OXFORD

Cerebral Cortex, 2024, **34**, bhae167
https://doi.org/10.1093/cercor/bhae167
Advance access publication date 23 April 2024
Original Article

The neural representations of valence transformation in indole processing

Laiquan Zou[1,†], Yue Qi[1,†], Lei Shen[2,3], Yanyang Huang[1], Jiayu Huang[1], Zheng Xia[2,3], Mingxia Fan[2], Wu Fan[4], Guo-bi Chai[4], Qing-zhao Shi[4], Qidong Zhang[4,*], Chao Yan[2,3,5,*]

[1]Chemical Senses and Mental Health Lab, Department of Psychology, School of Public Health, Southern Medical University, South Shatai Road 1023, Guangzhou 510515, China
[2]Key Laboratory of Brain Functional Genomics (MOE & STCSM), Shanghai Changning-ECNU Mental Health Center, School of Psychology and Cognitive Science, East China Normal University, North Zhongshan Road 3663, Shanghai 200062, China
[3]Shanghai Key Laboratory of Magnetic Resonance, East China Normal University, North Zhongshan Road 3663, Shanghai 20062, China
[4]Zhengzhou Tobacco Research Institute of CNTC, Fengyang Road 2, Zhengzhou 450001, China
[5]Key Laboratory of Philosophy and Social Science of Anhui Province on Adolescent Mental Health and Crisis Intelligence Intervention, South Jiuhua Road 189, Hefei 241002, China
*Corresponding authors: Chao Yan, Room 413, Building Junxiu, Shanghai Changning-ECNU Mental Health Center, School of Psychology and Cognitive Science, East China Normal University, North Zhongshan Road 3663, Shanghai 200062, China. Email: cyan@psy.ecnu.edu.cn; Qidong Zhang, Zhengzhou Tobacco Research Institute of CNTC, Fengyang Road 2, Zhengzhou 450001, China. E-mail: qdzhangcn@163.com
†Laiquan Zou and Yue Qi contributed equally.

Indole is often associated with a sweet and floral odor typical of jasmine flowers at low concentrations and an unpleasant, animal-like odor at high concentrations. However, the mechanism whereby the brain processes this opposite valence of indole is not fully understood yet. In this study, we aimed to investigate the neural mechanisms underlying indole valence encoding in conversion and nonconversion groups using the smelling task to arouse pleasantness. For this purpose, 12 conversion individuals and 15 nonconversion individuals participated in an event–related functional magnetic resonance imaging paradigm with low (low-indole) and high (high-indole) indole concentrations in which valence was manipulated independent of intensity. The results of this experiment showed that neural activity in the right amygdala, orbitofrontal cortex and insula was associated with valence independent of intensity. Furthermore, activation in the right orbitofrontal cortex in response to low-indole was positively associated with subjective pleasantness ratings. Conversely, activation in the right insula and amygdala in response to low-indole was positively correlated with anticipatory hedonic traits. Interestingly, while amygdala activation in response to high-indole also showed a positive correlation with these hedonic traits, such correlation was observed solely with right insula activation in response to high-indole. Additionally, activation in the right amygdala in response to low-indole was positively correlated with consummatory pleasure and hedonic traits. Regarding olfactory function, only activation in the right orbitofrontal cortex in response to high-indole was positively correlated with olfactory identification, whereas activation in the insula in response to low-indole was negatively correlated with the level of self-reported olfactory dysfunction. Based on these findings, valence transformation of indole processing in the right orbitofrontal cortex, insula, and amygdala may be associated with individual hedonic traits and perceptual differences.

Key words: indole; valence; fMRI; neural representations; humans.

Introduction

Indole and its congeners and derivatives are widely found in nature, primarily in animal feces (Anderson 1975), coal tar (Yamamoto et al. 1991), and natural flower oils (Edris et al. 2008), such as jasmine, bitter orange blossom, daffodil, vanilla, and others. Indole also contributes to the flavor of food and the aroma of perfumes. Accordingly, indole is used as flavor enhancer and fragrance in the food and cosmetics industries (Barden 2010).

Although the human nose recognizes indole and many of its derivatives at a low odor threshold (Zhou et al. 2016), the role of indole depends on its concentration. As shown by aroma extract dilution analysis in a study of volatile compounds extracted from Japanese green tea, indole has an animal-like odor in the range of flavor dilution from 3 to 243. However, at the flavor dilution of 729, indole exudes a floral-like odor (Hattori et al. 2003). In other words, indole is associated with a sweet and floral odor typical of jasmine flowers at low concentrations and with an unpleasant animal-like aroma at high concentrations (Zhou et al. 2019). Yet, unlike brewed tea, fresh tea leaves show no pleasant aroma as only ~14% of the indole extracted from fresh tea leaves is retained in green tea or oolong tea. In addition, in the experiment conducted by Grabenhorst et al. (2007), while some individuals find indole unpleasant, others do not, highlighting the subjective nature of experiencing its pleasantness. For this reason, the chemical synthesis of indole has been extensively studied by organic chemists (Gribble 2010; Taber and Tirunahari 2011; Clarke et al. 2019), but not the neural mechanisms of the underlying indole valence encoding, so we do not yet fully understand how the brain processes these opposite valences at different indole concentrations.

The hedonic or affective component of olfactory perception guides emotional responses, decision-making, and behaviors.

Received: January 16, 2024. **Revised:** March 18, 2024. **Accepted:** March 20, 2024

Extensive human imaging studies have implicated the neural coding of both positive or pleasant (Kjelvik et al. 2012) and negative or aversive (Rolls et al. 2003) olfactory stimuli in these processes and have identified key regions activated during the representation of the hedonic value of odors in the amygdala (Kjelvik et al. 2012) and in primary (Gottfried et al. 2002a; Zelano et al. 2007), and secondary olfactory areas, including the orbitofrontal cortex (OFC; Gottfried et al. 2002b; Wicker et al. 2003), the cingulate gyrus (De Araujo et al. 2005), and the insula (Soudry et al. 2011).

Emotion studies have long determined that emotional experiences are mainly characterized by 2 dimensions, namely valence and intensity (Russell 1980; Landau and Gleitman 1985), both of which presumably contribute to the experience independently but tend to correlate with each other, making it difficult to dissociate one from the other. Nevertheless, to disassociate valence from intensity, Anderson et al. (2003) created 4 stimuli using citral, valeric acid, and high and low concentrations and found that amygdala activation was associated with intensity, whereas OFC activation was associated with the valence of 2 odors (Anderson et al. 2003). In contrast, other authors noted that pleasant but not unpleasant odors activated a medial region of the rostral OFC (Rolls et al. 2003) and that the amygdala showed an intensity-by-valence interaction in olfactory processing (Winston et al. 2005). Combined, these findings suggest that distinct olfactory regions contribute to odor intensity and pleasantness and that affective representations of intensity and pleasantness depend on separable neural substrates.

Due to the interaction between pleasantness and intensity in emotional experiences (Winston et al. 2005), no study has been able to manipulate pleasantness at constant intensity. As mentioned above, indole is a particular flavor, with a sweet and floral odor typical of jasmine flowers at low concentrations and with an unpleasant, animal-like odor at high concentrations. Furthermore, various odors possess distinct molecular structures, leading to differential processing or perception by the human brain. Prior research has uncovered strong correlations between molecular features and perceptual qualities, such as pleasantness (Keller and Vosshall 2016). However, the existence of a universal rule governing this quantitative structure–odor relationship, as well as the extent to which these properties or chemical structures influence odor quality, remains unclear (Sharma et al. 2021). Therefore, indole is an ideal object of research for affective representations of pleasantness because changes in indole concentration can lead to changes in the perception of pleasantness independent of intensity, and low- and high-indole have the same molecular structure.

In a previous study, insula activation was correlated with hedonic traits. More specifically, bilateral insula activation was correlated with consummatory pleasure and hedonic traits when smelling pleasant odors, while right insula activation was correlated with increased consummatory pleasure and hedonic traits when smelling unpleasant odors (Zou et al. 2016).

In this study, we aimed to examine the neural mechanisms underlying the dissociation of valence and intensity and the valence processing of odor-specific indole. To dissociate the dimensions of intensity and valence, 4 stimuli were prepared, at high and low concentrations of indole, valeric acid and citral. At low concentrations (low-indole), indole has a fragrant odor, which is perceived by most people as pleasant. At high concentrations (high-indole), indole has a sweaty, rancid, and sickening odor, which is perceived by most people as unpleasant. In turn, citral and valeric acid were used as control conditions. Moreover, there may be a perceptual divergence in the pleasantness of indole. We sought to categorize participants into 2 groups: the conversion (CVN) group and the nonconversion (non-CVN) group. To enable brain activation to be directly related to the subjective affective value of the stimuli, valence ratings were assessed for each odor to correlate ratings with brain activation. Given the evidence from neurophysiological and neuroimaging experiments, we specifically examined regions previously implicated in valence processing, such as the OFC, the insula, and the amygdala. Considering these findings, we hypothesized that (i) the OFC, insula, and amygdala are activated during indole valence transformation at different concentrations and that (ii) right OFC, insula, and amygdala activation is associated with hedonic traits.

Materials and methods

Participants

Initially, 31 healthy participants participated in this study. Four participants were eventually excluded due to severe head motion. The remaining 27 participants (22 women and 5 men; mean age 21.67 ± 2.77 years) completed the study. This study was approved by the ethics committee of the East China Normal University (HR2-0096-2022), and all participants signed an informed consent form before starting their participation. No subject had a history of neurological, major medical, psychiatric disorder, or drug abuse. Prior to the scanning sessions, the participants were exposed to each odor and trained to use a visual analog scale (VAS) to rate the intensity and pleasantness of the odors. Due to the perceived difference in the pleasantness of low-indole across participants, the participants were divided into 2 groups, namely a valence CVN group ($n = 12$), defined by a score ≥ 4.5 on the pleasantness of low-indole, and a nonvalence CVN group ($n = 15$), defined by a score < 4.5 on the pleasantness of low-indole. The groups did not differ in terms of age [$t(25) = 1.91$; $P = 0.07$] or sex distribution [$\chi 2\ (1) = 0.50$; $P = 0.83$] but significantly differed in education [$t(25) = 2.38$; $P = 0.03$].

Stimuli

The functional magnetic resonance imaging (fMRI) experiment utilized 4 distinct odors: low-indole, high-indole, citral, and valeric acid. Low-indole and high-indole, both derived from indole (chemical formula: C8H7N; J&K Scientific), were diluted with ethyl alcohol and presented at concentrations of 0.0625% and 0.25%, respectively. Citral (chemical formula: C10H16O; J&K Scientific) was diluted with ethyl alcohol and presented at a concentration of 1%, while valeric acid (chemical formula: C5H10O2; Sigma-Aldrich) was diluted with ethyl alcohol and presented at a concentration of 99%. They were selected through several detailed psychophysical prestudies conducted with 8 other participants, based on a previous fMRI study. The odorants were delivered in the MRI scanner using an air dilution olfactometer—Sniff-0 (CyNexo, Trivignano Udinese, Italy, http://www.cynexo.com), which is a custom-built continuous airflow ten-channel computer-controlled olfactometer.

Experimental design

We used a procedure previously described in imaging studies of olfaction. The 4 odorant stimuli and a clean air stimulus (used as a baseline) were randomly delivered 15 times, controlling for trial history, based on an event-related design and in total of 3 runs and 75 trials. The odor air stream was maintained for 3,000 ms for all odorants, while clean air from the wash bottle was utilized during all other intervals. During each trial, an intertrial interval of 3,000 to 7,000 ms featured a stream of pure, odorless

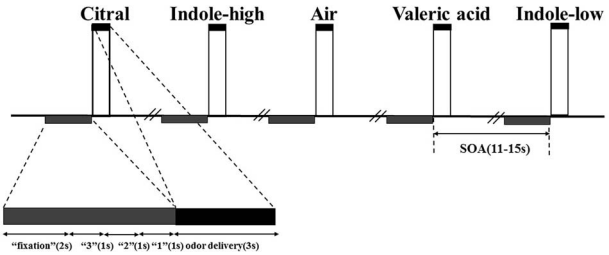

Fig. 1. Schematic diagram of the fMRI task. *Notes.* SOA: Stimulus onset asynchrony.

air (passed through water), ensuring the removal of the previous odorant before the next one was presented. Participants were instructed to maintain absolute stillness, breathe normally, and focus on smelling the odor. Following the 3,000-ms stimulation period, participants were prompted to indicate promptly and accurately whether an odorant was present by pressing one of two buttons (see Fig. 1). Individual intensity and valence estimations were obtained before the scan using separate VAS displayed on a screen, ranging from 0 (very pleasant/very strong) to 9 (very unpleasant/very weak). Participants underwent a practice session to familiarize themselves with the task before entering the MRI scanner.

Other assessments and questionnaires

All participants' basic olfactory function was assessed using the Sniffin' Sticks Threshold Test (Hummel et al. 1997), following a standardized procedure, including an odor threshold [16 phenyl ethyl alcohol (PEA) dilutions, a single staircase procedure, with No. 16 marking the lowest available odor concentration and, therefore, the best olfactory sensitivity] and identification (16 common odors and 4 alternative forced choices) test.

Additionally, we employed the self-reported olfactory dysfunction questionnaire (SODQ) as a reliable tool to screen for clinical olfactory disorders from a subjective standpoint (Liu et al. 2021). The SODQ comprises 10 items, with lower scores indicating better self-reported olfactory ability.

To measure trait–like anhedonia symptoms, we used the temporal experience pleasure scale (TEPS; Chan et al. 2012), which is a 20-item, self-report measure designed to capture anticipatory and consummatory pleasure. A higher score on the TEPS indicates a better ability to experience pleasure.

MRI data acquisition

Brain imaging data were collected on a 3.0-T SIEMENS PRISMA scanner with a 64-channel head coil at the Shanghai Key Laboratory of Magnetic Resonance, East China Normal University. FMRI data were axially collected using the following echo-planar imaging sequence: echo time = 30 ms, repetition time = 2,000 ms, flip angle = 90°, voxel size = 3∗3∗3 mm, field of view = 192 mm × 192 mm, axial slices = 33, slice thickness = 2 mm, and matrix = 64 × 64. A high-resolution structural brain image was acquired for anatomical registration (echo time = 3,690 ms, repetition time = 7,000 ms, flip angle = 90°, field of view = 192 mm × 192 mm, and matrix = 64 × 64). All participants wore earplugs during scanning. Their heads were fixed with a vacuum pillow and sponge pads to minimize head motion.

fMRI data analysis

The first 4 volumes from each functional scanning run were discarded. Functional imaging data were preprocessed and analyzed using Statistical Parameter Mapping software (SPM12, Wellcome Trust Centre for Neuroimaging, London, United Kingdom). The data were preprocessed in the following steps: the images were slice-time corrected and then realigned into the volume of each scanning sequence for movement correction. The head motion parameters, 3 transitions and 3 rotations, were calculated into the framewise displacement, a comprehensive and reliable index for head movement (Power et al. 2012). Participants with maximum head motion higher than 2 mm and 2 degrees were corrected and then excluded from the analysis [Artifact Detection Tools (ART)-based scrubbing]. A high–resolution brain structure image was registered to the Montreal Neurological Institute template, which produced a transformation matrix. Using this matrix, all functional brain images were normalized into a common standard atlas. Functional images were resampled into 3 × 3 × 3 mm and spatially smoothed to an 8-mm full-width-at-half-maximum Gaussian isotropic kernel.

Using percent signal change analysis, our goal was to discern the distinction between low-indole and high-indole stimuli. We identified the amygdala, insula, and OFC as the primary regions involved in odor valence processing. Regions of interest (ROIs) were delineated as 6-mm-radius spheres utilizing the MarsBaR utility (Brett et al. 2002), with coordinates for the amygdala (right: [24, 6, −27], left: [−24, 6, −27]), insula (right: [34, 22, 7], left: [−34, 22, 7]), and OFC (right: [40, 34, −8], left: [−40, 34, −8]), based on previous research (Small et al. 2005; Lombion et al. 2009; Zou et al. 2016). Subsequently, employing the Mars Bar 0.42 toolbox (Brett et al. 2002), we extracted the mean percentage signal change across voxels for ROI analysis, focusing on the contrast between low-indole and high-indole stimuli, as well as citral versus valeric acid for the valence-related areas.

The independent *t*-test was used to determine group differences in questionnaire scores and neuropsychological performances between the CVN and non-CVN groups. In addition, we performed Spearman's rank correlation analysis to determine relationships between percent signal changes and each participant's subjective pleasantness/TEPS/SODQ/odor identification/odor threshold scores on the clinical assessments using SPSS 20.0 (IBM, United States of America).

Results

Participants characteristics

The analysis of the demographic characteristics of the (CVN and non-CVN) groups showed no significant differences in gender and age between groups. Table 1 presents the demographic characteristics and scores of all participants.

Subjective ratings of hedonic traits and olfactory function

No significant difference in olfactory identification [$t(25) = 0.96$; $P = 0.35$], olfactory threshold [$t(25) = -0.42$; $P = 0.68$), SODQ sores [$t(25) = 1.40$; $P = 0.17$], and TEPS scores [$t(25) = 0.52$; $P = 0.16$] was found between the CVN and non-CVN groups (see Table 1).

Subjective ratings of pleasantness and intensity

Pleasantness ratings of low-indole were significantly higher than those of high-indole [$t(11) = 3.69$; $P < 0.01$], but no significant difference in intensity was found in the CVN group [$t(11) = -1.43$; $P = 0.18$; see Fig. 2]. In addition, neither pleasantness [$t(14) = 0.76$; $P = 0.46$] nor intensity ratings significantly differed in the non-CVN group [$t(14) = 0.35$; $P = 0.36$; see Fig. 2]. The pleasantness ratings

Table 1. Demographic and assessment of the participants.

	Group					
	All	CVN group	non-CVN group			
	$n = 27$ (mean ± SD)	$n = 12$ (mean ± SD)	$n = 15$ (mean ± SD)	$t/\chi 2$	p	Cohen's d
Age	21.67 ± 2.77	22.75 ± 3.44	20.80 ± 1.78	1.91	0.07	0.70
Gender (M/F)	5/22	2/10	3/12	0.50	0.83	
Education	15.48 ± 1.99	16.42 ± 2.02	14.73 ± 1.67	2.38	0.03	0.85
Threshold	14.43 ± 1.24	14.31 ± 1.21	14.52 ± 1.30	−0.42	0.68	−0.17
Identification	13.52 ± 1.12	13.75 ± 1.29	13.33 ± 0.98	0.96	0.35	0.38
SODQ	1.26 ± 2.78	2.08 ± 3.78	0.60 ± 1.45	1.40	0.17	0.53
TEPS total score	89.48 ± 11.10	90.75 ± 14.49	88.47 ± 7.84	0.52	0.61	0.21
TEPS-ant	46.78 ± 6.13	48.67 ± 7.39	45.27 ± 4.62	0.38	0.16	0.55
TEPS-con	42.70 ± 6.03	42.08 ± 7.80	43.20 ± 4.39	−0.47	0.64	−0.19

Notes: SODQ: self-reported olfactory dysfunction questionnaire; TEPS: temporal experience pleasure scale; ant: anticipatory pleasure; con: consummatory pleasure; CVN: Conversion group; non-CVN: Non-conversion group. Data from questionnaires are presented in terms of mean score (mean) and standard deviation (SD).

of citral were significantly higher than those of valeric acid in both the CVN [$t(11) = 2.38$; $P = 0.04$] and the non-CVN [$t(14) = 2.39$; $P = 0.03$] groups. No significant intensity difference between citral and valeric acid was found in the CVN [$t(11) = 1.17$; $P = 0.27$] and non-CVN [$t(14) = 0.71$; $P = 0.49$] group (see Fig. S1 in supplemental materials). Apart from the low-indole pleasantness scores, which were significantly higher in the CVN group than in the non-CVN group, no difference in pleasantness or intensity was identified between the 2 groups (see Fig. S2 in Supplemental Materials).

Neuroimaging results

Group differences in low-indole and high-indole

To compare neural activation in the low- and high-indole conditions, we analyzed the signal change percentage in the OFC, insula, and amygdala. Independence *t*-tests revealed a significant difference between the percent signal changes in the low- and high-indole conditions. In the CVN group, activation in the right lateral OFC was stronger with low-indole than with high-indole [$t(11) = 2.67$; $P = 0.02$], whereas no significant difference was observed in the non-CVN group [$t(14) = 1.58$; $P = 0.14$]. No significant difference in right medial OFC activation was found in the CVN group [$t(11) = -0.34$; $P = 0.74$] or in the non-CVN group [$t(14) = -1.54$; $P = 0.15$] either. Right insula activation was also higher with low-indole than with high-indole [$t(11) = 2.69$, $P = 0.02$] in the CVN group, but no significant difference was observed in the non-CVN group [$t(14) = 0.96$; $P = 0.35$]. Right amygdala activation was more responsive to low-indole than to high-indole in the CVN group [$t(11) = 2.30$; $P = 0.04$], with no significant difference in the non-CVN group [$t(14) = 0.99$; $P = 0.34$; see Fig. 3]. We also performed independence *t*-tests in citral and valeric acid, which showed that right lateral OFC activation was significantly higher with valeric acid than with citral [$t(14) = 2.34$; $P = 0.04$] in the non-CVN group, whereas no significant difference was observed in the CVN group [$t(11) = 0.03$; $P = 0.98$]. In the non-CVN groups, valeric acid induced a significantly stronger activation of the right insula than citral [$t(14) = 2.14$; $P = 0.05$], whereas no significant difference was observed in the CVN group [$t(11) = 1.25$; $P = 0.24$]. No significant difference in activation was identified in the right medial OFC [$t(11) = -0.34$; $P = 0.74$] or the amygdala, either in the CVN group [$t(11) = 0.43$; $P = 0.68$] or in the non-CVN group [$t(14) = -1.54$; $P = 0.15$; $t(14) = -0.12$; $P = 0.91$; see Fig. S3 in Supplemental Materials]. No brain region was significantly activated once the threshold was set to $P < 0.05$, with a false discovery rate correction.

Correlations between subjective pleasantness ratings, hedonic traits, olfactory function, and brain activation

In the CVN group, activation in the OFC was significantly correlated with the subjective pleasantness rating of high-indole ($r = 0.72$; $P < 0.01$), but not in the insula ($r = 0.21$; $P = 0.51$) or the amygdala ($r = 0.26$; $P = 0.42$). In the non-CVN group, no significant correlation between activation and pleasantness ratings was observed in any of the brain regions tested in this study (see Fig. 4).

In the CVN group, significant correlations of both low- and high-indole conditions with the anticipatory score of the TEPS were found in the insula ($r = 0.60$, $P = 0.04$; $r = 0.60$, $P = 0.04$) and the amygdala ($r = 0.70$,$P = 0.01$; $r = 0.70$, $P = 0.01$), but not in the OFC ($r = -0.08$, $P = 0.81$; $r = 0.08$, $P = 0.80$), respectively. In contrast, no significant correlation was observed in the non-CVN group (see Fig. 5).

In the CVN group, a notable correlation emerged between the consummatory pleasure score of the TEPS and the activation linked with low-indole in the amygdala ($r = 0.63$; $P = 0.03$). However, this correlation was not evident in the OFC ($r = 0.30$; $P = 0.34$) or insula ($r = 0.54$; $P = 0.07$). Conversely, no significant correlation was observed between high-indole and the consummatory pleasure score. Similarly, in the non-CVN group, such correlations were not significant (see Fig. 6).

Furthermore, we analyzed the correlation between brain activation and olfactory function, including olfactory identification, olfactory threshold, and the score of the SODQ. In the CVN group, no significant correlation was observed in the low-indole condition. However, there was a notable correlation between olfactory identification and neural activity linked to the high-indole condition in the OFC ($r = 0.73$; $P < 0.01$) and amygdala ($r = 0.58$; $P = 0.05$), but not in the insula ($r = 0.53$; $P = 0.08$). Once again, no significant correlation was observed in the non-CVN group (see Fig. 7).

In the CVN group, activity in the insula linked to the low-indole condition showed a significant correlation with the SODQ score ($r = 0.58$; $P = 0.05$), whereas no significant correlation was found in the OFC or the amygdala. Notably, no significant correlation was observed for high-indole. Consistently, in line with previous tests, no significant correlation was observed in the non-CVN group (see Fig. 8).

Discussion

In the present study, we aimed to explore the neural mechanism of indole valence transformation and the brain areas activated

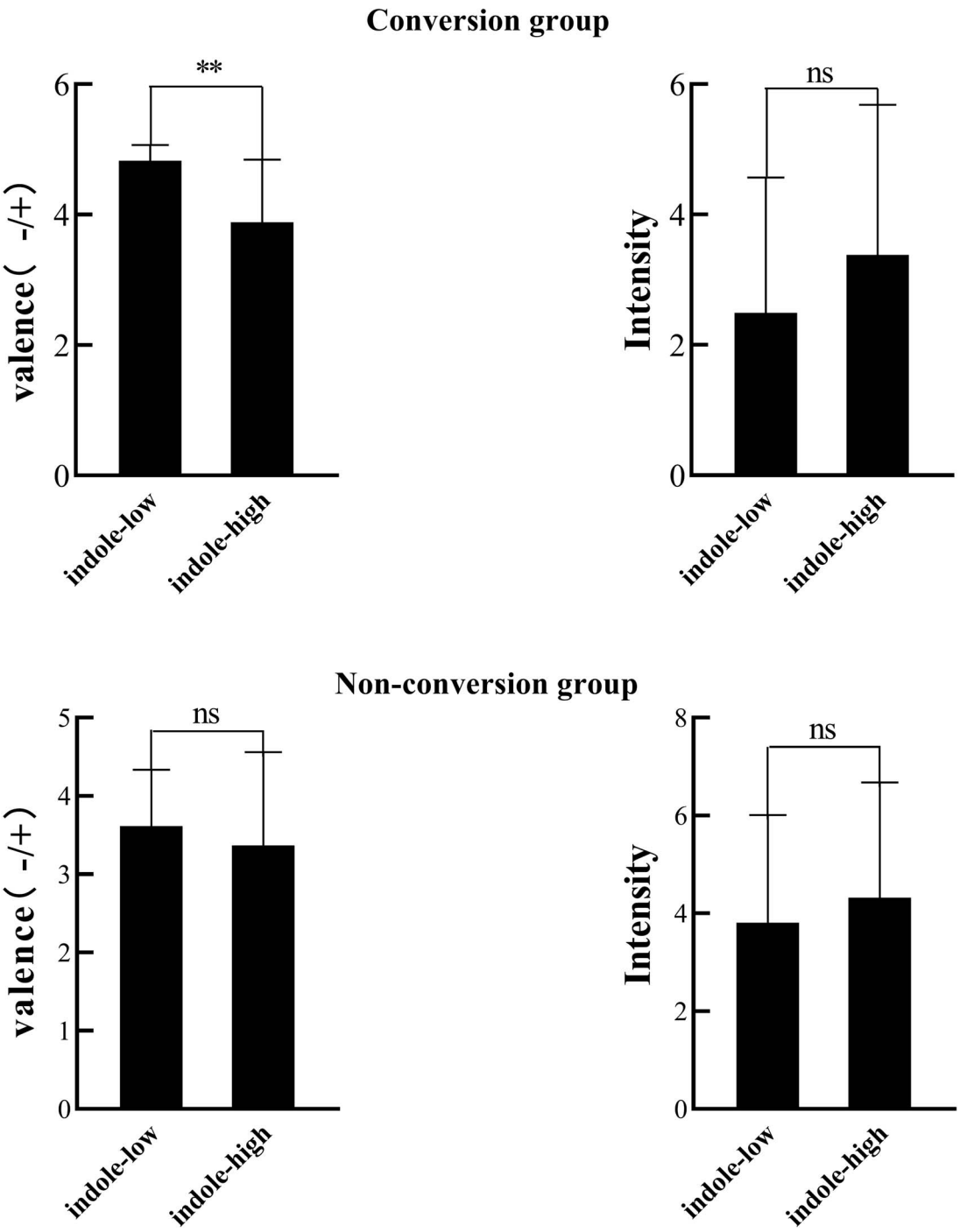

Fig. 2. Group differences in subjective pleasantness and intensity ratings of low- and high-indole between the Conversion group and Non-conversion group (mean ± SD). *$P < 0.05$, **$P < 0.01$, *ns*, $P \geq 0.05$.

by low- versus high-indole in CVN and non-CVN individuals. As expected, the change in indole concentration led to a change in the perception of pleasantness, and individuals in the CVN group showed more activation in the right lateral OFC, insula, and amygdala when comparing low- versus high-indole conditions, with no significant difference in the non-CVN group. Additionally, the indole valence transformation was associated with the individuals' hedonic traits.

Partly consistent with our hypothesis, our findings showed that the right OFC, insula, and amygdala were significantly activated in the indole valence transformation, which were more responsive to low- than high-indole in the CVN group. These findings have major implications for the interpretation of previous results (Anderson et al. 2003; Small et al. 2003), contradicting the assumption that the amygdala simply encodes representations of emotional intensity rather than emotional valence (Anderson and Sobel 2003; Hamann 2003).

The interplay between intensity and valence (Russell 1980) complicates the dissociation of neural representations and the determination of the precise contributions of the amygdala to

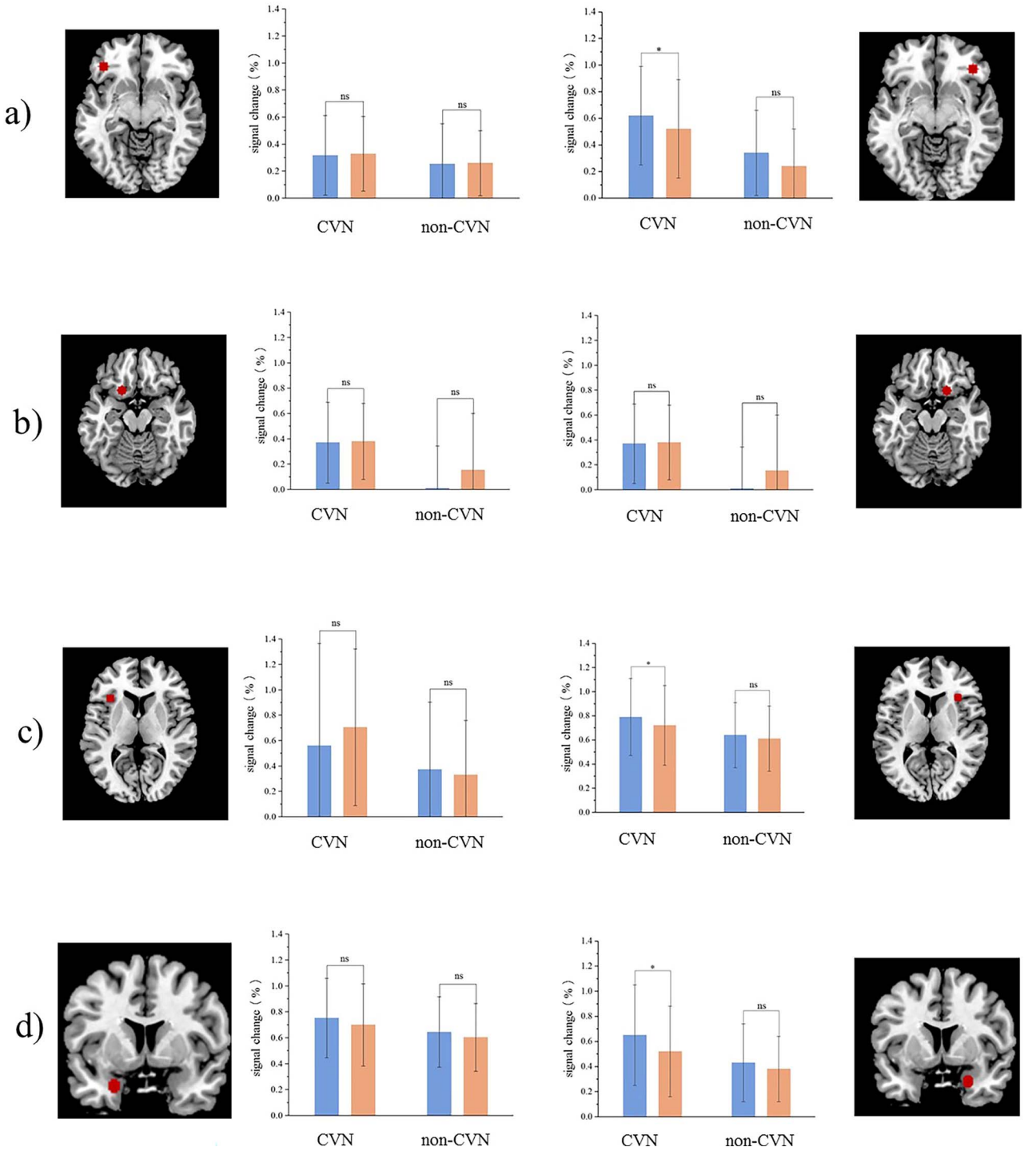

Fig. 3. Group differences in activation between low- and high-indole stimuli in a) lateral and b) medial OFC, c) insula, and d) amygdala. The left bar (blue) represents the low-indole condition, while the right bar (orange) represents the high-indole condition (the left hemisphere is on the left). *Notes.* CVN: Conversion group; non-CVN: Non-conversion group; OFC: orbitofrontal cortex. $^{*}P < 0.05$, $^{**}P < 0.01$, *ns*, $P \geq 0.05$.

their encoding. Notably, very unpleasant and very strong odors often appear conflated, consistent with findings from a prior study by Moskowitz and Gerbers (1974). Anderson et al. (2003) creatively manipulated valence and intensity independently, given that increasing physical intensity was strongly associated with the increase in the intensity of subjective and autonomic emotional responses (Bensafi et al. 2002). Instead of using low and high intensity levels of a pleasant odor and an unpleasant odor (Anderson et al. 2003), in our study, we dissociated valence from intensity using a unique odor (indole), which is ideal for independently manipulating subjective valence by varying the concentration without changing the physical intensity. Although we were able to circumvent the limitations of previous studies and to better characterize the response profile of the amygdala, chemosensory intensity (an inherent property of the stimulus) was identified as a substitute for arousal but not as a direct measure of arousal (the possible effect of the odorant on the subjective state; Bensafi et al. 2002; Winston et al. 2005).

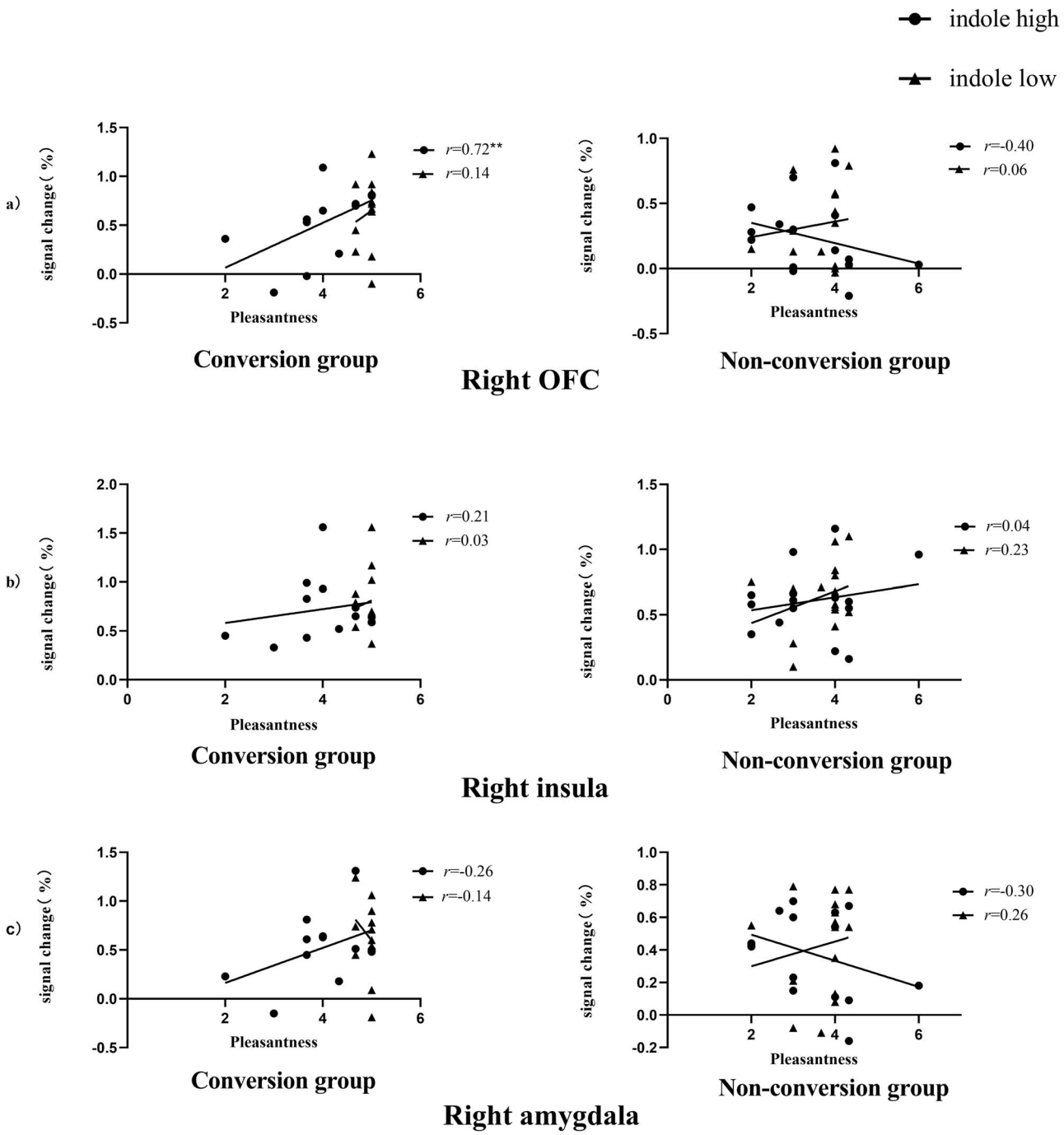

Fig. 4. The scatterplots show significant correlations between subjective pleasantness and signal change in the a) right OFC, b) insula, and c) amygdala. The Conversion group is shown on the left, and the Non-conversion group is shown on the right. *Notes.* OFC: orbitofrontal cortex.

We found that low-indole activated the lateral OFC more than high-indole, and subjective pleasantness ratings were positively correlated with the activation of this region, thus extending our understanding of the role of the lateral OFC in representing the unpleasantness of odors. Grabenhorst et al. (2007) demonstrated a correlation between the pleasantness ratings of indole and indole mixture in a more lateral part of the OFC, a finding consistent with our results. However, unlike their study, we did not observe a correlation with the pleasantness of the indole in the medial OFC. Previously, the lateral OFC was thought to modulate both decision-making and emotional processes (Mitchell 2011). Subjective pleasantness judgments were associated with activity in both the right medial and lateral OFC (Zou et al. 2016). However, while the medial OFC has been involved in representing pleasant stimuli and encoding pleasantness (Gottfried et al. 2002a; Rolls et al. 2003; Rolls and Grabenhorst 2008), this region was not activated in our study. This difference may be related to the uniqueness of indole, which is a complex odor, which tends to be rated as approximately neutral or positive at low concentrations, with a mean pleasantness score of only 4.91 even in the CVN group in our study. The neural correlates of odor hedonic processing observed in the present study—particularly in the insula—are consistent with results reported by Soudry et al. (2011) and Rolls (2019). The insula has also been involved in subjective awareness

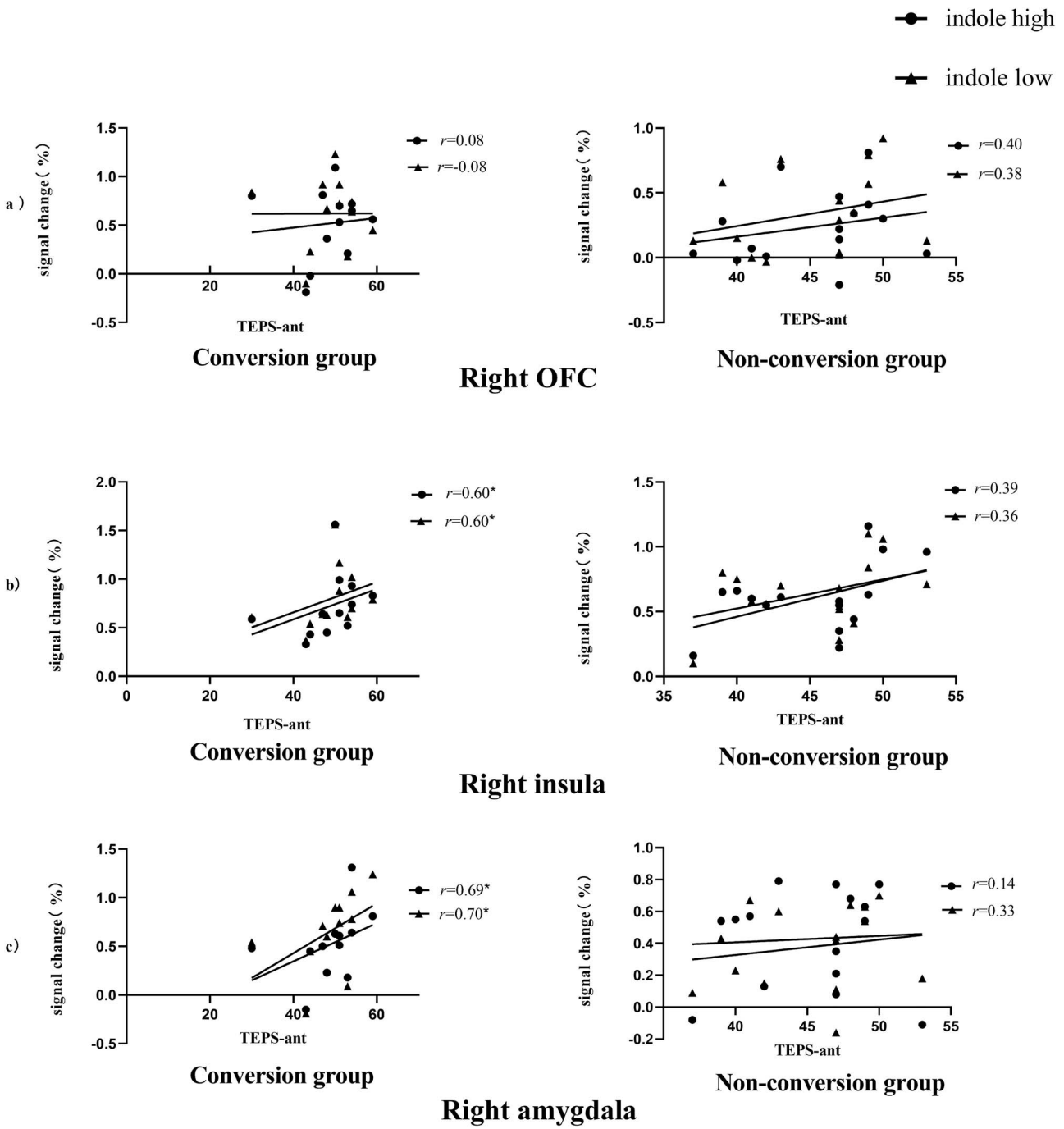

Fig. 5. The scatterplots show significant correlations between anticipatory hedonic traits measured using the TEPS and signal change in the a) right OFC, b) insula, and c) amygdala. The Conversion group is shown on the left, and the Non-conversion group is shown on the right. *Notes.* OFC: orbitofrontal cortex; TEPS-ant: anticipatory pleasure of the temporal experience pleasure scale.

of pleasantness (Craig 2004) whereby a pleasant odor induces stronger activation than a relatively unpleasant odor (Kühn and Gallinat 2012), thus playing a key role in hedonic judgment (Zou et al. 2016).

We also found that the right OFC, insula, and amygdala were significantly more active in indole valence transformation, with no difference in the left OFC, highlighting the predominant role of the right hemisphere in odor hedonic judgment. These findings corroborate the results of a previous meta-analysis by Kühn and Gallinat (2012), which pooled different types of rewarding stimuli. Therefore, lateralization may occur in hedonic evaluation.

Two interesting questions are whether changing the concentration of indole alters the participants' perception of pleasantness and whether valence transformation only occurs in the CVN group. Our results from the analysis of the relationships between brain activation and self-reported hedonic traits may provide some answers. The signal change induced by high-indole in the right insula and amygdala and by low-indole in the amygdala was positively correlated with the anticipatory score of the TEPS, while the signal change induced by low-indole in the right amygdala was positively correlated with consummatory pleasure and hedonic traits. These findings suggest that the higher the participants'

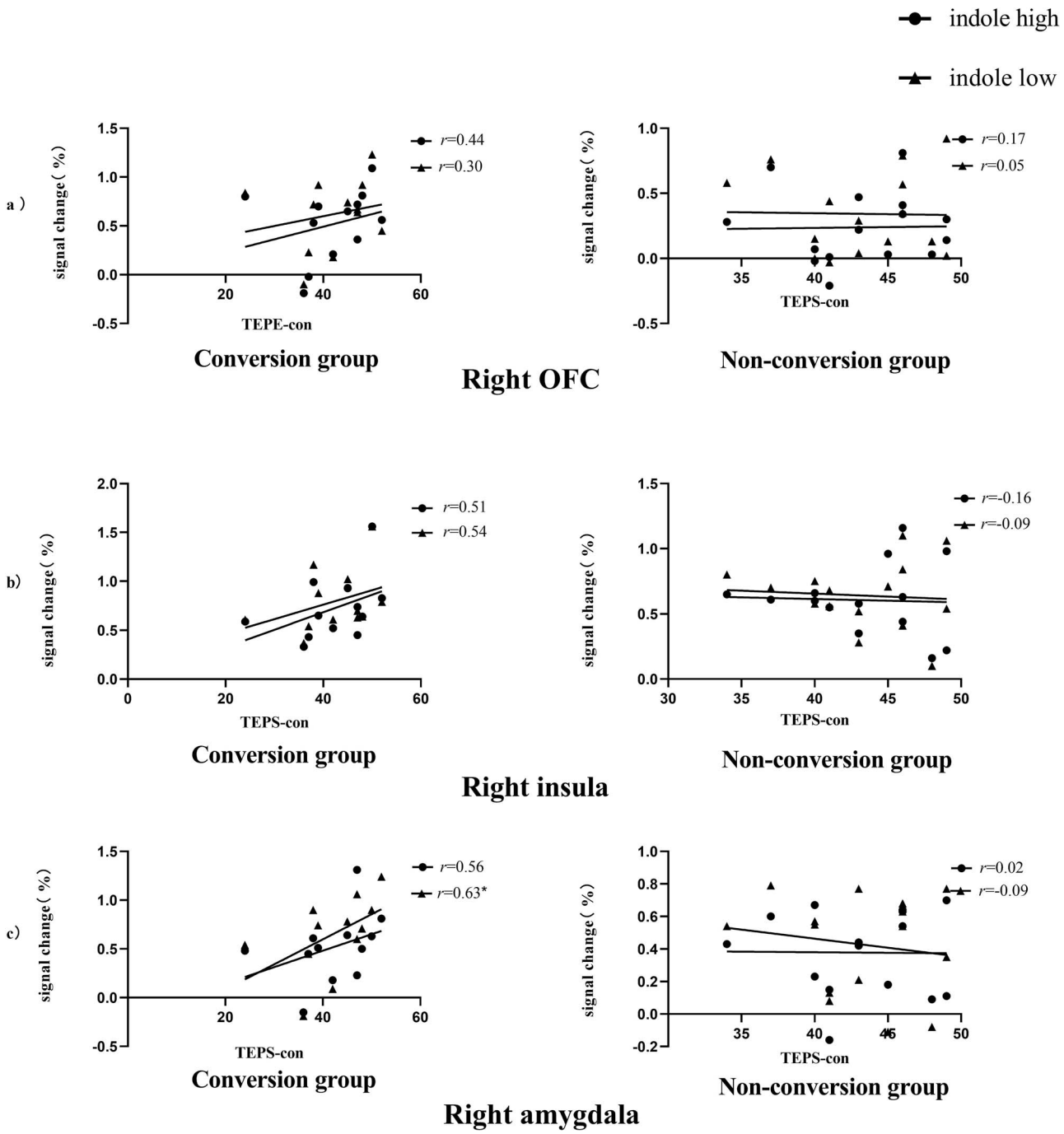

Fig. 6. The scatterplots show significant correlations between consummatory pleasure and hedonic traits measured using the TEPS and signal change in the a) right OFC, b) insula, and c) amygdala. The Conversion group is shown on the left, and the Non-conversion group is shown on the right. *Notes.* OFC: orbitofrontal cortex; TEPS-con: consummatory pleasure of the temporal experience pleasure scale.

ability to experience pleasure, the stronger the activation signals in the brain regions involved in hedonic processing, as shown by a study on the positive correlation between the ability to anticipate pleasure and activity in the insula (Rzepa and McCabe 2019).

The insula has also been involved in emotion processing, self-awareness and motor control (Chang et al. 2013), and specifically in anticipatory cues and approach (Kusumoto-Yoshida et al. 2015). In addition, Cano et al. (2022) showed a significant positive association between changes in anticipatory pleasure capability and regional gray matter volume increases in the right hippocampus and right amygdala. These results suggest that the more likely an individual is to experience anticipatory pleasure in response to olfactory stimuli, the more the insula and amygdala will be activated. So, the indole valence transformation in the CVN group may be linked to the individuals' hedonic traits. As such, the more likely an individual is to experience pleasure in response to indole, the more indole valence transformation will occur, especially in the amygdala, insula, and OFC, which are involved in hedonic processing.

Another explanation for our results may be related to the perceptual differences of indole. The changes in signal induced

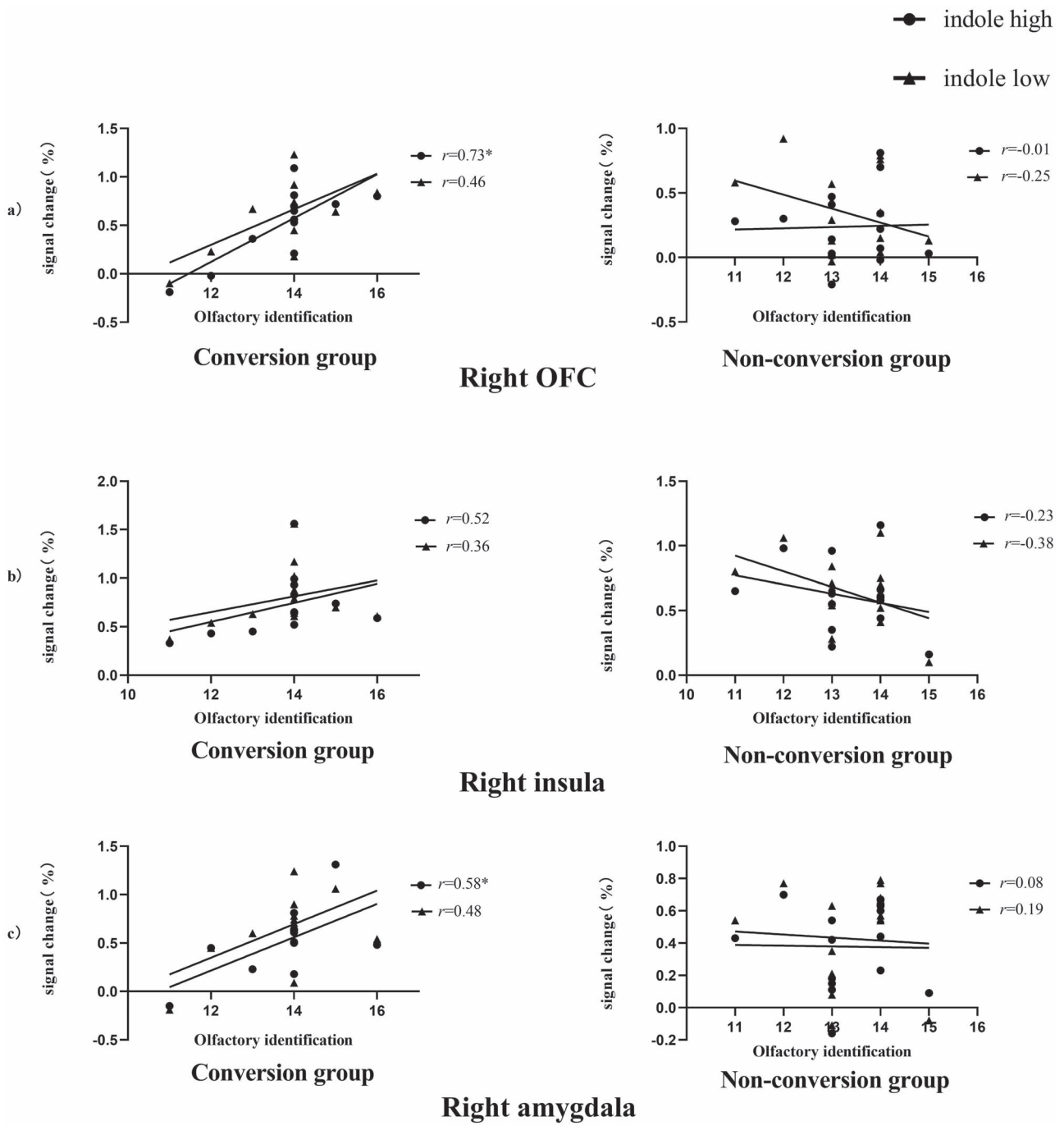

Fig. 7. The scatterplots show significant correlations between olfactory identification and signal change in the a) right OFC, b) insula, and c) amygdala. The Conversion group is shown on the left, and the Non-conversion group is shown on the right. *Notes*. OFC: orbitofrontal cortex.

by high-indole in the right OFC and amygdala were positively correlated with the individuals' olfactory identification in the CVN group, and the change in signal induced by low-indole in the right insula was negatively correlated with the SODQ scores. In particular, the pleasantness ratings of low-indole were significantly higher than those of high-indole, with no significant difference in pleasantness in the non-CVN group. The pleasantness of human olfactory perception is highly variable between individuals (Gilad and Lancet 2003), with large perceptual differences in the pleasantness and intensity of the specific odor (Menashe et al. 2003), which are mediated by olfactory receptors (ORs) encoded by >300 genes (Malnic et al. 2004). Furthermore, some authors have suggested that the heritability of hedonic perception may depend on the properties and chemical composition of the specific odorant, possibly because the hedonic perception of some odorants, such as androstenone (Keller et al. 2007) and cinnamon (Knaapila et al. 2007), is mediated by genetic factors. Thus, some individuals may like the smell of low-indole partly because they lack receptors to detect some of the more pungent volatiles of indole at the low concentration, whereas other individuals are repelled by this odor, even at low concentrations, because of the higher number of receptors that they express, suggesting that hedonic transformation is genetically individual. Future research should investigate individual genetic differences in the hedonic

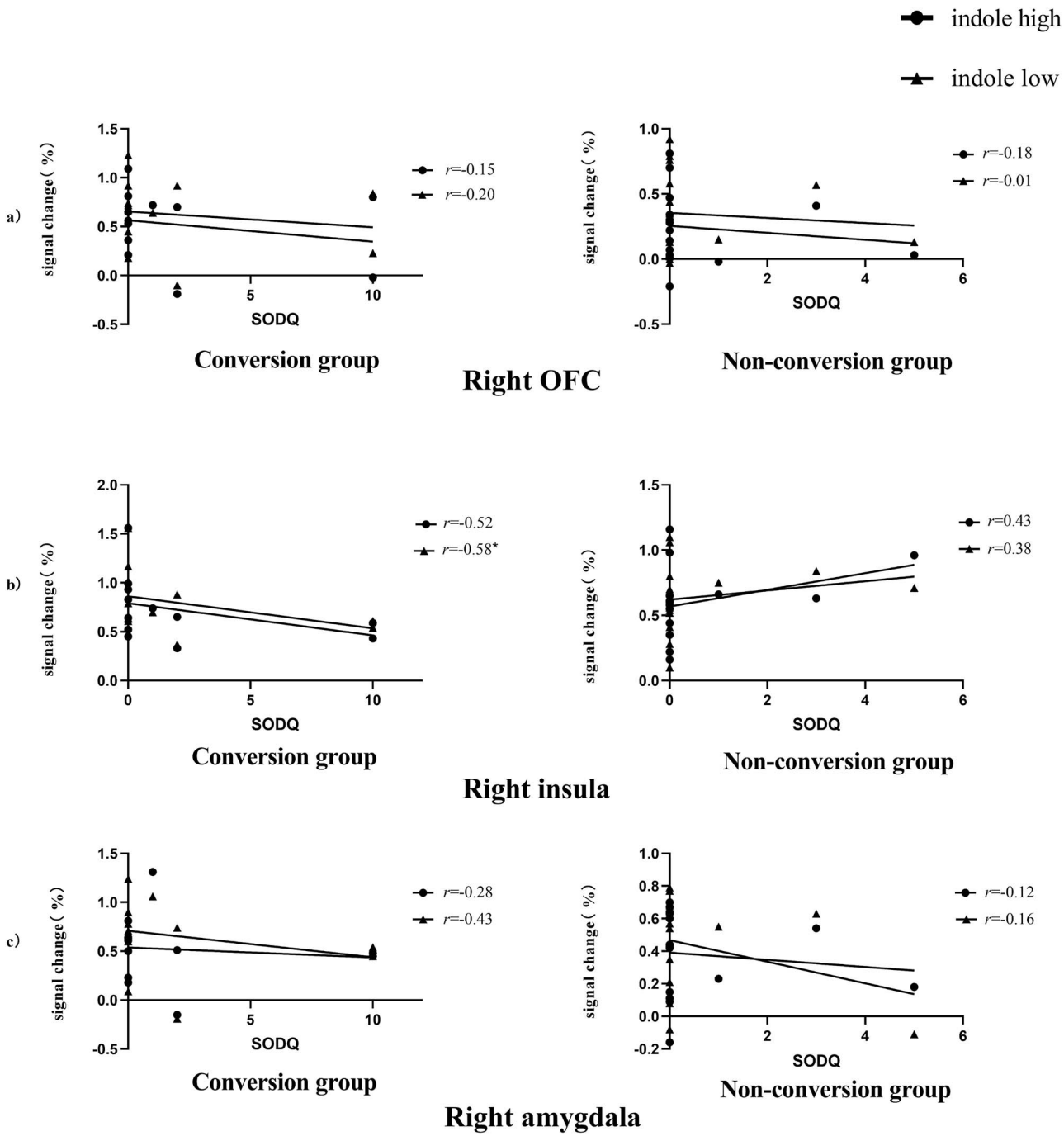

Fig. 8. The scatterplots show significant correlations between SODQ and signal change in the a) right OFC, b) insula, and c) amygdala. The Conversion group is shown on the left, and the Non-conversion group is shown on the right. *Notes.* OFC: orbitofrontal cortex; SODQ: self-reported olfactory dysfunction questionnaire.

response to indole and describe in depth relationships between individual OR disorders and defined cases of odor–specific olfactory threshold variability.

The present study has several limitations. First, the neural mechanism of indole valence transformation is not yet fully understood because we only selected 2 different concentrations of indole. Further studies should explore the continuous process of indole valence transformation using a parametric design. Second, the subjective ratings of intensity and pleasantness can also be combined with other methods, such as skin conductance, heart rate, respiration rate, and facial muscle activity, to further explore indole valence transformation. Another limitation is the relatively small sample size of each group, an issue that should also be addressed in future studies extending the research to a wider group of people to better understand indole valence transformation.

In conclusion, in line with a prior behavioral study of indole, changes in the concentration of indole lead to changes in valence. More specifically, differences in the right OFC, insula, and amygdala activation between low- and high-indole underlie a possible valence transformation in indole processing. This valence transformation may be associated with individual hedonic characteristics and perceptual differences. Taken together, our findings provide evidence suggesting valence transformation in

indole processing when subjective ratings are combined with brain activation patterns.

Author contributions

Laiquan Zou (Conceptualization, Formal analysis, Funding acquisition, Methodology, Writing—review & editing), Yue Qi (Formal analysis, Investigation, Methodology, Visualization, Writing—original draft), Lei Shen (Formal analysis, Investigation), Yanyang Huang (Investigation, Methodology), Jiayu Huang (Investigation, Methodology), Zheng Xia (Investigation), Mingxia Fan (Methodology, Project administration, Resources), Wu Fan (Project administration, Resources), Guo-bi Chai (Project administration, Resources), Qing-zhao Shi (Methodology, Resources), Qidong Zhang (Conceptualization, Funding acquisition, Project administration), and Chao Yan (Conceptualization, Funding acquisition, Project administration, Writing—review & editing).

Supplementary material

Supplementary material is available at *Cerebral Cortex* online.

Funding

This work was funded by National Natural Science Foundation of China [82371561; 32171084], the Natural Science Foundation of Shanghai [21ZR1421000], the scientific research program of innovation platform in State Tobacco Monopoly Administration and the State Key Program of National Natural Science Foundation of China [32130083], the East China Normal University and Health Joint Fund [2022JKXYD09003], and Open Research Fund of Shanghai Key Laboratory of Brain Functional Genomics (East China Normal University) [23SKBFGKF2].

Conflict of interest statement: None declared.

References

Anderson AK, Christoff K, Stappen I, Panitz D, Ghahremani DG, Glover G, Gabrieli JD, Sobel N. Dissociated neural representations of intensity and valence in human olfaction. *Nat Neurosci*. 2003:6(2):196–202. https://doi.org/10.1038/nn1001.

Anderson AK, Sobel N. Dissociating intensity from valence as sensory inputs to emotion. *Neuron*. 2003:39(4):581–583. https://doi.org/10.1016/S0896-6273(03)00504-X.

Anderson GM. Quantitation of tryptophan metabolites in rat feces by thin-layer chromatography. *J Chromatogr A*. 1975:105(2):323–328. https://doi.org/10.1016/S0021-9673(01)82261-5.

Barden TC (2010). *Indoles: industrial, agricultural and over-the-counter uses BT-heterocyclic scaffolds II: reactions and applications of indoles*. In Gribble GW, editor); p 31–46). Springer Berlin Heidelberg. https://doi.org/10.1007/7081_2010_48.

Bensafi M, Rouby C, Farget V, Bertrand B, Vigouroux M, Holley A. Autonomic nervous system responses to odours: the role of pleasantness and arousal. *Chem Senses*. 2002:27(8):703–709. https://doi.org/10.1093/chemse/27.8.703.

Brett M, Anton J-L, Valabregue R, Poline J-B. Region of interest analysis using an SPM toolbox. [abstract]. Presented at the 8th International Conference on Functional Mapping of the Human Brain, June 2–6, 2002, Sendai, Japan. *NeuroImage*. 2002:16. https://doi.org/10.1016/S1053-8119(02)90013-3.

Cano M, Lee E, Worthley A, Ellard K, Barbour T, Soriano-Mas C, Camprodon JA. Electroconvulsive therapy effects on anhedonia and reward circuitry anatomy: a dimensional structural neuroimaging approach. *J Affect Disord*. 2022:313:243–250. https://doi.org/10.1016/j.jad.2022.06.062.

Chan RCK, Shi Y, Lai M, Wang Y, Wang Y, Kring AM. The temporal experience of pleasure scale (TEPS): exploration and confirmation of factor structure in a healthy Chinese sample. *PLoS One*. 2012:7(4):e35352. https://doi.org/10.1371/journal.pone.0035352.

Chang LJ, Yarkoni T, Khaw MW, Sanfey AG. Decoding the role of the insula in human cognition: functional parcellation and large-scale reverse inference. *Cereb Cortex*. 2013:23(3):739–749. https://doi.org/10.1093/cercor/bhs065.

Clarke AK, Ho HE, Rossi-Ashton JA, Taylor RJK, Unsworth WP. Indole synthesis using silver catalysis. *Chem Asian J*. 2019:14(11):1900–1911. https://doi.org/10.1002/asia.201900309.

Craig AD. Human feelings: why are some more aware than others? *Trends Cogn Sci*. 2004:8(6):239–241. https://doi.org/10.1016/j.tics.2004.04.004.

De Araujo IE, Rolls ET, Velazco MI, Margot C, Cayeux I. Cognitive modulation of olfactory processing. *Neuron*. 2005:46(4):671–679. https://doi.org/10.1016/j.neuron.2005.04.021.

Edris AE, Chizzola R, Franz C. Isolation and characterization of the volatile aroma compounds from the concrete headspace and the absolute of Jasminum sambac (L.) Ait. (Oleaceae) flowers grown in Egypt. *Eur Food Res Technol*. 2008:226(3):621–626. https://doi.org/10.1007/s00217-007-0623-y.

Gilad Y, Lancet D. Population differences in the human functional olfactory repertoire. *Mol Biol Evol*. 2003:20(3):307–314. https://doi.org/10.1093/molbev/msg013.

Gottfried JA, Deichmann R, Winston JS, Dolan RJ. Functional heterogeneity in human olfactory cortex: an event-related functional magnetic resonance imaging study. *J Neurosci*. 2002a:22(24):10819 LP–10828. https://doi.org/10.1523/JNEUROSCI.22-24-10819.2002.

Gottfried JA, O'Doherty J, Dolan RJ. Appetitive and aversive olfactory learning in humans studied using event-related functional magnetic resonance imaging. *J Neurosci*. 2002b:22(24):10829–10837. https://doi.org/10.1523/JNEUROSCI.22-24-10829.2002.

Grabenhorst F, Rolls ET, Margot C, da Silva MAAP, Velazco MI. How pleasant and unpleasant stimuli combine in different brain regions: odor mixtures. *J Neurosci*. 2007:27(49):13532LP–13540LP. https://doi.org/10.1523/JNEUROSCI.3337-07.2007.

Gribble GW. *Heterocyclic scaffolds II: reactions and applications of indoles*. Vol. 26. Springer, Berlin; 2010. pp. 1–29. ISBN: 978-3-642-15733-2.

Hamann S. Nosing in on the emotional brain. *Nat Neurosci*. 2003:6(2):106–108. https://doi.org/10.1038/nn0203-106.

Hattori S, Takagaki H, Fujimori T. Evaluation of Japanese green tea extract using GC/O with original aroma simultaneously input to the sniffing port method (OASIS). *Food Sci Technol Res*. 2003:9(4):350–352. https://doi.org/10.3136/fstr.9.350.

Hummel T, Sekinger B, Wolf SR, Pauli E, Kobal G. 'Sniffin' Sticks': Olfactory Performance Assessed by the Combined Testing of Odour Identification, Odor Discrimination and Olfactory Threshold. *Chemical Senses*. 1997:22(1):39-52. https://doi.org/10.1093/chemse/22.1.39.

Keller A, Vosshall LB. Olfactory perception of chemically diverse molecules. *BMC Neurosci*. 2016:17:1–17. https://doi.org/10.1186/s12868-016-0287-2.

Keller A, Zhuang H, Chi Q, Vosshall LB, Matsunami H. Genetic variation in a human odorant receptor alters odour perception. *Nature*. 2007:449(7161):468–472. https://doi.org/10.1038/nature06162.

Kjelvik G, Evensmoen HR, Brezova V, Håberg AK. The human brain representation of odor identification. *J Neurophysiol*. 2012:108(2): 645–657. https://doi.org/10.1152/jn.01036.2010.

Knaapila A, Keskitalo K, Kallela M, Wessman M, Sammalisto S, Hiekkalinna T, Palotie A, Peltonen L, Tuorila H, Perola M. Genetic component of identification, intensity and pleasantness of odours: a Finnish family study. *Eur J Hum Genet*. 2007:15(5): 596–602. https://doi.org/10.1038/sj.ejhg.5201804.

Kühn S, Gallinat J. The neural correlates of subjective pleasantness. *NeuroImage*. 2012:61(1):289–294. https://doi.org/10.1016/j.neuroimage.2012.02.065.

Kusumoto-Yoshida I, Liu H, Chen BT, Fontanini A, Bonci A. Central role for the insular cortex in mediating conditioned responses to anticipatory cues. *Proc Natl Acad Sci*. 2015:112(4):1190–1195. https://doi.org/10.1073/pnas.1416573112.

Landau B, Gleitman L. Language and experience: evidence from the blind child. *Language*. 1985:62(62):95–106. https://doi.org/10.1002/acp.2350010109.

Liu X, Huang J, Tian P, Hu J, Zou L. Development of a self-reported olfactory dysfunction questionnaire (SODQ) to screen olfactory disorders in China. *Rhinology*. 2021:59. https://doi.org/10.4193/Rhin21.028.

Lombion S, Comte A, Tatu L, Brand G, Moulin T, Millot J. Patterns of cerebral activation during olfactory and trigeminal stimulations. *Hum Brain Mapp*. 2009:30(3):821–828. https://doi.org/10.1002/hbm.20548.

Malnic B, Godfrey PA, Buck LB. The human olfactory receptor gene family. *Proc Natl Acad Sci*. 2004:101(8):2584–2589. https://doi.org/10.1073/pnas.0307882100.

Menashe I, Man O, Lancet D, Gilad Y. Different noses for different people. *Nat Genet*. 2003:34(2):143–144. https://doi.org/10.1038/ng1160.

Mitchell DGV. The nexus between decision making and emotion regulation: a review of convergent neurocognitive substrates. *Behav Brain Res*. 2011:217(1):215–231. https://doi.org/10.1016/j.bbr.2010.10.030.

Moskowitz HR, Gerbers CL. Dimensional salience of odors. *Ann N Y Acad Sci*. 1974:237:1–16. https://doi.org/10.1111/j.1749-6632.1974.tb49839.x.

Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *NeuroImage*. 2012:59(3): 2142–2154. https://doi.org/10.1016/j.neuroimage.2011.10.018.

Rolls ET. The orbitofrontal cortex and emotion in health and disease, including depression. *Neuropsychologia*. 2019:128:14–43. https://doi.org/10.1016/j.neuropsychologia.2017.09.021.

Rolls ET, Grabenhorst F. The orbitofrontal cortex and beyond: from affect to decision-making. *Prog Neurobiol*. 2008:86(3):216–244. https://doi.org/10.1016/j.pneurobio.2008.09.001.

Rolls ET, Kringelbach ML, De Araujo IET. Different representations of pleasant and unpleasant odours in the human brain. *Eur J Neurosci*. 2003:18(3):695–703. https://doi.org/10.1046/j.1460-9568.2003.02779.x.

Russell JA. A circumplex model of affect. *J Pers Soc Psychol*. 1980:39(6):1161. https://doi.org/10.1037/h0077714.

Rzepa E, McCabe C. Dimensional anhedonia and the adolescent brain: reward and aversion anticipation, effort and consummation. *BJPsych Open*. 2019:5(6):e99. https://doi.org/10.1192/bjo.2019.68.

Sharma A, Kumar R, Ranjta S, Varadwaj PK. SMILES to smell: decoding the structure–odor relationship of chemical compounds using the deep neural network approach. *J Chem Inf Model*. 2021:61(2):676–688. https://doi.org/10.1021/acs.jcim.0c01288.

Small DM, Gregory MD, Mak YE, Gitelman D, Mesulam MM, Parrish T. Dissociation of neural representation of intensity and affective valuation in human gustation. *Neuron*. 2003:39(4):701–711. https://doi.org/10.1016/s0896-6273(03)00467-7.

Small DM, Gerber JC, Mak YE, Hummel T. Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron*. 2005:47(4):593–605. https://doi.org/10.1016/j.neuron.2005.07.022.

Soudry Y, Lemogne C, Malinvaud D, Consoli S-M, Bonfils P. Olfactory system and emotion: common substrates. *Eur Ann Otorhinolaryngol Head Neck Dis*. 2011:128(1):18–23. https://doi.org/10.1016/j.anorl.2010.09.007.

Taber DF, Tirunahari PK. Indole synthesis: a review and proposed classification. *Tetrahedron*. 2011:67(38):7195. https://doi.org/10.1016/j.tet.2011.06.040.

Wicker B, Perrett DI, Baron-Cohen S, Decety J. Being the target of another's emotion: a PET study. *Neuropsychologia*. 2003:41(2): 139–146. https://doi.org/10.1016/S0028-3932(02)00144-6.

Winston JS, Gottfried JA, Kilner JM, Dolan RJ. Integrated neural representations of odor intensity and affective valence in human amygdala. *J Neurosci*. 2005:25(39):8903–8907. https://doi.org/10.1523/JNEUROSCI.1569-05.2005.

Yamamoto Y, Sato Y, Ebina T, Yokoyama C, Takahasi S, Mito Y, Tanabe H, Nishiguchi N, Nagaoka K. Separation of high purity indole from coal tar by high pressure crystallization. *Fuel*. 1991: 70(4):565–566. https://doi.org/10.1016/0016-2361(91)90039-D.

Zelano C, Montag J, Johnson B, Khan R, Sobel N. Dissociated representations of irritation and valence in human primary olfactory cortex. *J Neurophysiol*. 2007:97(3):1969–1976. https://doi.org/10.1152/jn.01122.2006.

Zhou Y, Hallis SA, Vitko T, Suffet IHM. Identification, quantification and treatment of fecal odors released into the air at two wastewater treatment plants. *J Environ Manag*. 2016:180:257–263. https://doi.org/10.1016/j.jenvman.2016.05.046.

Zhou H-C, Hou Z-W, Wang D-X, Ning J-M, Wei S. Large scale preparation, stress analysis, and storage of headspace volatile condensates from Jasminum sambac flowers. *Food Chem*. 2019:286: 170–178. https://doi.org/10.1016/j.foodchem.2019.01.202.

Zou L-Q, van Hartevelt TJ, Kringelbach ML, Cheung EFC, Chan RCK. The neural mechanism of hedonic processing and judgment of pleasant odors: an activation likelihood estimation meta-analysis. *Neuropsychology*. 2016:30(8):970–979. https://doi.org/10.1037/neu0000292.