NEUROSCIENCE
RESEARCH ARTICLE

M. G. Peter et al. / Neuroscience 472 (2021) 1–10

Seeing Beyond Your Nose? The Effects of Lifelong Olfactory Sensory Deprivation on Cerebral Audio-visual Integration

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Abstract—Lifelong auditory and visual sensory deprivation have been demonstrated to alter both perceptual acuity and the neural processing of remaining senses. Recently, it was demonstrated that individuals with anosmia, i.e. complete olfactory sensory deprivation, displayed enhanced multisensory integration performance. Whether this ability is due to a reorganization of olfactory processing regions to focus on cross-modal multisensory information or whether it is due to enhanced processing within multisensory integration regions is not known. To dissociate these two outcomes, we investigated the neural processing of dynamic audio-visual stimuli in individuals with congenital anosmia and matched controls (both groups, $n = 33$) using functional magnetic resonance imaging. Specifically, we assessed whether the previously demonstrated multisensory enhancement is related to cross-modal processing of multisensory stimuli in olfactory associated regions, the piriform and olfactory orbitofrontal cortices, or enhanced multisensory processing in established multisensory integration regions, the superior temporal and intraparietal sulci. No significant group differences were found in the *a priori* hypothesized regions using region of interest analyses. However, exploratory whole-brain analysis suggested higher activation related to multisensory integration within the posterior superior temporal sulcus, in close proximity to the multisensory region of interest, in individuals with congenital anosmia. No group differences were demonstrated in olfactory associated regions. Although results were outside our hypothesized regions, combined, they tentatively suggest that enhanced processing of audio-visual stimuli in individuals with congenital anosmia may be mediated by multisensory, and not primary sensory, cerebral regions. **© 2021 The Authors. Published by Elsevier Ltd on behalf of IBRO. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).**

Key words: anosmia, multisensory integration, cross-modal, sensory loss.

INTRODUCTION

Absence of input from one sensory modality can lead to enhanced performance in remaining senses (Merabet and Pascual-Leone, 2010; Frasnelli et al., 2011). Although more sparsely studied, *integration* of input from remaining senses in sensory deprived individuals tend to not show the same behavioral benefits, compared to sensory intact individuals, as unimodal tasks often do (Collignon et al., 2009; Occelli et al., 2013; Hauthal et al., 2014; Scheller et al., 2021). In contrast to these observations, mainly based on auditory and visual sensory deprivation, olfactory sensory deprivation has been linked to unaltered or even decreased performance in the remaining chemical senses (Gudziol et al., 2001; Frasnelli et al., 2010; Landis et al., 2010), but also enhanced audio-visual integration performance (Peter et al., 2019). This suggests the existence of a compensatory mechanism that enables better utilization of the combined, rather than individual, available sensory input when the olfactory dimension is lost. Whether this ability is linked to cross-modal multisensory processing in

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https://doi.org/10.1016/j.neuroscience.2021.07.017
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regions associated with the absent sense, in line with the cross-modal unisensory processing demonstrated in blind and deaf (Bavelier and Neville, 2002), is not known.

We recently demonstrated that individuals with either acquired or congenital anosmia (complete olfactory sensory deprivation) demonstrated enhanced performance in an audio-visual simultaneity judgement task with simple perceptual stimuli when compared to healthy controls (Peter et al., 2019). Additional results further indicated greater multisensory enhancement in individuals with congenital anosmia when presented with more complex and degraded audio-visual object information; this was not the case, however, for individuals with acquired functional anosmia. While the mechanisms behind these sensory deprivation-related multisensory benefits are unknown, enhanced sensory performance in blind individuals is associated with both cross-modal processing and morphological reorganization in visual cortex (Collignon et al., 2007; Voss and Zatorre, 2012). Assuming that the same link between behavioral performance, functional processing, and morphological reorganization exists in congenital anosmia, the orbitofrontal cortex, a multisensory olfactory association region demonstrating morphological abnormalities in congenital anosmia (Frasnelli et al., 2013; Karstensen et al., 2018; Peter et al., 2020), could form a basis for the demonstrated behavioral alterations.

A prevailing notion has been that the brain consists of sensory specific regions processing unimodal sensory information and that this information is integrated with information from other sensory modalities in downstream, multisensory processing regions. There is, however, increasing consensus in the literature that regions previously thought of as unisensory in fact process input from multiple senses (Thesen et al., 2004; Ghazanfar and Schroeder, 2006). This multisensory character of early sensory processing regions is also evident in the olfactory system where the influence of non-olfactory sensory information has repeatedly been demonstrated within regions commonly viewed as part of the olfactory cortex when presented with odor-associated visual stimuli (Gottfried et al., 2004). Of particular relevance is a recent study demonstrating that activity within the posterior piriform cortex, a core olfactory region, increased with the number of senses carrying congruent, but not incongruent, object information. This suggests that information from non-olfactory sensory input is not merely processed within piriform cortex, but that there is actual *integration* of multisensory information taking place (Porada et al., 2019). The structures enabling cross-modal processing and multisensory integration within piriform cortex in individuals with normal olfactory abilities might help facilitate enhanced cross-modal processing and integration in individuals with anosmia who lack competing inputs from the olfactory system. Additionally, a surprising lack of morphological (Peter et al., 2020; Frasnelli et al., 2013; however see Karstensen et al., 2018) as well as functional connectivity (Peter et al., 2021) abnormalities in piriform cortex has been reported in individuals with congenital anosmia, suggesting that functional processing in this region is not eliminated despite the absence of olfactory input. It could therefore be hypothesized that enhanced cross-modal processing in piriform cortex forms a basis for the demonstrated increased audio-visual integration performance in individuals with lifelong olfactory sensory deprivation.

Although the demonstrated multisensory behavioral alteration in anosmia could be mediated by olfactory regions with high potential of enhanced cross-modal processing, it is also plausible that established audio-visual integration regions contribute to altered multisensory processing. Individual differences in audio-visual integration within individuals with intact sensory abilities have been linked to the superior temporal sulcus (Werner and Noppeney, 2010), a region that along with the intraparietal sulcus and prefrontal cortex are considered the main cortical sensory integration regions for auditory, visual, and tactile sensory information (Stein and Stanford, 2008). Of particular interest is the fact that both the superior temporal sulcus and intraparietal sulcus have been linked to temporal integration of audio-visual stimuli (Noesselt et al., 2012; Powers et al., 2012; Zmigrod and Zmigrod, 2015), an ability for which individuals with anosmia have demonstrated a clear benefit (Peter et al., 2019). Moreover, both of these established multisensory integration regions are associated with integration of olfactory information with information from other sensory modalities (Gottfried and Dolan, 2003; Regenbogen et al., 2017), potentially facilitating functional reorganization when olfactory input is absent. It could therefore be hypothesized that a complete absence of olfactory input promotes enhanced processing of multisensory stimuli in these two established multisensory regions.

Here, we assess two different, but not mutually exclusive, hypotheses: (I) Individuals with congenital anosmia demonstrate enhanced cross-modal processing of multisensory stimuli in cortical regions normally processing olfactory information, and (II) Individuals with congenital anosmia demonstrate enhanced processing of multisensory stimuli in traditional multisensory integration regions. To test these hypotheses, we assess whether the processing and integration of dynamic, audio-visual stimuli differs between individuals with congenital anosmia and matched controls using functional magnetic resonance imaging (fMRI).

EXPERIMENTAL PROCEDURES

Participants

A total of 68 individuals participated in the study: 34 were individuals with isolated congenital anosmia (ICA; lifelong complete olfactory sensory deprivation without a known cause or presumed related symptoms), and 34 were controls that were matched to the individuals with ICA in respect of age, sex, and level of education (Table 1). Inclusion in the ICA group was based on self-reported lifelong inexperience with olfactory perception in combination with an absence of known etiology of the sensory deprivation (e.g., head trauma early in life, endocrine problems indicating Kallmann syndrome). Functional anosmia was confirmed for the ICA group,

Table 1. Descriptive statistics per experimental group

	ICA [n = 33]	Control [n = 33]
Age [years]	34.2 (12.9)	34.4 (12)
Women; men [n]	21; 12	21; 12
Education [years]	14.1 (2.6)	14.2 (1.7)
TDI	10.9 (2.3)	35.3 (3.9)
TDI value range	7–15	28.5–42.5
Odor Threshold	1.2 (0.5)	8.6 (3.2)
Odor Discrimination	4.9 (1.6)	13.4 (1.6)
Odor Identification	4.8 (1.5)	13.3 (1.3)

Values presented as mean (standard deviation) except for TDI range which represents min and max values within each group. ICA = Isolated congenital anosmia, TDI = combined score from the Sniffin' Sticks olfactory sub-tests.

and normosmia for the control group, according to normative values within their respective age and sex group, using the full Sniffin' Sticks testing procedure (Hummel et al., 2007). This included olfactory detection threshold, odor quality discrimination, and cued odor identification ability. One individual from each group was excluded due to data corruption for a control participant and a discovered morphological deviation in one participant in the ICA group. This rendered a final sample size of 66 participants (Table 1).

Due to the rare clinical condition, data was collected at two different locations to increase sample size: Sweden and the Netherlands. A total of 44 participants (22 ICA patients and their matched control individuals) were recruited in Sweden and 22 participants (11 ICA patients and their matched control individuals) were recruited in the Netherlands. Because congenital anosmia is rarely studied, the assessed potential of discovering group differences, if present, are based on literature on sensory deprivation in more well-studied sensory modalities (blindness, deafness) using similar analysis approaches as in the current study. A sample size of 33 individuals with congenital sensory deprivation is comparably large and expected to sufficiently detect group differences of at least similar effect sizes as reported in the literature. The study was approved by ethical review authorities in both Sweden and the Netherlands. All participants provided signed informed consent prior to enrolment in the study.

Experimental design

Sensory stimuli and stimuli presentation. Presented stimuli consisted of 2 s long dynamic and matching audio and video clips previously used to assess behavioral multisensory integration performance (Regenbogen et al., 2016; Peter et al., 2019) as well as neural processing of multisensory integration using fMRI (Regenbogen et al., 2018). The audio and video clips depicted three familiar objects: flopping fish, fire, and lawn mower, and were obtained from Shutterstock (http://www.shutterstock.com). Editing of the raw stimuli is described in detail by Regenbogen and colleagues (2016). Based on the principle of inverse effectiveness, stating that the multisensory response is increased when the unisensory effectiveness is decreased (Stein and Meredith, 1993), the audio and video clips were degraded: A low level of noise (auditory pink noise resulting in −1 dB signal-to-noise ratio, 70% visual salt and pepper noise) was added to the stimuli to increase the dependency on multisensory integration to identify the presented objects, while still keeping the presented objects perceivable in the MR scanner based on participant performance in a previous fMRI study (Regenbogen et al., 2018).

Visual stimuli were presented on a MR compatible wide-screen monitor (NordicNeurolab, size 40″, contrast ratio 5000:1, refresh rate 120 Hz, white illuminance > 150cs/m^2) in Sweden, and on a custom-made screen using a projector in the Netherlands (Eiki, LC-XL100 LCD Projector); at both locations viewed via a head coil mounted mirror. Auditory stimuli were presented via MR-compatible headphones (Sweden: NordicNeurolab slim stereo headphones; the Netherlands: MR Confon).

Multisensory integration task. Prior to the scanning session, participants performed a short, computerized training session to increase familiarization with the stimuli. During scanning, stimuli were presented unimodally (only audio clip combined with a filled gray screen; only video clip with no audio) and bimodally (congruent audio and video clip simultaneously presented) in a randomized order. Each trial started with a fixation cross (jittered duration of 2000–3000 ms), followed by the stimulus presentation (2000 ms), a gray screen (100 ms), followed by the question "Which object was presented?" with the alternatives "fish", "fire", "lawnmower", and "nothing" presented (3000 ms). The trial ended with a gray screen (1800 ms; Fig. 1). Participants were instructed to use the response alternative "nothing" if they did not perceive an object and responded using a four-button diamond shaped response box. Responses were recorded merely to ensure that participants were awake and actively engaged in the task (mean accuracy auditory stimuli 63.3%, mean accuracy visual stimuli 97.2%, mean accuracy audio-visual stimuli 98.5%; no differences between individuals with ICA and controls according to independent samples t-tests, df = 64, all $ts \leq 0.51$, all $ps \geq 0.609$). A total of 99 trials were performed: 33 unisensory auditory (11 per object), 33 unisensory visual, and 33 bimodal audio-visual trials. E-prime 2.0 (Psychology Software Tools Inc., Sharpsburg, PA, USA) was used for stimulus presentation and response data collection.

Image acquisition

At both testing locations, a 3T Siemens Magnetom MR scanner was used (in Sweden, a Prisma scanner with a 20-channel head coil; in the Netherlands a Verio scanner with a 32-channel head coil) with identical scanning sequence protocols. Similar presentation devices and presentation scripts were used with the one difference being that each population received information in their own native language.

A structural T1-weighted image was collected using an MPRAGE sequence (TR = 1900 ms, TI = 900 ms,

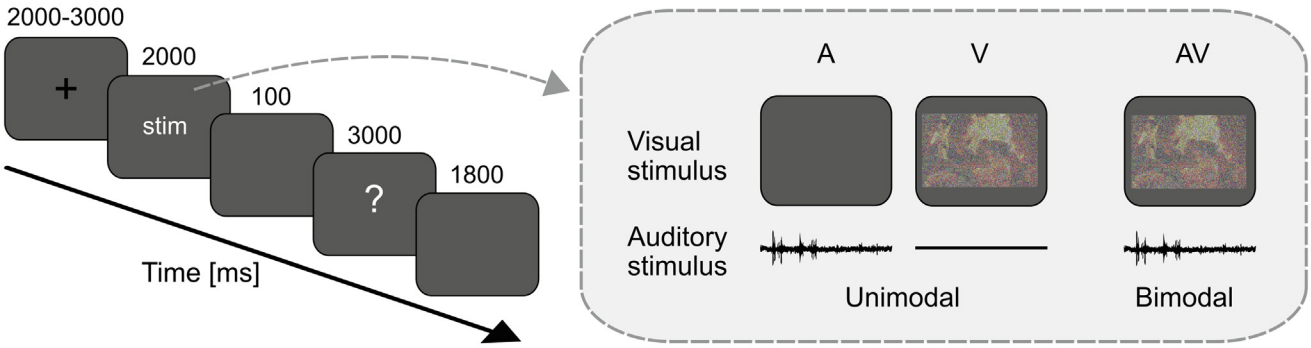

Fig. 1. Presentation timeline and sensory stimuli. Presentation timeline with values in milliseconds [ms]. Not shown are the options presented to the participants on the object identification slide, here indicated with a question mark. Stimuli were presented either unimodally (audio or visual) or bimodally (audio-visual) in a randomized order. A = auditory, V = visual, AV = audio-visual.

TE = 2.52 ms, flip angle = 9, voxel size 1 mm isotropic, 176 slices, FOV = 256 mm), and functional data was collected using a BOLD fMRI sequence consisting of a 16:36 minutes long T2*-weighted echo-planar imaging sequence (TR = 2000 ms, TE = 22 ms, flip angle = 70, voxel size 3 mm isotropic, 41 slices, FOV = 228 mm, interleaved acquisition). Two additional functional sequences and a T2-weighted sequence, results from which are not reported here, were collected during the same session.

Image analysis

Preprocessing. Preprocessing was done using SPM12 (Wellcome Trust Centre for Neuroimaging, UCL; https://www.fil.ion.ucl.ac.uk/spm/) in MATLAB 2019b (TheMathWorks, Inc., Natick, Massachusetts, USA) and included the following processing steps for each subject: slice timing correction to account for the temporal delay between acquisition of the slices in a volume, spatial realignment to account for motion between different volumes, coregistration of the anatomical to the mean functional image, normalization of the functional and anatomical images to MNI space using the unified segmentation approach as implemented in SPM12 (Ashburner and Friston, 2005), and finally, smoothing of the functional images with an 8 mm full width at half maximum Gaussian kernel. As a quality control to ensure that potential group differences in motion would be unlikely to give rise to group differences in future analysis, mean framewise displacement (Power et al., 2012) was calculated for each individual separately and compared between groups. No significant group difference in mean framewise displacement was found when assessed with a non-parametric Mann-Whitney U test (median ICA 0.119 mm, median control 0.132 mm, $U_{33,33}$ = 498, p = .436).

Regions of interest. To test our two directed hypotheses, we created four regions of interest (ROI); namely, piriform cortex and orbitofrontal cortex to assess potential differences in areas associated with olfactory processing (Fig. 2A), as well as the superior temporal sulcus and the intraparietal sulcus to assess potential differences in regions associated with multisensory integration (Fig. 2D).

We derived the two olfactory processing regions based on a published olfactory activation likelihood analysis (Seubert et al., 2013a). To restrict the extension of the regions to only encompass core olfactory regions, the piriform ROI was restricted by an anatomical piriform ROI based on manual delineation of the region, as described by Porada et al. (2019). The ROI for orbitofrontal cortex was restricted to lie within the frontal pole and orbitofrontal regions of the Harvard-Oxford cortical structural atlas, further described by Seubert and colleagues (Seubert et al., 2013b).

In line with the olfactory ROIs, the two multisensory processing regions were created based on a combination of functional and atlas-based data. First, to create whole-brain maps of predicted multisensory activation focusing on our two hypothesized multisensory regions, the search phrases ‘‘audiovisual multisensory integration superior temporal sulcus” and ‘‘audiovisual multisensory integration intraparietal sulcus” were used in NeuroQuery (Dockès et al., 2020). NeuroQuery is an interactive online meta-analyses tool of neuroimaging studies that produces a map of the most relevant brain regions based on a text query using a base of 13,459 published neuroimaging studies. In short, NeuroQuery predicts relevant brain areas by modeling relatedness of terms in the vocabulary of these studies and subsequently uses a regression model to link term occurrences to neural activations using supervised machine learning techniques. The resulting whole-brain maps were then thresholded at z = 9 and spatially restricted using superior temporal sulcus and intraparietal sulcus ROIs from the AICHA atlas (Joliot et al., 2015), resulting in our multisensory ROIs.

Statistical analysis

Stimuli specific functional activity were modeled in SPM12 using a general linear model on the subject level; once with unsmoothed data for extraction of mean values within ROIs, and once with the smoothed functional data for exploratory whole-brain analysis. Three regressors of interest were modeled: the onset of auditory stimuli (A), visual stimuli (V), and audio-visual

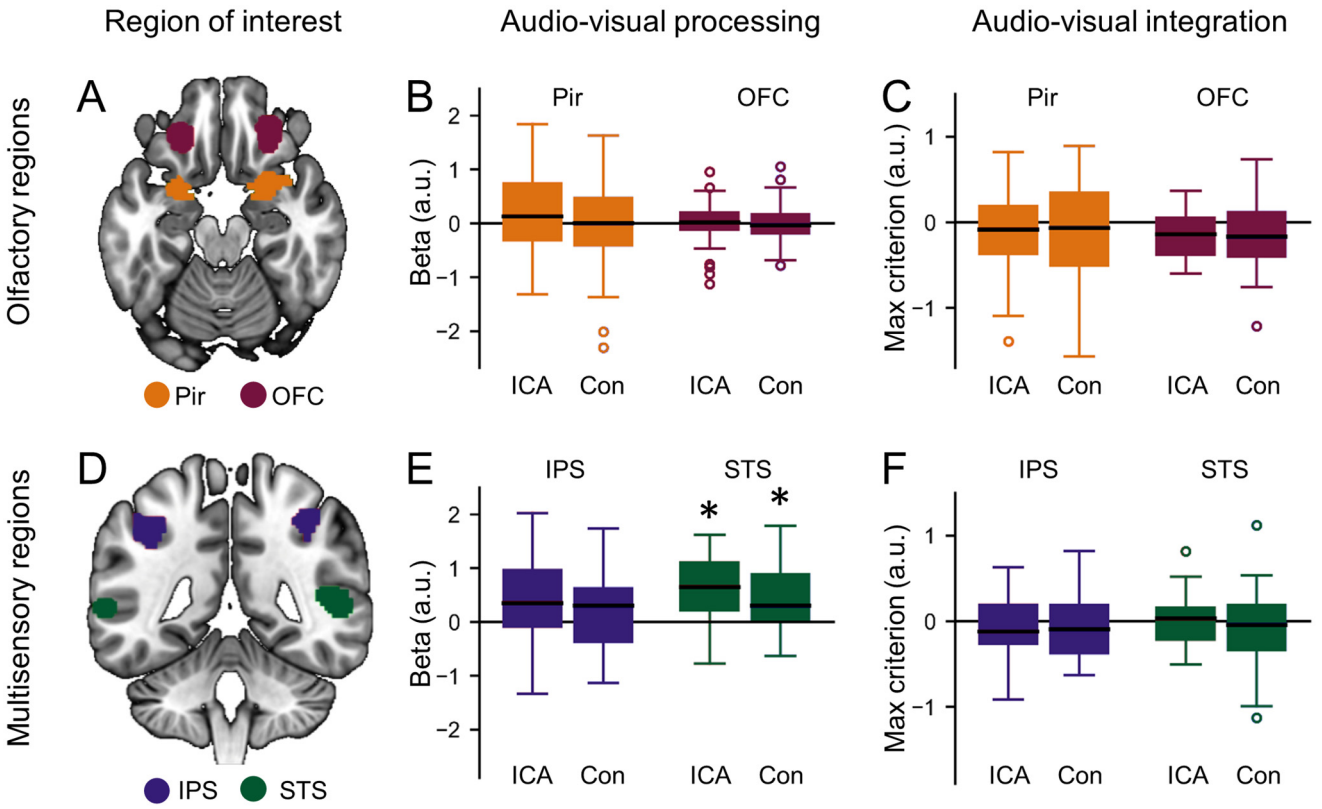

Fig. 2. Region of interest-based analysis results. **(A)** Axial view of the brain where colored areas indicate ROIs delineating olfactory processing areas. Orange color indicates piriform cortex (Pir) and red color indicates orbitofrontal cortex (OFC) ROIs. **(B)** Beta values, in arbitrary units (a.u.), for audio-visual processing within olfactory regions, displayed for each group separately. **(C)** Difference in beta values, for multisensory integration, according to the maximum criterion, of bimodal audio-visual stimuli within olfactory processing regions, displayed for each group separately. **(D)** Coronal view of the brain where colored areas indicate ROIs delineating multisensory processing regions. Purple color indicates intraparietal sulcus (IPS) and green color indicates superior temporal sulcus (STS) ROIs. **(E)** Beta values for basic audio-visual processing within multisensory processing regions, displayed for each group separately. **(F)** Difference in beta values, for multisensory integration, according to the maximum criteria, of bimodal audio-visual stimuli within multisensory processing regions, displayed for each group separately. In all box plot panels, box borders represent 25% and 75%, values with black horizontal line indicating median. Whiskers indicate the furthest data points within 1.5 interquartile range above/below the boxes and outlier values are marked with circles. In all graphs, dashed lines represent implicit baseline and * represents significant different from baseline, when corrected for multiple comparison using Bonferroni adjusted p-value of $p < .00625$. ICA = isolated congenital anosmia, Con = Control.

stimuli (AV). Additionally, two regressors of no interest were included to reduce the unexplained variance in the model: the onset of the fixation cross and the onset of the response period. All five regressors were modeled as stick functions and convolved with the canonical hemodynamic response function, as implemented in SPM12. To reduce the potential effects of participant motion, the six realignment parameters were further added as regressors in the model. Low frequency drift was removed using a high-pass filter (cutoff 128 s) and serial correlations was accounted for by a first-order autoregressive model AR(1). On the subject level, we assessed potential effects of each sensory condition, namely A, V, and AV, against the implicit baseline, and computed the maximum criterion as an indication of multisensory integration (Beauchamp, 2005; Stevenson et al., 2014): AV > max(A,V).

ROI-based analysis. To assess potential group differences in audio-visual processing and audio-visual integration in olfactory and established multisensory integration regions, mean betas within the four ROIs for A, V, and AV were extracted from unsmoothed data for each participant using the MarsBaR toolbox (Brett et al., 2002). The max criterion was computed region-wise. Group comparisons were performed using Mann-Whitney *U* tests due to non-normally distributed data. As a control measure, significance of effects within groups were tested using one-sample Wilcoxon signed rank tests, with a Bonferroni adjusted *p*-value of $p < .00625$ to indicate significant effect, based on the eight tests done for each contrast.

Whole-brain analysis. Because potential anosmia-associated alterations in cerebral processing or integration of audio-visual stimuli are as of today unknown, we further aimed to unrestrictedly explore whether group differences exist in regions beyond the *a priori* defined ROIs by conducting whole-brain mass-univariate group comparisons. Specifically, betas for AV and maximum criterion were compared between groups with two-sample *t*-tests including age, sex, and scanning

site as nuisance covariates. A family-wise error (FWE) corrected statistical threshold of $p < .05$ was first applied to correct for multiple statistical comparisons, followed by the more liberal statistical threshold of $p < .001$ (uncorrected) with a minimum cluster size of 10 to decrease the incident of spurious differences.

RESULTS

No evidence of increased multisensory processing in ICA within pre-defined ROIs

We first assessed whether there were statistical differences between groups in our predefined ROIs (Fig. 2A) in respect of bimodal processing of congruent audio-visual stimuli within regions associated with olfactory processing. There were, however, neither significant group differences in processing of audio-visual stimuli (Fig. 2B), nor multisensory integration of audio-visual stimuli within the defined olfactory areas according to the maximum criterion (Fig. 2C) at $p < .05$, uncorrected for multiple comparison (Supplementary Tables S1, S2). In addition, we wanted to determine whether olfactory regions are involved in multisensory processing or integration of non-olfactory bimodal stimuli *per se*, in line with the cross-modal processing demonstrated in blind individuals. However, the activation in olfactory processing regions was not significantly greater than the implicit baseline in either ROI or group (Fig. 2B, C; Supplementary Tables S3, S4).

We subsequently assessed whether there were differences between the two groups in processing of the bimodal stimuli within regions commonly associated with multisensory integration, the superior temporal sulcus and intraparietal sulcus. Similar to what we described above, there were no statistical differences between the two groups in either processing of audio-visual stimuli (Fig. 2D, Supplementary Table S1) or multisensory integration of audio-visual stimuli within the defined multisensory integration regions (Fig. 2E, Supplementary Table S2), according to the maximum criterion, at $p < .05$ uncorrected for multiple comparison. Finally, we wanted to assess whether our multisensory ROIs demonstrated audio-visual processing or integration. Indeed, we found that the superior temporal sulcus (ICA $p = 1.36 \cdot 10^{-4}$; Control $p = 1.1 \cdot 10^{-4}$), but not the intraparietal sulcus, showed significant positive audio-visual activation (Fig. 2E, Supplementary Table S3). However, we did not find evidence of significant integration of the bimodal sensory stimuli, according to the maximum criterion (Fig. 2F, Supplementary Table S4).

Exploratory analyses indicate potential differences in neural processing

Although the evidence does not support atypical processing of audio-visual stimuli in individuals with ICA in our *a priori* defined ROIs, the possibility exists that our ROIs do not capture potential group differences. Therefore, in addition to our targeted analyses reported above, we also assessed potential group differences using exploratory whole-brain analysis. First, we assessed whether there were significant differences in multisensory integration of the bimodal stimuli using the maximum criterion definition. There was no significant difference between groups when applying a conservative $p < .05$, FWE whole-brain correction for multiple comparisons. However, when using a more liberal statistical threshold of $p < .001$, combined with a minimum cluster size of 10 voxels to reduce spurious findings, we found that ICA individuals, when compared to matched controls, demonstrated significantly greater activity in the left posterior superior temporal sulcus and in the left pre/postcentral gyrus (Fig. 3, Table 2).

In addition, we assessed whether there were potential differences between the two groups in respect of basic processing of bimodal audio-visual stimuli. As demonstrated above for the maximum criterion, there was no significant difference between groups when applying FWE correction of obtained results. However, likewise, when using a statistical threshold of $p < .001$ and a minimum cluster size of 10 voxels, we found that ICA individuals demonstrated enhanced audio-visual processing in a cluster in the left and right cingulate gyrus (Table 2).

In all exploratory analyses above, the reverse contrast (i.e., Control > ICA) did not produce any significant difference, independent of whether using statistical thresholds of whole-brain correction for multiple comparisons or a non-corrected threshold of $p < .001$.

DISCUSSION

Lifelong absence of input into a sensory system generally leads to enhanced cross-modal processing of other sensory modalities within regions normally devoted to the deprived sense. Here, we aimed to determine whether similar cross-modal functional reorganization occurs for multisensory processing in individuals with isolated congenital anosmia (ICA), a group that has demonstrated enhanced multisensory integration performance (Peter et al., 2019), or whether altered processing of multisensory stimuli in established multisensory integration regions could be a potential neural basis of the enhanced performance. No group differences in the *a priori* hypothesized olfactory or multisensory regions were demonstrated. However, exploratory whole-brain analysis uncorrected for multiple comparisons indicated enhanced activation related to multisensory integration in a region in close proximity to our hypothesized multisensory ROI in the superior temporal sulcus, a region repeatedly associated with multisensory integration (Stein and Stanford, 2008), in individuals with ICA.

In contrast to our hypotheses, there were no significant group differences in either audio-visual processing or audio-visual integration, indicated by the maximum criterion, in the four ROIs. However, enhanced multisensory integration activity in the left superior temporal sulcus, just posteromedial to one of our two predefined multisensory ROIs, was present in individuals with ICA in a whole-brain voxel-wise

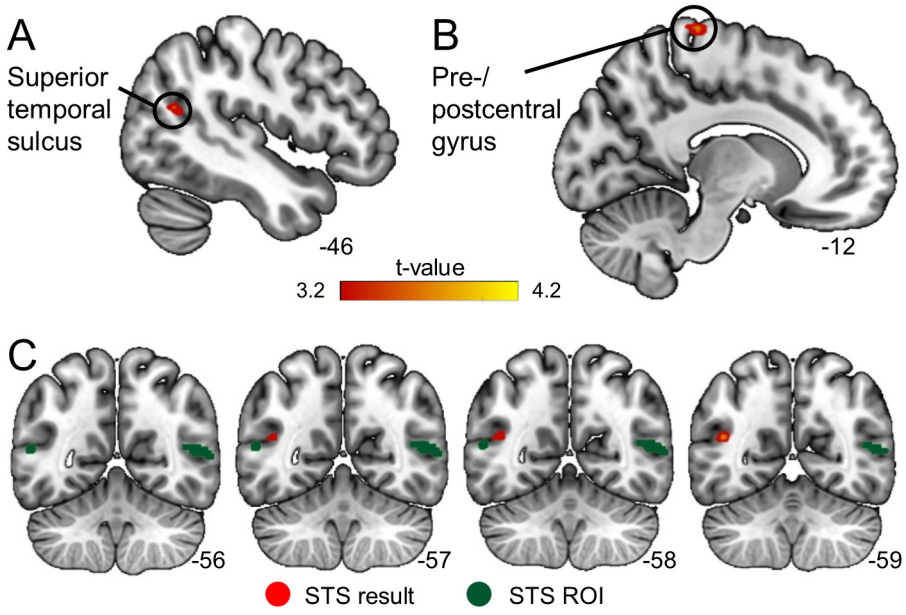

Fig. 3. Exploratory whole-brain analyses of group differences in audio-visual integration. **(A)** Individuals with isolated congenital anosmia (ICA) demonstrated increased activity related to multisensory integration, as indicated by the maximum criterion, in the left superior temporal sulcus (STS), compared to controls. **(B)** Individuals with ICA demonstrated increased activity related to multisensory integration in the left pre-/postcentral gyrus. **(C)** The STS cluster in which individuals with ICA demonstrated increased audio-visual integration is located in close proximity to the a priori chosen multisensory region of interest (ROI). Results are displayed on the MNI152 template at a significance level of $p < .001$, uncorrected, with a minimum cluster size of 10 voxels. Numbers in panels represent MNI *x*-coordinates in figure **(A)** and **(B)**, and MNI *y*-coordinates in figure **(C)**.

analysis. The same contrast furthermore revealed enhanced multisensory integration activity in the left pre-/postcentral gyri in individuals with ICA, whereas no region of decreased multisensory integration was demonstrated for the ICA group compared to the control group. Before discussing potential interpretations of these findings, it is important to note that the results did not remain statistically significant after whole-brain family-wise error correction for multiple comparison and should therefore be interpreted with caution. We do, however, argue that the fact that enhanced multisensory integration activity in individuals with ICA was indicated in the superior temporal sulcus so close to our predefined multisensory ROI is of high interest when considering our hypotheses. The superior temporal sulcus is a multisensory region associated with audio-visual object and speech processing (Stein and Stanford, 2008), and, in line with our results, linked to integration of information about and identity of objects (Beauchamp et al., 2004). It has furthermore been demonstrated that multisensory integration in the superior temporal sulci as well as in the left pre-/postcentral gyri can be predicted by multisensory performance benefits of degraded audio-visual stimuli (Werner and Noppeney, 2010), results indicating regions remarkably similar to where we demonstrate group differences in audio-visual integration. While Werner et al. (2010) suggests that multisensory integration effects in the posterior temporal sulcus may induce multisensory perceptual benefits, the link between integration performance and integration effects in the pre-/postcentral gyri was interpreted as multisensory facilitation of response selection, an explanation plausible also for our results, in that object identity was indicated using a right-hand button press. In addition to the link between multisensory integration in the superior temporal sulcus and multisensory performance benefits, the superior temporal sulcus has been shown to be sensitive to temporal, but not spatial, shifts between auditory and visual stimuli (Macaluso et al., 2004; Powers et al., 2012), a capacity highly relevant for the most clearly demonstrated multisensory behavioral advantage for individuals with anosmia: temporal integration, manifested as increased asynchrony detection of simple auditory and visual stimuli in a simultaneity judgement task (Peter et al., 2019). Based on our exploratory results indicating enhanced multisensory integration in the superior temporal sulcus in individuals with ICA, along with the links between superior temporal sulcus activity, temporal integration, and multisensory performance benefits, we here speculate that the multisensory behavioral benefits previously demonstrated by individuals with complete olfactory sensory deprivation are mediated by enhanced multisensory integration in the superior temporal sulcus. Based on the exploratory nature of our analysis, future studies focusing on the activity within the superior temporal sulcus while designed to assess behavioral performance are, how-

Table 2. Results from exploratory whole-brain analysis

Region	*x*	*y*	*z*	Cluster size[b]	*t*[c]	*p*[d]
ICA > Control (maximum criterion)						
Left superior temporal sulcus, horizontal posterior segment	−42	−61	20	15	4.17	$4.89 \cdot 10^{-5}$
Left pre-/postcentral gyrus	−12	−31	77	14	3.92	$1.12 \cdot 10^{-4}$
ICA > Control (AV)						
Cingulate gyrus	0	−16	32	22	4.19	$4.56 \cdot 10^{-5}$

All results significant at $p < .001$with a minimum cluster size of 10 voxels. ICA – isolated congenital anosmia, AV – audio-visual stimuli. [b] Cluster size as number of voxels. [c] *t*-value for peak voxel in cluster. [d] *p*-value for peak voxel. Region defined based on 'Atlas of the Human Brain' (Mai et al., 2015).

ever, necessary to confirm the here formulated hypothesis.

The mechanisms behind altered multisensory integration processing in individuals with ICA are unknown, but it could be speculated that a lifelong absence of olfactory input to the superior temporal sulcus, a region that in addition to audio-visual integration also has been linked to visual-olfactory integration (Gottfried and Dolan, 2003; Novak et al., 2015), facilitates integration of remaining sensory input. Analogous with sensory deprivation leading to increased cross-modal processing in early sensory processing regions of the deprived sensory modality, non-human animal studies have demonstrated altered neural populations in multisensory regions as a result of early sensory deprivation. Specifically, the studied multisensory regions demonstrate more neurons responding to remaining sensory modalities, compared to non-deprived animals (Hyvärinen et al., 1981), and a preserved or even slightly increased number of multisensory neurons integrating remaining senses (Wallace et al., 2004; Carriere et al., 2007) potentially forming a basis for altered multisensory integration processing and performance. In addition to the potential olfactory deprivation induced alteration in neural population in multisensory regions, it is furthermore plausible that a lifelong absence of one sensory modality results in greater attentional capacity for the remaining senses. A shift in attentional focus would likely be caused by less attentional division due to a lack of olfactory awareness and, potentially, a greater necessity to gather as much sensory information as possible from remaining sensory input when input from one sensory modality is lacking. Furthermore, while olfactory deprivation seems to have a negative effect on the trigeminal and gustatory senses (Ptito et al., 2014), i.e. the other constituents of the tightly bound flavor network, it is noteworthy that olfactory sensory information does not provide much input to the temporal and spatial dimensions that auditory, visual, and tactile information share. While an absence of visual input could interfere with the alignment of the auditory and tactile frames of reference, potentially impeding integration (Occelli et al., 2013), olfactory deprivation should not have the same detrimental effect on audio-visual integration (Peter, 2020). We therefore speculate that a neural reorganization of multisensory regions combined with an attentional shift towards the remaining sensory modalities contribute to enhanced audio-visual integration performance and processing in individuals with ICA.

In line with the exploratory analysis of audio-visual integration, exploratory analysis of audio-visual processing did not reveal any regions with either increased or decreased processing in individuals with ICA when correcting for multiple testing using family-wise error correction. However, using a more liberal statistical threshold, individuals with ICA demonstrated enhanced processing of bimodal stimuli in a cluster covering parts of both left and right cingulate gyrus. Whether this unexpected result in a region not commonly associated with multisensory processing is a spurious finding remains to be determined. It is, however, interesting to note that we did not find any regions in which increased processing of the multisensory stimuli was indicated in healthy control participants compared to individuals with ICA.

In addition to a lack of group differences in the olfactory ROIs, no regions clearly associated with olfactory processing were revealed in the whole-brain analysis. Albeit enhanced performance in remaining senses has been linked to cross-modal processing in regions normally associated with visual processing in blind individuals (Gougoux et al., 2005; Collignon et al., 2007), we here find no evidence of altered cross-modal audio-visual processing or integration in regions normally associated with olfactory processing in individuals with ICA, despite a reported behavioral benefit (Peter et al., 2019). Specifically, these results do not support the hypothesis of enhanced multisensory cross-modal processing in olfactory regions as a potential explanation of the lack of structural and functional connectivity abnormalities in piriform cortex in individuals with ICA (Peter et al., 2020; 2021). The functional role of olfactory regions in individuals with lifelong anosmia is yet to be determined.

The lack of group differences in *a priori* defined ROIs, despite indications of alterations in close proximity to a multisensory ROI, could potentially be due to a combination of limitations. First, it is important to acknowledge the difficulties in clearly demonstrating multisensory integration using fMRI due to the spatial limitations of the technique: there are multiple neural populations in one voxel, and even if some neurons demonstrate clear multisensory integration, others will likely only respond to one sensory modality, hence attenuating the signal of interest (Stevenson et al., 2014). Based on this difficulty, we implemented experimental design and analysis approaches to enhance multisensory integration effects and facilitate the detection of multisensory integration. To enhance multisensory integration effects based on the principle of inverse effectiveness (Stein and Meredith, 1993), the presented stimuli were degraded by overlaying them with a low level of noise. Note, however, that we here aimed to enhance the multisensory BOLD-signal, not behavioral measures of multisensory integration. Hence, in contrast to the behavioral study upon which our hypotheses are built (Peter et al., 2019), speeded responses were not used because they would risk motor confounds in the data, and the presented stimuli were masked to a lesser extent to ensure high object recognition for both uni- and bimodal stimuli. Consequently, a ceiling effect in performance with close to perfect object identification accuracy was demonstrated for both audio-visual and visual stimuli, albeit highest for the audio-visual stimuli. Importantly, this absence of indications of behavioral multisensory integration effects are not equivalent to a lack of multisensory responses in the brain, as multisensory neurons are expected to respond to multisensory stimuli even though the corresponding unisensory stimuli are informative enough to give rise to high behavioral performance. To further increase the probability of detecting multisensory integration in the brain, the maximum criterion was used as an indication of integration instead of the superadditive

criterion, i.e., a multisensory activity significantly greater than the combined activity of the two unisensory stimuli, because the latter has been demonstrated to be very conservative (Beauchamp, 2005; Stevenson et al., 2014). Finally, the study is inherently limited by the rarity of the population studied, thereby reducing the likelihood of detecting small effects.

In conclusion, the current study does not support the notion that individuals with ICA exhibit cross-modal functional reorganization of multisensory processing in olfactory associated regions. We can, however, demonstrate that individuals with ICA show increased multisensory integration in the superior temporal sulcus, a multisensory processing region in which we hypothesized to find group differences, albeit the result was not significant at a conservative statistical threshold. We conclude that lifelong olfactory sensory deprivation might be associated with compensatory processing in multisensory regions, as opposed to cross-modal-based functional reorganization. However, these results need to be replicated in an independent sample given the lack of confirmed results within our *a priori* defined cerebral regions.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

ACKNOWLEDGEMENTS

This work was supported by the Knut and Alice Wallenberg Foundation (KAW 2018.0152) and the Swedish Research Council (2017-02325), awarded to J. N.L. The use of the 3T MRI facility in the Netherlands has been made possible by Wageningen University & Research Shared Research Facilities.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroscience.2021.07.017.

(Received 24 February 2021, Accepted 16 July 2021)
(Available online 24 July 2021)