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The relation between flocculus volume and tinnitus after cerebellopontine angle tumor surgery

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ABSTRACT

Purpose: Chronic tinnitus is a common symptom after cerebellopontine angle (CPA) tumor removal. Sometimes, the tinnitus is gaze-modulated. In that case, patients can change the loudness or pitch of their tinnitus by ocular movements. During tumor removal by a retrosigmoid craniotomy, the cerebellar flocculus is manipulated by the surgical approach to access the tumor. The flocculus has been associated with tinnitus in rats, and is involved in eye-gaze control. This suggests that the flocculus may have a role in gaze-modulated tinnitus after CPA tumor removal. In order to investigate this hypothesis, the relation between the flocculus volume and the characteristics of postoperative tinnitus was studied.

Results: A single-center cohort of 51 patients completed a questionnaire after CPA tumor removal. The questionnaire asked for the effect of eye movements on tinnitus and included the Tinnitus Functional Index (TFI). Tinnitus was present in 36 patients (71% of 51), of which 29 (81% of 36) described gaze-modulation. The median TFI was 22 (range 0–85). A postoperative MRI-scan of sufficient quality was available in 34 cases. The volumes of the (para)flocculi ipsilateral and contralateral to the surgery, and the ratio of these volumes were similar between patients with and without tinnitus. The TFI correlated with the volume of both ipsi- and contralateral (para)flocculus ($r_s(23) = .516$, $p = .008$ and $r_s(23) = .430$, $p = .032$). The ipsilateral-to-contralateral volume ratio of the (para)flocculi volumes was significantly lower in patients that could modulate the loudness of their tinnitus by eye gaze, compared to patients that could not ($t(23) = 3.337$, $p = .003$).

Conclusions: The lack of a relation between flocculus volumes and the presence of tinnitus, combined with the significant correlation between tinnitus severity and flocculus volumes, suggests that the flocculus may not be the primary source of tinnitus, but is likely to mediate tinnitus severity. The reduced ipsi-to-contralateral volume ratio in patients with gaze-modulated tinnitus suggests that atrophy of the flocculus on the surgery side triggers cross-modal interactions leading to modulation of tinnitus.

1. Introduction

Chronic tinnitus is often experienced after cerebellopontine angle (CPA) tumor removal. In 19–36% of cases, the tinnitus is gaze-modulated, where its perceptual characteristics are modulated by a horizontal or vertical gaze deviation from a neutral head position (Baguley et al., 2006; Biggs and Ramsden, 2002; Van Gendt et al., 2012). Sometimes, the tinnitus is only audible during lateral gaze, usually referred to as gaze-induced tinnitus (Baguley et al., 2006; Biggs and Ramsden, 2002; Cacace et al., 1994; Whittaker, 1982). Gaze-modulated tinnitus (GMT) is mainly described after vestibular schwannoma removal, after which 19% of patients report tinnitus (Baguley et al., 2006). It is reported that surgical removal of any space-occupying lesion affecting the 8th cranial nerve can lead

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Abbreviations: CPA, cerebellopontine angle; FL, flocculus; GMT, gaze-modulated tinnitus; PFL, paraflocculus; RS, retrosigmoid approach; TFI, tinnitus functional index; TL, translabyrinthine approach; UBC, unipolar brush cell; VOR, vestibulo-ocular reflex; VS, vestibular schwannoma

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to GMT (Coad et al., 2001). It does not require a complete surgical transection of the 8th cranial nerve to occur (Coad et al., 2001).

The pathophysiology of gaze-modulated tinnitus, as well as tinnitus in general, is poorly understood. One hypothesis involves neural sprouting out of the para-abducens nucleus or medial longitudinal fasciculus into the auditory pathway, possibly at a cochlear nucleus level (Baguley et al., 2006; Biggs and Ramsden, 2002; Whittaker, 1982). An alternative hypothesis assumes that auditory gain and eye movement are linked because of the unmasking of a previously inhibited pathway. The de-afferentiation associated with VS removal may disinhibit this pathway (Biggs and Ramsden, 2002). However, these hypotheses are not supported by experimental clinical observations.

Chen et al. (2017) hypothesized that the eye-movement circuitry of the paraflocculus may be involved in modulating tinnitus. The paraflocculus recently received attention with regard to the pathophysiology of tinnitus in rats. A manganese enhanced magnetic resonance imaging (MEMRI) study of rats with psycho-physical evidence of chronic tinnitus showed increased neural activity in the auditory brainstem and also the ipsilateral paraflocculus. Normal rats exposed to a tinnitus-like sound showed a similar increased activity in the auditory system, but did not show an increased activity in the paraflocculus (Brozoski et al., 2007). Because tinnitus does not always develop after acoustic trauma, it has been suggested that the paraflocculus, like limbic regions, performs a gating or regulatory role for suppression of unwanted noise. If these regions are comprised, the noise-cancellation mechanism breaks down and tinnitus occurs (Rauschecker et al., 2010). It has been suggested that the paraflocculus has a tonic inhibitory effect on hyperactivity in the inferior colliculus in animals with hearing loss, but not on spontaneous firing rates in normal hearing animals (Vogler et al., 2016). So, the paraflocculus carries out a protective role for tinnitus after hearing loss according to these studies. Contradictory, ablation of the paraflocculus eliminated chronic tinnitus in rats, but it was less successful in the prevention of de novo onset of tinnitus. Taken together, this suggests that the paraflocculus may play an important, but a non-obligatory role in tinnitus. Once established as a tinnitus generator, the paraflocculus presumably becomes a necessary component in maintaining the condition (Bauer et al., 2013a).

The flocculus and paraflocculus are manipulated during retrosigmoid removal of a CPA tumor to expose the root entry zone of the cranial nerves. This might compromise the functions of the flocculus and possibly the paraflocculus, which include involvement in the vestibulo-ocular reflex and optokinetic response, controlling saccade adaptation, pursuit adaptation and gaze-holding, vestibulo-ocular reflex adaptation, gain, up/down asymmetry and direction (Beh et al., 2016; Matsuno et al., 2016). It could be that surgical manipulation or compression by the tumor causes atrophy of the flocculus and paraflocculus. Given the roles of the flocculus in tinnitus in rats (Bauer et al., 2013a, 2013b; Brozoski et al., 2007) and in gaze control (Beh et al., 2016; Matsuno et al., 2016), we hypothesized that atrophy of the flocculus related to a CPA tumor or its removal, may lead to gaze-modulated tinnitus. In order to test this hypothesis, we conducted an explorative, retrospective study to correlate the volume of the bilateral flocculi with perceptual characteristics of tinnitus after tumor removal.

2. Methods

2.1. Patients

A single-center cohort of 70 patients with previous surgery for a unilateral CPA tumor was assessed with a written questionnaire, in which the characteristics of possible postoperative tinnitus and the Tinnitus Functional Index (TFI) were evaluated. A total of 56 completed questionnaires were returned (80% response rate), of which five were excluded (Table 1). One had a cholesteatoma instead of a CPA tumor; one did not understand what tinnitus is; and three patients underwent both RS and TL removal of the CPA tumor.

Postoperative MRI-scans were available in 42 patients. Eight out of 42 patients were excluded from further analysis because of poor-quality imaging: On 2 scans the overall image quality was insufficient; on 1 scan the flocculus and paraflocculus were not displayed completely; and on 5 scans the brainstem structures were substantially distorted, which prohibited proper identification of the (para)flocculi.

2.2. MRI

The 3D-volume of the bilateral (para)flocculus was determined for all included patients on the post-operative MRI (0.7–7.6 months after surgery). MRI was performed using a 1.5 T whole body system (Siemens, Erlangen, Germany). Imaging of the CPA region was performed including a Constructive Interference in Steady State (CISS) sequence. The CISS sequence uses a strong T2-weighted 3D gradient echo technique that produces high resolution isotropic images. The parameters of the CISS sequence are: TR 6 ms; TE 2,53 ms; slice thickness 0.7 mm; 52 slices per slab; slab thickness 120 mm; matrix 256 × 256; 1 average; bandwidth 425 Hz/Px; 4:31 total scan time. For 2 patients, the 3D T2-CISS was performed on a 3.0 T MRI system (Siemens, Erlangen, Germany). Analysis of the MRI scans was performed using iPlan 3.0 cranial software (BrainLab AG, Munich, Germany). The volume of both right and left flocculi and paraflocculi was evaluated on the axial, coronal and sagittal T2-CISS reconstructed images by authors LMM and PJL. (Fig. 1). Both were blinded to the outcomes of the tinnitus questionnaire.

No distinction could be made between the flocculus and paraflocculus on the available MRI sequence. Therefore, the combination of flocculus and paraflocculus is called (para)flocculus in this report. In 5 patients, no T2-CISS sequence was available and therefore a 3D-T1 sequence was used to evaluate the (para)flocculi. After visual evaluation of the images, the (para)flocculi were manually outlined and automatically filled to calculate the volume of the (para)flocculi. A ratio was computed by dividing the volume of the flocculus ipsilateral to surgery side by the contralateral volume.

2.3. Statistics

Variables studied were age, sex, surgery side, years since surgery, presence of current tinnitus, presence of tinnitus loudness modulation by eye gaze (i.e. GMT), TFI scores and the volume of the bilateral (para)flocculi. Descriptive statistics were calculated for each group of patients. Frequencies were calculated for sex, surgery side, current tinnitus and GMT. Median and range were calculated for age, years since surgery and the TFI scores. Mean and standard deviation were calculated for the volume of the (para)flocculi.

Independent *t*-tests were used to determine whether statistically significant differences were present in the volume of the ipsi- and contralateral (para)flocculi and the ipsi-to-contralateral ratio between being able to gaze-modulate the tinnitus and not being able to. Also, independent samples *t*-tests were used for the volume of both the ipsi- and contralateral (para)flocculi and the ipsi-to-contralateral ratio between tinnitus and no tinnitus. For every independent samples *t*-test the assumption of normality was tested by Shapiro-Wilk test and the assumption of homogeneity of variances was tested by Levene test for equality of variances. A spearman's rank-order correlation was run to assess the relationship

Table 1
Characteristics of patients.

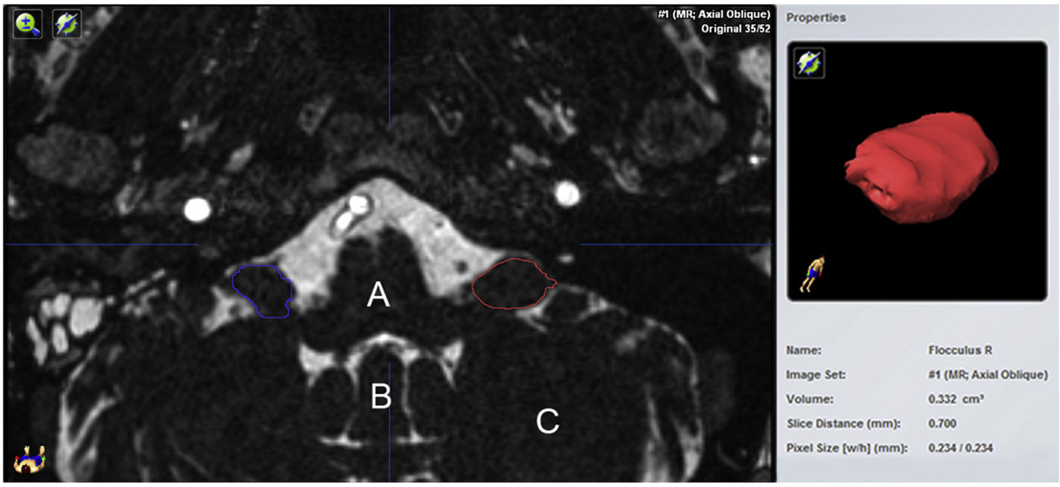
Characteristics	Values					
	All patients			Patients with post-surgery scans		
	TL (n = 11)	*RS (n = 40)*	*TL + RS (n = 51)*	*TL (n = 2)*	*RS (n = 32)*	*TL + RS (n = 34)*
Age						
Range	40-81y	27-77y	27-81y	40-70y	27-71y	27-71y
Median	64y	54.5y	57y	55y	54.5y	54.5y
Mean	62.9y	54.7y	56.5y	55y	53.8y	53.9y
Sex						
Male	7 (64%)	19 (48%)	26 (51%)	0	15 (47%)	15 (44%)
Female	4 (36%)	21 (52%)	25 (49%)	2 (100%)	17 (53%)	19 (56%)
Surgery side						
Left	8 (73%)	19 (48%)	27 (53%)	2 (100%)	16 (50%)	18 (53%)
Right	3 (27%)	21 (52%)	24 (47%)	0	16 (50%)	16 (47%)
Tumor size, long axis (mm)						
Range	7–22	35	7–46	12–13	11–46	11–46
Median	13	27	24	13	26	25
Mean	14	27	24	13	26	25
Tumor size, short axis (mm)						
Range	5–15	10–42	5–42	7–8	10–42	7–42
Median	8	20	19	8	20	20
Mean	8	22	19	8	21	21
Time since surgery						
Range	3m-6y	3m-6y	3m-6y	10m-3y	3m-6y	3m-6y
Median	3y	2y	3y	2y	2y	2y
Mean	2.9y	2.6y	2.6y	2y	2.3y	2.3y
Preoperative tinnitus						
Yes	10 (91%)	16 (40%)	26 (49%)	2	14 (44%)	16 (47%)
No	1 (9%)	24 (60%)	25 (51%)	0	18 (56%)	18 (53%)
Currently tinnitus						
Yes	10 (91%)	26 (65%)[b]	36 (71%)[b]	2	23 (72%)[c]	25 (74%)[a,c]
No	1 (9%)	14 (35%)[b]	15 (29%)[b]	0	9 (28%)[c]	9 (27%)[a,c]
	Patients with tinnitus			Tinnitus patients with scans		
	TL (n = 10)	*RS (n = 26)*	*TL + RS (n = 36)*	*TL (n = 2)*	*RS (n = 23)*	*TL + RS (n = 25)*
Current TFI score						
Range	10–85	0–71	0–85	30–40	7–71	7–71
Median	30	20	22	35	20	24
Mean	35	30	31	35	32	33
GMT						
Yes	6 (60%)	23 (88%)	29 (81%)	1 (50%)	20 (87%)	21 (84%)
No	4 (40%)	3 (12%)	7 (19%)	1 (50%)	3 (13%)	4 (16%)

Abbreviations: TL, translabyrinthine approach; RS, retrosigmoid approach, TFI, tinnitus functional index; GMT, gaze-modulated tinnitus.

[a] Because of rounding the total is 101%.

[b] Two patients had postoperative tinnitus, but it resolved.

[c] One patient had postoperative tinnitus, but it resolved.



Name: Flocculus R
Image Set: #1 (MR; Axial Oblique)
Volume: 0.332 cm³
Slice Distance (mm): 0.700
Pixel Size [w/h] (mm): 0.234 / 0.234

Fig. 1. Delimitation of the Left (blue) and right (red) (para)flocculus. A, medulla oblongata; B, inferior cerebellar vermis; C, cerebellar hemisphere.

between TFI scores and the volume of the ipsi- and contralateral (para)flocculi. A paired-samples *t*-test was used to determine whether there was a statistically significant difference between the ipsi- and contralateral volume of the (para)flocculus for both tinnitus and no tinnitus.

Data are mean ± standard deviation, unless otherwise stated. A *p* value < .05 was considered statistically significant. For all statistical analyzes, SPSS version 23 (IBM Corp. in Armonk, NY) software was used.

2.4. Ethics statement

As concluded by the Medical Ethics Committee of the University Medical Center Groningen (METc 2016/435), this study was not subjected to the Dutch Law Medical-Scientific Research with Humans (Wet Medisch-wetenschappelijk Onderzoek met Mensen, WMO). The study was conducted in accordance with the Declaration of Helsinki and applicable Dutch laws.

3. Results

3.1. Questionnaire results

Postoperative tinnitus was present in 38 patients (75%), of which 36 still have tinnitus. These 36 patients had a TFI score range of 0–85 (median 22) and 29 of these patients experienced GMT (81%).

3.2. Tinnitus v.s. no tinnitus

Within the group of patients with a sufficient scan (n = 34, 67%), (para)flocculus volumes were on average .24 ± .09 cm^3 ipsilateral to the surgery and .29 ± .07 cm^3 on the contralateral side. The ipsilateral (para)flocculus was smaller than the contralateral (para) flocculus, in both patients with and without tinnitus (with tinnitus: $t(24) = 2.543$, $p = .018$; without tinnitus: $t(8) = 2.675$, $p = .028$).

The ipsi- and contralateral (para)flocculus volumes were not significantly different between subjects with tinnitus in comparison to those without (ipsi: $t(32) = 1.131$, $p = 0.27$; contra: $t(32) = .742$, $p = 0.46$) (Fig. 2). Also, the ipsi-to-contralateral volume ratio was similar between the groups ($t(32) = .993$, $p = .33$).

3.3. The TFI-score

A scatterplot of the TFI score and the volumes of the (para) flocculi was made. This was done for both the ipsi- and contralateral (para)flocculus and the ipsi-to-contralateral volume ratio (Fig. 3).

Preliminary analysis showed the relationship of both to be monotonic, as assessed by visual inspection of both scatterplots. There appeared to be one bivariate outlier in the plot of the TFI and the ipsilateral (para)flocculus. Nonetheless, since this was no data error, it was taken into account in the analysis.

There was a positive correlation between the TFI score and the volume of the ipsilateral (para)flocculus ($r_s(23) = .516$, $p = .008$). Similarly, between the TFI score and the volume of the contralateral (para)flocculus there was also a positive correlation ($r_s(23) = .430$, $p = .032$). There was no correlation between the TFI score and the

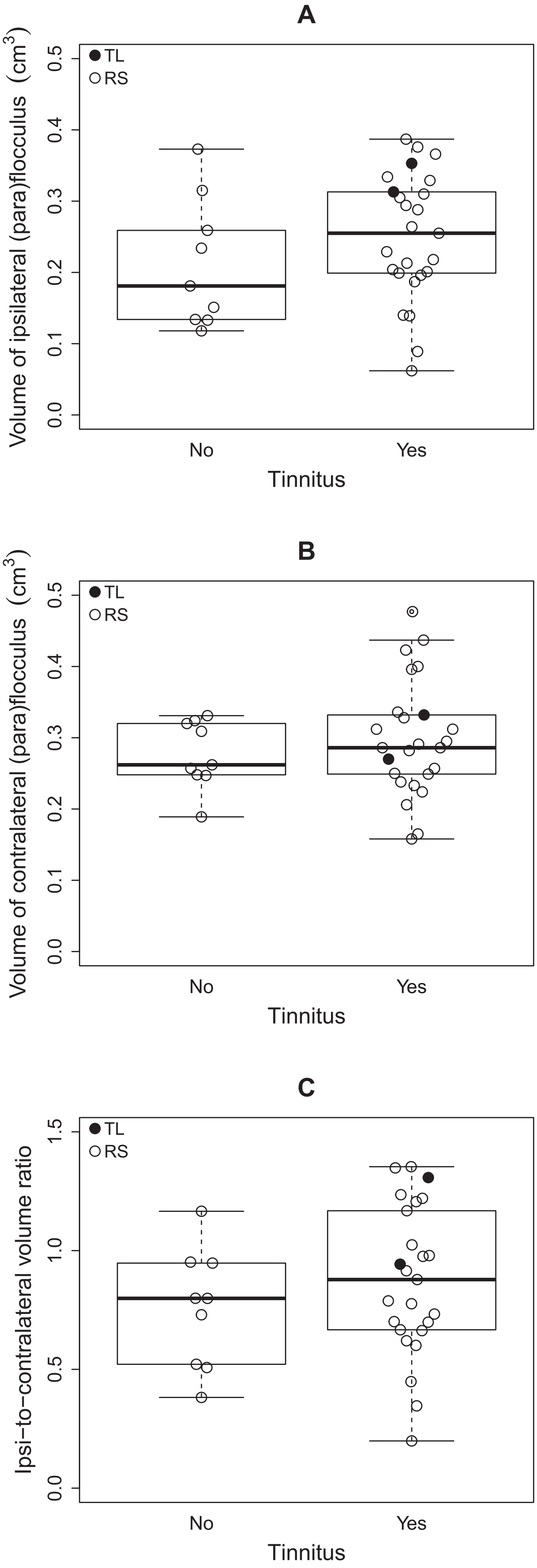


Fig. 2. Relation between the presence of tinnitus and (para)flocculus volume. (A) (para)flocculus ipsilateral to the surgery. (B) (para)flocculus contralateral to the surgery. (C) Ipsi-to-contralateral volume ratio. No difference was present in both ipsi- and contralateral (para)flocculus volumes between tinnitus and no tinnitus (ipsi: $t(32) = 1.131$, $p = 0.27$; contra: $t(32) = .742$, $p = 0.46$). Also, the ipsi-to-contralateral volume ratio was similar between the groups ($t(32) = .993$, $p = .33$).

ipsi-to-contralateral volume ratio the (para)flocculus ($r_s(23) = .241$, $p = .245$).

No relation was found for both the volume of the ipsilateral and contralateral (para)flocculus and age or gender.

Because of the positive correlation between (para)flocculus volumes and the TFI, it may be interesting to look at the TFI values of the patients whose (para)flocculi could not be delimited on the scans because of substantial distortion (2. Methods). 4 out of 5 patients had tinnitus postoperatively. In 1 the tinnitus resolved after some time and in 2 the TFI score was negligible (0 and 4). Only in 1 patient the TFI score was high (38). In this patient the (para) flocculus was at least partly visible, but we were too unsure to include it in the analysis. So, in most of these patients with much compression the TFI was very low.

3.4. GMT

GMT was present in 29 of these 36 patients with tinnitus (81%). In patients with an MRI scan, tinnitus was currently present in 25 out of 34 patients (74%) and 21 of these patients (84%) experienced GMT.

(Para)flocculus volumes were not significantly different between subjects with GMT and those without (ipsi: $t(23) = 1.466$, $p = .16$; contra $t(23) = 1.612$, $p = .12$) (Fig. 4a, b). However, the ipsi-to-contralateral volume ratio was significantly smaller in patients with GMT than in patients without ($t(23) = 3.337$, $p = .003$, Fig. 4c).

4. Discussion

The relation between tinnitus characteristics and (para)flocculus volume was investigated in patients who underwent surgery for a cerebellopontine angle tumor. Tinnitus was reported by 71% of the patients, of which 81% described their tinnitus to be modulated by lateral eye gaze (GMT, gaze-modulated tinnitus). There was no difference in (para)flocculus volumes between patients with and without tinnitus. In patients with tinnitus, the (para)flocculus volume on the surgery side and on the contralateral side showed a positive correlation with the tinnitus functionality index (TFI). The ipsilateral-to-contralateral (para)flocculus volume ratio was smaller in patients with GMT as compared to those where tinnitus was not modulated by eye gaze. To our knowledge this is the first study showing a relation between flocculus characteristics and tinnitus in humans.

The high prevalence of GMT in our patient cohort is remarkable. Other studies of GMT reported a prevalence of only 19–36% (Baguley et al., 2006; Biggs and Ramsden, 2002). This difference can be considered in the context of our hypothesis that atrophy of the flocculus may lead to gaze-modulated tinnitus. Baguley et al. and Biggs & Ramsden described patients that underwent tumor surgery via the translabyrinthine route (TL). In this surgery, the tumor is accessed via a surgical route through the vestibulum. The amount of manipulation of the flocculus is presumably related to size of the tumor to be removed. Small tumors reside mainly in the internal auditory canal, with little or no protrusion into the cerebral space (tumor classification according to Koos, classes 1–2) (Koos et al., 1998). Removal of a small tumor via the TL route presumably involves no or minimal manipulation of the flocculus. Larger tumors (Koos classification 3–4) extend into the intracranial space with

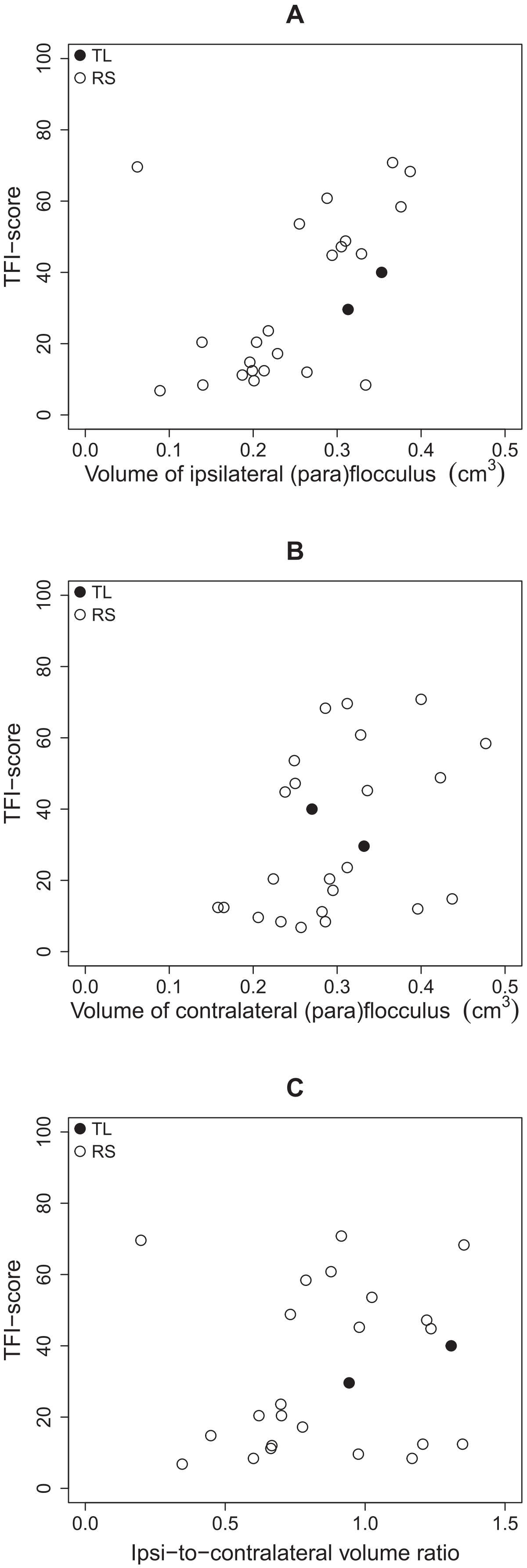


Fig. 3. Relation between the TFI-score and (para)flocculus volume. (A) (para)flocculus ipsilateral to the surgery. (B) (para)flocculus contralateral to the surgery. (C) Ipsi-to-contralateral volume ratio. There was a positive correlation between the TFI score and the volume of both the ipsi- and contralateral (para)flocculus (ipsi: $r_s(23) = .516$, $p = .008$; contra: $r_s(23) = .430$, $p = .032$). There was no correlation between TFI-score and the ipsi-to-contralateral volume ratio ($r_s(23) = .241$, $p = .245$).

possible compression of the cerebellar structures. Removal of a larger tumor via the TL route will involve manipulation of the flocculus. The subjects described by Biggs & Ramsden had tumor sizes ranging from 0.11 to 4.5 cm. Thus, the amount a flocculus manipulation must have varied between subjects, ranging from none in those with small tumors to substantial in those with larger tumors.

In contrast to the patients described by Biggs and Ramsden and by Baguley et al., most of our patients underwent a retrosigmoid surgical (RS) approach, where the tumor is accessed via a route posterior to the inner ear. This approach leaves the vestibulum and cochlea intact. However, it nearly always requires manipulation of the flocculus which may cover the tumor and vestibulocochlear nerve. In order to access the tumor, the flocculus needs to be pushed aside. The difference in prevalence of gaze-modulated tinnitus between patients who underwent TL (Baguley, Biggs and Ramsden) and RS (this work) surgery, suggest that the amount of manipulation of the flocculus during the surgery contributes to the development of post-surgical GMT.

Assuming that flocculus volume is related to the functional properties of the flocculus, our results suggest that the flocculus is probably not responsible for the presence of tinnitus, but plays a role in mediating tinnitus severity. This may correspond to the finding in an animal model of tinnitus, where the effectiveness of flocculus ablations to remove tinnitus depends on details of the experimental protocol (Bauer et al., 2013a): tinnitus was only removed if flocculus ablation occurred after tinnitus induction (rather than before inducting tinnitus). Apparently, the flocculus is not the primary generator of tinnitus, but may modulate existing tinnitus.

Besides possible auditory functions of the flocculus and paraflocculus (Azizi et al., 1985; Gacek, 1973; Morest et al., 1997), these structures are primarily involved in eye movement control (Beh et al., 2016; Matsuno et al., 2016). The flocculus is crucial to vestibulo-ocular reflex (VOR) gain and direction (Beh et al., 2016). The flocculus receives the primary and secondary vestibular afferents as mossy fibers and sends Purkinje cell axons to VOR relay neurons (Ito, 2006). Purkinje cells in the flocculus directly inhibit relay neurons of the VOR in vestibular nuclei and a lesion in the flocculus or inferior olive abolishes adaptive gain changes in the VOR (Fukuda et al., 1972; Ito, 2006). Moreover, the flocculus/paraflocculus optimizes the performance of the neural integrator to maintain eccentric gaze stability (Beh et al., 2016). In patients with large CPA tumors extensive oculomotor abnormalities occur, probably because of compression of the flocculus. Gaze nystagmus (beating to the side of deviation) was the most common oculomotor abnormality (Nedzelski, 1983).

In our patient cohort, the volume ratio of the flocculus on the ipsilateral surgery side and the contralateral non-surgical side, was smaller in patients with GMT as compared to those where tinnitus was not-modulated by gaze. This difference was statistically significant despite the unexpectedly small number of patients with non-modulated tinnitus. In a normal population, the paraflocculus and flocculus, respectively, have similar volumes on both sides of the brain (Tagliavini and Pietrini, 1984). Hence, the smaller volume ratios in our GMT patients likely represent atrophy caused by the tumor or the surgery. The correspondence between small volume ratios and GMT, support our primary hypothesis that atrophy of the flocculus leads to GMT. This suggests that atrophy of the flocculus

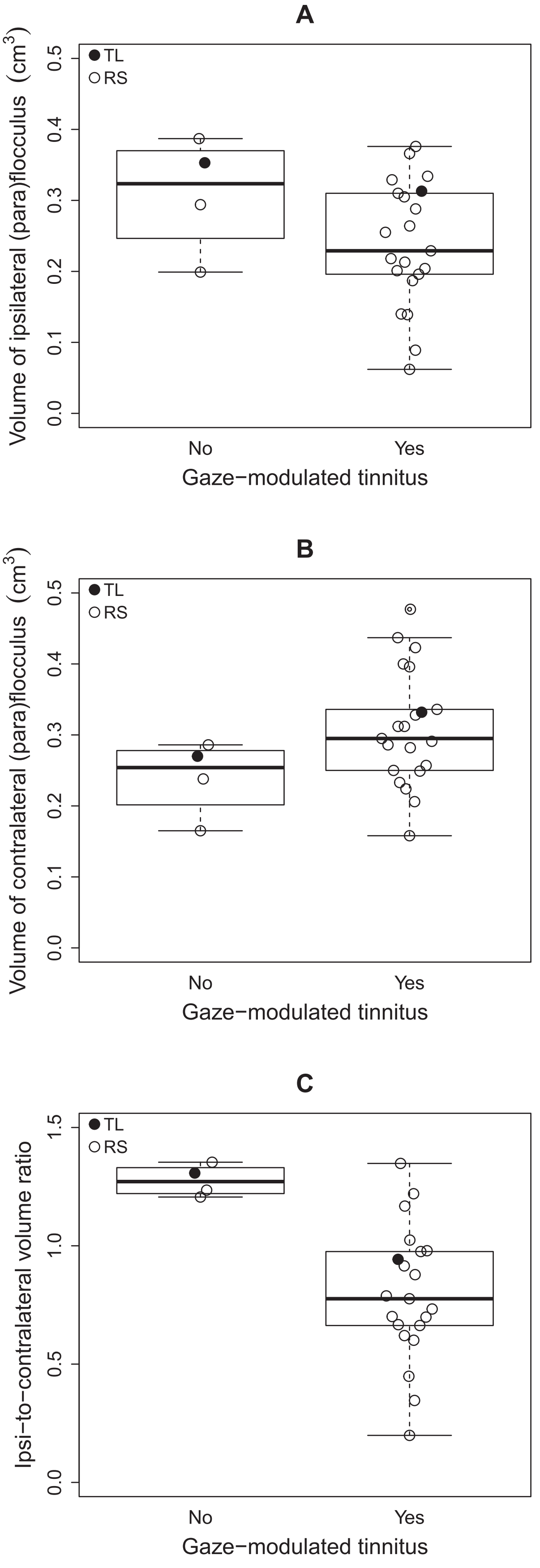


Fig. 4. Relation between the presence of gaze-modulated tinnitus and (para)flocculus volume. (A) (para)flocculus ipsilateral to the surgery. (B) (para)flocculus contralateral to the surgery. (C) Ipsi-to-contralateral volume ratio. No difference was present between the ipsi- and contralateral (para)flocculus volume, respectively, and the presence of gaze-modulated tinnitus. The ipsi-to-contralateral volume ratio was significantly smaller in patients with gaze-modulated tinnitus as compared to those without gaze modulation ($t(23) = 3.337$, $p = .003$).

creates an abnormal cross-modal interaction, whereby the normal function of the flocculus in gaze-control now becomes associated with changes in tinnitus.

The exact auditory function of the (para)flocculus remains unclear. The cerebellum controls afferent signals which involves error correction and signal gain adjustment, using multimodal inputs and feedback information (Brozoski et al., 2013). Because the (para) flocculus receives direct input from the cochlea via the auditory nerve and descending input from the auditory cortex (Bauer et al., 2013a), it could also have a role in error correction and signal gain adjustment in the auditory system. Brozoski et al. (2007) exposed rats to high-level sound to induce tinnitus. In the exposed rats with tinnitus there was a significant Mn^{2+} accumulation in the paraflocculi lobe of the cerebellum, but when an external tinnitus-like sound was presented to normal rats there was not. This suggests that the paraflocculus is involved in chronic tinnitus, but not in the processing of an external sound. Furthermore, Bauer et al. (2013a) showed that ablation of the flocculus resolved tinnitus, but did not prevent the onset of tinnitus. This appears to be consistent with the result of the present study, where flocculus volume did not relate to the presence of tinnitus (Fig. 2), but if present, tinnitus and (para)flocculus volume were correlated (Fig. 3).

What could be the possible tinnitus-related mechanisms in the flocculus? The flocculus, ventral paraflocculus and the transition zone between the flocculus and ventral paraflocculus contain a high density of Unipolar Brush Cells (UBCs) (Bauer et al., 2013b; Sekerková et al., 2004). UBCs are a class of excitatory, glutamatergic interneurons (Sekerková et al., 2004), which receive inputs from mossy fibers and form synapses with granule cells and other UBCs (Bauer et al., 2013b). They have been hypothesized to comprise a fan-out feed-forward excitatory network (Mugnaini Enrico, 2011). In one class of UBCs, spontaneous activity increases when mossy fibers are turned off (Bauer et al., 2013b; Rousseau et al., 2012). Infusion of a glutamatergic antagonist into the paraflocculus decreased evidence of tinnitus in rats (Bauer et al., 2013b) and reduced spontaneous neural activity in the paraflocculus, dorsal cochlear nucleus and the anterior ventral cochlear nucleus (Brozoski et al., 2013). Moreover, normal hearing rats displayed tinnitus-like behavior after similarly applied glutamatergic agonists (Bauer et al., 2013b). These results endorse the hypothesis that elevated output of UBCs and increased glutamatergic transmission contribute to the pathophysiology of tinnitus (Bauer et al., 2013b). If the flocculus indeed contributes to tinnitus, it could be that atrophy of the flocculus, as caused by a tumor or the tumor surgery, decreases the tinnitus severity. Possibly, this explains that patients with smaller (para)flocculi are less bothered by their tinnitus (Fig. 3).

Never before the characteristics of tinnitus have been related to MRI scans of the (para)flocculus in humans. Our results strongly suggest a role of the (para)flocculus in the mechanism of GMT and in mediating the severity of tinnitus. Nevertheless, this study also has some weaknesses. No distinction could be made between the flocculus and paraflocculus on the available MRI sequences. With more advanced MRI sequences, it may be possible to make a distinction between the flocculus and paraflocculus. Also, the clinical scans that were available, visualized only a limited scan volume. MRI scans which include a larger brain volume will allow the assessment of the volume of other brain structures and total brain volume. In addition, high resolution MRI scan will allow for a detailed analysis of the number and size of the folia of the (para) flocculus, which are highly variable across humans (Tagliavini and Pietrini, 1984). Then, it can be tested whether the correlations described in this study are specific for the (para)flocculus. A prospective study with a scan procedure tailored to the research questions is desirable to overcome these limitations.

5. Conclusion

The lack of a relation between flocculus volumes and the presence of tinnitus, combined with the significant correlation between tinnitus severity and flocculus volumes, suggests that the flocculus may not be the primary source of tinnitus, but is likely to mediate tinnitus severity. The reduced ipsi-to-contralateral volume ratio in patients with gaze-modulated tinnitus suggests that atrophy of the flocculus on the surgery side triggers cross-modal interactions leading to modulation of tinnitus.

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