Structural neural connectivity of the vestibular nuclei in the human brain: a diffusion tensor imaging study

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Abstract
Many animal studies have reported on the neural connectivity of the vestibular nuclei (VN). However, little is reported on the structural neural connectivity of the VN in the human brain. In this study, we attempted to investigate the structural neural connectivity of the VN in 37 healthy subjects using diffusion tensor tractography. A seed region of interest was placed on the isolated VN using probabilistic diffusion tensor tractography. Connectivity was defined as the incidence of connection between the VN and each brain region. The VN showed 100% connectivity with the cerebellum, thalamus, oculomotor nucleus, trochlear nucleus, abducens nucleus, and reticular formation, irrespective of thresholds. At the threshold of 5 streamlines, the VN showed connectivity with the primary motor cortex (95.9%), primary somatosensory cortex (90.5%), premotor cortex (87.8%), hypothalamus (86.5%), posterior parietal cortex (75.7%), lateral prefrontal cortex (70.3%), ventromedial prefrontal cortex (51.4%), and orbitofrontal cortex (40.5%), respectively. These results suggest that the VN showed high connectivity with the cerebellum, thalamus, oculomotor nucleus, trochlear nucleus, abducens nucleus, and reticular formation, which are the brain regions related to the functions of the VN, including equilibrium, control of eye movements, conscious perception of movement, and spatial orientation.

Key Words: nerve regeneration; vestibular nuclei; neural connectivity; diffusion tensor tractography; cerebellum; oculomotor nucleus; neural regeneration

Introduction

The vestibular nuclei (VN), located at the pons and medulla oblongata, consists of four subnuclei: superior nucleus, inferior nucleus, medial nucleus, and lateral nucleus (Barmack, 2003; Haines, 2006; Augustine, 2008; Siegel et al., 2010). It receives and carries various types of sensory information, including eye movement, direction or speed of head movement, and body orientation in space to control the movements (Barmack, 2003; Haines, 2006; Augustine, 2008; Siegel et al., 2010; zu Eulenburg et al., 2012). The VN is known to have neural connection with various brain regions including the cerebellum, thalamus, cerebral cortex, oculomotor nucleus, trochlear nucleus, abducens nucleus, insula, and hypothalamus (Henkel and Martin, 1977; Montgomery, 1988; Akbarian et al., 1994; Shiroyama et al., 1999; Barmack, 2003; Horowitz et al., 2005; Haines, 2006; Augustine, 2008; Markia et al., 2008; Siegel et al., 2010; Kirsch et al., 2016). The connected brain regions and their relation to functions can be summarized as follows: cerebellum - equilibrium; oculomotor nucleus - control of eye movements; thalamus and cerebral cortex - conscious perception of movement and spatial orientation (Barmack, 2003; Haines, 2006; Augustine, 2008; Siegel et al., 2010; zu Eulenburg et al., 2012; Hitier et al., 2014). As a result, an injury to VN can result in vertigo and sensorimotor dysfunction, including loss of equilibrium, poor perception of body movement and eye movement (Barmack, 2003; Haines, 2006; Augustine, 2008; Siegel et al.,

2010; Hitier et al., 2014).

Electrophysiologic and tracer techniques have been used in many animal studies reporting on the neural connectivity between the VN and various brain regions (Henkel and Martin, 1977; Montgomery, 1988; Faugier-Grimaud and Ventre, 1989; Barmack et al., 1993; Akbarian et al., 1994; Shiroyama et al., 1999; Horowitz et al., 2005; Markia et al., 2008). However, these techniques are limited to application in the live human brain, and the connectivity of the VN in the human brain has not been clearly elucidated. Recently developed diffusion tensor tractography (DTT), derived from diffusion tensor imaging (DTI), is a technique used to reveal the structural neural connectivity in three-dimensional visualization by detection of the translational displacement of water molecules (Parker and Alexander, 2005; Behrens et al., 2007). In particular, the probabilistic tracking method enables estimation of local uncertainty in fiber orientation of each voxel (Parker and Alexander, 2005; Behrens et al., 2007). In other words, it considers the distribution of underlying fiber structure. Accordingly, probabilistic DTI tractography has been widely used to investigate the neural connectivity between neural structures in the human brain, including the amygdala, lateral geniculate body and red nucleus (Muthusamy et al., 2007; Nucifora et al., 2007; Jang and Kwon, 2014; Kwon and Jang, 2014). However, few studies are reported on the structural neural connectivity of the VN in the human brain (Kirsch et al., 2016). In this study, we attempted to investi-
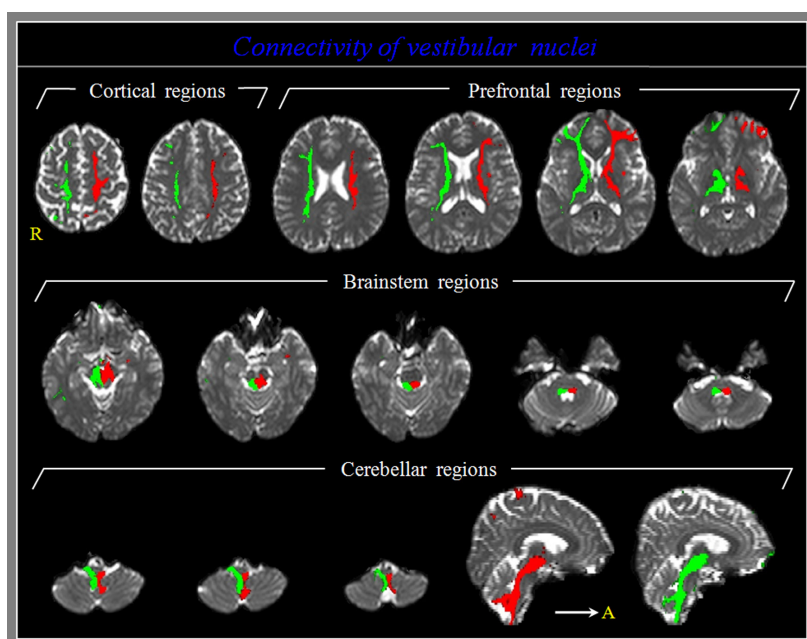
Figure 1 Diffusion tensor tractography for the structural neural connectivity of the vestibular nuclei in a healthy participant.
Cortical regions: Primary motor cortex, primary somatosensory cortex, premotor cortex, posterior parietal cortex; prefrontal regions: lateral prefrontal cortex, ventromedial prefrontal cortex, orbitofrontal cortex, thalamus; brainstem regions: hypothalamus, oculomotor nucleus, trochlear nucleus, abducens nucleus, reticular formation; cerebellar region: cerebellum. Colors: Frequency of connectivity. R: right; A: anterior.

gate the structural neural connectivity of the VN in normal subjects using DTT.

Participants and Methods

Participants
Thirty-seven healthy participants (22 males and 15 females; mean age of 37.8 years (range 20–56 years) with no previous history of neurological, physical, or psychiatric illness were included in this study *via* bulletin board notices. All participants understood the purpose of this study and provided written informed consent prior to participation and the study protocol was approved by the Institutional Review Board of Yeungnam University Hospital, Republic of Korea (YUMC 2017-07-065-011).

Data acquisition
A 1.5 T Philips Gyroscan Intera system (Philips Ltd, Best, The Netherlands) equipped with a 6-channel head coil with a single-shot spin echo planar imaging sequence was used for acquisition of DTT data. For each of the 32 non-collinear, diffusion-sensitizing gradients, 70 contiguous slices were acquired parallel to the anterior commissure-posterior commissure line. Imaging parameters of DTT were as follows: acquisition matrix = 96×96; reconstructed to matrix = 192×192; field of view = 240×240 mm^2; repetition time = 10,398 ms; echo time = 72 ms; parallel imaging reduction factor = 2; echo-planar imaging factor = 59; b = 1000 s/mm^2; number of excitations = 1; and a slice thickness of 2.5 mm.

Probabilistic fiber tracking
The Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB) Software Library (FSL; www.fmrib.ox.ac.uk/fsl) was used to analyze diffusion-weighted imaging data. Eddy current correction was applied to correct the head motion effect and image distortion. Fiber tracking was performed using probabilistic tractography, and applied

in the default tractography option in FMRIB Diffusion Software (5000 streamline samples, 0.5 mm step lengths, curvature thresholds = 0.2) (Smith et al., 2004; Behrens et al., 2007). The fiber tracking method was used to generate 5000 streamline samples from each seed region of interest with relection of both dominant and non-dominant orientation of diffusion in each voxel in each individual (Smith et al., 2004; Behrens et al., 2007). The fiber tracking method has an advantage in the evaluation of the structural neural connectivities of neural structures. In the seed region of interest, the VN was isolated using the following adjacent structures: the reticular formation (RF, antero-medial boundary), restiform body (lateral boundary), and posterior margin of the medulla (posterior boundary) (Naidich and Duvernoy, 2009). Out of 5,000 streamline samples generated from the seed voxel, results of fiber tracking were visualized at the threshold of 5, 50, and 100 streamlines through each voxel for analysis. Connectivity represented the percentage of connection in the hemispheres of 37 healthy subjects.

Determination of connectivity between the VN and targeted brain regions
Connectivity was defined as the incidence of connection between the VN and each of the following brain regions: the primary motor cortex, premotor cortex, primary somatosensory cortex, posterior parietal cortex, lateral prefrontal cortex, ventromedial prefrontal cortex, orbitofrontal cortex, thalamus, hypothalamus, oculomotor nucleus, trochlear nucleus, abducens nucleus, and reticular formation and cerebellum.

Statistical analysis
SPSS 15.0 software (SPSS, Chicago, IL, USA) was used for statistical analysis. The chi-square test was performed to determine the difference in connectivity of the VN between the right and left hemispheres. A level of $P < 0.05$ was considered statistically significant.

Table 1 Incidence of connectivity (%) between the vestibular nuclei and targeted brain regions in healthy subjects

| Targeted brain region | Threshold (streamline) | | | | | | | | |
| | 5 | | | 10 | | | 15 | | |
	Rt	Lt	Rt/Lt average	Rt	Lt	Rt/Lt average	Rt	Lt	Rt/Lt average
Cerebellum	100	100	100	100	100	100	100	100	100
Thalamus	100	100	100	100	100	100	100	100	100
Oculomotor nucleus	100	100	100	100	100	100	100	100	100
Trochlear nucleus	100	100	100	100	100	100	100	100	100
Abducens nucleus	100	100	100	100	100	100	100	100	100
Reticular formation	100	100	100	100	100	100	100	100	100
M1	94.6	97.3	95.9	83.8	83.8	83.8	70.3	78.4	74.3
S1	89.2	91.9	90.5	67.6	70.3	68.9	64.9	64.9	64.9
PMC	83.8	91.9	87.8	54.1	51.4	52.7	40.5	40.5	40.5
Hypothalamus	83.8	89.2	86.5	64.9	64.9	64.9	54.1	54.1	54.1
PPC	78.4	73.0	75.7	27.0	27.0	27.0	24.3	21.6	23.0
lPFC	70.3	70.3	70.3	27.0	27.0	27.0	13.5	21.6	17.6
vmPFC	51.4	51.4	51.4	29.7	24.3	27.0	21.6	18.9	20.3
OFC	43.2	37.8	40.5	29.7	18.9	24.3	21.6	16.2	18.9

Rt: Right; Lt: left; M1: Primary motor cortex; S1: primary somatosensory cortex; PMC: premotor cortex; PPC: posterior parietal cortex; lPFC: lateral prefrontal cortex; vmPFC: ventromedial prefrontal cortex; OFC: orbitofrontal cortex.

Results

A summary of the structural neural connectivity of the VN is shown in **Table 1** and **Figure 1**. The VN showed 100% connectivity with the cerebellum, thalamus, oculomotor nucleus, trochlear nucleus, abducens nucleus, and reticular formation, irrespective of thresholds. In contrast, regarding the threshold of 5, 50, and 100 streamlines, the VN showed connectivity with the primary motor cortex (95.9%, 83.8%, and 74.3%), primary somatosensory cortex (90.5%, 68.9%, and 64.9%), premotor cortex (87.8%, 52.7%, and 40.5%), hypothalamus (86.5%, 64.9%, and 54.1%), posterior parietal cortex (75.7%, 27.0%, and 23.0%), lateral prefrontal cortex (70.3%, 27.0%, and 17.6%), ventromedial prefrontal cortex (51.4%, 27.0%, and 20.3%), and orbitofrontal cortex (40.5%, 24.3%, and 18.9%), respectively. In all targeted brain regions, no significant difference in connectivity of the VN was observed between the right and left hemispheres ($P > 0.05$).

Discussion

In this study, probabilistic tracking was used to investigate the structural neural connectivity of the VN in the normal human brain. According to our findings, the VN showed 100% connectivity with brain regions (cerebellum, thalamus, oculomotor nucleus, trochlear nucleus, abducens nucleus, and reticular formation) related to the functions of the VN (equilibrium, control of eye movements, conscious perception of movement, and spatial orientation) irrespective of thresholds, as well as high connectivity (over 70%) with the sensory-motor cortex (primary motor cortex, primary somatosensory cortex, premotor cortex, and posterior parietal cortex), hypothalamus, and lateral prefrontal cortex at the threshold of 5 streamlines.

Tracer technique has been used in many animal studies reporting on the connectivity of the VN (Henkel and Martin, 1977; Montgomery, 1988; Faugier-Grimaud and Ventre, 1989; Barmack et al., 1993; Akbarian et al., 1994; Shiroyama et al., 1999; Horowitz et al., 2005; Markia et al., 2008). Using tracer technique, Henkel and Martin (1977) demonstrated connection of the major afferent fibers of the VN with the cerebellum in 300 rats and that the superior VN terminated at the ipsilateral trochlear nucleus and oculomotor nucleus. Subsequently, Akbarian et al. (1994), who reported that the VN connected the premotor and parietal cortex in five monkeys, suggested that these connectivities might affect the vestibulocular reflex. Horowitz et al. (2005) reported connection of the VN with various brain regions, including the thalamic nucleus, hypothalamus, oculomotor nucleus, and cerebellum in 24 hamsters. Markia et al. (2008) recently reported on the connectivity between the VN and paraventricular nucleus of hypothalamus using a monosynaptic retrograde tracer technique in six rats. Our results appear to be consistent with those of the previous studies.

To the best of our knowledge, only one study involving the human brain has been reported on the structural neural connectivity of the VN using DTT (Kirsch et al., 2016). Kirsch et al. (2016) examined the functional and structural connectivity between the VN and ipsilateral and contralateral parieto-insular vestibular cortex in 24 normal subjects using DTT and functional MRI. They found two ipsilateral pathways of the VN to the parieto-insular vestibular cortex, a direct pathway without the thalamus and an indirect pathway with the thalamus in either the posterolateral or paramedian nuclei, and with regard to the contralateral pathways, the VN connected with the parieto-insular vestibular cortex *via* the posterolateral thalamus (Kirsch et al., 2016). Compared to Kirsch's study, our study investigated the structural neural connectivity of the VN to almost all brain regions and adopted three different thresholds to determine the potential and tendency of connectivity between the VN and all brain regions. We believe our findings would be helpful for clinicians

who are engaged in research on neurological diseases in terms of diagnosis, treatment plan, and prognosis prediction.

In conclusion, in this study, we investigated the structural neural connectivity of the VN in normal human subjects and found that the VN showed high connectivity with brain regions related to the functions including equilibrium, eye movements, conscious perception of movement, and spatial orientation. We believe that the methods used in this study to investigate the structural neural connectivity of the VN in the live human brain, as well as the corresponding results, would provide valuable information for clinicians and researchers studying the VN. However, several limitations of this study should be considered (Parker and Alexander, 2005; Yamada et al., 2009; Fillard et al., 2011). First, although the VN is composed of four subnuclei, we found no connectivity between specific subnuclei of the VN and each brain region. Second, because DTT cannot discern the direction, the afferent and efferent fibers could not be divided between the VN and brain regions. Third, when using probabilistic DTT tractography, crossing fibers in a voxel or partial volume effect throughout the white matter of the brain can cause both false positive and negative results (Yamada et al., 2009; Fillard et al., 2011). Therefore, to overcome these limitations, in-depth studies, as well as studies regarding clinical application of our results for patients with brain injury, are encouraged.

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