INTRODUCTION

In order to keep up with the demands of a changing environment, our brains adapt quickly and efficiently. This is best demonstrated by gray and white matter structure changes in brain regions associated with practicing specific motor or cognitive skills. Such findings have been reported by many cross-sectional studies done over the past decade comparing trained experts to non-experts. Now termed as experience-dependent plasticity, the process is thought to be active throughout our lives (1, 2).

For instance, various voxel-based morphometry (VBM) studies comparing brains of musicians to non-musicians found increased gray matter density (GMD) in several brain regions in musicians, including the cerebellum, auditory, and motor cortices (3, 4). Skilled golfers exhibited significant gray matter density increases in left ventral premotor cortex, anterior cingulate cortex, and the supplementary eye field. We posit that the identified regions might be associated with cognitive and motor processes related to flying, such as joystick control, visuo-vestibular interaction, and oculomotor control.

**Keywords:** voxel-based morphometry (VBM), experience-dependent plasticity, anterior cingulate cortex (ACC, RCZa), ventral premotor cortex (PMv), supplementary eye field, vestibulo-ocular reflex, vestibular habituation

Glider flying is a unique skill that requires pilots to control an aircraft at high speeds in three dimensions and amidst frequent full-body rotations. In the present study, we investigated the neural correlates of flying a glider using voxel-based morphometry. The comparison between gray matter densities of 15 glider pilots and a control group of 15 non-pilots exhibited significant gray matter density increases in left ventral premotor cortex, anterior cingulate cortex, and the supplementary eye field. We posit that the identified regions might be associated with cognitive and motor processes related to flying, such as joystick control, visuo-vestibular interaction, and oculomotor control.

**Keywords:** voxel-based morphometry (VBM), experience-dependent plasticity, anterior cingulate cortex (ACC, RCZa), ventral premotor cortex (PMv), supplementary eye field, vestibulo-ocular reflex, vestibular habituation
Voxel-based morphometry is a method used to automatically analyze differences in local brain anatomy. Psychophysical tests have shown that fighter pilots have superior cognitive control as compared to non-pilots as measured by the Eriksen Flanker task. The same study also found differences in white matter radial diffusivity (derived from diffusion weighted imaging) between fighter pilots and non-pilots in the right dorsomedial frontal region and parietal lobe. These studies predict that compared to non-pilots, pilots may have changes in GMD in brain regions related to vestibular habituation, motor learning, sensorimotor integration, and cognitive control. Additionally, the results of the flight simulator fMRI studies suggest that these brain regions include but are not limited to the ventral premotor cortices, inferior parietal lobule, supplementary motor area, and few areas in occipital and temporal lobe.

In the present study, we wanted to investigate the structural correlates of flying a glider by analyzing gray matter differences between glider pilots and non-pilots using VBM. Unlike the previous study done to detect changes in white matter structure between fighter pilots and non-pilots, we looked at gray matter and did not use any masks to restrict our search.

**MATERIALS AND METHODS**

**ETHICS STATEMENT**
All subjects gave written informed consent for experimental procedures approved by the ATR Human Subject Review Committee in accordance with the principles expressed in the Declaration of Helsinki.

**SUBJECTS**
Thirty right-handed subjects participated in this study. The hand- edness of the subjects was determined using a questionnaire based on Edinburgh Handedness Inventory. Fifteen of the subjects were glider pilots recruited from nearby gliding clubs. The pilots were all well experienced with a mean in-air flight experience of 34.2057 h (SE 5.35), where an average glider flight lasts 10–15 min. All pilots reported using their right hand to control the joystick. All subjects in the control non-pilot group had experience with driving or flying related video games. Age and sex between the two groups was balanced. Pilots had a mean age of 22.4 years (SE 2.13) while the control non-pilot group had a mean age of 22.4 years (SE 0.49). Age effects were also controlled for by including age as a confounding regressor in the statistical model. There were 13 males and 2 females in both the pilot and control groups. All subjects were Japanese, came from similar educational and socioeconomic backgrounds, and had no history of neurological, head trauma, or psychiatric disorders.

**IMAGE ACQUISITION**
High-resolution anatomical scans were acquired with T1 weighting (TE = 3.06 ms, TR = 2.25 s, matrix size = 256 × 256, voxel size = 1 mm × 1 mm × 1 mm) were acquired on a Siemens Trio 3 T scanner at the ATR Brain Activity Imaging Center.

**VOXEL-BASED MORPHOMETRY ANALYSIS**
Voxel-based morphometry is a method used to automatically analyze differences in local brain anatomy. T1 weighted structural MR images were used as an input to the VBM pipeline. All T1 images were processed using SPM8 (Wellcome Department of Cognitive Neurology, UCL), running under MATLAB 7.13 on a Linux platform (The Mathworks, Natick, MA, USA). VBM was performed using the VBM extension present in SPM8. The preprocessing involved following steps:

1. After checking raw images for artifacts and setting the origin to Anterior Commissure (AC), they were segmented into GM, WM, and CSF using unified segmentation (18) (New Segment toolbox in SPM8).
2. All images were then warped to a study specific template created using the DARTEL registration algorithm (19) in SPM8.
3. To preserve the original GM volume, flow fields generated by DARTEL in the previous step were combined to generate Jacobian scaled GM images.
4. Warped and Jacobian scaled images were transformed to Montreal Neurological Institute (MNI) space and smoothed by a Gaussian kernel of 8 mm FWHM. The smoothed images were then used for statistical analysis.

General linear model as implemented in SPM8 was used for all statistical analysis. Differences in GMD between the two groups were analyzed using one-way ANCOVA. Data were corrected for global brain volume by dividing each voxel by the total intracranial volume and age was added as a regressor of no interest. Voxel-wise statistical parametric maps showing differences in GMD between pilot and non-pilot groups were generated by setting the voxel level threshold at t > 4.94, p < 0.05 [corrected for multiple comparisons using false discovery rate (FDR)]. The initial localization of brain regions that were found significant was done using SPM Anatomy toolbox (20) localization was further refined based on anatomical parcelation literature as mentioned in the discussion below.

**RESULTS**
Statistical analysis showed that compared to non-pilots, pilots had significantly higher GMD in the left ventral premotor area (IPMv) and right anterior cingulate cortex (rACC) (Table 1; Figures 1A,B), p < 0.05 FDR corrected for multiple comparisons. Lowering the threshold to p < 0.0001 uncorrected, showed

| Area          | Number of voxels | Peak co-ordinate (x,y,z) | Brodmann area |
|---------------|------------------|--------------------------|---------------|
| FDR corrected (p < 0.05) |                  |                          |               |
| Left PMCv     | 63               | −58.5, 6, 3              | 6             |
| Right ACC     | 101              | 9, 42, 24                | 9             |
| Uncorrected (p < 0.0001) |              |                          |               |
| Right SEF (SEF) | 71              | 6, −10, 54               | 6             |

PMCv, ventral premotor cortex; ACC, anterior cingulate cortex; SEF, supplementary eye field.
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FIGURE 1 | Statistical parametric maps showing differences in gray matter density between pilots and non-pilots. The maps were overlaid on the MNI aligned 3D image of a selected single subject and rendered on the average brain surface provided in SPM8. (A) Right anterior cingulate cluster \( (p < 0.05 \text{ FDR corrected}, \text{cluster threshold} = 25) \). (B) Left ventral premotor cortex cluster \( (p < 0.05 \text{ FDR corrected}, \text{cluster threshold} = 25) \). (C) Right supplementary eye field \( (p < 0.0001 \text{ uncorrected}, \text{cluster threshold} = 25) \).

Another cluster in the right supplementary eye field (rSEF) within the supplementary motor area, where pilots had a higher GMD compared to the non-pilot group (Table 1; Figure 1C). No regions were found to have significantly lower GMD in pilots (uncorrected \( p < 0.0001 \)).

Individual GMD values within the pilot group extracted from peak voxels of the two significant clusters showed no significant correlation \( (p > 0.05) \) with the number of hours of in-air flight experience.

DISCUSSION

To the best of our knowledge, our study is the first to demonstrate structural differences in the gray matter of glider pilots. We show that pilots have increased GMD in regions that can all be grouped under the premotor areas of the frontal lobe, regions that influence various kinds of motor output through projections to the primary cortex and spinal cord (21). Because of the complexity of the skill and paucity of previous work on the neuroanatomical correlates of flying, it is difficult to say precisely what role these brain regions play in this particular skill. However, based on a literature review, plausible interpretations for their involvement are discussed below; the interpretations are speculative but informative.

VENTRAL PREMOTOR CORTEX

As per a recent parcelation of the lateral premotor cortex, our lPMv blob lies in the cluster corresponding to area F5 in macaque (22). In the literature, this area has been repeatedly shown to be involved in grasping and manipulation of objects, as well as conditional motor learning (23, 24). Learning-dependent activity has been shown to occur in this region as subjects acquire new visuomotor associations to manipulate a joystick (25). In the aforementioned VBM
A more relevant involvement of ACC in flying comes from a study by Ahamed et al. Glider pilots gray matter differences in the supplementary eye field (SEF) are probably involved with the abovementioned oculomotor monitoring and prediction (31, 32). Amongst all the head and full-body rotations involved in flying, pilots require a fine-tuned oculomotor control to control their eye movements so that the visual image is stable on the retina. We believe that SEF is one of the areas that fulfill this role. SEF is also reported to be involved in suppression of nystagmus, which in turn is related to vestibular habituation (9, 10). This interpretation is strengthened by the fact that the flight simulator studies, which had no vestibular component reported no functional activation in this region.

SUPPLEMENTARY EYE FIELD

The cluster found in the supplementary motor area can be localized to a specialized region called the supplementary eye field (SEF) (30). Across human and monkey studies, this region has been reported to be involved in various aspects of oculomotor control, such as learning oculomotor transformations, smooth pursuit, and cognitive control of the oculomotor system like performance monitoring and prediction (31, 32). Amongst all the head and full-body rotation involved in flying, pilots require a fine-tuned oculomotor system to control their eye movements so that the visual image is stable on the retina. We believe that SEF is one of the areas that fulfill this role. SEF is also reported to be involved in suppression of nystagmus, which in turn is related to vestibular habituation in pilots (12, 13). Further support to this interpretation comes from the fact that this area was also found active in previous flight simulation fMRI studies (9, 10). Thus, the increase in GMD in the SEF is probably involved with the abovementioned oculomotor functions crucial to flying.

Evidently, the brain regions found significant in the present study could be responsible for physiological and perceptual processes involved in flying, such as motor learning, vestibular habituation, and cognitive control. The lack of correlation between in-air flight experience and GMD of the brain structures found significant may have several reasons. The lack of a significant correlation may be explained by the fact that habituation is a fast process and by the time a pilot is good enough to fly a real glider on his own, his eyes and vestibular senses are already well habituated. An additional explanation may be that in-air flight experience in our study is not a sensitive measure of differences in individual skill. It may be the case that our sample size is not large enough to capture such small differences in skill-related experience that is thought to be reflected by greater GMD in specific cortical regions. It should be pointed out that the aforementioned study, which looked at trained fighter pilots also did not find any correlations between flying hours and white matter changes (15). To the best of our knowledge, the neural correlates of vestibular habituation are not very well known; accordingly one of the key insights of this study is the possible involvement of ACC and SEF in the process of vestibular habituation.

CONCLUSION

The results of our study show that glider pilots have increased GMD in ventral premotor cortex, anterior cingulate cortex, and supplementary eye field, which are associated with sensorimotor learning, visual–vestibular interaction, and oculomotor control, respectively. Further studies are needed to evaluate the degree to which performance of flight-related tasks can be predicted from GMD in these regions and the longitudinal pattern of the changes.

ACKNOWLEDGMENTS

We would like to thank fMRI and MEG technicians Yasushi Shimada, Ichiro Fujimoto, Hiroaki Mano, and Hironori Nishimoto at the Brain Activity Imaging center at ATR as well as Yuka Furukawa for assisting in running the experiments. For assistance in recruiting pilots for this experiment, we would like to thank Yasushi Morikawa, Erika Matsumoto, and the Ohno Glider Club. We would also like to thank Ben Seymour for his feedback on the initial drafts of the manuscript. This research was supported in part by a contract with the National Institute of Information and Communications Technology, Japan, entitled, “Multimodal integration for brain imaging measurements,” by KAKENIH, Grant-in-Aid for Scientific Research(C) (21500321), and by a contract with the Ministry of Internal Affairs and Communications entitled, “Novel and innovative R&D making use of brain structures.”

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Conflict of Interest Statement: The 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.

Received: 30 April 2014; accepted: 12 November 2014; published online: 28 November 2014.

Citation: Ahamed T, Kawanabe M, Ishii S and Callan DE (2014) Structural differences in gray matter between glider pilots and non-pilots. A voxel-based morphometry study. Front. Neurol. 5:248. doi:10.3389/fneur.2014.00248

This article was submitted to Sports Neurology, a section of the journal Frontiers in Neurology.

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