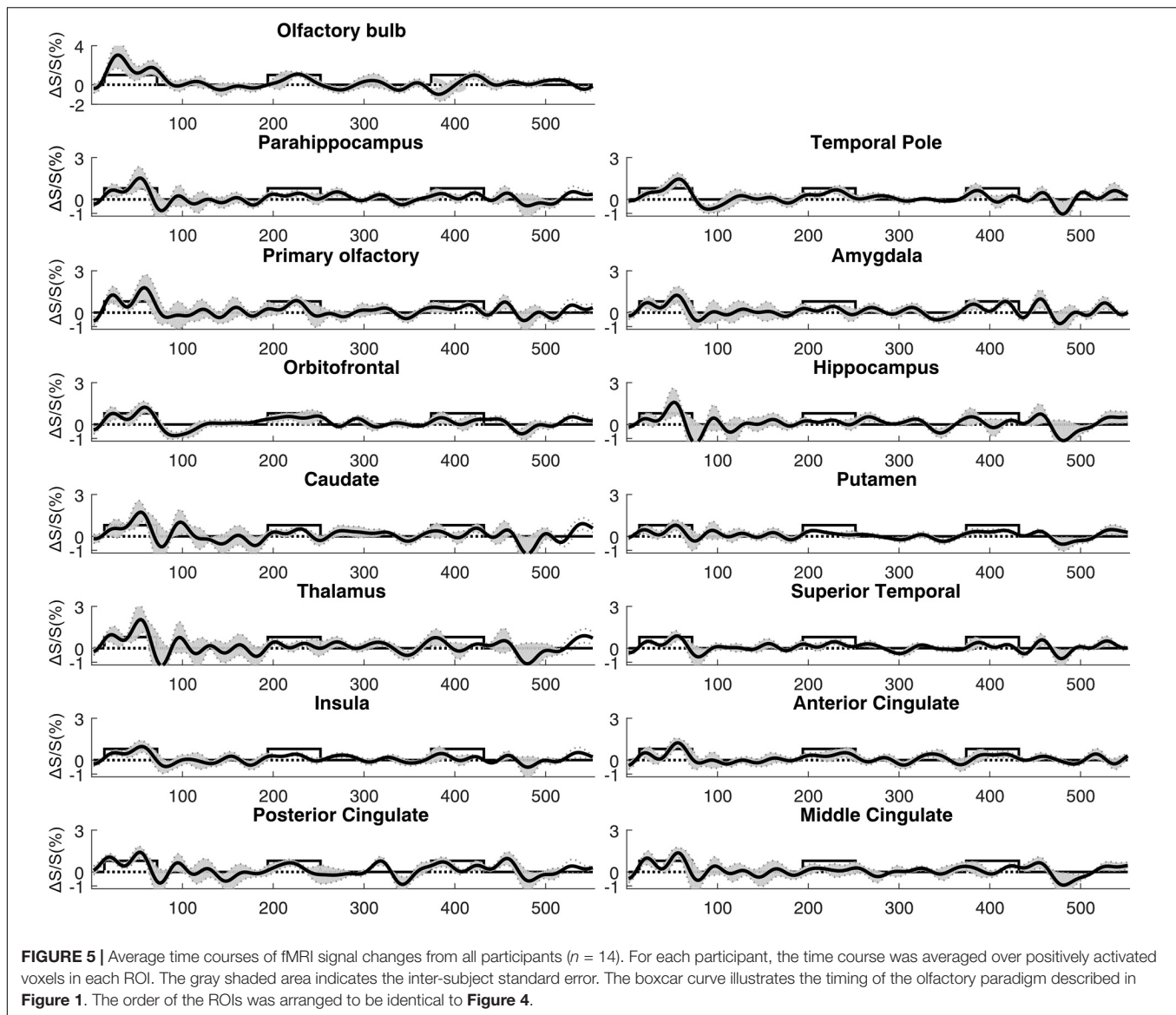


FIGURE 4 | Representative positive activation maps from T2prep BOLD fMRI scans during the olfactory stimulation from one participant. The t -scores of significantly positively activated voxels are overlaid on the T2prep BOLD images from the same participant. Two slices were shown for each ROI. But the entire activated cluster in each ROI covered more slices.

Reproducibility

In all participants ($n = 14$), the same T2prep BOLD fMRI scans and analysis were repeated using the same functional paradigm once to assess its reproducibility. The second T2prep BOLD scan (re-scan) was performed on the same scanner in 3–6 weeks after the first scan for each participant. Dice coefficients between the maps of activated voxels from the scan and re-scan of the same subjects were calculated to evaluate the reproducibility of spatial locations of the activated clusters (Sair et al., 2016). The value of a Dice coefficient ranges from 0, indicating no spatial overlap between the scan and re-scan results, to 1, indicating complete overlap. Intraclass correlation coefficient (ICC) was calculated to evaluate the reproducibility of $\Delta S/S$ between the scan and re-scan results of T2prep BOLD fMRI in the same subjects. In each ROI, activated voxels (positively or negatively) from the first scan were



overlaid on the second scan from the same subject. The ICC of $\Delta S/S$ from these same voxels were calculated for each ROI and each subject. The definition of ICC in a textbook (Rosner, 2011) was adopted. The procedure used here is identical to that in our previous reproducibility studies (Landman et al., 2011).

RESULTS

Comparison of GRE EPI BOLD and T2prep BOLD fMRI

Figure 3 shows the typical image quality of GRE EPI BOLD and T2prep BOLD fMRI from one subject. The T1-weighted MPRAGE images serve as an anatomical reference with minimal distortion and dropout. In the GRE EPI BOLD images, the susceptibility artifacts in the OB were substantial, showing signal dropout and geometric distortion caused by the nearby cavities.

These artifacts were significantly reduced in T2prep BOLD images from the same subject. On average, the OB can be clearly depicted on 4 ± 2 ($n = 5$) slices of T2prep images, and the group-averaged size of the OB was 23 ± 5 ($n = 5$) voxels or $77 \pm 16 \text{ mm}^3$ ($n = 5$) on T2prep images, consistent with literature values for healthy human subjects (Herzallah et al., 2013; Alarabawy et al., 2016). Similar results were obtained on MPRAGE images (4 ± 2 slices and 22 ± 7 voxels, $n = 5$). On GRE EPI images, the OB can only be identified on 1 ± 1 ($n = 5$) slices and 5 ± 3 ($n = 5$) voxels, significantly less than T2prep and MPRAGE ($P < 0.01$). Table 1 shows the quantitative CNR results. T2prep showed significantly greater CNR than GRE EPI in the OB, orbitofrontal cortex and the temporal pole. In regions that are less affected by susceptibility effects, GRE EPI showed similar or better CNR than T2prep. In middle cingulate, GRE EPI showed significantly greater CNR than T2prep. The number of voxels in the OB reported in Table 1 is small as it is a very small

TABLE 2 | Quantitative T2prep BOLD fMRI results from positively activated voxels from all subjects ($n = 14$).

ROI	Activated voxels (#)	$\Delta S/S$ (%)*			P^{**}	Relative difference (%)***	
		1st block	2nd block	3rd block		2nd vs. 1st	3rd vs. 1st
Olfactory bulb	14.8 ± 5.3	2.2 ± 0.7	0.8 ± 0.5	-0.2 ± 0.5	0.02	-65 ± 56	-110 ± 45
Parahippocampus	244.1 ± 87.8	0.8 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.02	-75 ± 42	-80 ± 45
Temporal Pole	766.0 ± 267.3	0.8 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	0.03	-43 ± 31	-80 ± 40
Primary olfactory	65.0 ± 23.3	1.3 ± 0.6	0.2 ± 0.1	0.2 ± 0.1	0.02	-78 ± 54	-81 ± 52
Amygdala	54.0 ± 19.8	0.7 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.05	-62 ± 66	-67 ± 59
Orbitofrontal	248.0 ± 83.1	0.8 ± 0.3	0.5 ± 0.1	0.2 ± 0.1	0.02	-39 ± 38	-68 ± 32
Hippocampus	203.7 ± 70.3	0.7 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.02	-71 ± 42	-81 ± 48
Caudate	181.0 ± 57.4	1.1 ± 0.4	0.2 ± 0.1	0.2 ± 0.2	0.01	-79 ± 35	-79 ± 22
Putamen	237.7 ± 80.7	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.06	-23 ± 69	-48 ± 62
Thalamus	220.4 ± 80.8	1.2 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	0.01	-87 ± 41	-88 ± 41
Superior temporal	482.0 ± 177.2	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.02	-35 ± 32	-79 ± 36
Insula	432.0 ± 145.4	0.6 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.03	-66 ± 47	-75 ± 41
Anterior cingulate	269.2 ± 95.2	0.8 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.02	-61 ± 31	-80 ± 44
Posterior cingulate	284.0 ± 94.4	0.9 ± 0.3	0.4 ± 0.1	0.0 ± 0.1	0.02	-50 ± 27	-90 ± 29
Middle cingulate	383.3 ± 144.0	0.8 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.02	-71 ± 30	-88 ± 42

Mean ± standard error.

* $\Delta S/S$ averaged over the 1st, 2nd, and 3rd blocks during the olfactory paradigm shown in **Figure 1** was calculated separately.

** P values from one-way repeated-measures analysis of variance (ANOVA) to examine differences of $\Delta S/S$ among the three blocks of the olfactory paradigm.

***Relative difference = $100 \times (\Delta S/S \text{ 2nd or 3rd block} - \Delta S/S \text{ 1st block}) / \text{group mean of } \Delta S/S \text{ 1st block } \%$.

The number of voxels in the olfactory bulb is small as it is a very small region.

region. Typical activation maps from T2prep BOLD and GRE EPI BOLD fMRI scans are shown in **Figure 4** and **Supplementary Figure S2**, respectively.

Olfactory fMRI Results Using T2prep BOLD fMRI

Figure 4 shows a representative activation map from one participant. Positively activated voxels in the OB and associated olfactory regions were overlaid on the T2prep BOLD images. The average number of positively activated voxels in the OB was 15 ± 5 ($n = 14$) on T2prep images. The group averaged time courses of relative signal changes ($\Delta S/S$) during the paradigm from positively activated voxels in each ROI are displayed in **Figure 5**. **Table 2** summarizes the quantitative results ($\Delta S/S$) from positively activated voxels in each ROI. As the same olfactory stimulation was repeated for three blocks (**Figure 1**), $\Delta S/S$ from each block was calculated and compared. In all ROIs except for the putamen, $\Delta S/S$ decreased significantly ($P < 0.05$) during the 2nd and 3rd blocks compared to that during the 1st block. In putamen, $\Delta S/S$ showed a similar decreasing trend but failed to reach significance ($P = 0.06$). The magnitude of $\Delta S/S$ in the OB during the 1st block was greater than that in the other regions. Within the 1st block, the time course of $\Delta S/S$ in the OB showed an initial increase during the first half of the block (~ 30 s) followed by a substantially smaller $\Delta S/S$ during the second half of the block. In all the other ROIs, $\Delta S/S$ within the 1st block showed an initial increase during the first half of the block (~ 30 s) followed by a second peak with similar magnitude during the second half of the block. The rising and decaying times for both peaks from the time courses were similar among all regions. **Supplementary Figure S4** shows the map of positively activated

voxels combined from all participants ($n = 14$) after individual maps were normalized to the MNI space.

Negatively activated voxels were also observed in all ROIs shown in **Figure 2**. By definition in section “Materials and Methods,” negatively and positively activated voxels are two mutually exclusive subsets of voxels in each ROI. **Figure 6** shows a representative activation map from one participant. Negatively activated voxels in the OB and associated olfactory regions were overlaid on the T2prep BOLD images. The average number of negatively activated voxels in the OB was 8 ± 5 ($n = 14$). The group averaged time courses of $\Delta S/S$ during the paradigm from negatively activated voxels in each ROI are displayed in **Figure 7**. **Table 3** summarizes the quantitative results ($\Delta S/S$) from negatively activated voxels in each ROI. Compared to positively activated voxels, in all ROIs, the absolute values of $\Delta S/S$ during the 1st block were smaller for negatively activated voxels. $\Delta S/S$ during the 1st, 2nd, and 3rd blocks were not substantially different for negatively activated voxels. Within the 1st block, time courses of $\Delta S/S$ averaged from the negatively activated voxels in most ROIs showed a bi-phasic pattern similar to that from positively activated voxels. **Supplementary Figure S5** shows the map of negatively activated voxels combined from all participants ($n = 14$) after individual maps were normalized to the MNI space.

Tables 4, 5 summarize the reproducibility results for T2prep BOLD fMRI. The spatial locations of the positively and negatively activated clusters in all ROIs showed a good reproducibility with Dice coefficients ranging from 0.87 to 0.93. The relative signal changes ($\Delta S/S$) in all ROIs and all blocks showed comparable reproducibility (ICC) to BOLD fMRI measures assessed in our previous 3T study (Landman et al., 2011).

DISCUSSION

We demonstrated that the whole brain T2prep BOLD fMRI technique can detect functional activations in response to olfactory stimulation in primary and secondary olfactory regions in healthy human subjects at 7T. Neuronal activation triggered cerebral blood flow (CBF) increase during odor stimulation has been demonstrated in the rodent OB using two-photon imaging and electrophysiology recordings (Chaigneau et al., 2007). Although functional activities in the OB have been measured in various animal studies (Xu et al., 2000, 2003, 2005; Boyett-Anderson et al., 2003; Cross et al., 2004; Schafer et al., 2005; Martin et al., 2007; Jia et al., 2014; Li et al., 2014; Poplawsky and Kim, 2014; Berns et al., 2015; Zhao et al., 2015, 2016, 2017; Murphy et al., 2016; Muir et al., 2019), to our knowledge, this is the first fMRI study to detect functional activation in the OB in human subjects using whole brain BOLD fMRI. Previous reports that detected functional activation in the OB are either animal studies (Xu et al., 2000, 2003, 2005; Boyett-Anderson et al., 2003; Cross et al., 2004; Schafer et al., 2005; Martin et al., 2007; Jia et al., 2014; Li et al., 2014; Poplawsky and Kim, 2014; Berns et al., 2015; Zhao et al., 2015, 2016, 2017; Murphy et al., 2016; Muir et al., 2019) or human scans with partial brain coverage (Fournel et al., 2020). T2prep BOLD was originally developed at 7T (Hua et al., 2014) to minimize susceptibility artifacts commonly seen in EPI based fMRI methods that are more prominent at higher magnetic fields, and was later on applied at 3T for fMRI in individuals with metallic head implants (Miao et al., 2020) and in patients undergoing presurgical brain mapping (Hua et al., 2017). In brain regions that are less affected by susceptibility artifacts such as the visual and motor cortices, GRE EPI BOLD still has better sensitivity. However, when susceptibility artifacts become prominent in some frontal and temporal areas and regions near metal objects, the sensitivity of GRE EPI BOLD drops substantially, whereas the BOLD sensitivity is largely preserved in the entire brain in T2prep BOLD (Hua et al., 2014, 2017; Miao et al., 2020). Our data in this study showed that several olfactory regions that are significantly affected by susceptibility artifacts in EPI due to nearby air cavities and bone structures had significantly enhanced CNR in T2prep BOLD compared to GRE EPI. These regions include the OB, orbitofrontal cortex and temporal cortex. In other regions less affected by susceptibility artifacts, CNR values were similar for both methods, or higher in GRE EPI. Although the current study was performed at 7T, the same methodology has been implemented on 3T human MRI systems as well (Hua et al., 2017; Miao et al., 2020). In addition to healthy subjects, we have also been using the same methodology on 3T and 7T in clinical populations with olfactory deficits such as individuals with Parkinson's disease, Alzheimer's disease, and schizophrenia.

Habituation to olfactory stimuli is a well-known phenomenon (Pellegrino et al., 2017) in which an attenuation of responses to prolonged and/or repeated olfactory stimulation is observed. In this study, a relatively long (60 s) olfactory stimulation was repeated three times in each participant in order to evaluate habituation effects in different brain regions. To detect functional

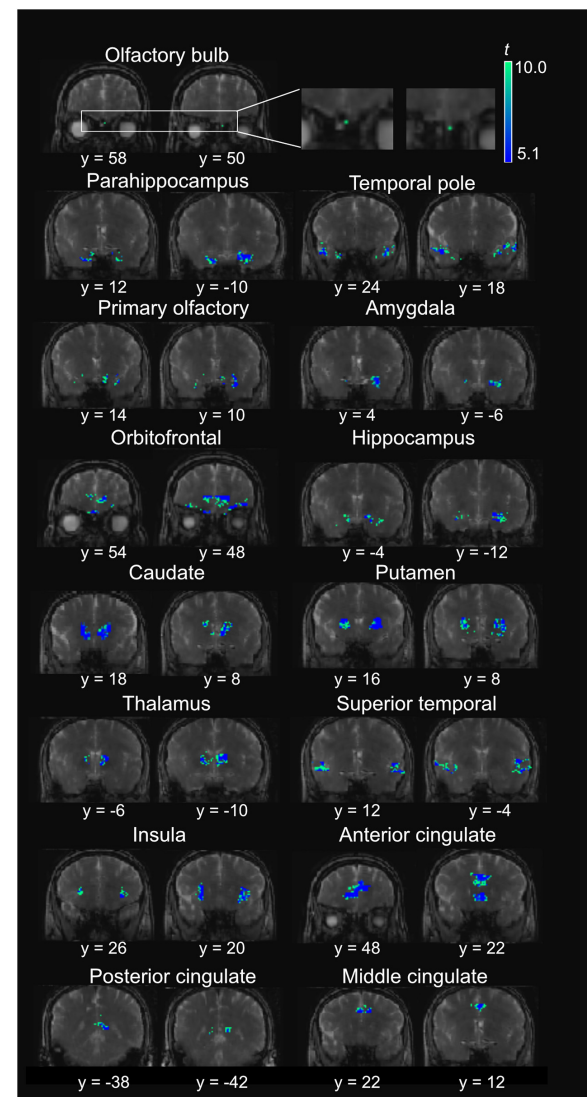


FIGURE 6 | Representative negative activation maps from T2prep BOLD fMRI scans during the olfactory stimulation from one participant. The t -scores of significantly negatively activated voxels are overlaid on the T2prep BOLD images from the same participant. Two slices are shown for each ROI. But the entire activated cluster in each ROI covered more slices.

activation with anticipated habituation during the period of stimulation, the non-parametric KS two-sample test was adopted. Strong habituation effects may lead to greater signal variance during the stimulus-on periods than the stimulus-off periods. The KS statistic is considered to be highly sensitive to this difference in signal variance (Zhao et al., 1997), and therefore is more suitable for olfactory fMRI analysis than the commonly used GLM approach in most task based fMRI analysis, as demonstrated in previous human olfactory fMRI studies (Sobel et al., 2000; Poellinger et al., 2001). In our data from positively activated voxels, all regions investigated showed reduced responses in the 2nd and 3rd blocks compared to the 1st block. This is consistent with previous studies in humans and animals using imaging

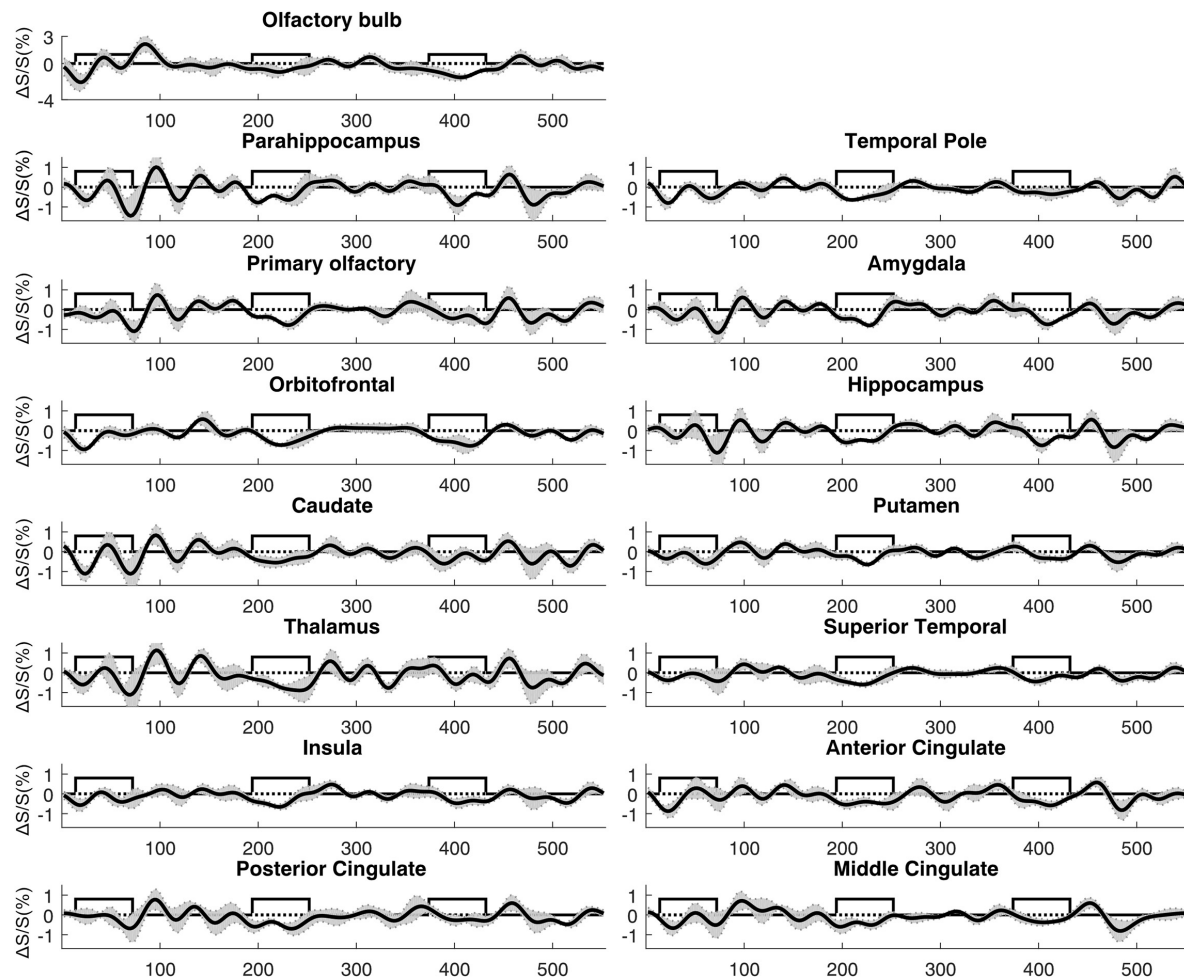


FIGURE 7 | Average time courses of fMRI signal changes from all participants ($n = 14$). For each participant, the time course was averaged over negatively activated voxels in each ROI. The gray shaded area indicates the inter-subject standard error. The boxcar curve illustrates the timing of the olfactory paradigm described in Figure 1. The order of the ROIs was arranged to be identical to Figure 6.

(Wilson, 1998; Yang X. et al., 1998; Sobel et al., 2000; Xu et al., 2000, 2003, 2005; Poellinger et al., 2001; Kida et al., 2002; Boyett-Anderson et al., 2003; Cross et al., 2004; Schafer et al., 2005, 2006; Martin et al., 2007; Jia et al., 2014; Li et al., 2014; Poplawsky and Kim, 2014; Berns et al., 2015; Zhao et al., 2015, 2016, 2017; Murphy et al., 2016; Muir et al., 2019) and electrophysiology recordings (Wilson, 1998; Chaudhury et al., 2010). Within the 1st block, all regions showed a bi-phasic pattern with two distinct peaks during the first and second halves of the 1st block. Comparing to the first peak, the second peak was much weaker in the OB than the other regions. The bi-phasic pattern has been reported in previous olfactory fMRI studies in rats (Zhao et al., 2016), where it was more prominent in higher olfactory regions such as the piriform cortex than in the OB, and was hypothesized to represent a post-inhibition excitation rebound (Zhao et al., 2016). Also, whether such bi-phasic pattern is affected by physiological noise sources warrant further investigation. In our data, the magnitude of the first peak was greater in the OB than in the other regions, but the time courses showed similar

shapes among all regions. Overall, we did not observe a significant difference in habituation effects between the OB and the other olfactory regions in the current human study, whereas many previous animal studies have shown less pronounced habituation effects in the OB compared to higher olfactory regions (Wilson, 1998; Yang Q.X. et al., 1998; Xu et al., 2000, 2005; Kida et al., 2002; Schafer et al., 2005, 2006; Chaudhury et al., 2010; Zhao et al., 2015, 2016, 2017). One possible reason may be the pulsed pattern during the stimulus-on period used in the olfactory stimulation paradigm in this study. The duration of a single pulse (1s) may be too short to differentiate the habituation effects in the OB and higher olfactory regions, whereas the hemodynamic responses from BOLD fMRI in response to consecutive pulses may overlap temporally due to a short inter-pulse interval (2s), which could mask any potential difference between the OB and higher olfactory regions. Other possible factors may include species differences (human in the current study, non-human primate, and rodent in previous studies), choice of odorant and its concentration, and the statistical approach used to

TABLE 3 | Quantitative T2prep BOLD fMRI results from negatively activated voxels from all subjects ($n = 14$).

ROI	Activated voxels (#)	$\Delta S/S$ (%)*			P^{**}	Relative difference (%)***	
		1st block	2nd block	3rd block		2nd vs. 1st	3rd vs. 1st
Olfactory bulb	8.1 ± 5.5	-0.8 ± 0.6	-0.5 ± 0.4	-0.9 ± 0.3	0.45	-38 ± 122	38 ± 62
Parahippocampus	134.6 ± 38.1	-0.4 ± 0.2	-0.5 ± 0.3	-0.3 ± 0.2	0.53	6 ± 82	-42 ± 42
Temporal pole	444.6 ± 153.9	-0.6 ± 0.3	-0.4 ± 0.3	-0.2 ± 0.2	0.29	-32 ± 55	-68 ± 31
Primary olfactory	39.7 ± 12.9	-0.5 ± 0.1	-0.5 ± 0.2	-0.2 ± 0.1	0.25	20 ± 45	-46 ± 35
Amygdala	34.3 ± 11.1	-0.5 ± 0.2	-0.5 ± 0.1	-0.3 ± 0.1	0.33	12 ± 62	-33 ± 42
Orbitofrontal	137.6 ± 51.8	-0.6 ± 0.1	-0.5 ± 0.2	-0.4 ± 0.1	0.58	-3 ± 52	-8 ± 38
Hippocampus	95.3 ± 32.3	-0.3 ± 0.1	-0.4 ± 0.2	-0.3 ± 0.1	0.46	102 ± 111	30 ± 66
Caudate	92.2 ± 31.0	-0.6 ± 0.3	-0.5 ± 0.2	-0.3 ± 0.2	0.29	-25 ± 51	-56 ± 41
Putamen	116.8 ± 44.7	-0.4 ± 0.1	-0.3 ± 0.1	-0.1 ± 0.1	0.06	-42 ± 36	-82 ± 22
Thalamus	149.7 ± 45.4	-0.6 ± 0.2	-0.6 ± 0.1	-0.2 ± 0.2	0.16	30 ± 50	-68 ± 42
Superior temporal	248.8 ± 87.8	-0.3 ± 0.1	-0.3 ± 0.2	-0.1 ± 0.1	0.55	52 ± 102	-40 ± 81
Insula	255.4 ± 91.3	-0.4 ± 0.2	-0.4 ± 0.2	-0.2 ± 0.1	0.46	25 ± 87	-52 ± 68
Anterior cingulate	145.2 ± 46.7	-0.4 ± 0.2	-0.5 ± 0.2	-0.3 ± 0.2	0.50	51 ± 79	5 ± 99
Posterior cingulate	170.1 ± 57.3	-0.3 ± 0.2	-0.5 ± 0.3	-0.2 ± 0.2	0.56	152 ± 189	6 ± 115
Middle cingulate	194.7 ± 56.7	-0.4 ± 0.2	-0.4 ± 0.2	-0.3 ± 0.2	0.77	-20 ± 121	-41 ± 92

Mean $\pm$ standard error.

* $\Delta S/S$ averaged over the 1st, 2nd, and 3rd blocks during the olfactory paradigm shown in **Figure 1** was calculated separately.

** P values from one-way repeated-measures analysis of variance (ANOVA) to examine differences of $\Delta S/S$ among the three blocks of the olfactory paradigm.

***Relative difference = $100 \times (\Delta S/S \text{ 2nd or 3rd block} - \Delta S/S \text{ 1st block})/\text{group mean of } \Delta S/S \text{ 1st block } \%$.

The number of voxels in the olfactory bulb is small as it is a very small region.

identify activated voxels during olfactory stimulation (KS and conventional GLM). To our knowledge, this is the first study to characterize BOLD fMRI signals in the OB in human subjects. Additional studies are needed to investigate these factors and their influence on habituation effects in the OB and cortical olfactory regions.

In all regions investigated in this study, including the OB, a substantial subset of voxels showed decreased fMRI signals upon olfactory stimulation. The magnitude of the signal responses from negatively activated voxels were smaller than those from positively activated voxels in corresponding regions, which made the habituation effects less prominent in negatively activated voxels. Such negative activations were observed from our data using the same functional analysis pipeline as positive activations, and also showed a good intra-subject reproducibility. Nevertheless, the physiological origin of the negative activations is unclear. Previous studies using electrical recordings have shown that different neurons in the primary olfactory cortex can show either increasing activity, decreasing activity, or a combination of both in response to the same odor stimulation (Tanabe et al., 1975; Nemitz and Goldberg, 1983; Wilson, 1998), which may be one of the plausible explanations for our data. Such negative activation has also been observed in the piriform cortex of rats (Zhao et al., 2017), which may be explained by the characteristics of “sparse coding” and “global inhibition” in the olfactory processing of piriform cortex (Poo and Isaacson, 2009). Alternatively, a few other theories have been proposed for negative BOLD activations (Huber et al., 2014; Mullinger et al., 2014), which warrants further investigation in subsequent studies combining fMRI with additional electrophysiological recording and imaging techniques.

It is important to exclude potential false positive voxels in fMRI studies. In the current study, we adopted a well-established pre-processing pipeline for human fMRI and the KS method established in previous human olfactory fMRI studies (Sobel et al., 2000; Poellinger et al., 2001) for functional analysis. An ICA based denoising approach (Salimi-Khorshidi et al., 2014) was employed to remove major confounding factors such as motion, breathing and cardiac pattern and other physiological noise. The T2prep BOLD fMRI results showed a good intra-subject reproducibility comparable to previous human BOLD fMRI studies for both the spatial pattern and the temporal profile of signal changes detected during the olfactory paradigm. We feel that a comprehensive evaluation of the neuronal origin of the fMRI signals is beyond the scope of this technical work. However, we are currently conducting a subsequent study where the EBG technique (Iravani et al., 2020) will be used to provide some validation for the neuronal origin of the fMRI signal changes measured in the OB.

The goal of the current study is to evaluate the T2prep BOLD fMRI method as one of the alternative approaches for improving fMRI signals in olfactory regions affected by significant susceptible artifacts. Many other techniques are available to improve signals in high susceptible regions such as spin echo (SE) EPI, spiral MRI, and gradient spin echo (GRASE) MRI. Parallel imaging and multiband techniques can substantially shorten the echo train in EPI readout, and thus mitigate distortions to some extent. Many methods have been developed to reduce dropouts in GRE EPI (Frahm et al., 1988; Cho and Ro, 1992; Ordidge et al., 1994; Constable, 1995; Ojemann et al., 1997; Yang et al., 1997, 2006; Yang Q.X. et al., 1998; Constable and Spencer, 1999; Glover, 1999; Cordes et al., 2000; Devlin et al., 2000; Stenger et al., 2000; Merboldt et al., 2001;

TABLE 4 | Reproducibility for positively activated voxels in T2prep BOLD fMRI ($n = 14$).

ROI	Dice* coefficient	ICC of $\Delta S/S^{**}$		
		1st block	2nd block	3rd block
Olfactory bulb	0.91 $\pm$ 0.06	0.90 $\pm$ 0.06	0.89 $\pm$ 0.05	0.88 $\pm$ 0.06
Parahippocampus	0.89 $\pm$ 0.08	0.83 $\pm$ 0.07	0.85 $\pm$ 0.06	0.83 $\pm$ 0.06
Temporal pole	0.93 $\pm$ 0.04	0.86 $\pm$ 0.07	0.86 $\pm$ 0.08	0.86 $\pm$ 0.08
Primary olfactory	0.93 $\pm$ 0.04	0.88 $\pm$ 0.05	0.89 $\pm$ 0.08	0.87 $\pm$ 0.08
Amygdala	0.87 $\pm$ 0.09	0.82 $\pm$ 0.08	0.88 $\pm$ 0.09	0.86 $\pm$ 0.07
Orbitofrontal	0.91 $\pm$ 0.06	0.89 $\pm$ 0.06	0.89 $\pm$ 0.06	0.88 $\pm$ 0.07
Hippocampus	0.93 $\pm$ 0.05	0.88 $\pm$ 0.06	0.89 $\pm$ 0.05	0.88 $\pm$ 0.05
Caudate	0.92 $\pm$ 0.07	0.86 $\pm$ 0.07	0.87 $\pm$ 0.08	0.88 $\pm$ 0.06
Putamen	0.89 $\pm$ 0.07	0.85 $\pm$ 0.08	0.82 $\pm$ 0.09	0.83 $\pm$ 0.09
Thalamus	0.88 $\pm$ 0.07	0.84 $\pm$ 0.06	0.84 $\pm$ 0.06	0.85 $\pm$ 0.08
Superior temporal	0.92 $\pm$ 0.06	0.85 $\pm$ 0.06	0.86 $\pm$ 0.06	0.88 $\pm$ 0.05
Insula	0.93 $\pm$ 0.06	0.88 $\pm$ 0.04	0.88 $\pm$ 0.05	0.87 $\pm$ 0.07
Anterior cingulate	0.87 $\pm$ 0.09	0.83 $\pm$ 0.07	0.83 $\pm$ 0.07	0.86 $\pm$ 0.08
Posterior cingulate	0.87 $\pm$ 0.09	0.83 $\pm$ 0.08	0.85 $\pm$ 0.08	0.85 $\pm$ 0.06
Middle cingulate	0.88 $\pm$ 0.10	0.83 $\pm$ 0.07	0.83 $\pm$ 0.07	0.86 $\pm$ 0.06

Mean $\pm$ standard error.

*Dice coefficients between the maps of positively activated voxels from the scan and re-scan of the same subjects were calculated to evaluate the reproducibility of spatial locations of the activated clusters. The value of a Dice coefficient ranges from 0, indicating no spatial overlap between the scan and re-scan results, to 1, indicating complete overlap.

**Intraclass correlation coefficient (ICC) of $\Delta S/S$ between the scan and re-scan results of T2prep BOLD fMRI in the same subjects.

TABLE 5 | Reproducibility for negatively activated voxels in T2prep BOLD fMRI ($n = 14$).

ROI	Dice* coefficient	ICC of $\Delta S/S^{**}$		
		1st block	2nd block	3rd block
Olfactory bulb	0.91 $\pm$ 0.06	0.91 $\pm$ 0.06	0.90 $\pm$ 0.06	0.90 $\pm$ 0.04
Parahippocampus	0.89 $\pm$ 0.07	0.86 $\pm$ 0.06	0.86 $\pm$ 0.07	0.85 $\pm$ 0.06
Temporal pole	0.92 $\pm$ 0.06	0.90 $\pm$ 0.07	0.88 $\pm$ 0.07	0.89 $\pm$ 0.07
Primary olfactory	0.92 $\pm$ 0.06	0.90 $\pm$ 0.06	0.90 $\pm$ 0.07	0.91 $\pm$ 0.06
Amygdala	0.87 $\pm$ 0.08	0.87 $\pm$ 0.06	0.86 $\pm$ 0.07	0.89 $\pm$ 0.06
Orbitofrontal	0.91 $\pm$ 0.07	0.89 $\pm$ 0.05	0.90 $\pm$ 0.05	0.89 $\pm$ 0.08
Hippocampus	0.92 $\pm$ 0.06	0.88 $\pm$ 0.05	0.88 $\pm$ 0.05	0.88 $\pm$ 0.06
Caudate	0.93 $\pm$ 0.06	0.89 $\pm$ 0.04	0.90 $\pm$ 0.05	0.87 $\pm$ 0.06
Putamen	0.88 $\pm$ 0.07	0.86 $\pm$ 0.06	0.84 $\pm$ 0.07	0.84 $\pm$ 0.07
Thalamus	0.88 $\pm$ 0.06	0.86 $\pm$ 0.07	0.86 $\pm$ 0.06	0.88 $\pm$ 0.08
Superior temporal	0.91 $\pm$ 0.06	0.89 $\pm$ 0.05	0.89 $\pm$ 0.06	0.89 $\pm$ 0.08
Insula	0.92 $\pm$ 0.05	0.88 $\pm$ 0.05	0.88 $\pm$ 0.05	0.87 $\pm$ 0.05
Anterior cingulate	0.87 $\pm$ 0.09	0.85 $\pm$ 0.07	0.85 $\pm$ 0.07	0.85 $\pm$ 0.07
Posterior cingulate	0.87 $\pm$ 0.08	0.85 $\pm$ 0.07	0.85 $\pm$ 0.05	0.88 $\pm$ 0.05
Middle cingulate	0.88 $\pm$ 0.09	0.87 $\pm$ 0.03	0.85 $\pm$ 0.08	0.85 $\pm$ 0.08

Mean $\pm$ standard error.

*Dice coefficients between the maps of negatively activated voxels from the scan and re-scan of the same subjects were calculated to evaluate the reproducibility of spatial locations of the activated clusters. The value of a Dice coefficient ranges from 0, indicating no spatial overlap between the scan and re-scan results, to 1, indicating complete overlap.

**Intraclass correlation coefficient (ICC) of $\Delta S/S$ between the scan and re-scan results of T2prep BOLD fMRI in the same subjects.

Deichmann et al., 2002, 2003; Gu et al., 2002; Wilson and Jezzard, 2003; Wilson et al., 2003; Heberlein and Hu, 2004; Robinson et al., 2004; Cusack et al., 2005; De Panfilis and Schwarzbauer, 2005; Juchem et al., 2006; Koch et al., 2006; Weiskopf et al., 2006, 2007; Du et al., 2007; Haines et al., 2010; Teeuwisse et al., 2012; Halai et al., 2014; Wastling and Barker, 2014) and distortion in EPI (Chang and Fitzpatrick, 1992; Weisskoff and Davis, 1992; Bowtell et al., 1994; Jezzard and Balaban, 1995; Morrell and Spielman, 1997; Robson et al., 1997; Wan et al., 1997; Reber et al., 1998; Chen and Wyrwicz, 1999, 2001; Jezzard and Clare, 1999; Kybic et al., 2000; Munger et al., 2000; Studholme et al., 2000; Andersson et al., 2001, 2003; Hutton et al., 2002; Ward et al., 2002; Zeng and Constable, 2002; Roopchansingh et al., 2003; Morgan et al., 2004; Zaitsev et al., 2004; Weiskopf et al., 2005; Gallichan et al., 2010; Holland et al., 2010; Chung et al., 2011; Oh et al., 2012; Visser et al., 2012; Ooi et al., 2013). It remains to be investigated in future studies which methods are most appropriate for specific applications.

The current study is designed to compare a new alternative method (3D T2prep BOLD) with the current method of choice for human fMRI (2D GRE EPI). Many aspects of the two methods differ from each other, which give rise to the different performance in each method. For instance, 3D and 2D acquisitions are intrinsically different. The spatial (voxel size) and temporal (TR) resolutions and the FOV were matched between the two methods for this comparison. Some other imaging parameters such as TE were optimized according to the BOLD theory for each method. The SENSE factor in 3D T2prep

(SENSE = 3×3) was matched with 2D GRE EPI (SENSE = 3) in the AP direction, but was higher in T2prep in the FH direction, which should reduce tSNR in T2prep BOLD compared to GRE EPI. The approximate Ernst angle (70°) was used in GRE EPI. A lower flip angle was used in T2prep BOLD (4°), mainly due to the specific absorption rate (SAR) limitation, which should also reduce tSNR in T2prep BOLD compared to GRE EPI. The coverage of GRE EPI was partial brain as the multiband technique is currently not available on our 7T system. This is certainly not viewed as a disadvantage for GRE EPI. Once the multiband technique is implemented on our 7T, GRE EPI will be able to achieve the same spatial and temporal resolution as T2prep BOLD with whole brain coverage, although more acceleration will lead to lower tSNR compared to the current GRE EPI scan.

There are several limitations in this initial technical study using T2prep BOLD for olfactory fMRI in humans. First, sniff is a common confounding factor in olfactory fMRI. In our study, we ensured that every participant received and practiced instruction to breathe passively through the nose and avoid sniffing before the start of each experiment. In future studies, additional procedures, such as a target sniffing pattern, will be implemented to better control sniffing patterns during the experiments (Sobel et al., 2000). Secondly, the respiration pattern may vary individually which may confound the olfactory fMRI data. A standard respiratory belt provided by the vendor of our MRI system was used to record the respiration pattern for each participant during the fMRI experiments, which was later regressed out from fMRI data during analysis. In subsequent

studies, one possible approach is to use a respiration-triggered olfactory fMRI technique (Wang et al., 2014) to provide a more precise estimate of the onsets of fMRI signals in response to olfactory stimulation. Thirdly, the olfactory stimulation paradigm can be re-designed to investigate how the various factors discussed above affect habituation in the OB and higher olfactory regions. Finally, the spatial resolution of 1.5 mm isotropic voxel in the current study is not sufficient to investigate layer dependent activities in the OB and other olfactory regions. Nevertheless, with the enhanced sensitivity from T2prep BOLD in the OB and other regions, fMRI with sub-millimeter spatial resolution focusing on the regions around the OB and primary olfactory cortex will be explored in subsequent studies.

CONCLUSION

The OB and several other olfactory regions are difficult to image with conventional EPI based BOLD fMRI methods due to significant susceptibility artifacts. We demonstrated that T2prep BOLD fMRI can be an alternative method to reduce artifact and enhance functional sensitivity especially in the OB. The signal characteristics during olfactory stimulation detected using T2prep BOLD fMRI are generally consistent with literature and showed a good intra-subject reproducibility comparable to previous human BOLD fMRI studies. The methodology demonstrated in this technical study is expected to be useful for olfactory studies on brain regions that are often inaccessible with conventional fMRI approaches in healthy human subjects and patients with olfactory dysfunction in neurodegenerative and neuropsychiatric diseases.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Johns Hopkins Institutional Review Board. The patients/participants provided their written informed consent to participate in this study.

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AUTHOR CONTRIBUTIONS

XM and AGP: organization and execution of the study, statistical analysis, writing of the manuscript, review, and critique of the manuscript. SR, DC, AYP, and LR: organization and execution of the study, review, and critique of the manuscript. DL: writing of the manuscript, review, and critique of the manuscript. PZ, SB, and DY: conception and design, review, and critique of the manuscript. VK: conception and design, organization and execution of the study, review, and critique of the manuscript. JH: conception and design, organization and execution of the study, statistical analysis, writing of the manuscript, review, and critique of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fnins.2021.723441/full#supplementary-material>

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Conflict of Interest: Equipment used in the study was manufactured by Philips Healthcare and Whiff LLC. PZ is a paid lecturer for the Philips Healthcare and has technology licensed to them. This arrangement has been approved by Johns Hopkins University in accordance with its conflict of interest policies.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Olfactory functional MRI (fMRI) using T2-prepared BOLD fMRI at ultra-high field (7T)

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Synopsis

The olfactory cortex is difficult to image with conventional EPI-fMRI due to susceptibility artifacts especially at 7T. T2-prepared (T2prep) fMRI is an alternative method to reduce such artifacts. Here, we show that T2prep-fMRI offers superior temporal signal-to-noise ratio in the olfactory cortex over EPI-fMRI at 7T. The signal characteristics detected using T2prep-fMRI during short (6s) and long (60s) olfactory stimulation were consistent with literature. During the long stimulus, fMRI signals in the olfactory cortex returned to baseline in 5-10s, and activation of secondary olfactory regions (insular, orbitofrontal) were observed, which can be attributed to habituation effects in the olfactory system.

Introduction

The olfactory cortex is difficult to image with conventional EPI-fMRI methods due to significant signal dropout and distortion caused by large susceptibility effects from the nearby temporal bone air especially petrous apex^{1,2}. Such susceptibility artifacts in the olfactory regions (including the entorhinal cortex and parahippocampal gyrus) have been reported at 3T and are exacerbated at higher field (7T). Recently, a T2-prepared (T2prep) fMRI sequence³⁻⁷ was proposed as an alternative fMRI method that can provide images free of signal loss and distortion in regions with large susceptibility artifacts, such as orbitofrontal and olfactory areas close to air cavities, and metallic implants. Here, we applied T2prep-fMRI to detect functional activation in olfactory-eloquent brain regions, and assessed its sensitivity in the olfactory regions. Olfactory functional experiments were performed at 7T with a short and a long stimulation paradigm to evaluate habituation effects in the olfactory system using T2prep fMRI.

Methods

All experiments were approved by the local IRB and all participants provided informed written consent. A custom-built multi-channel computer-controlled olfactometer (Whiff LLC, Swarthmore, PA) was used to deliver the odorants in precisely timed pulses. Phenyl ethyl alcohol (PEA) diluted in odorless mineral oil (50% v/v) was embedded in a constantly flowing humidified air stream at body temperature. Each participant was scanned during two different olfactory paradigms: a short stimulation paradigm with 16 blocks of alternating 6s PEA and 30s odorless mineral oil periods (10min); and a long stimulation paradigm with 3 blocks of alternating 60s PEA and 120s mineral oil (10min). fMRI experiments were performed on a 7T Philips scanner using T2prep-fMRI (TR/TE=2.0s/50ms, voxel=1.5mm isotropic, 84 slices, no gap) on 3 healthy subjects. GRE-EPI-fMRI (TR/TE=2.0s/22ms, voxel=1.5mm isotropic, 33 slices, no gap) was also performed on all subjects using only the long paradigm. Data analysis was performed using routines in SPM. A general linear model (GLM) was used for activation detection (adjusted P<0.05).

Results

Fig. 1 shows typical images from T2prep- and GRE-EPI-fMRI scans. The red arrows indicate the location of primary olfactory cortex. Temporal signal-to-noise ratios (tSNR) in the olfactory cortex were significantly higher in T2prep scans (105±26) compared to EPI scans (20±14, n=3, P<0.01). Given the higher tSNR in T2prep scans at 7T, the following analysis was focused on T2prep results. **Fig. 2** shows a representative activation map in the olfactory cortex detected using T2prep-fMRI during the short olfactory stimulation paradigm. As expected, the time course averaged over activated voxels from all subjects showed a signal increase during the stimulus period. However, little activation was detected in the olfactory cortex during the long paradigm (**Fig. 3**). The time course averaged over the olfactory cortex showed an initial increase for 5-10s during the stimulus and a quick return to baseline thereafter. Meanwhile, the long paradigm results showed significant activation in brain regions known to be associated with olfaction, including the insula and orbitofrontal cortex. **Fig. 4** shows an example of activation in the insula during the long stimulus. Different from the olfactory cortex, the time course averaged over activated voxels in the insula showed elevated signals across the entire period of olfactory stimulation. As activation detection in fMRI is highly dependent on the underlying signal model used in the GLM, we hypothesize that the main reason for the lack of activation in the olfactory cortex during the long paradigm is that the signal model used in the analysis expects a signal increase across the entire period of stimulus, whereas the actual signal increase only occurs in the first 5-10s of the stimulus (green shade in **Fig. 5**). To show this effect, we repeated the analysis with adjusted time period for signal increase in the GLM, and we found that comparable functional activation in the olfactory cortex can be detected during both the short and long paradigm in the same subjects (**Fig. 5**).

Discussion & Conclusion

T2prep-fMRI showed superior tSNR in the olfactory cortex compared to conventional GRE-EPI-fMRI at 7T. The fMRI signal characteristics detected using T2prep-fMRI during the short and long paradigms were consistent with previous studies in animals⁸⁻¹² (electrophysiology) and humans¹³⁻¹⁵. In particular, the quick return of fMRI signals in the olfactory cortex and the activation of secondary olfactory regions during the long paradigm can be attributed to well-known habituation effects in the olfactory cortex¹³⁻¹⁵. Our data also demonstrate the importance of the underlying signal models in GLM analysis. Further analysis of the data using less constrained approaches¹⁶ are underway.

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Figures

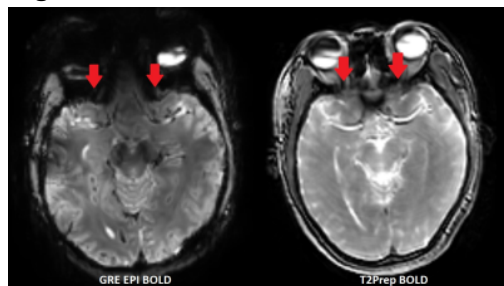


Figure 1: Representative images from a healthy subject comparing T2prep and conventional GRE EPI fMRI sequences. Signal dropout and distortion were observed in EPI images (red arrows), especially in the olfactory regions close to air-filled nasal cavities whereas such artifacts were negligible in T2prep images.

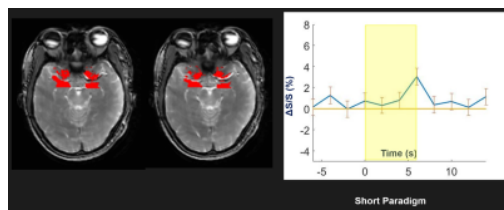


Figure 2: Functional MRI results from a healthy volunteer during a short (6s) olfactory stimulation. Functional activation in the olfactory cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red, left). The right panel shows the fMRI signals averaged over the activated voxels in the olfactory cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

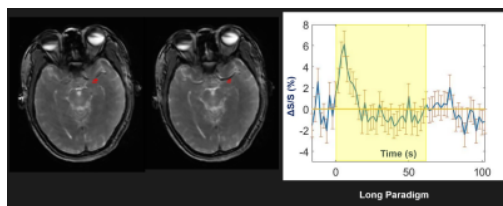


Figure 3: Functional MRI results from a healthy volunteer during a long (60s) olfactory stimulation. Functional activation in the olfactory cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red, left). Little activation was detected in both the left and right primary olfactory cortex (although voxels surpassing certain activation detection threshold may appear unilaterally). The right panel shows the fMRI signals averaged over the olfactory cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

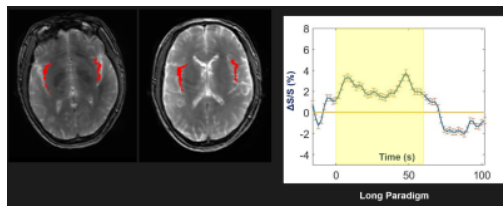


Figure 4: Functional MRI results from a healthy volunteer during a long (60s) olfactory stimulation. Functional activation in the insular cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red, left). The right panel shows the fMRI signals averaged over the activated voxels in the insular cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

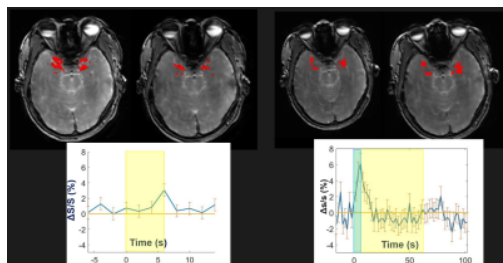


Figure 5: Functional MRI results from a healthy volunteer during a short (6s, left) and a long (60s, right) olfactory stimulation. Functional activation in the olfactory cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red). The bottom panels show the fMRI signals averaged over the activated voxels in the olfactory cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

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Synopsis: The olfactory cortex is difficult to image with conventional EPI-fMRI due to susceptibility artifacts especially at 7T. T2-prepared (T2prep) fMRI is an alternative method to reduce such artifacts. Here, we show that T2prep-fMRI offers superior temporal signal-to-noise ratio in the olfactory cortex over EPI-fMRI at 7T. The signal characteristics detected using T2prep-fMRI during short (6s) and long (60s) olfactory stimulation were consistent with literature. During the long stimulus, fMRI signals in the olfactory cortex returned to baseline in 5-10s, and activation of secondary olfactory regions (insular, orbitofrontal) were observed, which can be attributed to habituation effects in the olfactory system.

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METHODS: All experiments were approved by the local IRB and all participants provided informed written consent. A custom-built multi-channel computer-controlled olfactometer (Whiff LLC, Swarthmore, PA) was used to deliver the odorants in precisely timed pulses. Phenyl ethyl alcohol (PEA) diluted in odorless mineral oil (50% v/v) was embedded in a constantly flowing humidified air stream at body temperature. Each participant was scanned during two different olfactory paradigms: a short stimulation paradigm with 16 blocks of alternating 6s PEA and 30s odorless mineral oil periods (10min); and a long stimulation paradigm with 3 blocks of alternating 60s PEA and 120s mineral oil (10min). fMRI experiments were performed on a 7T Philips scanner using T2prep-fMRI (TR/TE=2.0s/50ms, voxel=1.5mm isotropic, 84 slices, no gap) on 3 healthy subjects. GRE-EPI-fMRI (TR/TE=2.0s/22ms, voxel=1.5mm isotropic, 33 slices, no gap) was also performed on all subjects using only the long paradigm. Data analysis was performed using routines in SPM. A general linear model (GLM) was used for activation detection (adjusted $P < 0.05$).

RESULTS: Temporal signal-to-noise ratios (tSNR) in the olfactory cortex were significantly higher in T2prep scans (105 ± 26) compared to EPI scans (20 ± 14 , $n=3$, $P < 0.01$). Given the higher tSNR in T2prep scans at 7T, the following analysis was focused on T2prep results. Within the short olfactory stimulation paradigm, the time course averaged over activated voxels from all subjects showed a signal increase during the stimulus period. However, little activation was detected in the olfactory cortex during the long paradigm. The time course averaged over the olfactory cortex showed an initial increase for 5-10s during the stimulus and a quick return to baseline thereafter. Meanwhile, the long paradigm results showed significant activation in brain regions known to be associated with olfaction, including the insula and orbitofrontal cortex. Different from the olfactory cortex, the time course averaged over activated voxels in the insula showed elevated signals across the entire period of olfactory stimulation. As activation detection in fMRI is highly dependent on the underlying signal model used in the GLM, we hypothesize that the main reason for the lack of activation in the olfactory cortex during the long paradigm is that the signal model used in the analysis expects a signal increase across the entire period of stimulus, whereas the actual signal increase only occurs in the first 5-10s of the stimulus. To show this effect, we repeated the analysis with adjusted time period for signal increase in the GLM, and we found that comparable functional activation in the olfactory cortex can be detected during both the short and long paradigm in the same subjects.

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Olfactory functional MRI (fMRI) using T2-prepared BOLD fMRI at ultra-high field (7T)

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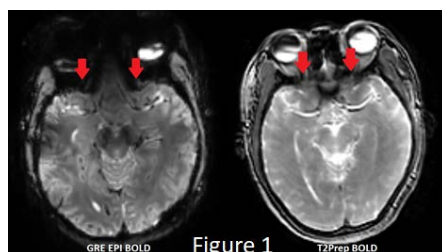


Figure 1: Representative images from a healthy subject comparing T2prep and conventional GRE EPI fMRI sequences. Signal dropout and distortion were observed in EPI images (red arrows), especially in the olfactory regions close to air-filled nasal cavities whereas such artifacts were negligible in T2prep

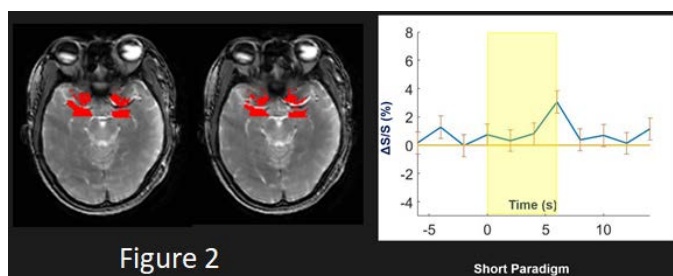


Figure 2: Functional MRI results from a healthy volunteer during a short (6s) olfactory stimulation. Functional activation in the olfactory cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red, left). The right panel shows the fMRI signals averaged over the activated voxels in the olfactory cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

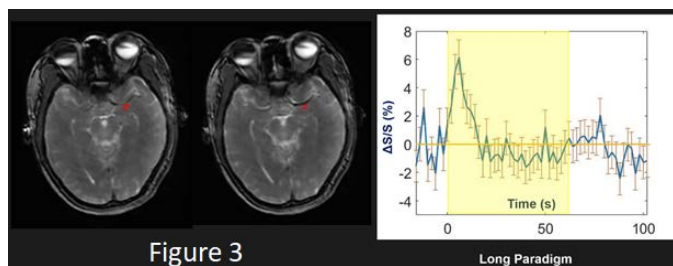


Figure 3: Functional MRI results from a healthy volunteer during a long (60s) olfactory stimulation. Functional activation in the olfactory cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red, left). Little activation was detected in both the left and right primary olfactory cortex (although voxels surpassing certain activation detection threshold may appear unilaterally). The right panel shows the fMRI signals averaged over the olfactory cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

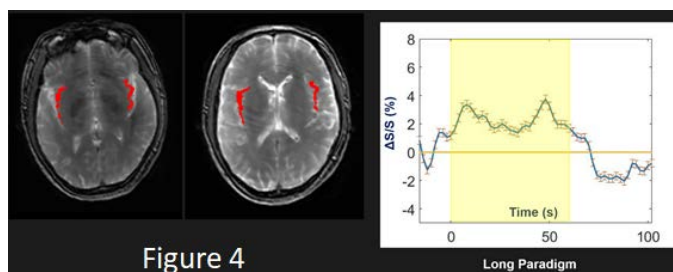


Figure 4: Functional MRI results from a healthy volunteer during a long (60s) olfactory stimulation. Functional activation in the insular cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red, left). The right panel shows the fMRI signals averaged over the activated voxels in the insular cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

Introduction: The olfactory cortex is difficult to image with conventional EPI-fMRI methods due to significant signal dropout and distortion caused by large susceptibility effects from the nearby temporal bone air especially petrous apex. Such susceptibility artifacts in the olfactory regions (including the entorhinal cortex and parahippocampal gyrus) have been reported at 3T and are exacerbated at higher field (7T). Recently, a T2-prepared (T2prep) fMRI sequence was proposed as an alternative fMRI method that can provide images free of signal loss and distortion in regions with large susceptibility artifacts, such as orbitofrontal and olfactory areas close to air cavities, and metallic implants. Here, we applied T2prep-fMRI to detect functional activation in olfactory-eloquent brain regions, and assessed its sensitivity in the olfactory regions. Olfactory functional experiments were performed at 7T with a short and a long stimulation paradigm to evaluate habituation effects in the olfactory system using T2prep fMRI.

Methods: All experiments were approved by the local IRB and all participants provided informed written consent. A custom-built computer-controlled olfactometer (Whiff LLC, Swarthmore, PA) was used to deliver the odorants in precisely timed pulses. Phenyl ethyl alcohol (PEA) diluted in odorless mineral oil (50% v/v) was embedded in a constant flow humidified air stream at body temperature. Each participant was scanned with two different olfactory paradigms: a short stimulation paradigm with 16 blocks of alternating 6s PEA and 30s odorless mineral oil periods (10min); and a long stimulation paradigm with 3 blocks of alternating 60s PEA and 120s mineral oil (10min). fMRI experiments were performed on a 7T Philips scanner using T2prep-fMRI (TR/TE=2.0s/50ms, voxel=1.5mm isotropic, 84 slices, no gap) on 3 healthy subjects. GRE-EPI-fMRI (TR/TE=2.0s/22ms, voxel=1.5mm isotropic, 33 slices, no gap) was also performed on all subjects using only the long paradigm. Data analysis was performed in SPM. A general linear model (GLM) was used for activation detection (adjusted $P < 0.05$).

Results: Temporal signal-to-noise ratios (tSNR) in the olfactory cortex were significantly higher in T2prep scans (105 ± 26) compared to EPI scans (20 ± 14 , $n=3$, $P < 0.01$). Given the higher tSNR in T2prep scans at 7T, the following analysis was focused on T2prep results. Within the short olfactory stimulation paradigm, the time course averaged over activated voxels from all subjects showed a signal increase during the stimulus period. However, little activation was detected in the olfactory cortex during the long paradigm. The time course averaged over the olfactory cortex showed an initial increase for 5-10s during the stimulus and a quick return to baseline thereafter. Meanwhile, the long paradigm results showed significant activation in brain regions known to be associated with olfaction, including the insula and orbitofrontal cortex. Different from the olfactory cortex, the time course averaged over activated voxels in the insula showed elevated signals across the entire period of olfactory stimulation. As activation detection in fMRI is highly dependent on the underlying signal model used in the GLM, we hypothesize that the main reason for the lack of activation in the olfactory cortex during the long paradigm is that the signal model used in the analysis expects a signal increase across the entire period of stimulus, whereas the actual signal increase only occurs in the first 5-10s of the stimulus. To show this effect, we repeated the analysis with adjusted time period for signal increase in the GLM, and we found that comparable functional activation in the olfactory cortex can be detected during both the short and long paradigm in the same subjects.

Discussion & Conclusion: T2prep-fMRI showed superior tSNR in the olfactory cortex compared to conventional GRE-EPI-fMRI at 7T. The fMRI signal characteristics detected using T2prep-fMRI during the short and long paradigms were consistent with previous studies in animals (electrophysiology) and humans. In particular, the quick return of fMRI signals in the olfactory cortex and the activation of secondary olfactory regions during the long paradigm can be attributed to well-known habituation effects in the olfactory cortex. Our data also demonstrate the importance of the underlying signal models in GLM analysis. Further analysis of the data using less constrained approaches are underway.

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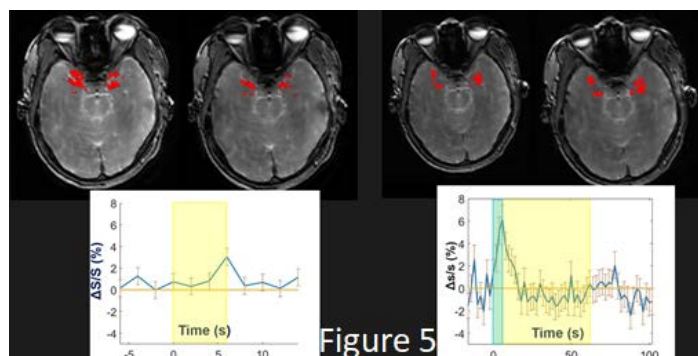


Figure 5: Functional MRI results from a healthy volunteer during a short (6s, left) and a long (60s, right) olfactory stimulation. Functional activation in the olfactory cortex detected with T2prep BOLD fMRI is highlighted on the fMRI images (red). The bottom panels show the fMRI signals averaged over the activated voxels in the olfactory cortex from all subjects as a function of time. Time = 0s indicates the start of stimulus, and the yellow shade depicts the duration of the stimulus.

Functional MRI in the olfactory bulb and associated olfactory regions in the human brain on 7T

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Target audience: Investigators and clinicians with an interest in fMRI on 7T, especially fMRI in the human olfactory bulb.

Purpose: To date, few studies using EPI-based BOLD fMRI can measure functional signal changes in the olfactory bulb (OB) in humans, mainly due to susceptibility artifacts. T2-prepared (T2prep) BOLD fMRI is an alternative approach developed especially for reducing susceptibility artifacts. In this study, functional activation in olfactory-eloquent brain regions, especially the OB, was assessed using T2prep-BOLD-fMRI¹.

Methods: Olfactory fMRI was performed on 7T in 14 healthy participants with written informed consents. The T2prep and conventional EPI-based BOLD fMRI were performed in each subject with the same spatial resolution (isotropic 1.5mm voxel) and TR (2 sec). The olfactory paradigm started with an OFF period of 60s with odorless mineral oil, followed by three blocks of a ON period of 60s with Phenyl-Ethyl-Alcohol and an OFF period of 120s with odorless mineral oil. An independent-component-analysis (ICA) based denoising approach was performed on the single-subject fMRI data using FSL6.0.1. A low-pass filter (<0.03 Hz) was applied. The Automated Anatomical Labelling atlas was used to identify the primary and secondary olfactory regions in the brain according to the literature. The OB was manually delineated on the MPRAGE images for each participant. Functional activation during olfactory stimulation in the whole brain was detected using the nonparametric Kolmogorov–Smirnov two-sample test (adjusted- $P < 0.01$).

Results: **Figure 1** shows the typical image quality of GRE-EPI-BOLD and T2prep-BOLD-fMRI from one subject. **Figure 2** shows a representative activation map from one participant. The group averaged time courses of $\Delta S/S$ during the paradigm from activated voxels in each region are displayed in **Figure 3**. All regions showed a habituation effect in that responses in the 2nd and 3rd blocks were reduced compared to the 1st block. Within the 1st block, all regions showed a bi-phasic pattern with two distinct peaks during the first and second halves of the 1st block. T2prep-BOLD-fMRI showed a good intra-subject reproducibility of the spatial locations of the activated clusters (Dice-coefficients 0.88-0.96) and $\Delta S/S$ (ICC>0.8), comparable to typical BOLD-fMRI measures reported in previous studies.

Discussion: We demonstrated that the whole-brain T2prep-BOLD-fMRI technique can detect functional activations in the OB and associated olfactory regions in healthy human subjects at 7T. Habituation effects and a bi-phasic pattern of fMRI signal changes during olfactory stimulation were observed in all regions. These signal characteristics are generally consistent with literature and showed a good intra-subject reproducibility comparable to previous human BOLD fMRI studies.

Conclusion: In conclusion, the methodology demonstrated in this study holds promise for future olfactory fMRI studies in the olfactory bulb and other brain regions that suffer from large susceptibility artifacts.

References: 1 Miao, X. *et al. Frontiers in Neuroscience* 15, doi:10.3389/fnins.2021.723441 (2021).

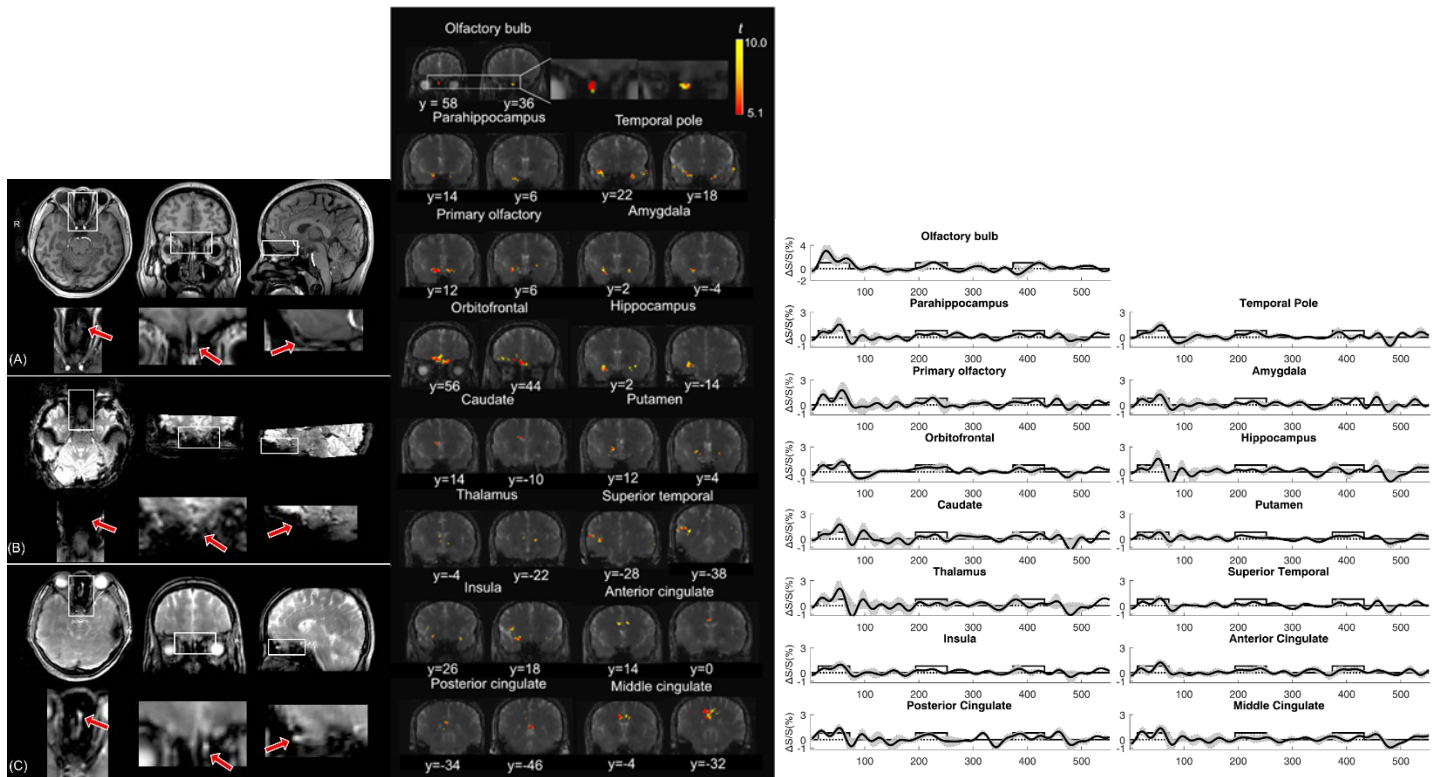


Figure 1: Typical MPRAGE (A), GRE EPI BOLD (B) and T2prep BOLD (C) images from one subject. The red arrows indicate the location of the olfactory bulb. The olfactory bulb can be clearly identified in the MPRAGE and T2prep images, but not in the GRE EPI images due to substantial susceptibility artifacts. All images are from the same location.

Figure 2: Representative activation maps during the olfactory stimulation from one participant. The t-scores of significantly activated voxels are overlaid on T2prep BOLD images. Two slices are shown for each region.

Figure 3: Average time courses of fMRI signal changes from all participants ($n=14$). For each participant, the time course was averaged over activated voxels in each region. The grey shaded area indicates the inter-subject standard error. The boxcar curve illustrates the timing of the olfactory paradigm. The order of the regions was arranged to be identical to Figure 2.

Functional activities detected in the olfactory bulb and associated olfactory regions in the human brain using T2-prepared BOLD fMRI at 7T

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Synopsis

Conventional EPI-based BOLD fMRI can be challenging in olfaction related brain regions, such as the olfactory bulb (OB), mainly due to the large susceptibility artifacts. To date, few studies have demonstrated successful fMRI in the human OB. T2-prepared (T2prep) BOLD fMRI is an alternative approach developed especially for reducing such susceptibility artifacts. Here, olfactory fMRI was performed on 7T in 14 healthy participants. T2prep-BOLD showed greater functional sensitivity than GRE-EPI-BOLD in the OB and associated olfactory regions. Habituation effects and a bi-phasic pattern of fMRI signal changes were observed using T2prep-BOLD, which showed a good intra-subject reproducibility.

Introduction

Olfactory deficits have emerged as an early risk factor in many brain diseases[1-3] and aging[4]. BOLD-fMRI using conventional EPI based sequences can be challenging in brain regions important for olfactory processing, such as the olfactory bulb and orbitofrontal cortex, mainly due to the signal dropout and distortion artifacts caused by large susceptibility effects. To date, few studies have demonstrated successful fMRI in the olfactory bulb in humans. T2-prepared (T2prep) BOLD-fMRI[5] is an alternative approach developed especially for performing fMRI in regions affected by large susceptibility artifacts. In this study, functional activation in olfactory-eloquent brain regions, especially the OB, was assessed using T2prep-BOLD-fMRI.

Methods

Olfactory fMRI scans were performed on 7T in 14 healthy participants (47±11yo, 8 females). All participants had normal olfactory functions as assessed by the University of Pennsylvania Smell Identification Test (UPSIT) score[6]. The following scans were performed in each participant: a) T2prep-BOLD-fMRI[7]: TR=2000ms, TE=50ms, voxel=1.5x1.5x1.5mm3, 84 slices; b) GRE-EPI-BOLD-fMRI: same resolution and TR, TE=22ms, flip-angle=70o, 33 slices; c) MPRAGE: TR/TI/TE=4500/563/1.81ms, voxel=1x1x1mm3. T2prep-BOLD-fMRI was repeated once within 3-6 weeks to assess its reproducibility. Olfactory paradigm: An enhanced model of computer-controlled olfactometer[8] was used to deliver the odorants in precisely timed pulses. The olfactory paradigm started with a stimulus-off period of 60s with odorless mineral oil, followed by three blocks of a stimulus-on period of 60s with Phenyl-Ethyl-Alcohol and a stimulus-off period of 120s with odorless mineral oil (total duration 10min). The relatively long stimulation period was chosen in this study to evaluate habituation effects in the olfactory system. All participants were instructed to breathe passively through the nose and avoid sniffing. Data analysis was performed mainly using the SPM12 software package and Matlab (2017a) routines. Realignment and spatial smoothing (kernel of 4mm) were performed for all fMRI images. An independent-component-analysis (ICA) based denoising approach [9] was performed on the fMRI data using FSL6.0.1, from which components related to motion and physiological noise were removed. Temporal filtering was performed using a low-pass filter with a cut-off frequency of 0.03Hz. The Automated Anatomical Labelling (AAL) atlas[10] was used to identify the primary and secondary olfactory regions in the brain according to the literature[11]. Since the olfactory bulb is not included in the AAL-atlas, it was manually delineated on the MPRAGE images for each participant. Functional activation during olfactory stimulation in the whole brain was detected using the nonparametric Kolmogorov-Smirnov (KS) two-sample test[12] (adjusted-P<0.01). Compared to the commonly used general linear model (GLM), previous olfactory fMRI studies[11, 13] have shown that the KS statistic may be more suitable for detecting functional activations with strong habituation effects. Relative signal changes ($\Delta S/S$) between the stimulus-on and stimulus-off periods were calculated for each voxel. Note that due to the transition period following the cessation of stimulus that usually lasts for 50-100% of the stimulus-on period [14, 15], only signals from the second half of the stimulus-off period were included when calculating $\Delta S/S$. Paired t-tests were performed to compare contrast-to-noise ratio (CNR) from GRE-EPI-BOLD and T2prep-BOLD-fMRI. Effect size was estimated with Cohen's d. One-Way Repeated-Measures Analysis of Variance (ANOVA) was conducted to examine differences of $\Delta S/S$ among the three blocks of the olfactory paradigm. All statistical tests were corrected for multiple comparisons by controlling the false-discovery rate (adjusted-P<0.05).

Results

Figure 1 shows the typical image quality of GRE-EPI-BOLD and T2prep-BOLD-fMRI from one subject. Table 1 shows the quantitative CNR results. T2prep showed greater CNR than GRE EPI in the olfactory bulb, orbitofrontal cortex and the temporal pole. Figure 2 shows a representative activation map from one participant. The group averaged time courses of $\Delta S/S$ during the paradigm from activated voxels in each region are displayed in Figure 3. Table 2 summarizes the quantitative results ($\Delta S/S$) from activated voxels in each region. As the same olfactory stimulation was repeated for three blocks, $\Delta S/S$ from each block was calculated and compared. All regions showed habituation effect that responses in the 2nd and 3rd blocks were reduced compared to the 1st block. Within the 1st block, all regions showed a bi-phasic pattern with two distinct peaks during the first and second halves of the 1st block. T2prep-BOLD-fMRI showed a good intra-subject reproducibility of the spatial locations of the activated clusters (Dice-coefficients 0.88-0.96) and $\Delta S/S$ (ICC>0.8), comparable to typical BOLD-fMRI measures reported in previous studies[16].

Discussion and Conclusion

We demonstrated that the whole-brain T2prep-BOLD-fMRI technique can detect functional activations in response to olfactory stimulation in primary and secondary olfactory regions in healthy human subjects at 7T. Functional activation was detected using T2prep-BOLD in the olfactory bulb and associated olfactory regions. Habituation effects[17] and a bi-phasic pattern of fMRI signal changes during olfactory stimulation were observed in all regions. These signal characteristics are generally consistent with literature and showed a good intra-subject reproducibility comparable to previous human BOLD fMRI studies. In conclusion, the methodology demonstrated in this study holds promise for future olfactory

fMRI studies in the olfactory bulb and other brain regions that suffer from large susceptibility artifacts.

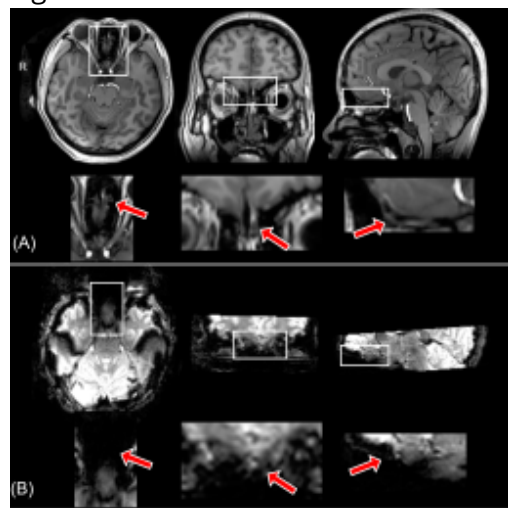
Acknowledgements

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Figures



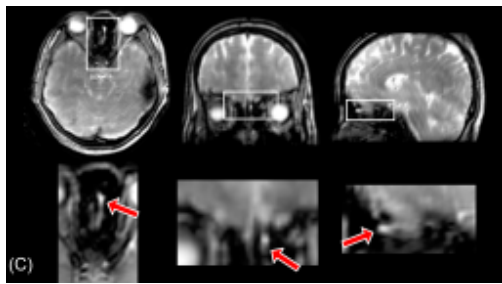


Figure 1: Typical MPRAGE (A), GRE EPI BOLD (B) and T2prep BOLD (C) images from one subject. The red arrows indicate the location of the olfactory bulb. The olfactory bulb can be clearly identified in the MPRAGE and T2prep images, but not in the GRE EPI images due to substantial susceptibility artifacts. All images are from the same location.

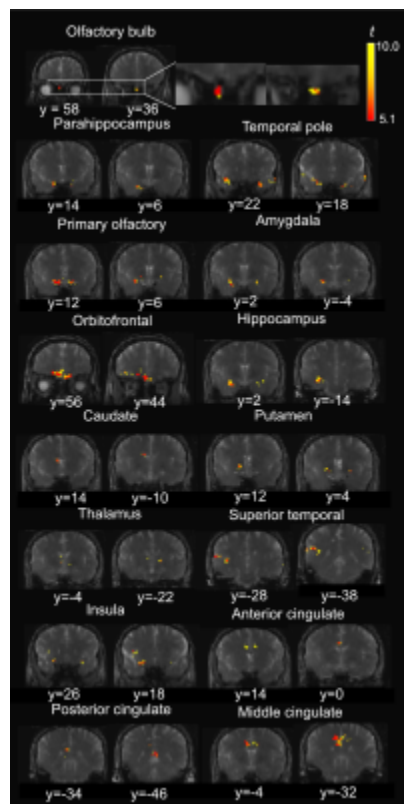


Figure 2. Representative activation maps during the olfactory stimulation from one participant. The t-scores of significantly activated voxels are overlaid on T2prep BOLD images. Two slices were shown for each region.

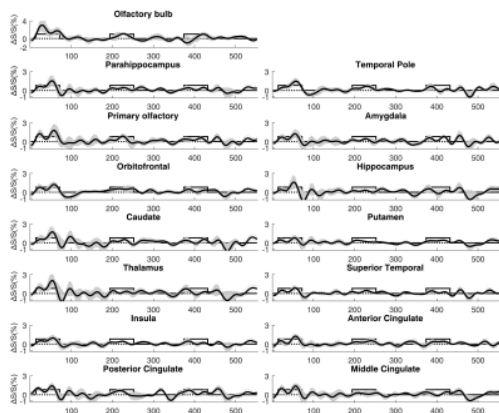


Figure 3. Average time courses of fMRI signal changes from all participants ($n=14$). For each participant, the time course was averaged over activated voxels in each region. The grey shaded area indicates the inter-subject standard error. The boxcar curve illustrates the timing of the olfactory paradigm. The order of the regions was arranged to be identical to Figure 2.

ROI	ROI size (F voxel)	T2prep BOLD	GRE EPI BOLD	P	Relative difference (%)	Effect size
Olfactory bulb	15.4 ± 5.1	1.3 ± 0.3	0.5 ± 0.1	0.02*	183.3	4.7
Preperigeniculate	266.1 ± 83.4	0.9 ± 0.2	0.8 ± 0.1	0.57	8.5	0.4
Temporal pole	795.0 ± 243.3	1.7 ± 0.3	0.4 ± 0.1	0.01*	395.5	5.3
Pirmary olfactory	67.0 ± 21.2	1.1 ± 0.3	1.1 ± 0.2	0.66	6.1	0.3
Amygdala	57.0 ± 18.0	0.5 ± 0.1	1.7 ± 0.7	0.15	-48.0	-2.4
Orbitofrontal	363.0 ± 98.0	0.9 ± 0.1	0.2 ± 0.1	<0.001*	417.7	7.9
Hippocampus	212.1 ± 71.7	0.9 ± 0.2	2.1 ± 0.5	0.16	-58.4	-3.4
Caudate	165.0 ± 56.5	1.1 ± 0.2	1.6 ± 0.4	0.44	-27.0	-1.4
Putamen	256.1 ± 91.4	0.7 ± 0.1	0.7 ± 0.2	0.62	6.9	0.3
Thalamus	344.8 ± 73.9	0.9 ± 0.2	1.5 ± 0.3	0.23	-59.4	-3.0
Superior temporal	498.8 ± 163.8	0.7 ± 0.2	0.3 ± 0.1	0.07	135.4	3.2
Insula	499.2 ± 149.4	1.0 ± 0.2	0.9 ± 0.1	0.71	6.6	0.4
Anterior cingulate	353.2 ± 95.0	0.9 ± 0.2	1.2 ± 0.2	0.24	-24.6	-1.4
Posterior cingulate	289.5 ± 97.0	0.7 ± 0.1	1.6 ± 0.4	0.12	-58.7	-3.0
Medial cingulate	413.3 ± 130.0	0.9 ± 0.1	2.3 ± 0.4	0.02*	-62.3	-4.8

Mean ± standard error. *P < 0.05.
The same voxels were used in both fMRI methods. The number of voxels in the olfactory bulb is small as it is a very small region.
Relative difference was defined as: $100 \times (\text{mean CNR in T2prep} - \text{mean CNR in EPI}) / (\text{mean CNR in EPI}) \%$.
Effect size was calculated using Cohen's d = $(\text{mean CNR in T2prep} - \text{mean CNR in EPI}) / s$, where s is the pooled standard deviation of the two groups.

Table 1. Comparison of contrast-to-noise ratio (CNR) between T2prep BOLD and GRE EPI BOLD fMRI.

TABLE 2 Quantitative T2prep BOLD fMRI results from positively activated voxels from all subjects (n = 14).							
ROI	Activated voxels (N)	ASIS (Hz)			P**	Relative difference (%)***	
		1st block	2nd block	3rd block		2nd vs. 1st	3rd vs. 1st
Olfactory bulb	14.8 ± 5.3	2.2 ± 0.7	0.8 ± 0.5	-0.2 ± 0.5	0.02	-65 ± 56	-110 ± 45
Preperigeniculate	244.1 ± 87.8	0.8 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	0.02	-75 ± 42	-80 ± 45
Temporal Pole	796.0 ± 267.3	0.8 ± 0.3	0.4 ± 0.1	0.2 ± 0.1	0.03	-43 ± 31	-80 ± 40
Pirmary olfactory	65.0 ± 23.3	1.3 ± 0.6	0.2 ± 0.1	0.2 ± 0.1	0.02	-79 ± 54	-87 ± 50
Amygdala	54.0 ± 19.8	0.7 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	0.05	-62 ± 36	-67 ± 39
Orbitofrontal	348.0 ± 85.1	0.8 ± 0.3	0.5 ± 0.1	0.2 ± 0.1	0.02	-39 ± 38	-69 ± 32
Hippocampus	203.7 ± 70.3	0.7 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.02	-71 ± 42	-87 ± 48
Caudate	161.0 ± 57.4	1.1 ± 0.4	0.2 ± 0.1	0.2 ± 0.2	0.01	-79 ± 35	-79 ± 22
Putamen	237.7 ± 80.7	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.1	0.06	-23 ± 69	-48 ± 62
Thalamus	323.4 ± 80.8	1.2 ± 0.4	0.1 ± 0.1	0.1 ± 0.1	0.01	-87 ± 61	-89 ± 41
Superior temporal	492.0 ± 177.2	0.4 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.02	-35 ± 32	-79 ± 36
Insula	430.0 ± 145.4	0.6 ± 0.2	0.2 ± 0.1	0.1 ± 0.1	0.03	-66 ± 47	-75 ± 41
Anterior cingulate	350.2 ± 95.2	0.8 ± 0.2	0.3 ± 0.1	0.1 ± 0.1	0.02	-61 ± 31	-80 ± 44
Posterior cingulate	294.0 ± 94.4	0.9 ± 0.3	0.4 ± 0.1	0.0 ± 0.1	0.02	-50 ± 27	-90 ± 29
Medial cingulate	365.3 ± 144.0	0.8 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	0.02	-71 ± 30	-88 ± 42

Mean ± standard error.
ASIS averaged over the 1st, 2nd, and 3rd blocks during the olfactory paradigm was calculated separately.
**P values from one-way repeated measures analysis of variance (ANOVA) to examine differences of ASIS among the three blocks of the olfactory paradigm.
***Relative difference = $100 \times (\text{ASIS 2nd or 3rd block} - \text{ASIS 1st block}) / \text{group mean of ASIS 1st block} \%$.
The number of voxels in the olfactory bulb is small as it is a very small region.

Table 2. Quantitative T2prep BOLD fMRI results from activated voxels from all subjects (n=14).

High-resolution functional MRI in the human olfactory bulb at 7T

Poster No:

1412

Submission Type:

Abstract Submission

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Introduction:

Olfactory deficits have emerged as an early risk factor in many brain diseases (Marin, Vilas et al. 2018) and aging (Doty and Kamath 2014). BOLD-fMRI using conventional EPI based sequences can be challenging in brain regions important for olfactory processing, such as the olfactory bulb (OB) and orbitofrontal cortex, mainly due to the large susceptibility effects. To date, few studies have demonstrated successful fMRI in the OB in humans. T2-prepared (T2prep) BOLD-fMRI (Hua, Qin et al. 2014) is an alternative approach developed especially for performing fMRI in regions affected by large susceptibility artifacts. In this study, functional activation in olfactory-eloquent brain regions, especially the OB, was assessed using T2prep-BOLD-fMRI.

Methods:

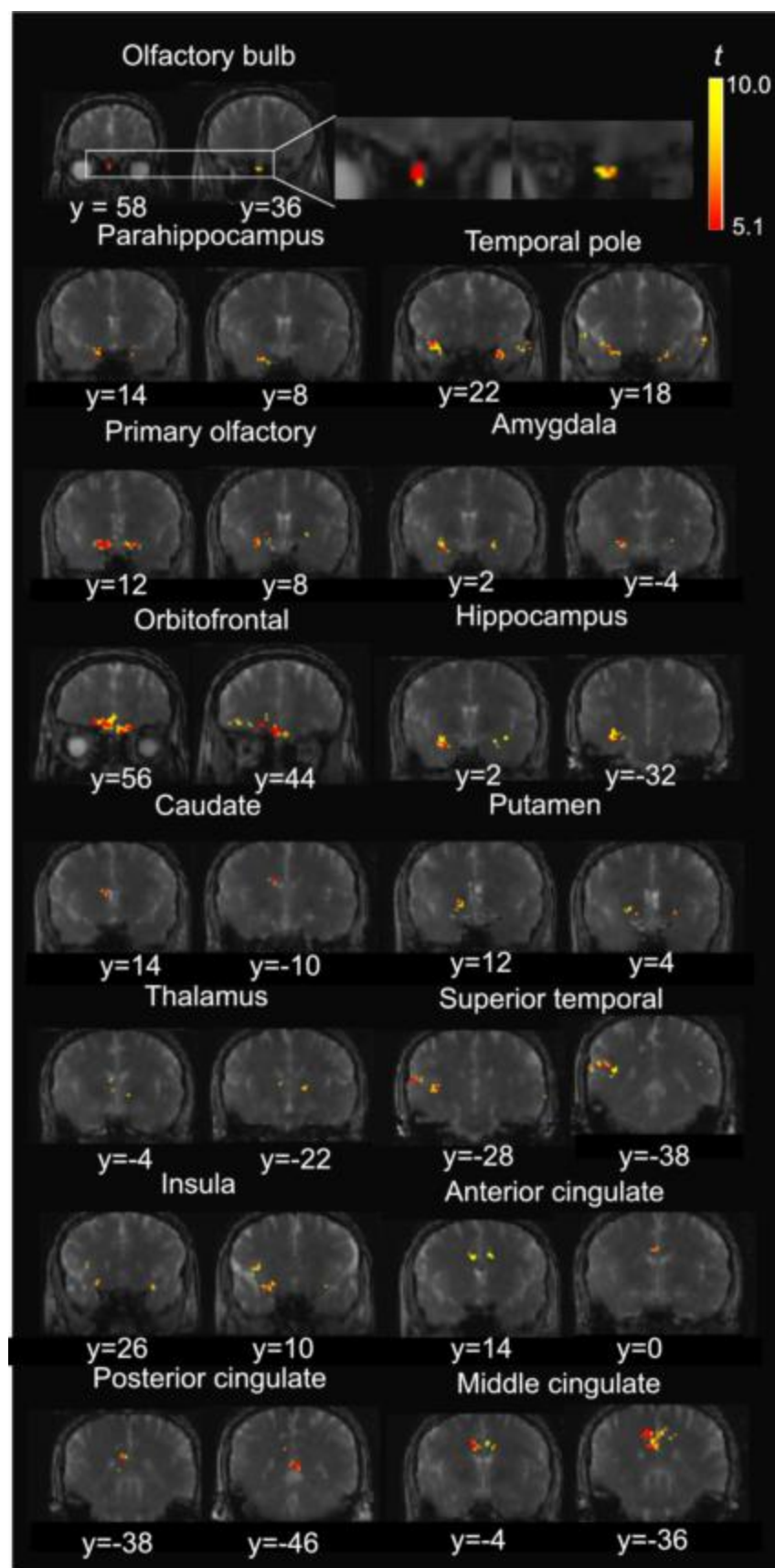
Olfactory fMRI scans were performed on 7T in 14 healthy participants (47 ± 11 yo, 8 females). All participants had normal olfactory functions as assessed by the University of Pennsylvania Smell Identification Test score (Doty, Shaman et al. 1984). The following scans were performed in each participant: a) T2prep-BOLD-fMRI (Miao, Paez et al. 2021): TR=2000ms, TE=50ms, voxel=1.5x1.5x1.5mm³, 84 slices; b) GRE-EPI-BOLD-fMRI: same resolution and TR, TE=22ms, flip-angle=70°, 33 slices; c) MPRAGE: TR/TI/TE=4500/563/1.81ms, voxel=1x1x1mm³. T2prep-BOLD-fMRI was repeated once within 3-6 weeks to assess its reproducibility. The olfactory paradigm started with a stimulus-off period of 60s with odorless mineral oil, followed by three blocks of a ON period of 60s with Phenyl-Ethyl-Alcohol and a OFF period of 120s with odorless mineral oil. Data analysis was performed mainly using the SPM12 software package and Matlab (2017a) routines. Realignment and spatial smoothing (kernel of 4mm) were performed for all fMRI images. An independent-component-analysis (ICA) based denoising approach was performed on the fMRI data using FSL6.0.1, from which components related to motion and physiological noise were removed. Temporal filtering was performed using a low-pass filter with a cut-off frequency of 0.03Hz. The Automated Anatomical Labelling atlas was used to identify the primary and secondary olfactory regions in the brain according to the literature (Poellinger, Thomas et al. 2001).

Functional activation during olfactory stimulation in the whole brain was detected using the nonparametric Kolmogorov–Smirnov (KS) two-sample test (adjusted- $P < 0.01$). Compared to the commonly used general linear model (GLM), previous olfactory fMRI studies (Sobel, Prabhakaran et al. 2000, Poellinger, Thomas et al. 2001) have shown that the KS statistic may be more suitable for detecting functional activations with strong habituation effects.

Results:

T2prep showed greater CNR than GRE EPI in the olfactory bulb, orbitofrontal cortex and the temporal pole

(data not shown). Figure 1 shows a representative activation map from one participant. The group averaged time courses of $\Delta S/S$ during the paradigm from activated voxels in each region are displayed in Figure 2.



(<https://files.aievolution.com/prd/hbm2201>)

Figure 1. Representative activation maps during the olfactory stimulation from one participant. The t-scores of significantly activated voxels are overlaid on T2-weighted ROI-D images. Two slices were shown for

/abstracts/abs_1341/fig1_hbm.jpg)

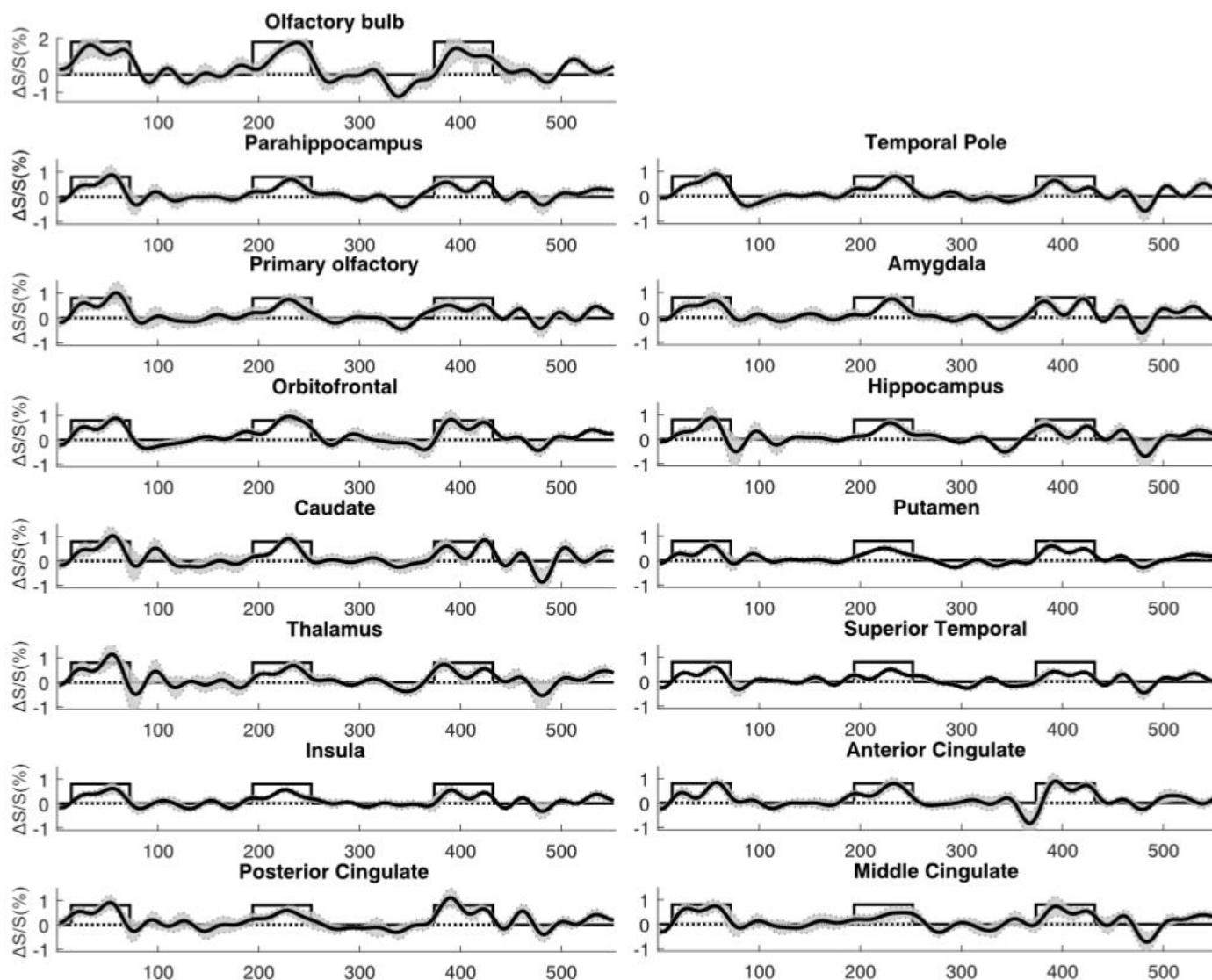


Figure 2. Average time courses of fMRI signal changes from all participants ($n=14$). For each participant, the time course was averaged over activated voxels in each region. The grey shaded area indicates the inter-subject standard error. The boxcar curve illustrates the timing of the olfactory paradigm.

(https://files.aievolution.com/prd/hbm2201/abstracts/abs_1341/fig2_hbm.jpg)

Conclusions:

We demonstrated that the whole-brain T2prep-BOLD-fMRI technique can detect functional activations in response to olfactory stimulation in primary and secondary olfactory regions in healthy human subjects at 7T. Functional activation was detected using T2prep-BOLD in the OB and associated olfactory regions. Habituation effects (Pellegrino, Sinding et al. 2017) and a bi-phasic pattern of fMRI signal changes during olfactory stimulation were observed in all regions. These signal characteristics are generally consistent with literature comparable to previous human BOLD fMRI studies. T2prep-BOLD-fMRI showed a good intra-subject

reproducibility of the spatial locations of the activated clusters (Dice-coefficients 0.88-0.96) and $\Delta S/S$ ($ICC > 0.8$), comparable to typical BOLD-fMRI measures reported in previous studies. In conclusion, the methodology demonstrated in this study holds promise for future olfactory fMRI studies in the OB and other brain regions that suffer from large susceptibility artifacts.

Novel Imaging Acquisition Methods:

BOLD fMRI ²

Perception, Attention and Motor Behavior:

Chemical Senses: Olfaction, Taste ¹

Keywords:

HIGH FIELD MR

Smell

Other - Olfaction, MRI artifacts, T2prep, habituation

^{1|2}Indicates the priority used for review

My abstract is being submitted as a Software Demonstration.

No

Please indicate below if your study was a "resting state" or "task-activation" study.

Task-activation

Healthy subjects only or patients (note that patient studies may also involve healthy subjects):

Healthy subjects

Are you Internal Review Board (IRB) certified? Please note: Failure to have IRB, if applicable will lead to automatic rejection of abstract.

Yes

Was any human subjects research approved by the relevant Institutional Review Board or ethics panel? NOTE: Any human subjects studies without IRB approval will be automatically rejected.

Yes

Was any animal research approved by the relevant IACUC or other animal research panel? NOTE: Any animal studies without IACUC approval will be automatically rejected.

Not applicable

Please indicate which methods were used in your research:

Functional MRI

For human MRI, what field strength scanner do you use?

7T

Which processing packages did you use for your study?

SPM

FSL

Provide references using author date format

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High resolution structural MRI reveals abnormalities in olfactory brain regions in early Parkinson’s disease (PD) patients

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TARGET AUDIENCE: Researchers who are interested in structural brain changes especially in olfactory brain regions in neurodegenerative disease.

PURPOSE: Structural changes in the olfactory bulbs (OB), piriform cortex (PC), and secondary olfactory regions have been reported in PD. In this study, high resolution structural MRI scans were performed in early PD patients and matched controls to systemically investigate structural abnormalities in the primary and secondary olfactory regions. The experiments were conducted on ultra-high magnetic field (7.0 Tesla or 7T) human MRI scanners. The relationship between MRI and behavioral olfactory measures was investigated.

METHODS: All MRI scans were performed on a 7T Philips MRI scanner. A cohort of 15 PD patients, and 15 healthy controls matched in age, sex, and education level were recruited for the study (Table 1). The University of Pennsylvania Smell Identification Test (UPSIT) was administrated to all participants to assess their behavioral olfactory functions. The following MRI scans were performed for each participant: 1) MPRAGE (voxel=1mm isotropic, TR/TE/TI=5ms/1.81ms/563ms); 2) T2-weighted scan (voxel=0.5mm isotropic, TR/TE=5000ms/58ms). Due to its small size and unique contrast, the OB was manually traced on the T2-weighted images. Thirteen additional regions of interest (ROI) in the cortex and subcortex were manually identified and segmented on the MPRAGE images. Figure 1 shows an example of the segmentation from one participant. Volumes of individual ROI were calculated using a custom Matlab script. Two-sample t-tests were performed to examine group difference in the volumes of individual ROI. Age and sex were accounted for as covariates. Correlations between volumes of ROIs and UPSIT scores were assessed with multiple regression with age and sex as covariates. Multiple comparisons were corrected with the false-discovery rate (adjusted P < 0.05).

RESULTS: The behavioral olfactory test (UPSIT) scores were significantly lower in early PD patients compared to controls, indicating impaired olfactory function in PD patients. Figure 2 shows the group comparison results of the measured volumes of the OB, primary (piriform cortex) and secondary olfactory regions in early PD patients and controls. The volume of OB did not show significant difference between PD and controls. The volumes of the primary olfactory (piriform) cortex were significantly lower in early PD patients. In contrast, the volumes of insula, thalamus, caudate and cingulate cortex were significantly higher in PD patients. Figure 3 shows the correlation analysis between region volumes and the UPSIT scores, which revealed significant positive correlations in the OB, piriform cortex and amygdala, and significant negative correlations in the insula, caudate and cingulate cortex.

DISCUSSION & CONCLUSION: We demonstrate significant structural abnormalities in the OB, primary and secondary olfactory brain regions in early PD patients measured by high resolution MRI at 7T. Regions with significant volume decrease in PD showed positive correlations with behavioral olfactory scores in the participants, and vice versa. Although the OB and amygdala did not show significant volume changes, their volumes were significantly associated with behavioral olfactory scores. Among all the regions investigated, the primary olfactory (piriform) cortex showed the most significant volume decrease in early PD patients. Interestingly, several brain regions such as the insula, thalamus, caudate and cingulate cortex showed an opposite change of volume compared to the piriform cortex. It is tempting to speculate that such differential effects in different brain regions might indicate compensatory mechanisms in the early stage of the disease. Despite the relatively small cohort, the structural changes were prominent in our data, which warrant further investigation with a larger cohort and a longitudinal design.

Funding: DoD (PD160104), NINDS (1R01NS108452), NIA (1R01AG064093), NIBIB (P41EB031771), NICHD (U54 HD079123).

Table 1: Demographics and behavioral test results from all study participants.

	Controls	PD	P
N	15	15	N/A
Sex (Female)	5	8	0.95
Age (year)	58.53±6.69	65.93±10.67	0.32
Education (year)	16.60±2.23	17.27±1.94	0.46
PD duration (year)	N/A	4.16±2.06	N/A
UPSIT	32.5±3.9	19.8±9.4	<0.01

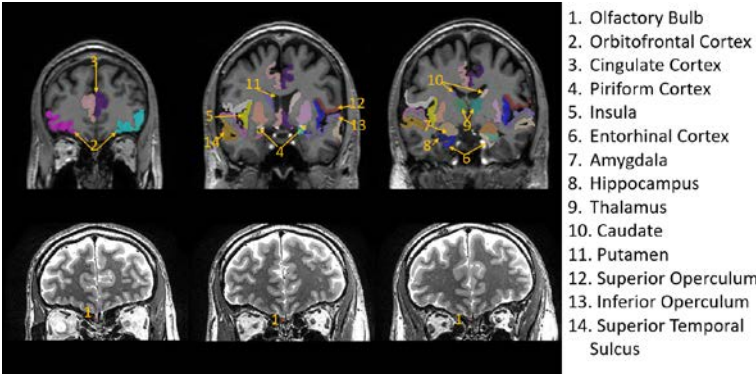


Figure 2. Group comparison of the volumes of the OB, primary (piriform) and secondary olfactory regions in the brains of early PD patients (n = 15) and controls (n = 15). Ln (Volume) was used for display due to the large difference in volumes from different regions. *: P < 0.05; **: P < 0.01.

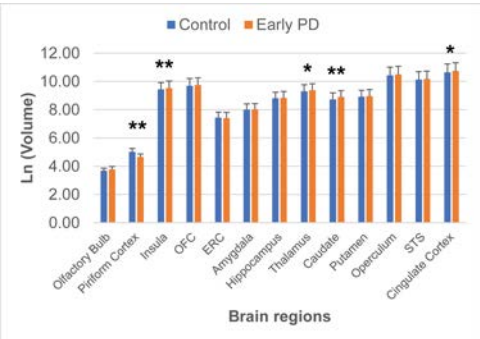
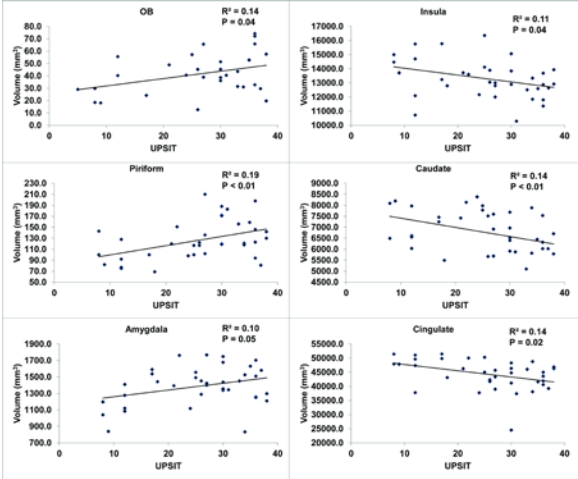


Figure 3. Correlation between volumes of different brain regions and the behavioral olfactory test scores (UPSIT) in all participants.



Structural abnormalities in olfactory brain regions in early Parkinson's disease (PD) patients measured by high resolution MRI at 7T

Adrian G. Paez,^{1,2} Suraj Rajan,^{3,4} Alex Y. Pantelyat,³ Liana I. Rosenthal,³ Andreia Faria,² Xinyuan Miao,^{1,2} Ted M. Dawson,³ Peter C. M. van Zijl,^{1,2} Vidyulata Kamath,⁴ Jun Hua^{1,2}

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INTRODUCTION: Parkinson's disease (PD) is a neurodegenerative disorder characterized by widespread impairments in motor and cognitive/behavioral functions. Olfactory deficits in PD are prevalent and reportedly present in up to 80-90% of sporadic PD cases(1-5). Such deficits can precede the onset of motor symptoms(6-8), and may be one of the earliest symptoms in PD(9). Postmortem pathological studies have demonstrated that Lewy bodies appear in the olfactory bulb (Braak Stage 1) before spreading to lower brainstem nuclei and the amygdala (Braak stages 2–3), and eventually substantia nigra and other midbrain regions (Braak stage 4: clinical disease stage), when motor functions become clearly affected(10). Olfactory loss has been linked to α -synuclein pathology, a major pathological hallmark of PD(11). Therefore, non-invasive and repeatable *in vivo* measures associated with olfactory deficits may be excellent candidates as quantitative *in vivo* biomarkers in early PD for tracking disease progression and evaluating treatment outcomes. In the human olfactory system, odorants are received by olfactory sensory neurons in the nasal epithelium,(12) which project first to glomeruli within the olfactory bulbs (OB) and then to piriform cortex (PC), also known as the primary olfactory cortex. Subsequently, neuronal networks in the orbitofrontal cortex (OFC), insular cortex, hippocampus, amygdala, and hypothalamus are involved to translate external odor information into behavioral responses(13). These regions are often referred to as secondary olfactory regions. Structural changes in the OB, PC and secondary olfactory regions have been reported in PD. In this study, high resolution structural MRI scans were performed in early PD patients and matched controls to systemically investigate structural abnormalities in the primary and secondary olfactory regions. The experiments were conducted on ultra-high magnetic field (7.0 Tesla or 7T) human MRI scanner to enhance the sensitivity. The relationship between MRI and behavioral olfactory measures was investigated.

METHODS: All MRI scans were performed on a 7T Philips MRI scanner. A cohort of 15 PD patients, and 15 healthy controls matched in age, sex, and education level were recruited for the study (**Table 1**). The University of Pennsylvania Smell Identification Test (UPSIT) (14,15) was administered to all participants to assess their behavioral olfactory functions. The following MRI scans were performed for each participant: 1) MPRAGE (voxel=1mm isotropic, TR/TE/TI=5ms/1.81ms/563ms); 2) T2-weighted scan (voxel=0.5mm isotropic, TR/TE=5000ms/58ms). Due to its small size and unique contrast, the OB was manually traced on the T2-weighted images. Thirteen additional regions of interest (ROI) in the cortex and subcortex were manually identified and segmented on the MPRAGE images. **Figure 1** shows an example of the segmentation from one participant. Volumes of individual ROI were calculated using a custom Matlab script. Two-sample t-tests were performed to examine group difference in the volumes of individual ROI. Age and sex were accounted for as covariates. Correlations between volumes of ROIs and UPSIT scores were assessed with multiple regression with age and sex as covariates. Multiple comparisons were corrected with the false-discovery rate (adjusted P < 0.05).

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UPSIT	32.5±3.9	19.8±9.4	<0.01

Figure 1: Regions-of-interest (ROI) evaluated in the study overlaid on structural images from one subject. Due to its small size and unique contrast, the olfactory bulb (#1) was traced on the T2-weighted images (bottom row). All the other ROIs were identified and segmented on MPRAGE images (top row). Note that only six coronal slices were provided to illustrate the locations of the ROIs, but many ROIs can cover more slices. Most olfactory regions are best viewed in the coronal plane.

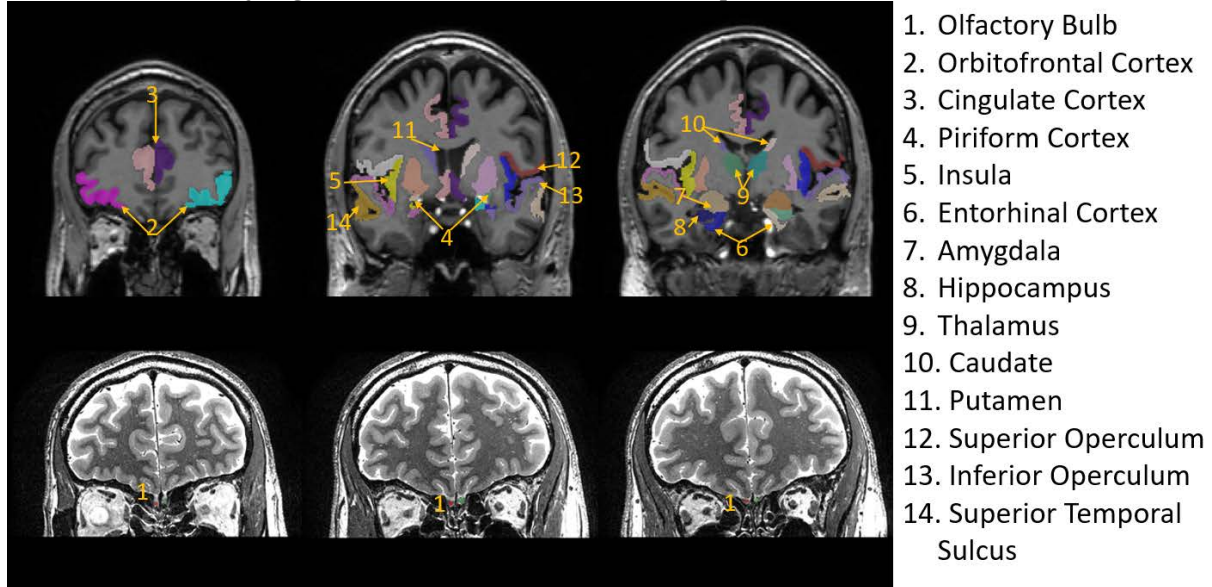


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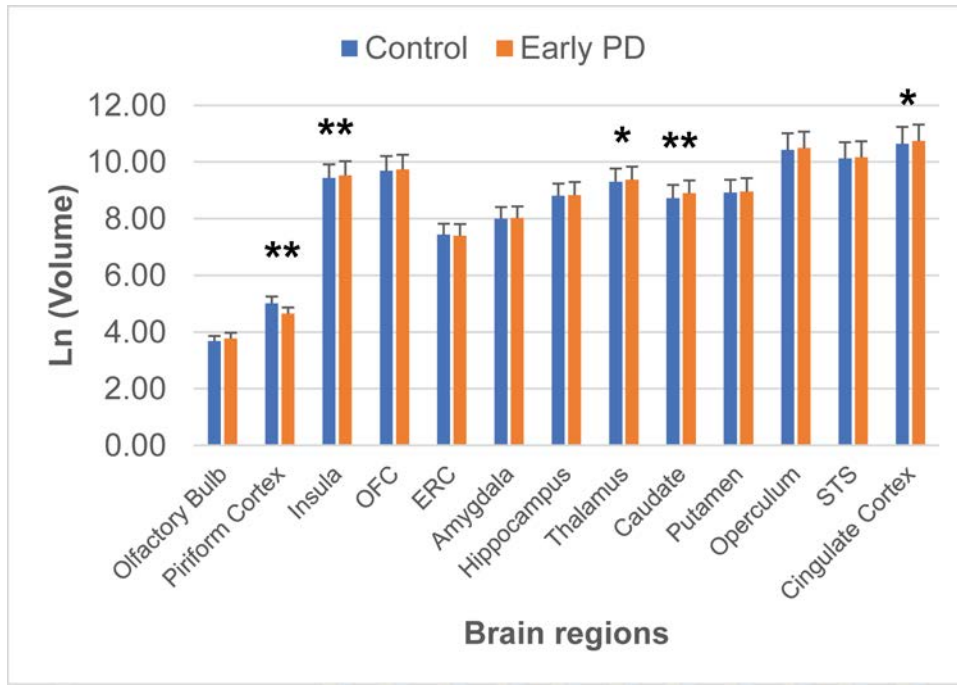
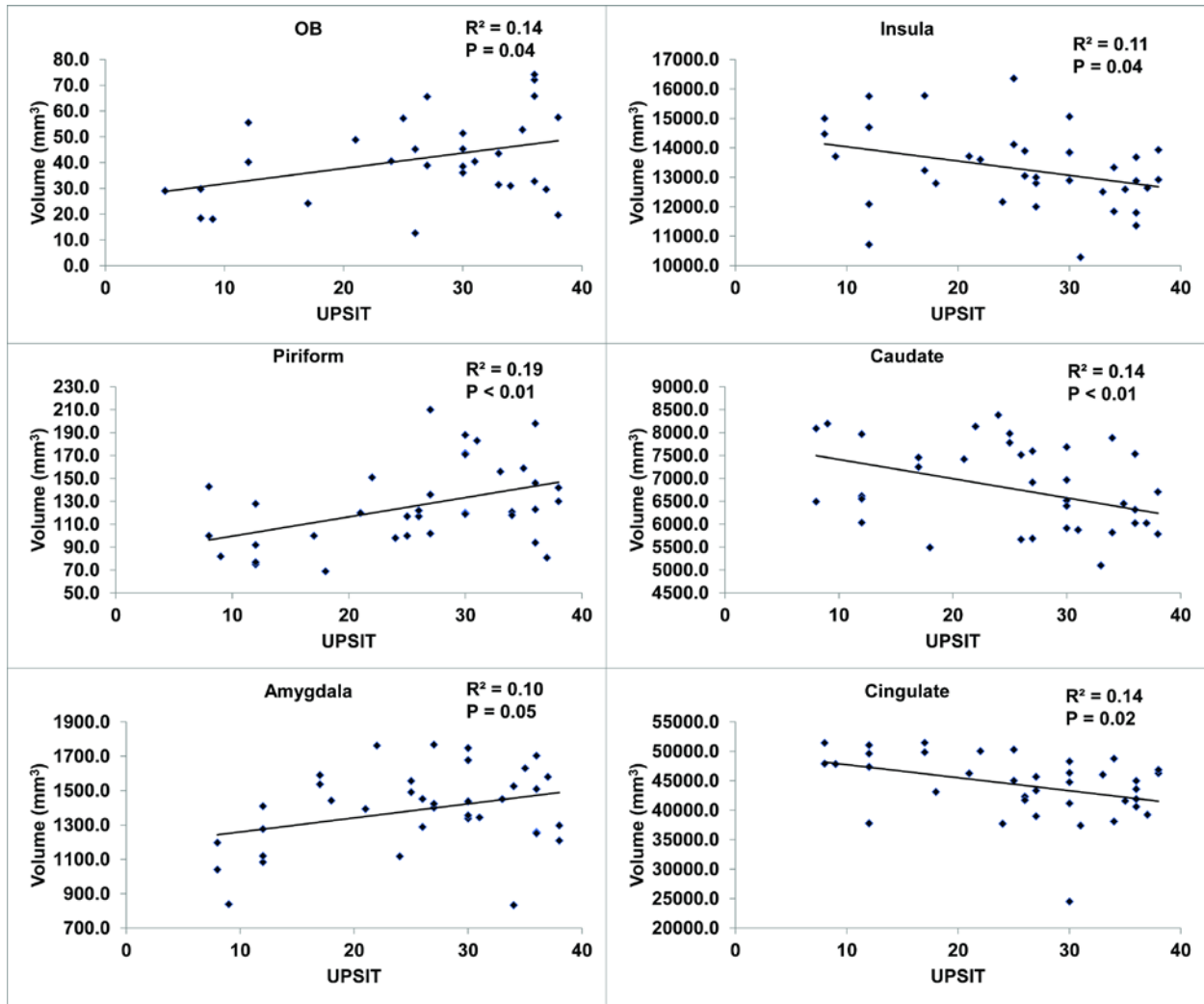


Figure 3. Correlation between volumes of different brain regions and the behavioral olfactory test scores (UPSIT) in all participants.



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Volumetric changes in olfactory regions in Parkinson's disease measured by high-resolution 7T MRI

Poster No:

2566

Submission Type:

Abstract Submission

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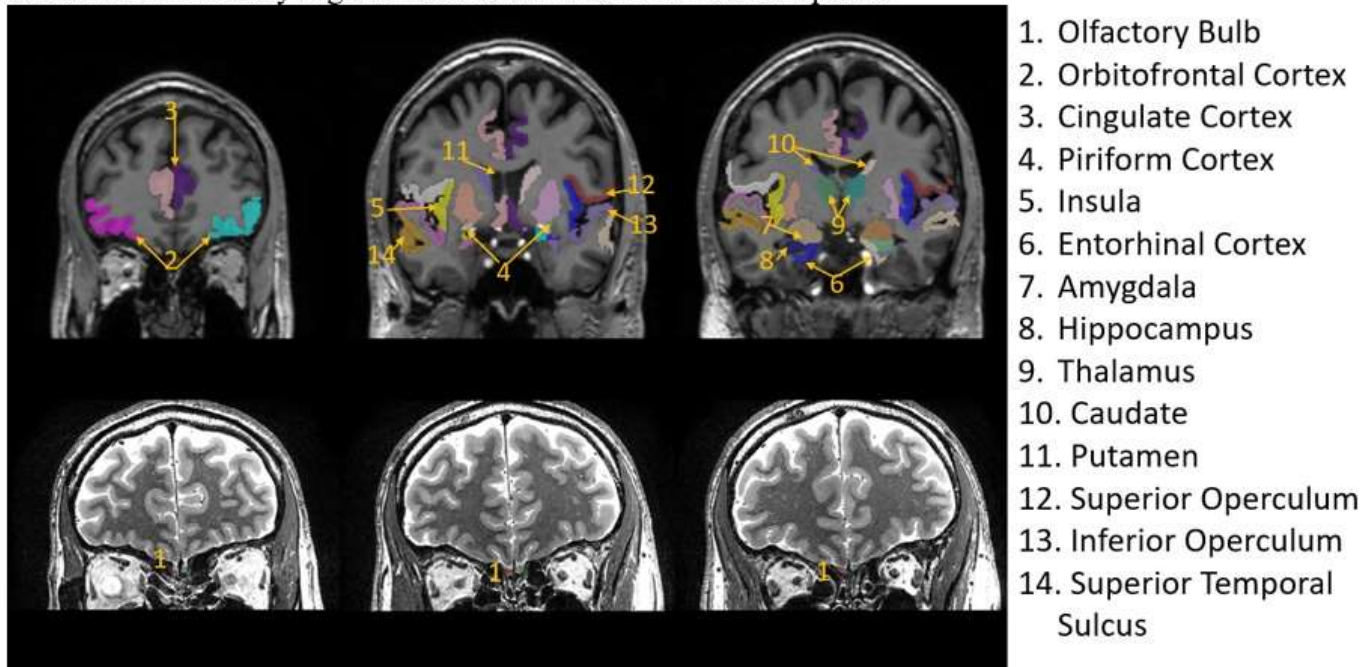
Introduction:

Parkinson's disease (PD) is a neurodegenerative disorder characterized by widespread impairments in motor and cognitive/behavioral functions. Olfactory deficits in PD are prevalent and reportedly present in up to 80-90% of sporadic PD cases¹⁻⁴. Such deficits can precede the onset of motor symptoms⁵⁻⁷, and may be one of the earliest symptoms in PD⁸. Postmortem pathological studies have demonstrated that Lewy bodies appear in the olfactory bulb (Braak Stage 1) before spreading to lower brainstem nuclei and the amygdala (Braak stages 2–3), and eventually substantia nigra and other midbrain regions (Braak stage 4: clinical disease stage), when motor functions become clearly affected⁹. Olfactory loss has been linked to α -synuclein pathology, a major pathological hallmark of PD¹⁰. Therefore, non-invasive and repeatable in vivo measures associated with olfactory deficits may be excellent candidates as quantitative in vivo biomarkers in early PD for tracking disease progression and evaluating treatment outcomes. In the human olfactory system, odorants are received by olfactory sensory neurons in the nasal epithelium, which project first to glomeruli within the olfactory bulbs (OB) and then to piriform cortex (PC), also known as the primary olfactory cortex. Subsequently, neuronal networks in the orbitofrontal cortex (OFC), insular cortex, hippocampus, amygdala, and hypothalamus are involved to translate external odor information into behavioral responses. These regions are often referred to as secondary olfactory regions. Structural changes in the OB, PC and secondary olfactory regions have been reported in PD. In this study, high resolution structural MRI scans were performed in early PD patients and matched controls to systemically investigate structural abnormalities in the primary and secondary olfactory regions.

Methods:

A cohort of 15 PD patients, and 15 healthy controls matched in age and sex were studied on a 7T Philips MRI scanner. The University of Pennsylvania Smell Identification Test (UPSIT) was administered to assess behavioral olfactory functions. The following MRI scans were performed for each participant: 1) MPRAGE (voxel=1mm isotropic, TR/TE/TI=5ms/1.81ms/563ms); 2) T2-weighted scan (voxel=0.5mm isotropic, TR/TE=5000ms/58ms). Fourteen regions of interest (ROI) were manually identified and segmented on the structural images. **Figure 1** shows an example of the segmentation from one participant. Volumes of individual ROI were calculated using a custom Matlab script. Two-sample t-tests were performed to examine group difference in the ROI volumes, and correlations between ROI volumes and UPSIT scores were assessed with multiple regression with age and sex as covariates. Multiple comparisons were corrected with the false-discovery rate.

Figure 1: Regions-of-interest (ROI) evaluated in the study overlaid on structural images from one subject. Due to its small size and unique contrast, the olfactory bulb (#1) was traced on the T2-weighted images (bottom row). All the other ROIs were identified and segmented on MPRAGE images (top row). Note that only six coronal slices were provided to illustrate the locations of the ROIs, but many ROIs can cover more slices. Most olfactory regions are best viewed in the coronal plane.

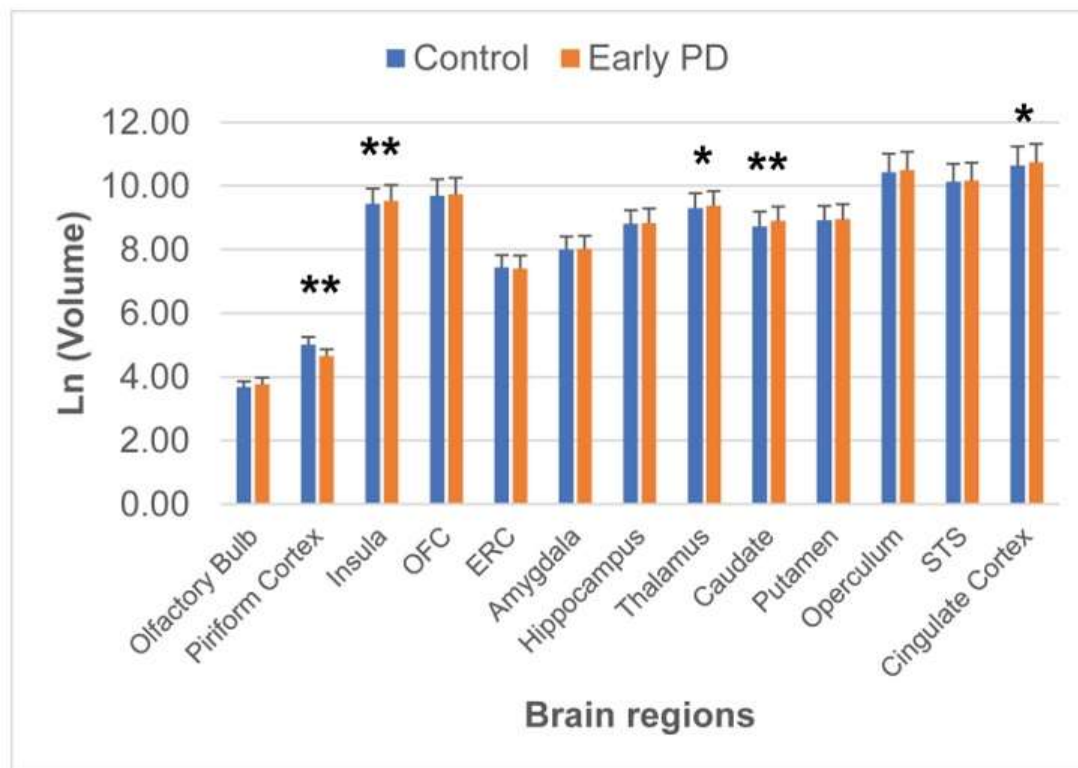


(https://files.aievolution.com/prd/hbm2201/abstracts/abs_2397/Figure1.jpg)

Results:

The behavioral olfactory test (UPSIT) scores were significantly lower in early PD patients compared to controls, indicating impaired olfactory function in PD patients. In the group comparison (**Figure 2**), the volume of OB did not show significant difference between PD and controls. The volumes of the primary olfactory (piriform) cortex were significantly lower in early PD patients. In contrast, the volumes of insula, thalamus, caudate and cingulate cortex were significantly higher in PD patients. In the correlation analysis between region volumes and the UPSIT scores, significant positive correlations in the OB, piriform cortex and amygdala, and significant negative correlations in the insula, caudate and cingulate cortex were observed (data not shown).

Figure 2. Group comparison of the volumes of the OB, primary (piriform) and secondary olfactory regions in the brains of early PD patients (n = 15) and controls (n = 15). Ln (Volume) was used for display due to the large difference in volumes from different regions. *: P < 0.05; **: P < 0.01.



(https://files.aievolution.com/prd/hbm2201/abstracts/abs_2397/Figure2.jpg)

Conclusions:

We demonstrate significant structural abnormalities in the OB, primary and secondary olfactory brain regions in early PD patients measured by high resolution MRI at 7T. Despite the relatively small cohort, the structural changes were prominent in our data, which warrant further investigation with a larger cohort and a longitudinal design.

Disorders of the Nervous System:

Neurodegenerative/ Late Life (eg. Parkinson's, Alzheimer's) ¹

Modeling and Analysis Methods:

Segmentation and Parcellation

Novel Imaging Acquisition Methods:

Anatomical MRI ²

Perception, Attention and Motor Behavior:

Chemical Senses: Olfaction, Taste

Keywords:

Cortex

Degenerative Disease

HIGH FIELD MR
MRI
Segmentation
Smell
STRUCTURAL MRI

^{1|2}Indicates the priority used for review

My abstract is being submitted as a Software Demonstration.

No

Please indicate below if your study was a "resting state" or "task-activation" study.

Other

Healthy subjects only or patients (note that patient studies may also involve healthy subjects):

Patients

Are you Internal Review Board (IRB) certified? Please note: Failure to have IRB, if applicable will lead to automatic rejection of abstract.

Yes

Was any human subjects research approved by the relevant Institutional Review Board or ethics panel? NOTE: Any human subjects studies without IRB approval will be automatically rejected.

Yes

Was any animal research approved by the relevant IACUC or other animal research panel? NOTE: Any animal studies without IACUC approval will be automatically rejected.

Not applicable

Please indicate which methods were used in your research:

Structural MRI
Neuropsychological testing

For human MRI, what field strength scanner do you use?

7T

Provide references using author date format

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