

Decreased resting perfusion in precuneus and posterior cingulate cortex predicts tinnitus severity

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ABSTRACT

Functional magnetic resonance imaging has been increasingly used to understand the mechanisms involved in subjective tinnitus; however, researchers have struggled to reach a consensus about a primary mechanistic model to explain tinnitus. While many studies have used functional connectivity of the BOLD signal to understand how patterns of activity change with tinnitus severity, there is much less research on whether there are differences in more fundamental physiology, including cerebral blood flow, which may help inform the BOLD measures. Here, arterial spin labeling was used to measure perfusion in four regions-of-interest, guided by current models of tinnitus, in a sample of 60 tinnitus patients and 31 control subjects. We found global reductions in cerebral perfusion in tinnitus compared with controls. Additionally, we observed a significant negative correlation between tinnitus severity and perfusion. These results demonstrate that examining perfusion from the whole brain may present a complementary tool for studying tinnitus. More research will help better understand the physiology underlying these differences in perfusion.

1. Introduction

Magnetic resonance imaging has been used to noninvasively study the neural correlates of subjective tinnitus in humans, with the goal of building an increasingly refined understanding of the mechanisms that underlie the variation in tinnitus perception alongside behavioral reactions to tinnitus, including emotional and attentional responses. While the body of neuroimaging studies on tinnitus continues to grow, implicating a wide-range of structural and functional changes that accompany tinnitus (see (Shamsavarani et al., 2019) for a comprehensive review), researchers have still struggled to come to a consensus regarding the primary models or mechanisms to explain tinnitus. This is often precipitated by competing or contradictory results between studies and a lack of ability to predict or diagnose tinnitus objectively without relying on subjective, self-report measures. Much of this difficulty is rooted in the heterogeneity of the condition, which varies in etiology, duration, perceptual quality, and severity of impairment. It is likely that this

variation is mapped onto the brain, and so varying samples of tinnitus patients reveal different pieces of the story according to their unique composition.

The most common class of theoretical models used to explain the mechanisms behind the perception of tinnitus are “gain theories” that usually explain tinnitus as a dysfunctional compensation for hearing loss (Sedley, 2019). How the dysfunctional gain is instantiated differs between models. Models that implicate limbic (Rauschecker et al., 2010) and attentional (Roberts et al., 2013; Husain, 2016) circuitry have been especially influential, since they incorporate the attentional and emotional impairment that often accompanies tinnitus. While it is true that hearing loss is a major predictor of tinnitus, it does not always co-occur with tinnitus (Savastano, 2008; Martines et al., 2010). This creates a difficult situation experimentally, since presumably hearing loss is not required for tinnitus. In fact, a majority of those with hearing loss do not develop tinnitus, but it is difficult to recruit large samples of participants who have tinnitus but no substantial hearing loss. Therefore, the

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effects of hearing loss and tinnitus on neuroimaging data are very difficult to separate. This is particularly true for areas of the brain that may reflect both changes due to hearing loss and changes due to tinnitus, such as the primary auditory cortices (Adjajian et al., 2014; Galazyuk et al., 2012).

Careful researchers may recruit a sample of participants matched for hearing loss who do not have tinnitus and use this group to control for effects that may be related to hearing loss, in order to highlight differences that are unique to tinnitus. An alternative approach is to focus an investigation on the severity of the tinnitus percept itself, which is not collinear with the degree of hearing loss. These investigations have largely implicated brain areas involved in attentional control and emotional mechanisms and their interactions with auditory pathways.

A promising route to probing overarching changes in brain function in the tinnitus condition is through measuring resting state functional connectivity (rsFC). Changes in the connectivity within the default mode network (DMN) in tinnitus patients have been demonstrated in multiple studies. A consistent pattern has emerged of reduced connectivity within the default mode network (Schmidt et al., 2013; Carpenter-Thompson et al., 2015a; Lanting et al., 2016; Leaver et al., 2016). Some studies have particularly implicated the precuneus as playing an important role in this reduced connectivity. Compared to a group of hearing-loss controls, patients with tinnitus showed decreased connectivity between the precuneus and the rest of the DMN, which became more pronounced with increased tinnitus severity (Schmidt et al., 2013). The same pattern was also observed with respect to tinnitus duration (Carpenter-Thompson et al., 2015a). Other researchers focusing on the connectivity with the auditory rest-state component also found the involvement of posterior cingulate and precuneus in the development of tinnitus distress (Burton et al., 2012).

The involvement of the precuneus meshes well with models that focus on changes in the relationship between attentional and DMN/limbic networks. As connectivity *within* the DMN decreases, connectivity *between* the DMN and attention networks and limbic areas tends to strengthen. These observations are typically interpreted as neural plasticity relevant to habituation to tinnitus through the cognitive control of emotion. Schmidt and colleagues (Schmidt et al., 2017) showed greater coupling between the precuneus and the dorsal attention network (DAN) in patients with tinnitus compared to hearing-loss controls. Mirroring the reduction in within-network connectivity in the DMN, between-network connectivity between the DMN and DAN increased as a function of tinnitus severity. Changes in connectivity between attention and limbic areas also seem to be involved. Co-activity between the left parahippocampal regions and the DAN has been observed (Schmidt et al., 2013), and altered connectivity with the amygdala has also been implicated as a predictor of tinnitus (Zimmerman et al., 2018). Additional attentional control networks may be involved. For example, early studies of the neural correlates of tinnitus using positron emission tomography (PET) found increased neuronal activity caused by tinnitus in the right middle frontal gyrus, which may implicate either the DAN or the frontoparietal networks (Mirz et al., 1999).

These networks, involved in attentional control and emotion, seem to interact in complex ways with auditory areas. In a study conducted by Maudoux et al. (2012a), increased connectivity was observed between left parahippocampal regions and auditory cortices, a result replicated by Schmidt et al. (2013). In a separate study, Maudoux et al. (2012b) showed multiple changes in connectivity with the auditory resting-state network in tinnitus across the brain, including increased connectivity with the brainstem, cerebellum, right basal ganglia, parahippocampal areas, right frontal, and right parietal areas. The researchers also observed decreases with the right primary auditory cortex, left fusiform gyrus, left frontal, and occipital regions.

While there are many studies that now use functional connectivity and the blood-oxygen level dependent (BOLD) signal to understand how patterns of functional activity change as a function of tinnitus and tinnitus severity, there is much less research on whether there are

differences in more fundamental physiology, including metabolic rate and cerebral blood flow. Early [¹⁸F]-deoxyglucose-PET (FDG-PET) studies found increased metabolism in the auditory cortices related to tinnitus (Arnold et al., 1996; Wang et al., 2000; Langguth et al., 2006; Eichhammer et al., 2007; Geven et al., 2014). However, these studies were geared toward testing the specific hypothesis of whether hyperactive metabolism in the auditory cortices may be responsible for the perception of tinnitus. Since then, theories around tinnitus have evolved and largely incorporate large cortical areas involved in attention and emotion, especially when explaining the severity of tinnitus, which reflects the actual impairment of the condition. Yet, the available literature on fundamental metabolic physiology in humans remains sparse. Using FDG-PET, Schecklmann and colleagues (Schecklmann et al., 2013) found increases in metabolism in areas related to duration of tinnitus in frontal and posterior cingulate regions, as well as increases in metabolism related to severity in the posterior temporal lobe. Mahmoudian et al. (2013) convincingly linked metabolism to cerebral perfusion in tinnitus patients by using single photon emission computerized tomography (SPECT) to measure perfusion and FDG-PET to measure metabolism and also found a significant correlation between the two measures.

Like the BOLD signal, which is typically used to determine functional connectivity, arterial spin labeling (ASL) is a completely non-invasive MR imaging technique, using arterial water as an endogenous tracer. However, arterial spin labeling offers some key differences from the BOLD signal that make it a potentially interesting marker to accompany the neuroimaging techniques that have previously been discussed. The biggest advantage is that absolute measures of blood flow can be quantified, and so values can be compared across subjects. The BOLD signal, in contrast, is a relative measure that must be expressed as a % change. So, it is impossible to compare the resting BOLD signal across subjects, unless it is first transformed into some meaningful relative relationship (as is the case with functional connectivity). Building on the earlier research using the more invasive PET and SPECT methods, ASL is a promising tool to investigate baseline differences in perfusion in tinnitus patients that may yield insights about neural changes to tinnitus, particularly with regard to contextualizing new findings regarding changes in rsFC in the tinnitus condition. However, there are currently no studies using ASL to assess regional differences in cerebral blood flow that may be used to further understand the physiology underlying tinnitus in the brain. Here, we explore the utility of using ASL as a non-invasive technique to investigate altered perfusion in tinnitus in four regions-of-interest: the dorsal middle frontal gyrus (DMFG), posterior superior temporal gyrus (PSTG), posterior cingulate cortex (PCC), and precuneus.

2. Materials and methods

2.1. Participants

91 participants (42 female) were recruited from Champaign-Urbana and the surrounding area with community advertisements in flyers, bulletins, and newspapers. The study was approved by the University of Illinois at Urbana-Champaign Institutional Review Board (IRB Protocol Number: 15955), and all subjects gave written informed consent prior to participation in the study. Subjects who met the following criteria were excluded from participation in the study: a history of traumatic brain injury, Meniere's disease, temporomandibular joint disorder, psychological disorders except for currently managed depression and/or anxiety, or a history of neurological disorders. All participants were fluent English speakers. Data were collected from two groups of participants: 31 controls with no tinnitus and 60 patients with chronic tinnitus (duration of tinnitus ≥ 6 months). In order to have a larger patient group to adequately represent the variance in tinnitus severity and hearing loss, the tinnitus group was designed to be larger than the control group. Demographic details are provided in Table 1.

Table 1
Subject demographics.

	Controls (N = 31, 17 female)		Tinnitus (N = 60, 25 female)		p-value
	M	SD	M	SD	
Age	47.03	11.33	52.33	11.21	0.036*
Beck's Depression Inventory	3.65	5.43	3.85	4.77	0.854
Beck Anxiety Inventory	1.64	1.99	2.45	3.07	0.189
Tinnitus Functional Index					
Total	N/A	N/A	23.10	19.40	N/A
Intrusive	N/A	N/A	37.33	21.36	N/A
Sense of control	N/A	N/A	33.72	22.93	N/A
Cognitive	N/A	N/A	19.89	19.66	N/A
Sleep	N/A	N/A	17.94	27.14	N/A
Auditory	N/A	N/A	26.56	28.43	N/A
Relaxation	N/A	N/A	28.22	27.39	N/A
Quality of life	N/A	N/A	14.17	20.28	N/A
Emotional	N/A	N/A	9.94	14.44	N/A
Mean hearing thresholds					
Right 250 Hz	11.77	5.71	13.50	6.66	0.223
Right 500 Hz	11.77	5.85	13.67	7.47	0.223
Right 1000 Hz	12.74	6.93	14.08	7.62	0.414
Right 2000 Hz	14.19	9.58	18.17	11.27	0.098
Right 3000 Hz	15.50	12.20	24.33	17.45	0.015*
Right 4000 Hz	16.93	13.89	28.08	19.00	0.005**
Right 6000 Hz	21.61	16.80	31.83	19.74	0.016*
Right 8000 Hz	21.77	20.68	33.00	22.59	0.023*
Right 9000 Hz	29.03	24.41	41.25	24.76	0.027*
Right 10000 Hz	29.52	25.70	43.42	25.65	0.016*
Right 11200 Hz	35.00	25.98	50.58	24.04	0.005**
Right 12500 Hz	43.06	26.35	59.25	22.81	0.003**
Right 14000 Hz	49.84	25.74	65.08	17.89	0.001**
Right 16000 Hz	45.65	16.06	50.08	14.80	0.191
Left 250 Hz	9.68	5.15	15.08	11.70	0.016*
Left 500 Hz	10.48	4.72	15.17	12.32	0.045*
Left 1,000 Hz	11.13	6.15	16.25	14.13	0.058
Left 2,000 Hz	11.77	8.42	20.5	14.31	0.002**
Left 3,000 Hz	14.68	9.12	29.08	19.67	<0.001**
Left 4,000 Hz	16.61	11.65	31.92	20.95	<0.001**
Left 6,000 Hz	20.65	16.52	35.67	21.66	0.001**
Left 8,000 Hz	20.81	19.15	35.42	23.98	0.004**
Left 9,000 Hz	27.26	25.16	44.33	23.91	0.002**
Left 10,000 Hz	28.71	25.17	47.75	24.96	<0.001**
Left 11,200 Hz	33.71	26.52	53.83	24.29	<0.001**
Left 12,500 Hz	41.61	28.91	60.33	24.48	0.002**
Left 14,000 Hz	49.68	25.36	64.25	20.19	0.004**
Left 16,000 Hz	42.90	17.36	50.75	14.78	0.026*

2.2. Measures

Tinnitus severity was classified according to the Tinnitus Function Index (TFI) (Meikle et al., 2012). TFI scores were collected for each subject at the time of the magnetic resonance imaging (MRI) scan. Scores for the total tinnitus severity and sub-domains of the TFI are presented in Table 1. In subsequent analysis, only the total TFI scores are utilized.

Audiological assessments were completed for each subject prior to the scan session. Mean hearing thresholds were measured from 0.25 to 16 kHz. The pure tone thresholds for each ear at each measured frequency are listed in Table 1. Pure tone averages (calculated at higher frequencies) (PTA_{HF}) were computed using bilateral thresholds at 2, 4, 6, and 8 kHz.

Depression was assessed using the Beck Depression Inventory (BDI-II) (Beck et al., 1988a). Anxiety was assessed using the Beck Anxiety Inventory (BAI) (Beck et al., 1988b). There was no significant difference in either depression or anxiety scores between the tinnitus and control group samples (Table 1).

2.3. MRI data acquisition

MRI data were collected on a 3T Siemens MAGNETOM Prisma MRI

scanner. Arterial spin labeling data were acquired using the PICORE Q2T pulsed ASL sequence with a transverse orientation (TR = 2500 ms, TE = 12 ms, flip angle = 90°, QUIPSS II (TI1 (bolus duration) = 800 ms, TI2 (inversion time) = 1800 ms, 9 slices, 4 mm × 4 mm × 8 mm voxel size, matrix size = 64 × 64, FOV = 256 mm × 256 mm). The total acquisition time was 3 min and 52 s producing 45 pairs of tag/control images, and a single image with the magnetization at equilibrium (M₀).

In addition, a high resolution T1-weighted MPRAGE image was collected and used for brain extraction and tissue segmentation (TR = 2300 ms, TE = 2.32 ms, flip angle = 8°, 192 slices, voxel size = 0.9 mm × 0.9 mm × 0.9 mm, FOV = 230 mm × 230 mm, matrix size = 256 × 256, using in plane acceleration (GRAPPA) factor = 2.).

2.3.1. ASL analysis

The ASL image data processing was performed using MATLAB (MathWorks, Natick, MA, USA), with SPM12 (Wellcome Department of Cognitive Neurology, UK) and ASLtbx (Wang et al., 2008). Tag and control images were realigned to the first image in the series for each subject. Then the M₀ and the ASL images were coregistered to the T1-weighted anatomical image for each subject. ASL images were motion corrected using an adapted ASL specific motion corrected method (Wang, 2012) and spatially smoothed with a 6 mm full-width-half-maximum (FWHM) kernel. Then each tag and control pair was subtracted to create perfusion-weighted images, which were used to create quantified maps of CBF (ml/100 g/min) using ASLtbx (Wang et al., 2008). The 4D CBF time series were masked to remove out-of-brain voxels and then coregistered to the MNI template in SPM12.

CBF was calculated according to equation (1),

$$\frac{\Delta M}{2\alpha M_{0b} T_{I1} e^{-\frac{T_{I2}}{T_{1b}}}} \quad (1)$$

where ΔM is the perfusion difference image, $\alpha = 0.9$ is the labeling efficiency, M_{0b} is the fully relaxed magnetization of arterial blood estimated using the M_0 image collected at the beginning of the ASL sequence, T_{I1} is the bolus duration, T_{I2} is the inversion time, and T_{1b} is the T1 of blood estimated at 1664 ms (Lu et al., 2004). A quantified CBF map from an example subject is presented in Fig. 3.

Regions-of-interest (ROIs) were chosen from the Harvard-Oxford atlas according to areas that had been identified by previous work as being involved in the tinnitus severity (Shahsavaran et al., 2019; Desikan et al., 2006). Four ROIs were chosen: the DMFG, thought to be involved in core attentional mechanisms that may play a role in habituation (Roberts et al., 2013; Husain, 2016; Schmidt et al., 2013, 2017); the PCC, a core area of the default mode network, where functional connectivity has been observed to change with tinnitus severity (Schmidt et al., 2013; Carpenter-Thompson et al., 2015a; Lanting et al., 2016; Leaver et al., 2016); the precuneus, whose interaction with both attention and default mode networks has been implicated in tinnitus (Schmidt et al., 2017); and the PSTG, where activity is thought to be involved in the generation of the tinnitus percept (Husain, 2016; Husain and Schmidt, 2014; Sedley et al., 2016).

Anatomical areas defined in MNI space were registered back to the individual subject's native ASL image space using the transformations from the previous coregistration steps. Fig. 4 depicts a visualization of the 3D ROIs transformed back onto the anatomical brain of a representative subject. Gray and white matter masks were also derived for each subject using the partial volume gray and white matter segmentations acquired using the FSL Fast tool (Zhang et al., 2001). These gray and white matter segmentations were thresholded at a value of 0.6. Perfusion values from the quantified CBF maps were averaged from the gray and white matter separately in each of the selected ROIs and over the whole brain.

2.3.2. Statistical analysis

Statistics were carried out using R (R Core Team, 2019). Welch's t-tests were completed to test for differences between the two groups,

except in the case of using a test of proportions when testing differences of a binary factor measures including hearing loss and sex. Pearson correlations were used to test for correlations between continuous variables.

Regression models were used to determine the best overall models for predicting perfusion. Nested models were compared using the F-statistic as a forward selection approach. The Akaike Information Criterion (AIC) (Akaike, 1974) was employed to select the most appropriate model between sets of nested models.

3. Results

3.1. Perfusion-related covariates

When analyzing group differences in perfusion, it is important to control for known confounding factors that may influence the dependent variable. In this case, it is known that perfusion is typically lower in males than in females, is lower in older age compared to younger age, and may be affected by hearing loss, particularly in the PSTG (Parkes et al., 2004; Ponticorvo et al., 2019).

Indeed, despite attempts in recruitment to match age and hearing between the tinnitus and control groups as best as we could, we observed significant differences in age ($t(60) = -2.12, p = 0.038$), where tinnitus participants ($M = 52.33$ years, $SD = 11.21$ years) were older than the controls ($M = 47.03$ years, $SD = 11.33$ years). We also observed differences in hearing loss between the two groups using both test of proportions of a binary measure of hearing loss [$\chi^2(1) = 7.321, p = 0.007$], where the proportion of tinnitus subjects with hearing loss (73%) was significantly higher than the proportion of control subjects with hearing loss (42%), and using t-tests of differences in PTA_{HF} [$t(78) = -3.69, p < 0.001$], where tinnitus subjects had significantly higher PTA_{HF} hearing thresholds ($M = 29.32, SD = 16.50$) than control subjects ($M = 18.04, SD = 12.17$). In contrast, sex was well balanced between groups ($\chi^2(1) = 0.946, p = 0.331$). Overall, there was a significant correlation between age and PTA_{HF} ($r(89) = 0.506, p < 0.001$).

Due to these group differences in age and hearing thresholds, we investigated if perfusion was influenced by age or hearing in our sample to examine whether it was necessary to include these variables as confounding factors in our subsequent analyses. In our sample, we did not observe a significant correlation between age or PTA_{HF} and perfusion globally in white or gray matter or within any of the ROIs that we explored (Table 2).

3.2. Effects of tinnitus and hearing loss on perfusion

Significant differences between tinnitus and controls were found in both global gray ($t(56) = 2.23, p = 0.030$) and white matter perfusion ($t(65) = 2.45, p = 0.017$; Table 3). In the gray matter, perfusion was about 6.4 mL/100 g/min higher in the control subjects ($M = 75.9$ mL/100 g/min, $SD = 13$ mL/100 g/min) than in the tinnitus patients ($M = 69.5$ mL/100 g/min, $SD = 12$ mL/100 g/min), representing a decrease of about 8%. Across groups, perfusion was significantly lower in the white matter than the gray matter ($t(90) = 29.88, p < 0.001$). The global white

Table 2
Correlations between perfusion and potential covariates.

	Correlation with age	p-value	Correlation with pure tone averages	p-value
Gray Matter CBF	-0.032	0.76	-0.069	0.52
White Matter CBF	0.002	0.98	-0.058	0.58
PSTG CBF	0.114	0.28	-0.043	0.68
DMFG CBF	0.011	0.92	0.002	0.99
Precuneus CBF	-0.161	0.13	-0.120	0.26
PCC CBF	-0.110	0.30	-0.162	0.12

Table 3
Perfusion.

	Controls (N = 31, 17 female)		Tinnitus (N = 60, 25 female)		p-value
	M	SD	M	SD	
Gray Matter CBF	75.92	13.47	69.47	12.33	0.030*
White Matter CBF	50.35	9.73	44.95	10.45	0.017*
PSTG CBF	55.69	13.11	52.95	13.68	0.354
DMFG CBF	36.23	12.51	33.94	12.20	0.324
Precuneus CBF	60.57	12.19	54.42	12.87	0.029*
Posterior cingulate CBF	63.09	13.59	57.65	11.76	0.064+

+ $p < 0.1$, * $p < 0.05$.

matter perfusion was about 5 mL/100 g/min higher in the tinnitus patients ($M = 50.4, SD = 9.7$) compared to the control subjects ($M = 45.0, SD = 10.45$), representing a decrease of about 11%.

In the regional analysis, there was no significant difference in perfusion between tinnitus patients and controls in either the PSTG or the DMFG. However, there was a significant difference in perfusion in both the gray matter in the precuneus ($t(64) = 2.24, p = 0.029$) and trending in the PCC ($t(54) = 1.89, p = 0.064$).

Given concerns regarding the significant differences in proportions between groups in the number of participants with hearing loss and age, we performed a multiple regression analysis including tinnitus group membership (TIN), hearing loss assessed either as a binary variable (HL; defined as >25 dB HL hearing threshold at any frequency from 0.25 to 8 kHz in either ear) or using the hearing thresholds (PTA_{HF}), and age (AGE) as independent predictors of CBF.

The following models were compared for each ROI separately. The use of “+” indicates that only the main effects of the predictors are modeled, whereas “*” indicates that both main effects and interaction terms are included.

Models using binary HL factor:

- 1) $CBF \sim TIN$
- 2) $CBF \sim TIN + HL$
- 3) $CBF \sim TIN * HL$
- 4) $CBF \sim TIN + HL + AGE$
- 5) $CBF \sim TIN * HL * AGE$

Models using PTA_{HF}:

- 1) $CBF \sim TIN$
- 6) $CBF \sim TIN + PTA_{HF}$
- 7) $CBF \sim TIN * PTA_{HF}$
- 8) $CBF \sim TIN + PTA_{HF} + AGE$
- 9) $CBF \sim TIN * PTA_{HF} * AGE$

Within each set of nested models, the models were compared using the F-statistic as a forward selection approach. The AIC was employed to select the most appropriate model between the best models from each set of nested models (i.e. using either PTA_{HF} or a binary factor of hearing loss).

In the global gray matter, global white matter, DMFG, and PSTG ROIs, the simplest model, including only tinnitus as a predictor, was the best, with more complex models failing to significantly improve the model's prediction of CBF. However, in both the precuneus ($F(1, 88) = 4.717, p = 0.033$) and the PCC ($F(1, 88) = 5.020, p = 0.028$), model 2 significantly outperformed model 1. Because including PTA_{HF} never improved the model, the AIC was never used to select the preferred model.

Including the binary hearing loss variable as a predictor of CBF in the precuneus and PCC reduced the previously significant effect of tinnitus to non-significance (precuneus: $t(88) = -1.466, p = 0.146$; PCC: $t(88) = -1.226, p = 0.223$). In contrast, in these two regions, there was a remaining significant effect of hearing status (precuneus: $\beta = -6.153$,

$t(88) = -2.182, p = 0.032$; PCC: $\beta = -6.336, t(88) = -2.295, p = 0.024$) with hearing loss predicting lower perfusion in both ROIs.

As stated earlier, sex was not significantly different between groups, but we did replicate the effect that females ($M = 77.43, SD = 14.14$) have greater global perfusion than males ($M = 66.73, SD = 9.66; t(71) = -4.144, p < 0.001$).

3.3. Regional perfusion predicts tinnitus severity

We analyzed the relationship of tinnitus severity and perfusion in each of the ROIs, with a particular interest in whether tinnitus severity predicted the extent of perfusion decreases in the precuneus and PCC, where the most significant differences in perfusion between subjects with tinnitus and controls were observed. Tinnitus severity did not correlate with age ($r(58) = 0.137, p = 0.296$), sex ($r(58) = -0.046, p = 0.726$), or pure tone averages ($r(58) = 0.212, p = 0.104$).

Perfusion was significantly negatively correlated with tinnitus severity in global gray matter ($r(58) = -0.273, p = 0.035$) and white matter ($r(58) = -0.267, p = 0.039$) CBF. Within the ROIs, tinnitus severity significantly predicted perfusion in both the precuneus ($r(58) = -0.291, p = 0.024$; Fig. 1) and the PCC ($r(58) = -0.357, p = 0.005$; Fig. 2), the two areas where perfusion differed between subjects with tinnitus and controls. There was also a significant correlation between perfusion and tinnitus severity in the PSTG ($r(58) = -0.288, p = 0.026$) and a trending relationship in the DMFG ($r(58) = -0.219, p = 0.093$).

Interestingly, controlling for the affect of global gray matter on tinnitus severity eliminated the ability for the perfusion in any particular region to significantly predict tinnitus severity (precuneus: $r(57) = -0.124, p = 0.349$; PCC: $r(57) = -0.242, p = 0.065$; PSTG: $r(57) = -0.156, p = 0.238$; DMFG: $r(57) = -0.077, p = 0.564$).

Again, we analyzed the more complex model including tinnitus severity and hearing loss in each ROI, given our finding that, particularly in the precuneus and PCC, hearing loss was a better predictor of perfusion than whether or not the subject has tinnitus. When we included hearing loss in the model, the significance level of the effect of tinnitus severity remained the same across all ROIs: global gray matter ($t(57) = -2.184, p = 0.033$), global white matter ($t(57) = -2.054, p = 0.045$), precuneus ($t(57) = -2.155, p = 0.035$), PCC ($t(57) = -2.681, p = 0.010$), PSTG ($t(57) = -2.257, p = 0.028$), DMFG ($t(57) = -1.750, p = 0.086$). Hearing loss was not a significant predictor in any ROI: global gray matter ($t(57) = 0.398, p = 0.692$), global white matter ($t(57) = -0.074, p = 0.941$), precuneus ($t(57) = -0.799, p = 0.428$), PCC ($t(57) = -1.359, p = 0.180$), PSTG ($t(57) = 0.093, p = 0.926$), DMFG ($t(57) = 0.457, p = 0.650$).

3.4. Effects of hearing loss on perfusion within experimental groups

When accounting for hearing loss, tinnitus severity continued to significantly predict perfusion in the same ROIs as when not accounting for hearing loss. However, the significant differences between tinnitus subjects and controls were lost when hearing thresholds were used as a covariate. This suggests the possibility that hearing loss may have an independent effect on perfusion besides tinnitus, but in our study this effect was confounded by the differences in the varying proportions of subjects with hearing loss in the tinnitus and control groups. Therefore, we investigated how hearing loss related to perfusion *within* each group to supplement the analysis.

In the group of control participants without tinnitus, precuneus CBF was higher in those with normal hearing ($M = 64.42, SD = 13.67$) than those with hearing loss ($M = 55.24, SD = 7.33; t(27) = 2.409, p = 0.023$). There was no significant difference in CBF depending on hearing loss in any other ROI (global gray matter: $t(26) = 1.355, p = 0.187$; global white matter $t(26) = 1.520, p = 0.141$; DMFG: $t(29) = 0.857, p = 0.399$; PSTG: $t(28) = 0.566, p = 0.576$; PCC: $t(29) = 1.529, p = 0.137$).

In the group of participants with tinnitus, there was no significant difference in perfusion based on hearing loss in any of the ROIs, although there was a trend in the PCC ($t(33) = 1.899, p = 0.066$) where, again, the group with no hearing loss had higher perfusion ($M = 61.90, SD = 9.80$) compared to those without hearing loss ($M = 56.10, SD = 12.13$). There was no significant difference in CBF depending on hearing loss in the other ROIs (global gray matter: $t(33) = -0.056, p = 0.956$; global white matter $t(34) = 0.444, p = 0.660$; DMFG: $t(30) = -0.188, p = 0.852$; PSTG: $t(27) = 0.265, p = 0.793$; precuneus: $t(42) = 1.367, p = 0.179$).

4. Discussion

The aim of this study was to investigate whether there were differences in cerebral perfusion in patients with tinnitus compared to controls, and whether perfusion predicted tinnitus severity in the tinnitus patients. We observed significant differences in perfusion between tinnitus patients and controls, both globally in gray and white matter, and regionally in the precuneus but not in the DMFG or PSTG. There was a trending, but non-significant difference in the PCC. Further, across the whole brain, decreased perfusion predicted higher tinnitus severity. Interestingly, the regional differences in perfusion were better explained by hearing loss than by the presence of tinnitus, but controlling for hearing loss did not change the observed relationships between tinnitus severity and perfusion within the tinnitus patients.

A consistent challenge of tinnitus research is the significant overlap

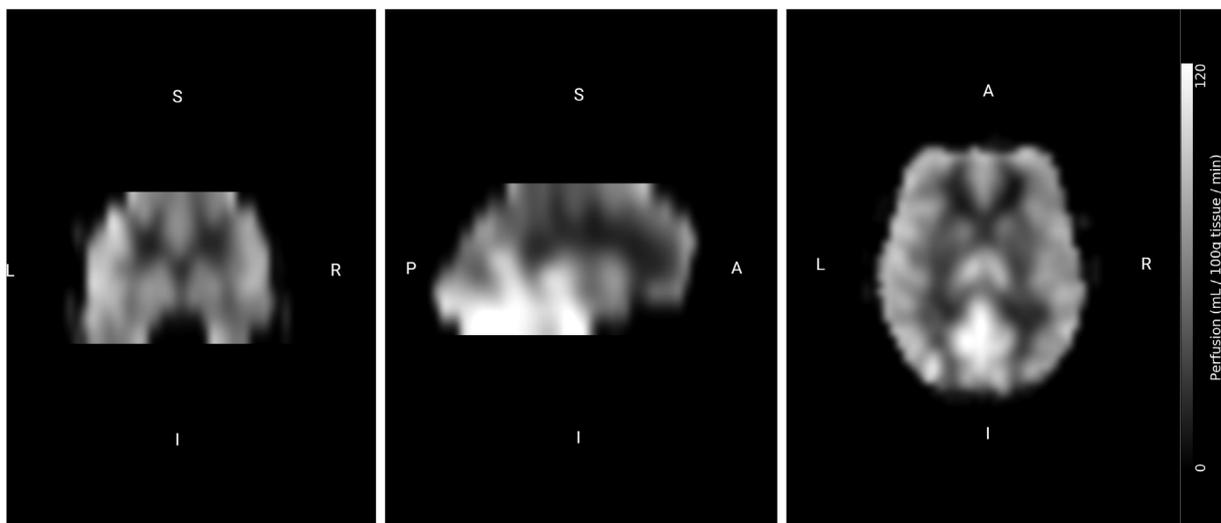


Fig. 1. Higher tinnitus severity, measured by Tinnitus Function Index, predicted lower perfusion in the precuneus.

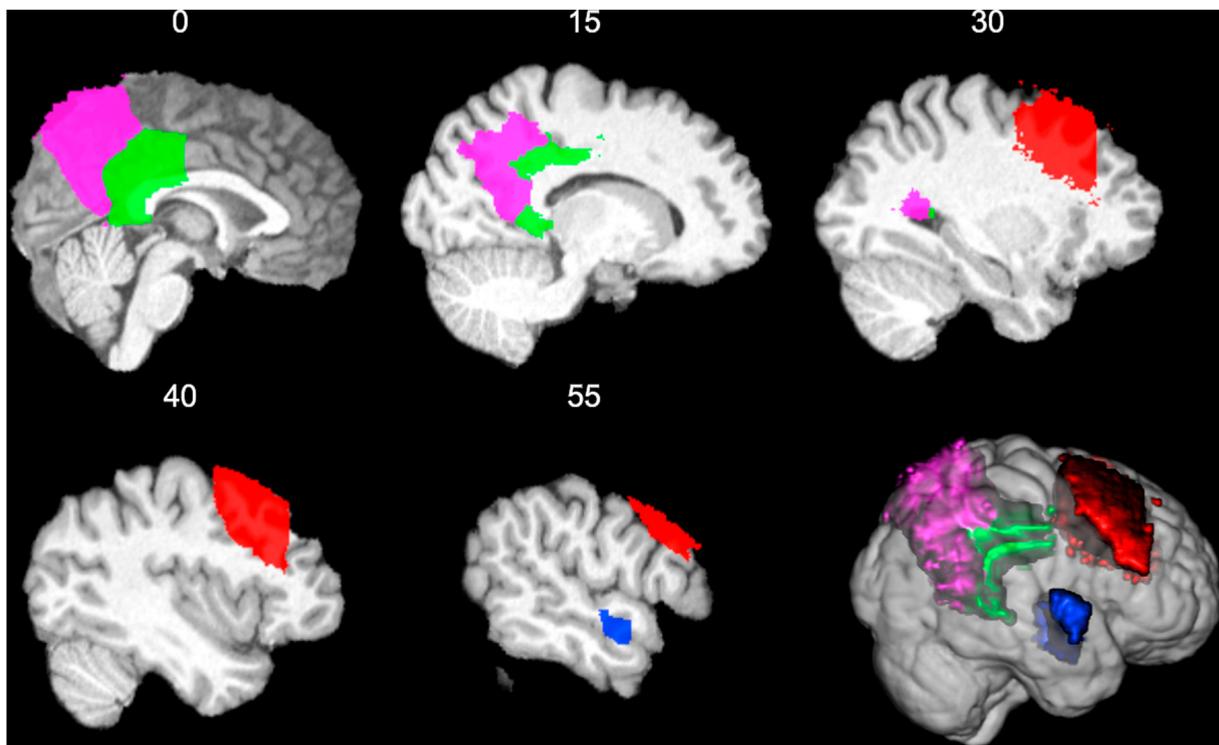


Fig. 2. Higher tinnitus severity, measured by tinnitus function index, predicted lower perfusion in the posterior cingulate.

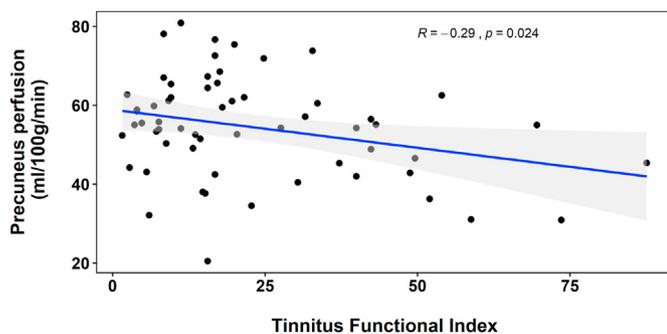


Fig. 3. An example of the mean perfusion image from one participant after quantification, showing the coronal, sagittal, and axial coverage.

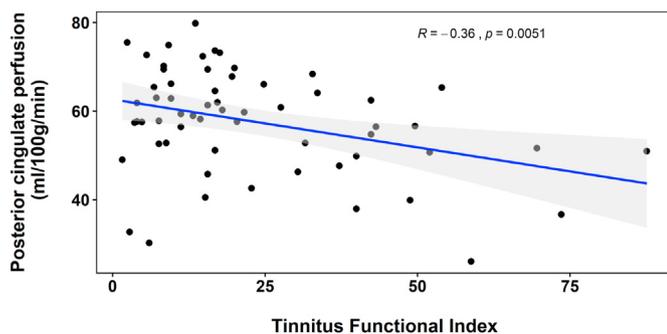


Fig. 4. Example of the regions of interest investigated in native anatomical space. The four regions include – Red: dorsal middle frontal gyrus, Blue: posterior superior temporal gyrus, Green: posterior cingulate cortex, and Fuchsia: precuneus.

between hearing loss and tinnitus, and their intertwining effects should be considered together. In the sample studied here, the proportion of

study participants who had hearing loss was significantly greater in the tinnitus group than the control group. We did a number of secondary analyses to better understand the overlap and differences between the effect of hearing loss and tinnitus on brain perfusion. Given past literature which has shown decreases in perfusion due to hearing loss (Ponicorvo et al., 2019), it is likely that at least some part of the perfusion differences between the tinnitus and control groups is related to the different proportions of hearing loss in the groups. An interesting outcome was that in at least the precuneus and PCC, the combination of hearing loss and tinnitus was better at predicting CBF than just tinnitus itself, which suggests that these variables may have some separate relationship with perfusion. It is possible that a reduction in perfusion may occur through separate mechanisms that influence the tinnitus percept. For example, perfusion may be reduced as an adaptation to hearing loss, which plays a mechanistic role in tinnitus, or perfusion may be reduced according to other mechanisms independent from hearing loss, which also play a mechanistic role in tinnitus. Additionally, within the tinnitus group, tinnitus severity predicts decreased perfusion even after controlling for the effect of hearing loss, which is suggestive that some aspect of tinnitus itself contributes to or at least relates with perfusion above and beyond hearing loss.

One of the most striking results from this paper points to a relationship between perfusion and tinnitus severity, where lower perfusion predicted greater tinnitus severity. This was true in areas where there were observed differences in perfusion between the tinnitus and control groups, but was also observed even in the PSTG, where we did not see significant perfusion differences between groups. There was an observable trend in the DMFG which followed the same pattern. Even after including hearing loss as a covariate, this relationship remained.

The ROIs explored in this study were chosen based on their likely involvement in the process of habituation and relationship to tinnitus severity. Current frameworks identify interacting neural networks that change in functional connectivity as tinnitus evolves. Much of these connectivity differences are thought to reflect habituation through the attentional control of emotion (Husain, 2016), although how the interactions between brain regions change is complex and seems to depend

on heterogeneous aspects of how the tinnitus progresses (e.g. laterality, severity, length of time) (Husain, 2016). The connectivity between regions of the brain, that are involved in attentional processing, such as the DMFG, and the default mode network appears to increase in patients with moderately severe tinnitus (Schmidt et al., 2017). At the same time, connectivity within the default mode network, such as the connectivity between the precuneus and the PCC, appears to decrease (Schmidt et al., 2013; Carpenter-Thompson et al., 2015a; Lanting et al., 2016; Leaver et al., 2016). It is unclear if these changes in connectivity are directly related to the decreases in perfusion that correlate with increases in tinnitus severity here. It is possible that in the ASL scan, tinnitus patients are more likely to be attending to their tinnitus and devoting less attention to the typical mind-wandering that is correlated with default-mode network activity. If this was the case, we would expect that the participants with tinnitus would have decreased activation in the default-mode network and increased activation in the dorsal attention or auditory networks compared to controls. In tinnitus patients, we observe the strongest decreases of perfusion relative to controls in nodes of the default mode network, consistent with this hypothesis. However, we also observe decreases in perfusion in nodes of the auditory and dorsal attention networks rather than increases, although these differences were non-significant. We also observe a global reduction in perfusion across all of the gray matter in tinnitus patients. Therefore, although the state-of-mind of the tinnitus patients may partially contribute to the results that we observe, the data suggests that the perfusion differences represent more than just differences in network activity.

It may be possible that an adaptation to tinnitus that decreases perfusion, perhaps reflecting overall resting activity or vascular differences, manifests as changes in connectivity, by reducing the evident opposition between attention and default mode networks, and by reducing the evident coherency within networks. The changes in connectivity could be due to a functional implementation of some beneficial adaptive strategy or alternatively due to reduced signal and increased noise affecting the correlation between seed regions. In a similar vein, recent studies have begun to investigate differences in the amplitude of low-frequency fluctuations in tinnitus (Chen et al., 2015), which could have both neural or vascular interpretations (e.g. reduced cerebrovascular reactivity (Golestani et al., 2016)). Recent studies have begun exploring generally how differences in neurovascular coupling or various differences in BOLD signal amplitude or noise levels may affect functional connectivity (Duff et al., 2018; Archila-Meléndez et al., 2020). Future work should prioritize understanding the relationship between any potential vascular explanations from neural explanations regarding brain changes in tinnitus.

Why decreases in perfusion are related to tinnitus is still unclear. It is possible that they are involved in the habituation process or the mechanism behind tinnitus itself. However, it might be expected that if tinnitus severity decreased with greater perfusion as part of the mechanism of habituation, then the tinnitus group would have *greater* overall perfusion than the controls. In other words, since tinnitus severity decreases with habituation, and tinnitus severity is anti-correlated with perfusion, we might expect that if increasing perfusion is somehow reflective of compensatory processes related to habituation, that overall, perfusion would be higher in tinnitus patients. This is not what we observe.

It is also possible that some hidden covariate is causing the difference. For example, Gispén et al. (2014) observed that hearing impairment was associated with lower levels of physical activity in older adults. It is possible that physical activity is the more proximal cause of the reduction in perfusion and may relate to the severity of tinnitus or hearing loss or both. Carpenter-Thompson and colleagues observed striking results showing that physical activity predicted lower levels of tinnitus severity (Carpenter-Thompson et al., 2015b). In another study, tinnitus patients with higher physical activity levels recruited more frontal activation in response to an affective sound listening task, and recruitment of more frontal activation was associated with reduced tinnitus severity

(Carpenter-Thompson et al., 2015c). In the future, physical activity level should be investigated more thoroughly, both as a potential predictor of tinnitus and hearing loss, since there may be dire health implications if individuals with tinnitus are significantly less active, but also as a potential therapeutic, since the direction of the relationship between physical activity and tinnitus severity may work in either direction. If there is some relationship with physical activity, the perfusion reduction that we are observing here may not be limited to tinnitus, but would have some mechanistic overlap with any condition that reduced physical activity.

This study is somewhat limited in its strength due to a few factors. First, as we noted in the results, the tinnitus and control groups were not perfectly balanced with age or hearing loss, which are known factors that affect perfusion. Even if it was possible to recruit a number of patients with tinnitus who did not have hearing loss or age-matched controls with hearing loss who did not have tinnitus, it is not trivial whether or not these samples should be considered truly representative of the population. It turned out that in our sample, age did not significantly predict perfusion. This is not entirely surprising given that the sample reported here had a somewhat restricted age-range, with most of the participants in middle-age. Previous reports using ASL, looking at samples with age-ranges over the entire lifespan, typically see small annual declines in CBF (~0.1–0.25 ml/100 g/min per year) and often see the oldest individuals disproportionately contributing to the slope (Parkes et al., 2004; Zhang et al., 2018; Lu et al., 2011). We tried to account for differences in hearing as best as we could by exploring in detail how hearing loss and tinnitus separately affected perfusion in our sample, while balancing our goal of recruiting an ecologically valid sample of participants. There is also the possibility that the control and tinnitus samples differed in either their engagement with the study, their experience in the scanning environment, or in other unexpected ways that may influence perfusion measures such as group differences in diurnal rhythms, physiological effects due to anxiety, heart rate, or caffeine consumption.

Another limitation has to do with the scope of our study. Due to considerations of statistical power, we approached the study with just two main questions: are there differences in perfusion between tinnitus patients and controls, and does perfusion relate with tinnitus severity in the tinnitus patients. We further examine the variation in these effects in a few particular ROIs that have been implicated in tinnitus in prior studies, and also conducted multiple analyses to clarify the contribution of known potential covariates in this data. We did not statistically correct for these multiple comparisons because the additional comparisons are meant only to help clarify and support the global results. The effects that we observed globally followed a similar pattern across all of our ROIs, even if the pattern was not significant in each ROI. Thus, the importance of “region” is still not completely clear and should not be over-emphasized in this study. After statistically controlling for the global effect, there is no significant relationship between tinnitus severity and perfusion in any of the individual regions. It may be that the pattern of decreased perfusion affecting tinnitus severity is a global effect that would be observed across the brain, but systemic noise in certain regions (e.g. due to smaller number of voxels) makes the effect appear stronger in certain regions than others. However, it is also possible that this effect of perfusion predicting tinnitus severity is only strong and significant in specific regions, which are involved in generating the perception of and reaction to tinnitus, and it just so happens that these regions comprise a high enough percentage of the total brain volume that the pattern is observed globally. Future research, designed to be powerful enough for a voxel-wise analysis, would be ideally suited to answer this question. Additionally, although ASL as a technique has been shown to be relatively reliable and correlated with other measures of cerebral perfusion (Parkes et al., 2004; Puig et al., 2020), the technique may be more sensitive to certain kinds of cerebrovascular factors, such as transit delays (Fan et al., 2017), that affect the quantification. Thus, conducting a future study using multiple methods to quantify cerebral perfusion, cerebral blood volume, and cerebral metabolism may reveal important

differences that help uncover the reasons for the effects observed in this study.

In many ways, the results from this study reveal more new questions than the questions that they answer. These results demonstrate that examining perfusion from the whole brain may offer an excellent tool for studying tinnitus with MRI methods and offer a complementary approach to task-related fMRI or functional connectivity. We saw here that cerebral perfusion, measured using arterial spin labeling, was significantly different between tinnitus patients and controls, observed both globally and in the precuneus, a core region whose function has been previously observed to play a role in tinnitus. Despite the inherent difficulty in separating the effects of hearing loss alone from the effects of tinnitus on perfusion, we observed a striking relationship where tinnitus severity increased as perfusion decreased, even in regions where there were not significant differences in perfusion between tinnitus patients and control subjects. More research is necessary to understand the physiology underlying these differences in perfusion and to understand why decreases in perfusion in brain regions implicated in tinnitus predict tinnitus severity so well.

Author contributions

Benjamin J. Zimmerman: conceptualization, methodology, data collection, analysis, writing the original draft, review and editing; Rafay A. Khan: data collection, review and editing; Sara A. Schmidt: data collection, review and editing; Yihsin Tai: data collection (audiology), review and editing; Somayeh Shahsavarani: data collection, review and editing; Fatima T. Husain: conceptualization, methodology, writing the original draft, review and editing, funding acquisition, supervision.

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Data availability statement

The datasets generated for this study are available on reasonable request to the corresponding author.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Peer Review Overview and Supplementary data

A Peer Review Overview and (sometimes) Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.crneur.2021.100010>.

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