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Singing training predicts increased insula connectivity with speech and respiratory sensorimotor areas at rest

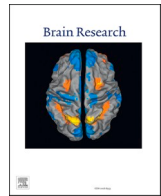A.M. Zamorano [a], R.J. Zatorre [b,c], P. Vuust [f], A. Friberg [d], N. Birbaumer [e], B. Kleber [e,f,*]

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ABSTRACT

The insula contributes to the detection of salient events during goal-directed behavior and participates in the coordination of motor, multisensory, and cognitive systems. Recent task-fMRI studies with trained singers suggest that singing experience can enhance the access to these resources. However, the long-term effects of vocal training on insula-based networks are still unknown. In this study, we employed resting-state fMRI to assess experience-dependent differences in insula co-activation patterns between conservatory-trained singers and non-singers. Results indicate enhanced bilateral anterior insula connectivity in singers relative to non-singers with constituents of the speech sensorimotor network. Specifically, with the cerebellum (lobule V-VI) and the superior parietal lobes. The reversed comparison showed no effects. The amount of accumulated singing training predicted enhanced bilateral insula co-activation with primary sensorimotor areas representing the diaphragm and the larynx/phonation area–crucial regions for cortico-motor control of complex vocalizations—as well as the bilateral thalamus and the left putamen. Together, these findings highlight the neuroplastic effect of expert singing training on insula-based networks, as evidenced by the association between enhanced insula co-activation profiles in singers and the brain's speech motor system components.

1 Introduction

Over the last two decades, research has demonstrated that the extensive practice and performance of fine motor, sensory, and multi-modal integration skills required for mastering a musical instrument can lead to significant functional and structural changes in the brain (Criscuolo et al., 2022; Klein et al., 2016; Schlaug, 2015). Musical training has since become a well-established model for studying experience-dependent neuroplasticity, revealing that adaptive changes in the brain are associated with the practiced musical action and its behavioral outcomes (e.g., enhanced performance), can occur in response to both short and long-term training, and are more pronounced when formal training starts during a sensitive period before the age of seven years (for review, see Herholz and Zatorre, 2012). Professionally trained singers,

on the other hand, have been understudied in this context. They are distinct from instrumentalists, as they often begin formal training later in life (Schirmer-Mokwa et al., 2015) and rely on a motor system for music production that has already experienced substantial maturation throughout speech motor development and informal singing experience (Stadler Elmer, 2011; Weiss-Croft and Baldeweg, 2015). Nevertheless, to achieve the increased precision demands in a musical framework, they still necessitate extensive deliberate practice, which makes conservatory trained singers an ideal model to examine experience-dependent neuroplasticity in the speech-motor system. By utilizing resting-state fMRI to investigate insula-based networks in both trained and untrained singers, and emphasizing the insula as a critical hub for sensorimotor integration in speech and song production networks (Fuertinger et al., 2015; Zarate, 2013), this study aims to advance our understanding of the

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impact of vocal training on neuroplasticity within these networks.

The brain regions supporting basic vocalizations have been well-described in primates (Jürgens, 2009), whereas higher-level aspects of vocal motor production have been extensively studied in the context of human speech (Price, 2012). This research has revealed a hierarchically organized structure–function relationship in which basic vocal patterns are generated in the brain stem and the periaqueductal gray, elicited by limbic inputs, whereas higher-level processes of volitional vocalizations are planned and performed in the cortex (Simonyan and Horwitz, 2011). Higher-level processes include motor planning and sequencing, prosody, and the integration of sensory feedback and motor information in real-time (Hickok, 2012; Kroger et al., 2022). Fundamentally, the same principles of vocal motor control and learning apply to both speech and singing (Zuk et al., 2022), yet there are also important differences in terms of higher-level processes. In singing, for instance, pitch control and timing are more structured and precise than in speech, and the use of prosody serves a different function (Zatorre and Baum, 2012). Thus, the main differences may rest in the increased demands placed on the same vocal motor system (Zuk et al., 2022). How the brain controls speech has been described and formalized by computational models (Parrell et al., 2019). One prominent model (Guenther and Vladusich, 2012) suggests that the dorsal speech-motor stream engages a sensory integration interface consisting of auditory, somatosensory, and inferior parietal cortices, which communicate via the arcuate fasciculus with the articulatory sensorimotor network in ventral primary- and pre-motor regions. The supplementary motor area, the basal ganglia, and the cerebellum moreover contribute to feedback and feedforward transformations that support the accurate performance of planned vocalizations (Guenther and Hickok, 2015). Although not explicitly incorporated in this model, the insula's role in integrating sensorimotor, emotional, and cognitive processes suggests that it may contribute to the overall functioning of the speech production network. Correspondingly, the brain areas that are sensitive to singing training overlap with those involved in speech-motor control. This includes the somatosensory, inferior parietal, and auditory cortices, as well as the cerebellum, the anterior cingulate cortex, and the anterior insula, which may serve a gating function for sensory feedback (Kleber et al., 2010; Kleber et al., 2016; Kleber et al., 2017; Zarate and Zatorre, 2008; Zarate, 2013).

The insular cortex is known to contribute to motor aspects of speech production (Ardila et al., 2014; Dronkers, 1996), as well as to various other aspects that are integral to vocal performance and music, such as sensory, emotional, motivational, and cognitive processes (Bamiou et al., 2003; Criscuolo et al., 2022; Gogolla, 2017; Nomi et al., 2018). Its broad functional roles involve the integration of bodily signals (Craig, 2002; Craig, 2009), homeostatic emotion regulation (Strigo and Craig, 2016), the detection and assessment of salient sensory inputs (Singer et al., 2009), and coordination of large-scale network dynamics underlying higher-level cognitive processes (Molnar-Szakacs and Uddin, 2022). The insula's more specific involvement in speech production is supported by its strong connectivity with constituents of the dorsal sensorimotor stream (Hickok, 2017; Remedios et al., 2009) and its involvement in vocalization-related autonomic and perceptual-motor processes (Craig, 2002; Craig, 2009). This involvement includes both linguistic and non-linguistic vocalizations, with a more left-lateralized contribution from the dorsal anterior insula to speech production (Ackermann and Riecker, 2010; Oh et al., 2014) and a right-hemispheric dominance in singing (Jeffries et al., 2003; Riecker et al., 2000). The latter may be due to the melodic elements of vocalizations (Oh et al., 2014), similar to reported brain asymmetries for speech and melody perception in the auditory cortex (Albouy et al., 2020). Task-based fMRI studies have reported experience-dependent changes in insular activation patterns for trained singers. These findings suggest that the right anterior insula plays a role in stabilizing vocal pitch when faced with sensory feedback perturbations (Kleber et al., 2013; Kleber et al., 2017), thereby supporting its proposed gating function for sensory feedback (Zarate, 2013). However, further research is needed to determine the long-term effects of vocal training on insula-based networks, especially in trained singers, as such studies are still scarce.

In the present study, we utilized resting-state fMRI (rs-fMRI) to assess experience-dependent neuroplastic changes in large-scale insular connectivity among trained singers and non-singers, an approach similar to previous investigations of functional network connectivity changes between musicians and non-musicians (Luo et al., 2012; Tanaka and Kirino, 2016a; Tanaka and Kirino, 2016b). This methodology is based on the premise that rs-fMRI captures the history of repeated task-dependent synchronized activation between brain regions (Guerra-Carrillo et al., 2014), rendering it a suitable tool for exploring the long-term effects of vocal training on insular networks. To investigate these effects, we utilized a seed-based approach (Fox and Raichle, 2007) to examine the temporally correlated spontaneous low-frequency blood oxygenation level-dependent fluctuations between the whole brain and a-priori defined regions of interests (ROI). These ROIs were chosen based on a tripartite insula subdivision model (Deen et al., 2011; Uddin et al., 2014) and encompassed the left and right posterior insula (PI), dorsal anterior insula (dAI), and ventral anterior insula (vAI) (available at https://bendeen.com/data/).

Previous research from our group using the same approach has shown training-specific insula co-activations with the somatotopic hand area in orchestra musicians (Zamorano et al., 2017), indicating that adaptive changes in the brain associated with the practiced musical action can also be observed during rest. Building on these findings, we aim to test the hypothesis that trained singers will exhibit enhanced insula connectivity compared to non-singers, specifically between the AI with constituents of the dorsal sensorimotor speech stream involved in vocal tract coordination. We predict that the observed connectivity in singers will show a high level of task-relevant structure–function specificity, which would provide additional support for the insula's role in the vocal motor system, and further evidence for the impact of vocal training on insula-based sensorimotor networks (Kleber et al., 2013; Kleber et al., 2017; Zarate, 2013).

2. Results

2.1. Pitch-matching accuracy

All individuals were able to complete the pitch matching task without difficulty. The mean deviation from target pitch across pitch-matching trials was 17.5 cent in singers (SD = 9.71, Median = 16) and 72.8 in non-singers (SD = 103, Median = 29.5). A linear mixed effects model (group = fixed effect; subjects and interval size = random effect) using log-transformed pitch-accuracy data (S-Fig. 1) indicated that singing expertise significantly improved pitch-matching accuracy ($\chi2(1) = 5.80$, p = 0.015). Re-transformation of the data in this model showed that deviation from target pitch was about 21.5 cent (±1.3 SD) lower in singers (15.2 cent) compared to non-singers (36.7).

2.2. Voxel-wise functional connectivity of insula subdivisions

Main connectivity patterns across all participants

Whole-brain connectivity patterns (S-Fig. 2) for the PI, dAI, and vAI (left and right, respectively) across participants were consistent with earlier reports (Deen et al., 2011; Uddin et al., 2014).

The left and right *posterior insula* (PI) connectivity (S-Fig. 2A) encompassed the ipsi- and contralateral insula subdivisions and adjacent operculae, but was otherwise mostly centered on sensorimotor regions (S1, S2, M1, and pre-motor cortices, SMA and pre-SMA), frontal speech motor areas (IFG, Broca's area), the posterior and mid cingulate cortex (PCC and MCC), the temporal (superior temporal, Heschl's, and fusiform gyrus), parietal and (superior and inferior, supramarginal gyrus, and the precuneus) occipital lobe (calcarine, lingual, and cuneus), and the cerebellum.

The left and right *dorsal anterior insula* (dAI) connectivity (S-Fig. 2B)

encompassed other ipsi- and contralateral insula subdivisions, adjacent rolandic and frontal operculae, as well as bilateral sensorimotor regions in the primary (S1) and secondary (S2) somatosensory and motor (M1) cortices, pre-motor cortex and supplementary motor area (SMA), the occipital (calcarine, lingual, and cuneus), temporal (superior temporal gyrus, Heschl's and fusiform gyrus, temporal pole) and parietal areas (supramarginal, superior and inferior parietal gyrus, and the pre-cuneus), the cerebellum, as well as the inferior frontal (IFC), dorsolateral prefrontal (DLPFC), and cingulate cortex (posterior, PCC; middle, MCC, and anterior, ACC). The left and right *ventral anterior insula* (vAI) con-nectivity patterns (S-Fig. 2C) also encompassed ipsi- and contralateral insula subdivisions, but were more focused on adjacent rolandic and frontal operculae, as well as bilateral regions in the frontal lobe (DLPFC, inferior frontal gyrus, IFG) and frontal limbic cortices (ventro anterior prefrontal cortex, VAPFC; orbitofrontal gyrus, OFC), the cingulate

cortex (ACC and MCC), SMA proper and pre-SMA, the temporal (e.g., Heschl's gyrus, temporal pole, superior, middle and inferior temporal gyrus) and the parietal lobes (supramarginal and angular gyrus, precuneus).

Subcortically, all three insula sub-regions showed co-activation patterns with the basal ganglia (putamen, pallidum and caudate) and the thalamus.

2.3. Differences in insula connectivity between trained singers and non-singers

T-contrasts comparing connectivity maps between singers and non-singers revealed increased functional connectivity for the bilateral dAI and the right vAI in singers (Fig. 1 and Table 1).

The **left and right dAI** (Fig. 1A, Table 1) showed increased

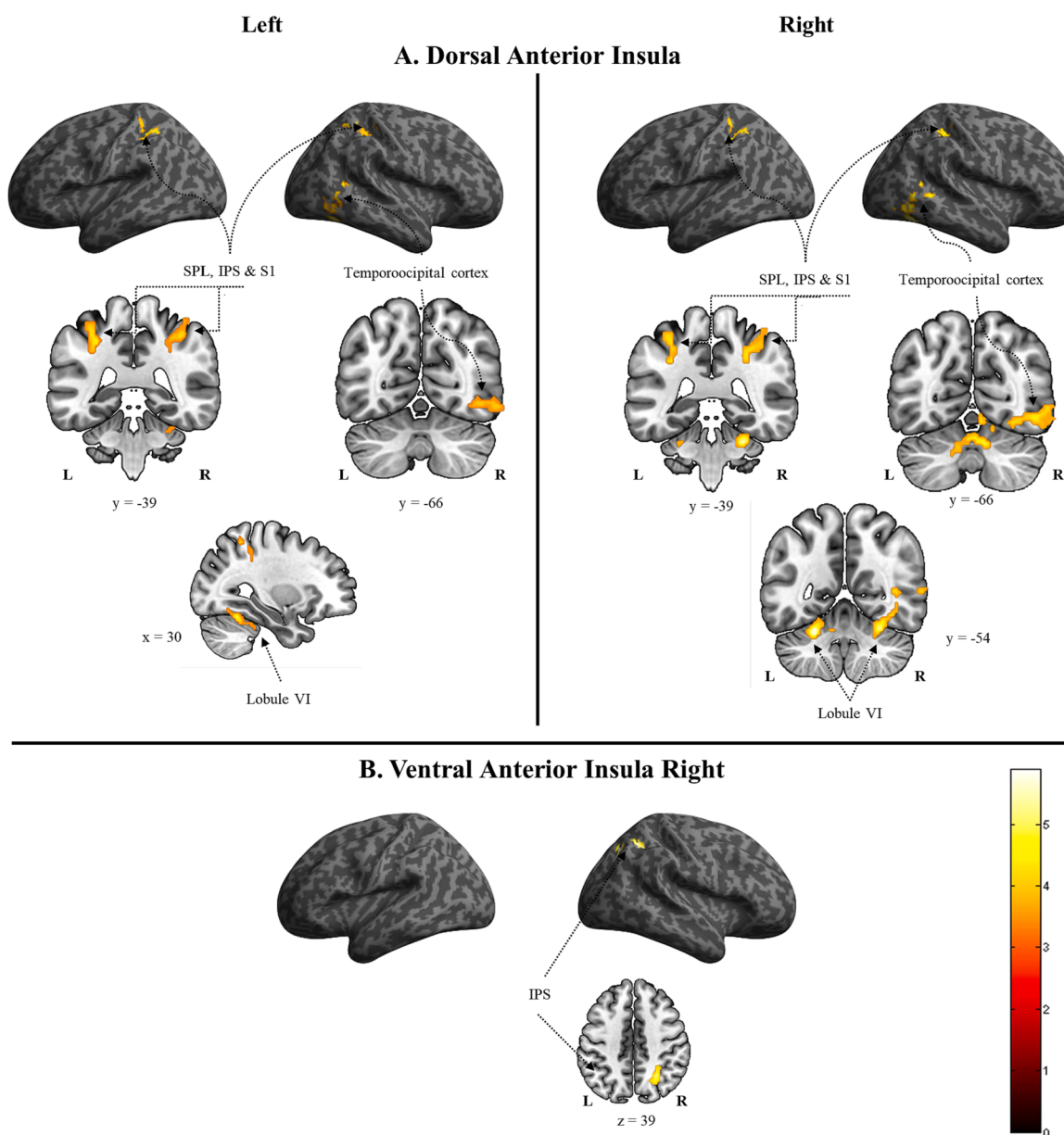**Fig. 1.** T-maps showing significant group differences in functional insula connectivity during resting-state in singers. Only results surviving a cluster-extent based significance threshold of p < 0.05 (FWE corrected) are shown. Detailed information is provided in Table 1. Abbreviations: S1, primary somatosensory cortex; ITG, inferior temporal gyrus; IPS, intraparietal sulcus; Lobule VI, cerebellar lobules.

Table 1
Regions with increased insula connectivity in singers compared to non-singers.

Seed		Left						Right					
Co-activated region	Side	MNI Coordinates						MNI Coordinates					
		Cluster	x	y	z	t-value	d_unb	Cluster	x	y	z	t-value	d_unb
Dorsal Anterior Insula													
Parietal													
IPS & SPL *(Areas hIP3 & 7PC)*	R	79 [a]	33	−51	57	4.67	1.8	60	30	−42	45	4.23	1.7
IPS & S1 *(Areas hIP2 & 2)*	R	79 [a]	39	−39	48	4.40	1.7	60	39	−36	51	4.22	1.7
SPL & S1 *(Areas 7PC & 1)*	R	79 [a]	42	−39	60	4.01	1.6	–	–	–	–	–	–
SPL & S1 *(Areas 5L & 2)*	L	78 [a]	−33	−42	54	4.90	1.9	63	−33	−42	54	4.56	1.8
Temporooccipital													
LOC *(Area hOc41a)*	R	181 [a]	57	−66	−9	5.35	1.8	403 [a]	48	−69	−9	5.00	2.0
Cerebellum													
Lobule VI	R	181 [a]	30	−54	−21	5.25	2.1	403 [a]	27	−54	−24	5.04	2.0
Lobule V	R		30	−36	−30	4.04	1.6	403 [a]	27	−39	−30	5.37	2.1
Lobule VI	L	53	−24	−54	−24	5.35	2.1	98 [a]	−24	−54	−24	5.67	2.2
Lobule V	L	53	−27	−36	−33	4.86	1.95	98 [a]	−27	−42	−27	4.07	1.6
Vermis VI	L	–	–	–	–	–	–	403 [a]	−3	−66	−24	4.23	1.7
Ventral Anterior Insula													
Parietal													
IPS *(hIP3)*	R	–	–	–	–	–	–	75	27	−57	39	4.25	1.7

MNI coordinates and local maxima of whole-brain differences (t-contrasts) in insula-based network connectivity during resting state in singers compared to non-singers. Rsults shown survived an SPM12 cluster-extent threshold of p < 0.05 (FWE corrected). T-values of significantly activated peak-voxels refer to MNI co-ordinates ([a] = same cluster). Brodmann Areas (BA) labeling utilized the Automatic Anatomic Labeling toolbox (AAL; 2002). Probabilistic cytoarchitectonic maps for structure–function relationships in standard reference space (in brackets) were assigned using the Anatomy Toolbox (Eickhoff et al., 2005). Abbreviations: d_{unb}, effect size as unbiased Cohen's d (Cohen, 1988); S1, primary somatosensory cortex; SPL, superior parietal lobe; LOC, lateral occipital cortex; IPS, intra parietal sulcus.

functional connectivity with the right superior parietal and the bilateral primary somatosensory cortex (S1), the cerebellum (lobule IV, V and VI) and the inferior temporal gyrus.

The **right vAI** (Fig. 2B, Table 1) showed increased functional connectivity with the right intraparietal sulcus.

The left vAI and the PI yielded no significant differences between singers and non-singers, neither did any of the reversed comparison (non-singers minus singers) across all ROIs.

2.4. Regression results

Regression analyses aimed to determine correlations between insula ROI connectivity maps with (i) behavioral pitch-matching accuracy and (ii) accumulated singing training (Table 2). Pitch-matching accuracy showed no significant correlations with insula connectivity maps across participants. However, accumulated musical training in trained singers was significantly correlated with insula connectivity maps (Fig. 2, Table 2):

The **right PI** connectivity maps (Fig. 2A) showed a positive correlation with accumulated training with right S1 in the somatotopic representation of the trunk.

The **left dAI** connectivity maps (Fig. 2B) showed positive correlations with accumulated singing training in the bilateral representation of the diaphragm and the right hemispherical representation of the larynx within M1 and S1, as well as in the bilateral thalamus and the left putamen. The **right dAI** maps showed positive correlations with accumulated training in the left hemispherical representation of the diaphragm within M1 and S1.

3 Discussion

In this resting-state fMRI study, we present novel insights into the impact of conservatory-level singing training on insula-based networks at rest. Comparing singers to non-singers, our results demonstrate experience-dependent enhancements in insular connectivity, particularly between the dAI and sensorimotor areas as well as higher-level brain regions. Singers exhibited increased connectivity with sensorimotor speech networks, and accumulated vocal training correlated positively with connectivity in motor and somatosensory cortices,

including the somatotopic representations of the larynx and the diaphragm/pelvic floor. These findings suggest that expert singing training may have use-dependent neuroplastic effects on insula-based networks involved in speech motor control and sensorimotor integration. By providing new evidence for a role of the insula in vocal and musical performance, this study lays the foundation for further research aimed at exploring the specific mechanisms underlying these changes.

3.1. Insula connectivity across groups

Previous studies identified numerous functional and structural connections between the insula and cortical and subcortical brain regions involved in sensory, emotional, motivational and cognitive processes (Gogolla, 2017). Based on functional segregation of co-activation patterns during resting-state, a tripartite organization of insula subdivisions has been described, containing both unique and overlapping functional profiles of the posterior insula (PI), the dorsal anterior insula (dAI), and the ventral anterior insula (vAI) (Uddin et al., 2014). These functional profiles are supported by an underlying structural organization (Nomi et al., 2018). Consistent with previous findings (Deen et al., 2011; Zamorano et al., 2017; Zamorano et al., 2019), the co-activation maps reported in this study reflect a "cognitive" frontoparietal profile for the dAI, a more "affective" profile for the vAI, and a more sensorimotor centered profile for the PI (S-Fig. 2).

3.2. Between-group differences in insula connectivity

Direct group comparisons revealed that trained singers showed enhanced insula-based connectivity compared to non-singers, whereas the reverse comparison did not show any significant differences. This is a novel finding that underscores the unique impact of singing training on insula-based networks.

Dorsal anterior insula connectivity between singers and non-singers

The pattern of enhanced left dAI co-activation in singers was focused on the bilateral cerebellum (lobules V & VI) and the superior parietal lobe (SPL), with clusters extending into the intraparietal sulcus (IPS) and the primary somatosensory cortex (S1). The right dAI showed a comparable co-activation profile (Fig. 1).

Lobule VI of the cerebellum is a somatotopically organized

Regression accumulative training in singers
A. Right Posterior Insula

B. Dorsal Anterior Insula
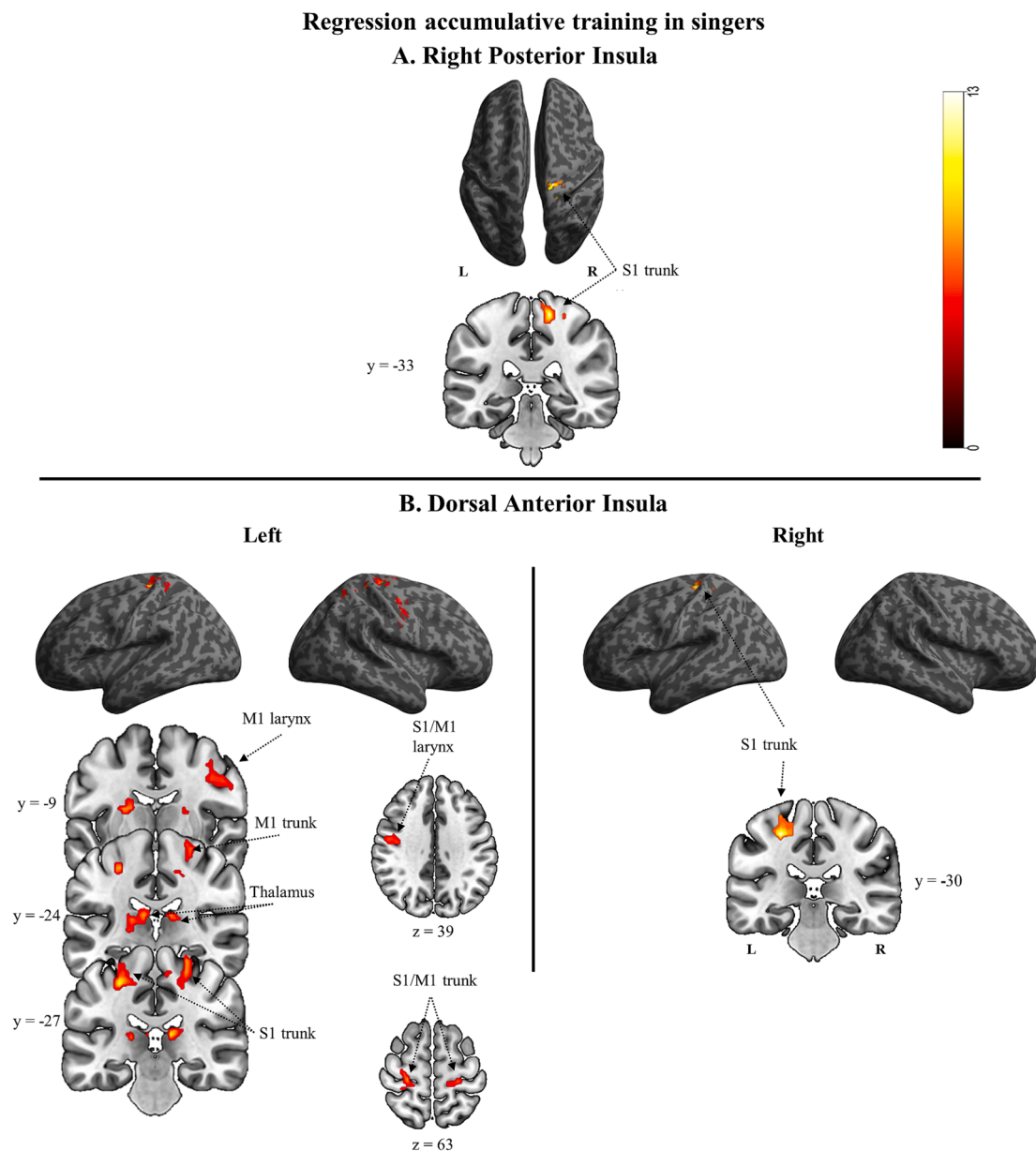
Fig. 2. Regression analyses for individual insular ROIs assessing connectivity maps in relation to the total amount of vocal training in professional singers. Only results surviving a cluster-extent based significance threshold of p < 0.05 (FWE corrected) are shown. Detailed information is provided in Table 2. Abbreviations: M1, primary motor cortex; S1, primary somatosensory cortex.

sensorimotor region that receives and sends signals involved in the control of speech and song production (Brown et al., 2006; Grodd et al., 2001; Guell et al., 2018; O'Reilly et al., 2010; Stoodley and Schmahmann, 2010). Its functional contribution to vocalization processes (Ackermann, 2008; Callan et al., 2007; Mathiak et al., 2002; Stoodley et al., 2012) is consistent with reports that cerebellar lesions in this area can lead to speech motor disorders (Ackermann et al., 2007). Both task- and rs-fMRI studies have linked lobules IV and V to predictive mechanisms of sensorimotor and cognitive aspects of speech motor control (Argyropoulos, 2016; Baumann et al., 2015; Moberget and Ivry, 2016). These findings align with the insula's role as a central hub within the functional speech connectome, which includes the cerebellum (Fuertinger et al., 2015). The current study's dAI cerebellar co-activation coordinates closely match those reported during overt singing (Kleber et al., 2010; Kleber et al., 2013) and vowel production (Brown et al., 2008; Brown et al., 2009), and task-fMRI has linked cerebellar sensorimotor activation to singing accuracy and experience (Kleber et al.,

2017). This is consistent with reports that cerebellar neuroplasticity is sensitive to musical training (Baer et al., 2015; Olszewska et al., 2021). Together, these findings suggest that long-term professional singing training may have lasting effects on insula-based networks involved in speech motor control and sensorimotor integration.

However, because the cerebellum has a domain-general role in motor coordination (Swinnen and Wenderoth, 2004), it is not possible to claim that these effects are specific to (singing) training of the vocal system. In fact, a previous rs-fMRI study reported increased insular connectivity with the cerebellum in a cohort of trained pianists (Luo et al., 2012). The presence of somatotopically plausible co-activations with the cerebellum during resting-state in highly homogeneous samples of singers and pianists suggests the potential impact of musical expertise on these co-activations. However, our previous resting-state fMRI study with professional orchestra musicians did not exhibit similar co-activation profiles, possibly due to the larger variability in participants' musical training (i.e., wind, brass, strings, and percussion

Table 2
Correlations between insula connectivity maps with accumulated music training.

Seed		Left					Right				
Co-activated region	Side	MNI Coordinates					MNI Coordinates				
		Cluster	x	y	z	t-value	Cluster	x	y	z	t-value
Posterior Insula											
Sensorimotor											
S1-trunk (Area 3b)	R	–	–	–	–	–	52	15	–33	63	10.63
Dorsal Anterior Insula											
Sensorimotor											
SPL (Area 7a)	R	136 [a]	24	–54	51	13.44					
M1-trunk (Area 4a)	R	136 [a]	21	–30	54	7.98	–	–	–	–	–
S1-trunk (Area 3a)	R	136 [a]	27	–39	60	7.49	–	–	–	–	–
M1-trunk (Area 4p)	L	93 [a]	–27	–30	51	13.20	–	–	–	–	–
S1-trunk (Area 3a)	L	–	–	–	–	–	63 [a]	–27	–30	51	9.34
S1-trunk (Area 3a)	L	93 [a]	–24	–30	69	6.45	63 [a]	–27	–30	66	5.55
M1-larynx (Area 4p)	R	63 [a]	51	–9	36	5.75	–	–	–	–	–
S1-larynx (Area 3b)	R	63 [a]	42	–12	48	6.01	–	–	–	–	–
Subcortical											
Thalamus	R	54 [a]	12	–27	12	10.59	–	–	–	–	–
Thalamus	R	54 [a]	21	–12	12	7.29	–	–	–	–	–
Thalamus	L	33	–9	–24	15	7.85	–	–	–	–	–
Putamen	L	54	–24	9	12	9.15	–	–	–	–	–

MNI coordinates and local maxima of from regression analyses testing correlations between insular subdivision connectivity maps with accumulated singing training. Results shown survived a cluster-extent based threshold of p < 0.05 (FWE). T-values of significantly activated peak-voxels correspond to MNI coordinates (a = same cluster). Brodmann Areas (BA) labeling utilized the Automatic Anatomic Labeling toolbox (AAL; 2002). Probabilistic cytoarchitectonic maps for structure–function relationships in standard reference space (brackets) were assigned using the Anatomy Toolbox (Eickhoff et al., 2005). Abbreviations: dunb, effect size as unbiased Cohen's d (Cohen, 1988); M1, primary motor cortex; S1, primary somatosensory cortex; SPL, superior parietal lobe. Somatotopic representations of activations in the primary motor and somatosensory cortex were determined based on the literature and confirmed through direct cluster comparisons with task-fMRI singing data from our prior studies (pitch matching, Kleber et al., 2013; song production, Kleber et al., 2010) and based functional localizer fMRI data from an ongoing (unpublished) study in our lab (i.e., rhythmic finger tapping and rhythmic vocalization).

instruments) (Zamorano et al., 2017). These prior results were instead centered on the dAI's vital role in salience detection and cognitive processes, such as task-based attention switching, inhibition, and error awareness (Molnar-Szakacs and Uddin, 2022; Uddin, 2015).

Apart from the cerebellum, the dAI co-activation in trained singers also extended to the bilateral SPL, IPS, and S1. The SPL is crucial for integrating multiple perceptual signals with action to perform high-level cognitive functions required for interacting with the world (Passarelli et al., 2021), and its own signals are associated with movement intentions and goals by using sensory information for goal-directed movements, such as the perception of one's own body. Increased SPL activation in musicians has been linked to heightened sensorimotor control, increased attention, and working memory load (Foster and Zatorre, 2010; Pallesen et al., 2010), which may explain the findings in trained singers.

At its ventral border, the IPS is known to be a multimodal convergence zone that integrates multiple cognitive functions (Guipponi et al., 2013). Although its association with numeric cognitions is most consistent (Stripeikyte et al., 2021), rs-fMRI has linked functional connectivity of the anterior IPS (hIP2 and hIP1) more strongly to frontal attentional and premotor regions (including the insula), and the posterior IPS (hIP3) with occipital brain areas (Uddin et al., 2010), contributing to visual object representations and coordinating visuomotor actions. Additionally, the IPS has demonstrated a significant role in processing fine-grained discrete pitch relations in song as compared to speech prosody (Merrill et al., 2012), including the transposing of musical pitch information (Foster and Zatorre, 2010). In the current study, the SPL activation clusters that extended anteriorly into S1 may correspond to a second somatosensory homunculus in which tactile, visual, and/or auditory receptive fields are anchored to the same body part (Serino, 2019).

Ventral anterior insula connectivity in singers versus non-singers

In contrast to the dAI, the vAI has been primarily associated with affective processing (Craig, 2009; Craig, 2010). However, our study found that left vAI co-activation in singers also extended to the intraprietal sulcus (IPS), which is more commonly linked to frontoparietal attentional control systems (Kleber et al., 2017; Uddin, 2015; Zarate, 2013). This suggests that singing experience may have an impact on these attentional networks in addition to affective processing, and supports previous studies that have reported changes in attentional processing and executive function in musicians (Pallesen et al., 2010; Roden et al., 2013).

3.3. The influence of accumulated singing training on insula co-activation maps

Experienced singers showed a positive correlation between the total amount of accumulated vocal training and increased insula co-activation with the sensorimotor speech network's core constituents. The corresponding coordinates aligned with the cortical representations of the trunk/diaphragm (left dAI-M1/S1 bilaterally; right dAI-S1 left; right PI-S1 left) and the larynx/phonation area (left dAI-M1/S1 right). These functional associations were confirmed through direct comparisons with previous singing-related peak and cluster activation data (Kleber et al., 2010; Kleber et al., 2013), as well as functional localizer data from an ongoing (unpublished) fMRI study that included isolated hand, voice, and foot movements. In addition, left dAI co-activation encompassed the bilateral thalamus and the left putamen.

The cortical areas subserving the sensorimotor and related cognitive control of speech are known to interact with the anterior insula (Battistella et al., 2018), likely by integrating sensory and visceral inputs necessary for coordinating the respiratory and vocal tract muscles (Ackermann and Riecker, 2010; Dronkers, 1996; Oh et al., 2014). The motor cortical "larynx/phonation" area, which coordinates the interaction of respiratory, laryngeal, and articulatory muscle groups, is responsible for voluntary vocalizations (Belyk and Brown, 2017; Kumar et al., 2016; Loucks et al., 2007). An voluntary breathing area has also been reported in the somatotopic representation of the trunk and diaphragm (Eickhoff et al., 2008; Takai et al., 2010), both regions of which overlap with the locations found in the current study. Furthermore, the putamen receives strong projections from M1 during speech production (Simonyan and Horwitz, 2011) and displays enhanced activation during

vocalization in trained singers (Kleber et al., 2010; Segado et al., 2018).

The insula also contributes to the spinothalamocortical pathway, in which the lamina I projects afferent information from body tissues via the thalamus to both the somatosensory area 3a and the PI (Craig, 2002; Craig, 2009; Craig, 2010). The signals are then re-represented, integrated, and evaluated in the anterior insula, contributing to cognitive, emotional, and sensorimotor processes by comparing predictions about expected bodily signals with the actual sensory inputs (Molnar-Szakacs and Uddin, 2022). The somatosensory larynx and trunk representations process bodily information from respiratory, laryngeal, and articulatory actions (Bouchard et al., 2013; Conant et al., 2018), guiding the vocal motor system during the preparation and coordination of vocalizations (Bouchard et al., 2013; Simonyan and Horwitz, 2011; Tremblay et al., 2003). Recent studies have demonstrated a causal involvement of larynx S1 in vocal pitch control during singing using repetitive TMS (Finkel et al., 2019). Given the somatotopic correspondence, we propose that the repeated co-activation of insula-based vocal sensorimotor networks linked to singing experience may trigger neuroplastic processes. An alternative explanation may be that the conscious awareness of breathing-related signals in singers might even be enhanced during vocal rest, thus reflecting the importance of the respiratory system for singing voice "support" (Herbst, 2017).

3.4. Limitations

A known limitation of rs-fMRI studies is that participants are exposed to continuous scanner noise, which can reduce the robustness and the replicability of functional connectivity findings within the somatosensory, auditory, and motor networks (Andoh et al., 2017). Despite this, the replication of previous large-scale connectivity profiles across participants (Deen et al., 2011; Uddin et al., 2014) and the long-range functional correspondence between the dAI and somatotopic vocalization areas in trained singers increases our confidence in the current results. Moreover, residual head-motion effects after artifact correction tend to decrease rather than increase long-distance correlations (Satterthwaite et al., 2012). Additionally, our group's previous research with orchestra musicians demonstrated enhanced insula connectivity with the somatotopic hand area (Zamorano et al., 2017), indicating that training-related adaptive changes in insula-based networks are specific to the practiced musical action. However, an important consideration is that the increased activation of the insula network in singers may reflect a result of mastering a series of abilities required for singing, rather than a specific effect of vocal training on the brain. For example, singing requires not only the coordination of respiratory and vocal tract muscles but also emotional expression, the integration of auditory feedback, and the ability to monitor and adjust pitch and rhythm. Thus, it is possible that the increased activation of the insula network in singers is a reflection of the dynamics of all of these abilities. Future research with larger samples should therefore investigate the specific contributions of musical expertise to insular connectivity, including comparisons between professional singers, instrumentalists, and professionals who extensively use their voice in their work (e.g., actors, voiceover artists).

3.5. Conclusions

This study examined the impact of conservatory-level singing training on insula-based co-activation maps at rest. The results of this research reveal experience-dependent enhancements in insular connectivity in singers. Specifically, between the dAI with sensorimotor and higher-level brain regions, suggesting that singing training may affect both cognitive and motor aspects of the vocal system. Together, the current study adds to a growing body of literature associating functional neuroplasticity involving the insula with musical performance expertise (Criscuolo et al., 2022), and contributes to our understanding of the insula's involvement in vocal sensorimotor control. However, further research, including task-based fMRI studies, is required to elucidate the

specific mechanisms underlying these changes and to determine the extent to which the insula moderates vocalization-related sensory integration.

4. Experimental procedure

4.1. Participants

A total of 25 right-handed subjects without reported history of neurological or psychiatric disease participated in this study. Participants were subdivided in two groups based on their singing expertise. Professional singers (n = 12, 6 female, 32.7 ± 8.6 yrs) with music-conservatory level education took their first formal singing lesson at the average age of 16 years (±6.7) and accumulated on average 12,957 h of singing experience (range: 1456–38220). Non-singers (n = 13, 6 female, 28.1 ± 7.3 yrs) consisted of University of Tübingen medical school students, who neither received prior singing training nor reported any involvement in occasional singing activities (e.g., choirs, informal rock bands etc.). Among the non-singer participants, five reported having prior formal training on various musical instruments. The instruments, age range, and accumulated hours were as follows: flute (age 8–11, 312 h), guitar (age 18–20, 208 h), piano (age 17–18, 52 h), accordion (age 8–13, 520 h), and cello (age 12–18, 1248 h). Considering the limited musical experience in this group and that none of them had played any instruments in the five years preceding the study, we determined that controlling for this factor was not necessary. All participants were informed about the details of the study and provided written consent. The study was conducted under a protocol approved by the research ethics board of the University of Tübingen.

4.2. Behavioral experiments

Pitch-matching accuracy

Prior to rs-fMRI, a behavioral pitch-matching task was performed to assess experience-dependent differences in singing accuracy between trained singers and non-singers. During this task, a total of 54 pseudorandomized musical target intervals were presented via headphones using Max/MSP software to control the experiment (Cycling 74, San Francisco, California, USA). Upon each presentation, participants were prompted to reproduce the pitch of two tones with their singing voice. The first tone always started at the fundamental frequency of 311.13 Hz for females (D#4 in musical notation) and 155.565 Hz for males (D#3 in musical notation). The second tone was either the same (4×) or differed from the first tone by one (6×), three (12×), five (12×), six (12×) or seven (8×) semitones, with an equal number of ascending and descending intervals. Each tone was played with a duration of 900-ms, separated by a 200-ms gap. Vocal reproduction was recorded and saved in wave format for offline automated analyses of pitch-matching accuracy.

Pitch-matching accuracy was defined by the deviation between the target tones presented via headphones and the tones sung by the participants. In a first step, the deviation was estimated in cents (one semitone corresponds to 100 cents) using a custom-made script within the CUEX performance analysis system (Friberg et al., 2005) runing in Matlab (The MathWorks, Inc., Natick, Massachusetts, United States). In a second step, statistical analyses were performed to assess the effect of singing expertise on pitch-matching accuracy. As the 54 pitch-matching responses per subject and the responses for same interval sizes could not be regarded as independent observations, a generalized linear mixed model was fit using the lmer function provided by the lme4 package (Bates et al., 2015) in R (v.3.6.1; R Development Core Team, 2019). The full model included 'group' (singers *vs* non-singers) as fixed effect, and 'subject' and 'interval size' as random effects [m_full <- lmer(pitch_devation ~ group + (1|subject_nr) + (1|interval_size), REML = FALSE)]. The statistical significance of the full model was assessed from Chi square distribution and p values estimated with a likelihood ratio test

(Barr et al., 2013) by comparing the full to a null model without the fixed factor 'group' [m_null <- lmer(pitch_deviation ~ 1 + (1|subject_nr) + (1|interval_size), REML = FALSE)]. As the residuals were found to be non-normal and there was evidence of heteroscedasticity of variances, the data were log transformed (log10) before statistical analysis. We confirmed that the transformed values produced comparable results to non-transformed values.

4.3. Resting-state fMRI acquisition

Resting-state data were acquired over a period of 7.5 min with the eyes closed. Participants were instructed to stay awake and to think of nothing in particular. Magnetic resonance imaging was performed using a 3-Tesla whole body MRI Scanner (Siemens MAGNETOM Prisma™ 3 T, Erlangen, Germany). For each subject, 225 echo-planar volumes were acquired (repetition time, 2000 ms; echo time, 30 ms; matrix dimensions, 64 × 64; field of view, 1260 mm; 30 transversal slices; slice thickness, 4 mm; flip angle, 90 degrees) The structural imaging data for anatomical reference consisted of T1-weighted images (mprage, repetition time, 2300 ms; echo time, 4.18 ms; matrix dimensions, 512 × 512; field of view, 256 × 256 mm; 176 slices; slice thickness, 1 mm; flip angle, 9 degrees).

4.4. fMRI data preprocessing

Following our previous approach (Zamorano et al., 2017; Zamorano et al., 2019), functional images were preprocessed with the Data Processing Assistant for Resting-State fMRI (DPARSF; Chao-Gan and Yu-Feng, 2010), based on the Statistical Parametric Mapping software package (SPM12; https://www.fil.ion.ucl.ac.uk/spm) and the Data Processing & Analysis of Brain Imaging toolbox (DPABI; https://rfmri.org/DPABI DPARSF_V6.1_141101). We discarded the initial 10 volumes from each participant's dataset before preprocessing. Following slice-time correction and co-registration, gray and white matter were segmented from co-registered T1 images using the unified segmentation model (Ashburner and Friston, 2005). The resulting parameter file was used to normalize the functional images (3 mm^3 voxel size) to standard Montreal Neurological Institute (MNI) stereotactic space, which were subsequently smoothed with an isotropic Gaussian kernel (FWHM: 6 mm^3). Post-hoc physiological noise control was performed by including nuisance regression parameters, consisting of white matter, CSF, and the Friston-24 head motion parameters (Friston et al., 1996), which account for a wider range of motion-related effects on the rs-fMRI signal (Yan et al., 2013). Following the description by (Van Dijk et al., 2012) as implemented in the BRANT toolkit (Xu et al., 2018), the average head motion displacement between singers and non-singers was computed (0.07- and 0.06-mm translation; 0.032- and 0.033-degree rotation) and differences were non-significant: $t(21) = 1.172$, $p = 0.25$ and $t(21) = -0.328$, $p = 0.75$. Likewise, the average number of movements > 0.1 mm between the group of singers (39.5) and non-singers (26.3), as well as the mean framewise displacement (FD; 0.13 and 0.11) were not significantly different: $t(21) = 0.934$, $p = 0.36$ and $t(21) = 0.857$, $p = 0.57$. WM and CSF masks were generated using SPM's tissue probability maps (empirical thresholds: 90 % for WM mask and 70 % for CSF mask). No global signal regression was performed to avoid introducing BOLD signal distortions (Murphy et al., 2009). A temporal filter (0.006–0.1 Hz) was applied to reduce low frequency drifts and high frequency physiological noise.

4.5. Functional connectivity analysis

Statistical analyses were performed in SPM12 to assess voxel-wise connectivity maps of posterior, ventral anterior and dorsal anterior insula ROIs in each hemisphere. The six insula ROIs consisted of parcellated insular subdivisions (posterior, PI; dorsal anterior, dAI; and ventral anterior insula, vAI) in MNI stereotactic space based on the

clustering of functional connectivity patterns during resting state. The ROI template images were kindly provided by Dr. Ben Deen (https://bendeen.com/data/). A visual representation is provided in S-Fig. 3.

First, the main connectivity patterns were determined across all participants (N = 25) by entering z-transformed connectivity maps into one-sample t-tests for each insula ROI (S-Fig. 2). Significance threshold for voxel-wise statistics was set to $p < 0.05$ familywise error corrected (FWE) to validate our results against previously published patterns (Deen et al., 2011).

To assess group differences in insula-connectivity maps between singers and non-singers, independent two-sample t-tests were computed for each insula ROI. A cluster-extent based thresholding method was employed to increase sensitivity for detecting true activations in studies with moderate sample sizes. Following the recommendations for cluster-extent thresholding in fMRI data analyses (Woo et al., 2014), we first set a stringent cluster-defining threshold of $P = 0.001$ before estimating the FWE corrected ($p < 0.05$) cluster-extent threshold by Gaussian Random Field method, as implemented in SPM12. Effect sizes using Cohen's d ($d = 2 t / sqrt(df)$) were computed and adjusted [unbiased Cohen's d; $d_{unbiased} = d (1 - (3 / 4df - 1))$] to control for overestimation of the effect size due to the moderate sample size (Cohen, 1988).

T-values of significantly activated peak-voxels within these clusters are reported as MNI coordinates. Anatomical regions were determined with the Automated Anatomical Labeling atlas (aal, Tzourio-Mazoyer et al., 2002) and the Anatomy Toolbox (Eickhoff et al., 2005). The somatotopic representation of activation clusters in the primary motor and somatosensory cortex was determined through comparison with task-fMRI singing data from our prior studies (pitch matching, Kleber et al., 2013; song production, Kleber et al., 2010) and unpublished functional localizer data from our lab (rhythmic finger tapping and rhythmic vocalization).

4.6. Regression analysis

Two independent regression analyses were performed in SPM12 to correlate insula ROI connectivity maps with (i) behavioral pitch-matching accuracy across all participants (i.e., log-transformed deviation from target pitch) and (ii) accumulated hours of singing training in professional singers using the cluster-based FWE correction method detailed above.

CRediT authorship contribution statement

A.M. Zamorano: Methodology, Investigation, Formal analysis, Writing – original draft, Writing – review & editing. **R.J. Zatorre:** Methodology, Writing – review & editing. **P. Vuust:** Writing – review & editing. **A. Friberg:** Writing – review & editing, Formal analysis. **N. Birbaumer:** Writing – review & editing, Supervision. **B. Kleber:** Conceptualization, Methodology, Investigation, Writing – review & editing, Supervision.

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.

Data availability

Data will be made available on request.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.brainres.2023.148418.

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