CORTEX 165 (2023) 119–128

Available online at www.sciencedirect.com

ScienceDirect

Journal homepage: www.elsevier.com/locate/cortex

Research Report

From nose to brain: The effect of lemon inhalation observed by whole brain voxel to voxel functional connectivity

Charlotte Martial [a,b,*], Anne-Lise Poirrier [c], Laurence Pottier [c], Héléna Cassol [a], Sepehr Mortaheb [d], Rajanikant Panda [a], Mateo Lopez [e], Thibault Perrin [e], Antoine Boilevin [e,f], Olivia Gosseries [a,b] and Steven Laureys [a,b,g]

[a] Coma Science Group, GIGA-Consciousness, University of Liège, Avenue de L'hôpital, 11, 4000, Liège, Belgium
[b] Centre Du Cerveau[2], University Hospital of Liège, Avenue de L'hôpital, 11, 4000, Liège, Belgium
[c] ENT Department, University Hospital of Liège, Avenue de L'hôpital, 11, 4000, Liège, Belgium
[d] Physiology of Cognition, GIGA-CRC in Vivo Imaging Research Unit, University of Liège, Allée Du 6 Août 8, 4000, Liège, Belgium
[e] Valeo, Carlab Health & WellBeing, 100 Rue de Courcelles, 75017, Paris, France
[f] Université Bordeaux Montaigne, UFR Humanités, Département Design Bordeaux, Esplanade des Antilles Domaine Niversitaire, 33607, Pessac, France
[g] Joint International Research Unit on Consciousness, CERVO Brain Research Centre, CIUSS, University Laval, 2301 Av. D'Estimauville, QC, G1E 1T2, Québec, Canada

ARTICLE INFO

Article history:
Received 13 December 2022
Reviewed 5 January 2023
Revised 7 February 2023
Accepted 6 April 2023
Action editor Ilona Croy
Published online 16 May 2023

Keywords:
Odor
Lemon
MRI
Global functional connectivity
Alertness

ABSTRACT

Lemon fragrance is known for its stimulating properties, but its mechanisms of action are not well known yet. This study aimed to examine the effect of lemon essential oil inhalation on healthy participants' alertness level and their neural correlates using magnetic resonance imaging (MRI). Twenty-one healthy men underwent functional MRI scans in different conditions: a resting state condition, a condition where they were exposed to passive lemon smelling (alternating exposure to lemon and breathing fresh air), and a control condition without lemon fragrance diffusion –the order of the last two conditions being randomized. Alertness levels were assessed immediately after each condition using the Karolinska Sleepiness Scale. Voxel-wise whole-brain global functional connectivity and graph theory analyses were computed to investigate brain functional connectivity and network topology alterations. After lemon fragrance inhalation, we observed a higher level of alertness as compared to resting state –but not compared to control condition. During lemon fragrance inhalation, we found increased global functional connectivity in the thalamus, paralleled by decreased global connectivity in several cortical regions such as precuneus, postcentral and precentral gyrus, lateral occipital cortex and paracingulate gyrus. Graph theory analysis revealed increased network integration in cortical regions typically involved in olfaction and emotion processing such as olfactory bulb, hypothalamus and thalamus, while decreased network segregation in several regions of the posterior

* Corresponding author. Coma Science Group, GIGA-Consciousness, University of Liège, Liège, Belgium.
E-mail address: cmartial@uliege.be (C. Martial).
https://doi.org/10.1016/j.cortex.2023.04.012

part of the brain during olfaction as compared to resting state. The present findings suggest that lemon essential oil inhalation could increase the level of alertness.

1. Introduction

Of all the senses, olfaction is the most underutilized sense in humans as well as the least well understood by scientists. While the current pandemic reminds us how important it is to study olfactory (dys)functions, with the highly contagious COVID-19 virus affecting frequently olfactory functions (Izquierdo-Dominguez, Rojas-Lechuga, Mullol, & Alobid, 2020), scientists still cannot offer a complete and satisfying theory for the complex process of smelling (from nose to brain) (Gane, 2010).

It is known that certain odors, such as lavender or lemon, can modulate in a specific way mood, cognition and emotion (Herz, 2009; Kadohisa, 2013; Zucco, Paolini, & Schaal, 2009; for a recent review, see Kontaris, East, & Wilson, 2020), causing associated changes in brain waves, heart and respiratory rate, and blood pressure (e.g., Han, Mukdad, Long, & Lopez, 2020; Park, Kwon, Choi, Park, & Yoon, 2018; Sayorwan et al., 2012, 2013). Interestingly, one can observe that the neuroanatomy associated to olfaction and the one associated to the emotional state are closely linked, with some regions heavily overlapping in both neuroanatomical circuits, such as the hippocampus, the amygdala and the olfactory cortex itself (Kontaris et al., 2020). In the brain, the olfactory system functions as follows: Olfactory sensory neurons can directly connect with brain cortex networks (Mori, 2014), with the olfactory bulb as the first relay center of the olfactory system (i.e., regions receiving direct synaptic inputs from projection neurons in the olfactory bulb; Wilson & Sullivan, 2011) that projects the information to various cortical and subcortical brain regions, including the amygdala and the anterior olfactory nucleus (Cleland & Linster, 2003). Importantly, olfactory sensory information may be sent to higher association areas, such as the orbitofrontal cortex, the thalamus, and the hypothalamus. However, to date, the complex neurophysiological process that translates chemical inputs into the subjective experience of odor perception is not well understood.

More specifically, lemon is known for its stimulating and exciting properties, and for its associated physiological measurements modulation (Kiecolt-Glaser et al., 2008; Kikuchi, Yamagughi, Tanida, Abe, & Uenoyama, 1992; Keville & Green, 1995; Warrenburg, 2005; Martin & Cooper, 2007), as well as for stress-alleviating effect (Komiya, Takeuchi, & Harada, 2006; Lehrner, Eckersberger, Walla, Pötsch, & Deecke, 2000). Indeed, some empirical studies observed lemon oil's effects on mood using self-report standardized questionnaires (e.g., Kiecolt-Glaser et al., 2008). It has been hypothesized that lemon inhalation may boost norepinephrine release (Kiecolt-Glaser et al., 2008). Assessing the potential of lemon fragrance is interesting, since it may be, for example, used as a non-pharmacological intervention having several advantages (e.g., safety, natural product, cheaper) over the pharmacological ones.

The present study aimed to investigate alertness modulation and neural correlates effects of lemon essential oil inhalation using standardized questionnaires and functional magnetic resonance imaging (fMRI) in a sample of healthy young adult men. To explore the brain dynamic alteration, we performed data driven approaches of voxel-to-voxel wise global (whole-brain) functional connectivity and network topological alteration using graph theory analyses.

2. Materials and methods

2.1. Participants

A sample of young men (between 21 and 30 years old) were recruited via media advertising and word-of-mouth. Exclusion criteria (established prior to data acquisition) were left-handed, a history of brain trauma, neurosurgical or psychiatric disorder, olfactory dysfunction, addiction, asthma, nasal polyps, claustrophobia, MRI contraindications, smoker, allergic skin reaction to fragrances and scores at the "Sniffin' Sticks" test (Kobal et al., 1996) falling below the 25th percentile (see below for details). We did not perform a power analysis.

The study was approved and carried out in accordance with the recommendations of the ethics committee of the Faculty of Medicine of the University of Liège. All participants completed a written informed consent in accordance with the Declaration of Helsinki and its later amendments. No part of the study procedures and analyses was pre-registered prior to the research being conducted. We report how we determined our sample size, all data exclusions (if any), all inclusion/exclusion criteria, whether inclusion/exclusion criteria were established prior to data analysis, all manipulations, and all measures in the study.

2.2. Procedure

Participants were instructed not to eat or drink anything other than water, as well as not to use nose spray 2 h before the experiment. After completing the informed consent, each participant underwent an olfactory examination administrated by an Ear-Nose-Throat (ENT) specialist, including the standard "Sniffin' Sticks" test (Kobal et al., 1996). The purpose of the odor test was to exclude a participant with incidental olfactory dysfunction. Odorants were presented with pen-like devices and testing comprised three components, namely odor threshold, odor discrimination and odor identification. The three subsets of this test were compared to olfactory percentile charts. This olfactory examination was conducted

on the same day and before the fMRI session. All fMRI sessions were performed between 10.30 am and 12.30 am. Just prior to the fMRI exam, the participant was administrated questionnaires including socio-demographic questions, the Positive and Negative Affect Schedule (PANAS; Watson, Clark, & Tellegen, 1988) and the State-Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, & Lushene, 1970; Spielberger, 1983). During the fMRI session, structural (T1-weighted) MRI images were first acquired. Then, four functional sequences were acquired: (1) a resting state; (2) "odor imagery" condition: a mental imagery task in which they were asked to imagine smelling an odor; (3) "olfactory" condition: an olfactory task in which they were invited to smell the citrus fragrance; and (4) a control condition consisting of the exact same procedure as the olfactory task except that the citrus fragrance was not diffused. The last two sequences were randomized. Participants were randomly assigned to one of the two protocol orders (olfactory condition followed by the control condition vs. the control condition followed by the olfactory condition). For all functional sequences, instructions stated to normally breathe through the nose and to keep eyes closed (to get comparable sequence instructions with the "odor imagery" condition necessarily requesting eyes closed). During the olfactory condition sequence, 12 alternating periods of olfactory stimulation (lasting 6 s) and rest (i.e., no stimulation at all) (lasting 24 s) were acquired. The olfactory stimulation was delayed for 13 s after the start of the sequence acquisition. The odor stimulus was presented thanks to a MRI-compatible diffuser. Immediately after each functional sequence, we administrated the Karolinska Sleepiness Scale (KSS; Akerstedt & Gillberg, 1990) verbally, inside the scanner. Additional questions were also asked for the mental imagery task and the olfactory task (see details in the next section), inside the scanner too. After the fMRI session, they were invited to fill in the PANAS again, as well as the state subscale of the STAI. The relevant findings concerning the odor imagery condition will be reported elsewhere since this condition was added and used for another project. Fig. 1 illustrates the study design.

3. Materials

3.1. *ENT exam*

The standard "Sniffin' Sticks" test (Kobal et al., 1996; Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997) is a widely used test aiming to evaluate olfactory performance, including three subtests: olfactory threshold, odor discrimination and odor identification. A total score can be calculated by summing the three subscores, ranging from 1 to 48 points. Scores of the individuals are compared to standard values recently updated by Oleszkiewicz, Schriever, Croy, Hahner, and Hummel (2019) (group C including people aged 21–30). These normative data can be used in clinical practice, as well as in scientific quantitative assessment of olfactory performance.

3.2. *Questionnaires*

3.2.1. *The Positive and Negative Affect Schedule*

The PANAS is a 20-item self-report questionnaire used to assess positive and negative affect (Watson et al., 1988; see Supplementary Material 1). This questionnaire can be used in both clinical and non-clinical setting. The questionnaire includes a positive affect subscore (corresponding to the sum of 10 items' responses) assessing the level of pleasurable engagement and subjective experience of happiness and a negative affect subscore (corresponding to the sum of 10 items' responses) reflecting the level of subjective distress and unpleasurable engagement. Participants are asked to rate on a five-point Likert scale (ranging from 1 = "Never or very slightly" to 5 "Extremely") how they feel with regards to each item describing an emotion or a mood.

3.2.2. *The Karolinska Sleepiness Scale*

The KSS (Akerstedt & Gillberg, 1990; see Supplementary Material 2) is a self-reported measure of sleepiness. Scores range on a visual analogue scale (VAS) from 1 = "extremely

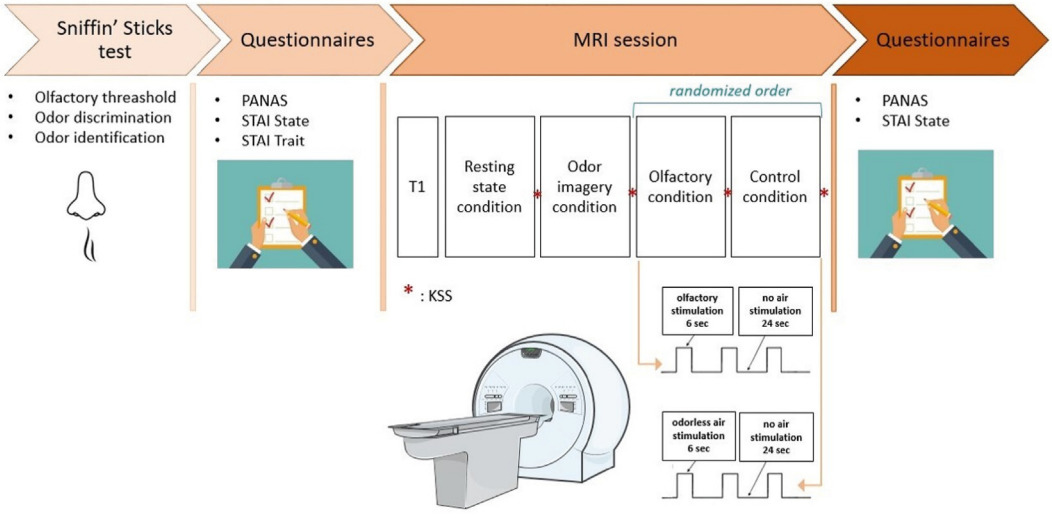

Fig. 1 – Study design. PANAS = the Positive and Negative Affect Schedule; STAI = the State Trait Anxiety Inventory; KSS= Karolinska Sleepiness Scale.

alert" to 9 = "extremely sleepy, fighting sleep", with 7, 8 and 9 representing a high level of sleepiness. The KSS has been validated using vigilance measures and electroencephalography, and permits to describe changes in drowsiness over time (Akerstedt & Gillberg, 1990; Kaida et al., 2006).

3.2.3. *The State Trait Anxiety Inventory*

The STAI (Spielberg et al., 1970; Spielberger, 1983; Barnes, Harp, & Jung, 2002; see Supplementary Material 3) is a validated self-report questionnaire containing two subscales of 20 items each: (1) the anxiety state (STAI form Y-1) measure assessing how the individual feel about anxiety "right now, at this moment", via a 4-point response choice (1 = "not at all", 2 = "somewhat", 3 = "moderately so", and 4 = "very much so"), and (2) the anxiety trait (STAI form Y-2) measure evaluating how the individual "generally feel" about anxiety, via a 4-point response choice (1 = "almost never", 2 = "sometimes, 3 = "often", and 4 = "almost always"). Scores can be calculated by summing all items (thus ranging from 20 to 80); higher scores indicating greater anxiety. Forty is considered as the cut-off suggesting probable clinical level of anxiety.

3.2.4. *Additional questions*

Two additional questions were asked for the mental imagery task and the olfactory task: to rate to what extent the odor was pleasant (1 = "extremely unpleasant" to 7 = "extremely pleasant") and to what extent the odor was intense (1 = "completely imperceptible" to 7 = "very intense").

3.3. *Fragrance diffuser*

The natural lemon essential oil organic (*citrus limon peel oil*) supplied by *Naissance* corporation was used. Lemon essential oil administration was performed by an electrical MRI-compatible diffuser developed by the Valeo company. A fan (RL65-21/12H Model; 12 V) connected to a power supply (B&K Precision Model BK1550) that we can easily monitor, diffused the essential oil via a stream of air (see Fig. 2). The fragrance was diffused through a polytetrafluoroethylene (PTFE) pipe to avoid smell contamination over time, going out of the pipe at the speed of 3.5 m/sec. In the MRI room, the pipe was attached to a belt worn by the participant on the torso. A small bag of paraffin beads was imbued with the fragrance and put just before the olfactory condition in the cartridge in order to be diffused. Another similar diffuser system was also used in the control condition to reproduce the exact same conditions as the olfactory condition but without fragrance diffusion. This permits that the participants also felt the light breeze of the diffusion air even if no fragrance was diffused.

4. MRI data

4.1. *Data acquisitions*

All structural and functional images were acquired on a 3 T Siemens Magnetom Vida scanner at the University Hospital Center of Liège (Belgium). A high-resolution T1-weighted image was acquired: T1-weighted 3D gradient echo images using 176 slices, repetition time = 2500 ms, echo time = 2.27 ms, voxel size = $1 \times 1 \times 1$ mm^3, flip angle = 4°, field of view = 240×256 mm^2). Multislices T2-weighted fMRI images (sequence of 6.13 min) were obtained: 300 volumes, 39 slices, voxel size = $3.0 \times 3.0 \times 3.0$ mm^3, repetition time = 728 ms, echo time = 30 ms, flip angle = 35°, field of view = 192 mm, number of voxel = $64 \times 64 \times 39$, delay = 0, slice order = interleaved descending.

4.2. *Data pre-processing*

Data preprocessing was performed using a locally developed pipeline using Statistical Parametric Mapping (SPM) 12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/) and Artifact Detection Tools (ART) (http://web.mit.edu/swg/

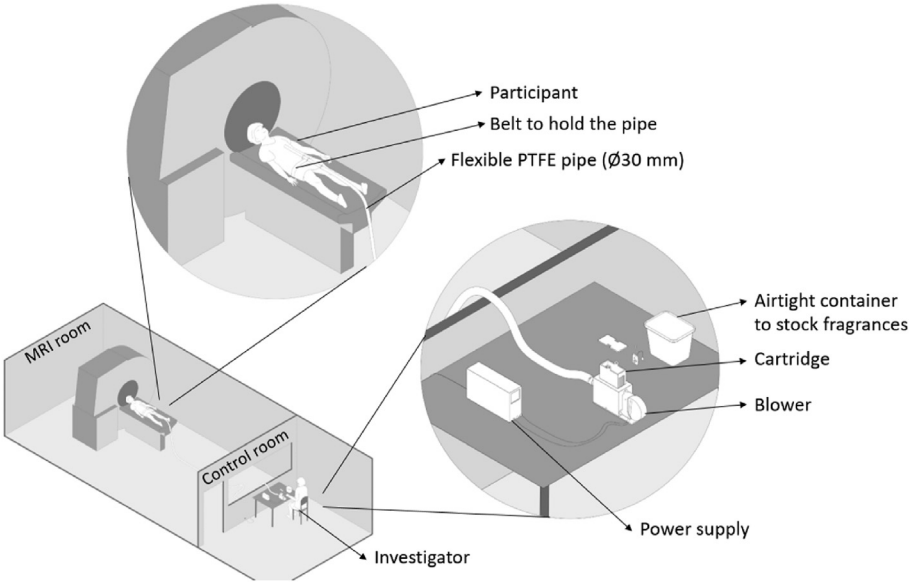

Fig. 2 – Schema of the diffuser system in the MRI session. PTFE = polytetrafluoroethylene.

software.htm). We performed slice timing correction, followed by the realignment of all the functional volumes to the first volume and, in a second pass, to their average. Estimated motion parameters were then used for artifact detection. An image was defined as an outlier or artifact image if the maximum voxel displacement (composite motion measure in ART) was greater than 2 mm from the previous frame or if the global mean intensity in the image was greater than 3 standard deviations (SDs) from the mean image intensity for the entire scans. Realigned functional images were registered to the bias-corrected structural image in the participant space (rigid-body transformation with normalized mutual information cost function). After extracting white matter (WM), grey matter (GM), and cerebrospinal fluid (CSF) masks, all the data and masks were normalized into the standard stereotaxic Montreal Neurological Institute (MNI) space (MNI152 with 2 mm resolution), and functional data were smoothed using a Gaussian kernel of 6-mm full width at half-maximum. WM and CSF masks were further eroded by one voxel. For noise reduction, we modeled the influence of the noise as a voxel specific linear combination of multiple empirically estimated noise sources by deriving the first five principal components from WM and CSF masked functional data separately. These nuisance regressors together with detected outlier volumes, effect of rest/task period, motion parameters and their first-order derivative were regressed out from the voxel-level time series and the denoised time series were temporally band-pass filtered (.008–.09 Hz) using CONN's denoising procedure. We have regressed out task effect in CONN denoising as per standard recommendation of CONN toolbox.

To assess the brain connectivity in a data driven approach, voxel-wise whole-brain global functional connectivity (GFC) was conducted using the CONN-functional connectivity toolbox. The GFC is a measure of global coherence that computes the average connectivity of every seed voxel to the entire brain. Constant and linear trend regressors were used to create a design matrix in the first-level general linear model (GLM). The designed GLM was fitted to the smoothed data.

Further, to assess the network topological organizations, we performed graph theory analyses using CONN toolbox. First, we extracted the time series of BOLD signal using ALL atlas for 100 brain region of interests (ROIs) consisting of cortical and subcortical regions. Then, we constructed the brain connectivity matrix using ROIs-to-ROIs Pearson correlation. Followed by, the connectivity matrix was thresholded and binarised. On the binarised connectivity matrix, we calculated brain network integration and segregation using global and local efficiency respectively (see Whitfield-Gabrieli & Nieto-Castanon, 2012 for details).

5. Statistics

5.1. Behavioral data

All variables were tested for normality ($P < .05$) with the Shapiro–Wilk test. Data are expressed as means (±SD) or median (interquartile range [IQR]) accordingly. We performed repeated measures ANOVA to compare the scores of all participants before and after the MRI session. We conducted a repeated measures ANOVA to compare the KSS scores of each functional sequences, followed by post-hoc Tukey's pairwise comparisons –if significant.

5.2. MRI data

5.2.1. Global functional connectivity

To assess between conditions difference, first the Fisher's z-transformations of the connectivity maps were derived for all conditions and all subjects. Differences in GFC between conditions were estimated using two sample paired *t*-tests. Results were considered significant at whole-brain cluster-level corrected α value of .05 for voxel wise P value of FDR-corrected, and minimum cluster extent threshold of 20 contiguous voxels (Bharath, Panda, Saini, Sriganesh, & Rao, 2017). Finally, we performed correlations between connectivity effect size of significant GFC and participants' KSS scores, as well as with the two additional questions (pleasantness and intensity ratings).

5.2.2. Graph theory analyses

Network integration and segregation differences between resting state and olfactory condition were estimated with two sample paired *t*-tests with FDR-correction at $p < .05$.

6. Results

Twenty-one healthy men (mean age 24 ± 3 years) were included in the present study. All variables were normally distributed, except the two PANAS- variables, the STAI Trait, the STAI State acquired before the MRI session, and the KSS for the resting state and for the control condition.

6.1. ENT exam results

The participants presented 10.5 ± 3.01 for odor threshold, 13.6 ± 1.50 for odor discrimination, 12.9 ± 1.56 for odor identification, and 37 ± 3.07 for the total score of the Sniffin' Sticks test. Seven participants scored between the 25th and the 50th percentiles. Eight subjects were between the 50th and the 75th percentiles. Three participants scored between the 75th and the 50th percentile and three subjects were above the 90th percentile of olfactory performance. The individual scores allow to consider all participants' olfactory performance as normal according to recent updated normative data published by Oleszkiewicz et al. (2019). No participant was excluded due to the Sniffin' Sticks test.

6.2. Questionnaires

Table 1 presents the scores of the PANAS and the STAI, before and after the MRI session. The individual scores of the PANAS allow to consider all participants' positive and negative affect scores as normal. All participants exceeded the proposed cut-off score (i.e., >40) for both STAI subscores, suggesting a relatively high level of anxiety in all participants. No statistical difference was found between the participants' response before and after the MRI session.

Table 1 – Total scores of the PANAS and the STAI for the whole sample before and after the MRI session.

Questionnaires (min–max total score)		Before the MRI $n = 21$	After the MRI $n = 21$	Statistics	P-value
PANAS	PANAS+ (0–50) *Median (IQR)*	34 (29–36)	33 (29–35)	$F = .925$	.34
	PANAS- (0–50) *Median (IQR)*	11 (10–14)	11 (10–12)	$F = .036$	.85
STAI	Trait score (20–80) *Median (IQR)*	47 (46–48)	/	/	/
	State score (20–80) *Median (IQR)*	49 (47–52)	48 (46–52)	$F = 3$	.09

PANAS = the Positive and Negative Affect Schedule; STAI = the State Trait Anxiety Inventory; IQR = interquartile range.

Table 2 presents the KSS scores for all participants. The interaction of the repeated measures ANOVA was significant and Tukey's pairwise comparisons showed that participants had lower scores for the olfactory task condition as compared with the resting state condition. No other differences were observed for the KSS.

7. MRI results

7.1. Global functional connectivity

We observed brain regions with increased and other brain regions with decreased GFC during olfactory condition as compared to resting state baseline condition (Fig. 3). More precisely, we found increased GFC in the thalamus. During olfactory condition, decreased GFC was observed in the precuneus, postcentral and precentral gyrus, lateral occipital cortex and paracingulate gyrus (see Supplementary Table 1). The other contrasts did not show significant difference. See Supplementary Figure 1 for connectivity of each condition.

We observed a significant positive correlation between KSS scores and precuneus connectivity reduction ($p = .005$; $r = .58$) for olfaction condition. No other significant correlations were observed.

7.2. Graph theory analyses (network topology)

Our analyses revealed that during olfaction, global brain network integration increased while network segregation decreased. A significantly increased network integration was observed in primary and secondary visual cortex, hypothalamus, olfactory bulb, thalamus, caudate nucleus, intracalcarine cortex, brainstem, angular gyrus, inferior parietal sulcus, ventral tegmental area and pallidum (see Fig. 4 and Supplementary Table 2). No decreased network integration was observed. In parallel, a significantly decreased network segregation was observed in several areas of the posterior part of the brain, such as primary visual cortex, visual association area, supracalcarine cortex, intracalcarine cortex, and occipital lobe, as well as in transverse temporal gyrus, planum polare and primary motor cortex (see Supplementary Table 2). No increased network segregation was observed.

8. Discussion

The present study sought to explore alertness modulation and neural correlates effects of lemon essential oil inhalation in a sample of healthy young adult men. After lemon inhalation, the participants reported higher level of self-alertness assessed via the KSS as compared to the resting state condition (but not as compared to the control condition), suggesting stimulating and exciting properties of the fragrance. This is consistent with previous empirical studies that have observed stimulating properties of the lemon (e.g., Keville & Green, 1995; Kiecolt-Glaser et al., 2008; Kikuchi et al., 1992; Martin & Cooper, 2007; Warrenburg, 2005). We did not find differences regarding the level of alertness with the control condition, though. Therefore, this does not rule out the possibility that the expectation of the participants to smell an odor may play a role, since the control condition was the exact same procedure as the olfactory condition except that they also felt a light breeze of the diffusion air even if no fragrance was diffused. Another hypothesis is that it is due to an order effect, since only the olfactory and control conditions were randomized.

Table 2 – The KSS scores, odor pleasantness and intensity ratings for each functional conditions.

Questionnaire (min–max total score)	Resting state $n = 21$	Odor imagery $n = 21$	Olfactory task $n = 21$	Control condition $n = 21$	Statistics	P-value
KSS (1–9) *Median (IQR)*	4 (5–3)	4 (5–3)	3 (4–2)	3 (5–3)	$F = 3.40$	**.023**
Odor pleasantness rating (1–7) *Median (IQR)*	/	5 (5–6)	6 (5–7)	/	$W = 5$	**.006**
Odor intensity rating (1–7) *Median (IQR)*	/	3 (2–4)	6 (5–7)	/	$W = 2$	**<.001**

KSS= Karolinska Sleepiness Scale; IQR = interquartile range.

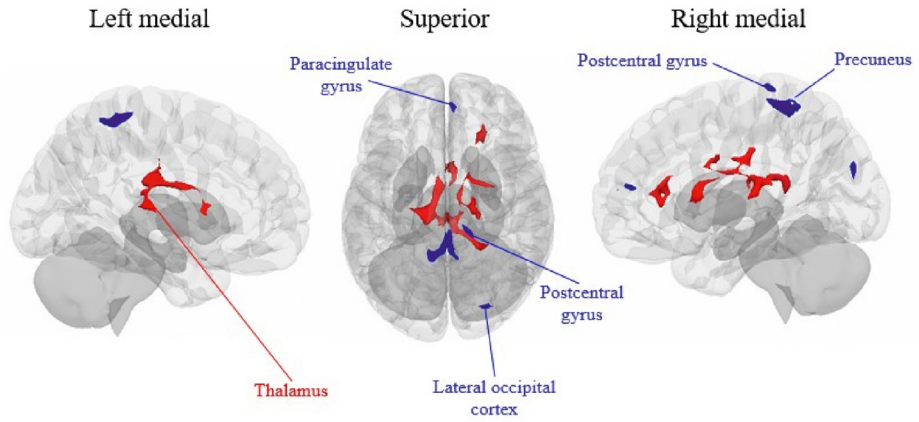

Fig. 3 – Voxel-wise whole brain global functional connectivity (GFC). Differences between the resting state and olfactory conditions considered significant at $p < .05$, voxel-wise whole-brain correction (FDR corrected) (presented on the MNI template brain). Red color represents increased connectivity and blue color represents decreased connectivity during olfactory condition as compared to resting state condition.

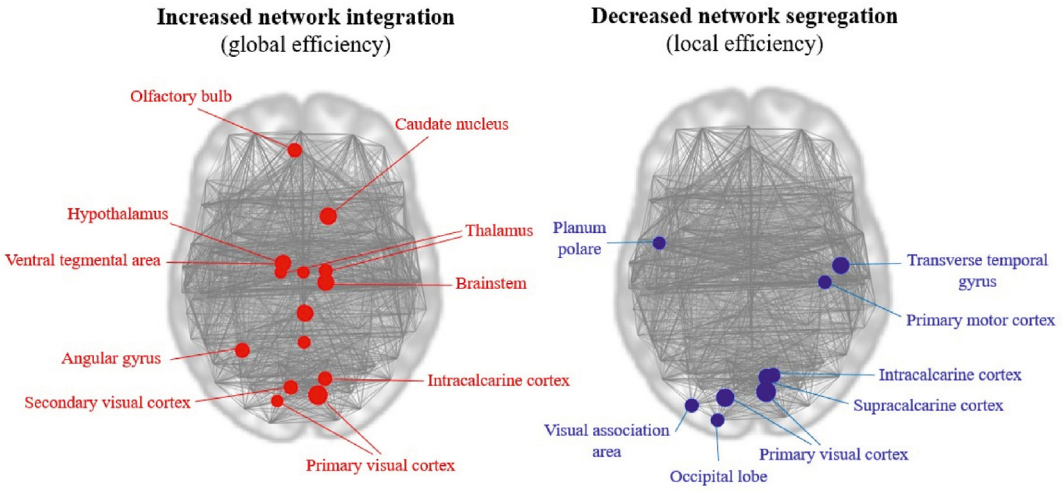

Fig. 4 – Graphical representation of graph theory results. During olfaction, global brain network integration increased (red color) while network segregation decreased (blue color), considered significant at FDR-correction at $p < .05$.

During olfaction condition, fMRI connectivity analyses showed increased global connectivity in one subcortical region, the thalamus, paralleled by decreased global connectivity in several cortical regions: the precuneus, postcentral and precentral gyrus, lateral occipital cortex and paracingulate gyrus. Interestingly, several studies showed that patients with thalamic damage presented deficits in odor identification and discrimination (e.g., Sela et al., 2009; Tham, Stevenson, & Miller, 2011; Zobel et al., 2010). More generally, it is now known that the thalamus is an important gateway for the transfer of sensory information to the cortex (Oh et al., 2014; Sherman & Guillery, 1996). Nevertheless, the implication and role of the thalamus in the olfactory circuit is highly debated but still underexplored in olfaction literature (see Courtiol & Wilson, 2015).

Interestingly, we found a significant positive correlation between KSS scores and precuneus connectivity reduction for olfaction condition. In other words, the more participants scored high on the KSS, thus suggesting increase of sleepiness, the more reduction we observed in precuneus connectivity. Precuneus has been often associated with general vigilance (i.e., not directed attention) in functional studies (Gusnard & Raichle, 2001) and its activity decreases during sleep (Maquet, 2004). The subjective ratings regarding the pleasantness and intensity of the odor did not show significant correlation with brain activity.

In parallel, the graph theory analysis revealed increased integration and decreased segregation in several subcortical and cortical structures during olfaction as compared to resting state. More specifically, increased network integration was observed in regions such as primary and secondary visual cortex, hypothalamus, olfactory bulb, thalamus, caudate nucleus, intracalcarine cortex, brainstem, angular gyrus, inferior parietal sulcus, ventral tegmental area and pallidum. These findings are consistent with previous research recorded during passive smelling, highlighting the unique role of the olfactory bulb in olfaction (Mori, 2014; Wilson & Sullivan, 2011). Regions typically involved in the expression and regulation of

emotional states have also been found, such as hypothalamus and brainstem. Decreased network segregation was found in several areas of the posterior part of the brain, such as primary visual cortex, visual association area, supracalcarine cortex, intracalcarine cortex, and occipital lobe, as well as in transverse temporal gyrus, planum polare and primary motor cortex.

All participants showed a relatively high level of anxiety, which is not surprising in the context of the COVID-19 pandemic. Young adults have been particularly affected by the restrictions set up to contain the COVID-19 virus, notably due to the lack of peer connections and emotional support (Magson et al., 2020; Saraswathi et al., 2020). However, we did not explicitly ask them the reasons of this relatively high level of anxiety.

While research still needs to be carried out to precise the specific effects of certain odors on human and their underlying mechanisms, the interest for multisensory interface design is growing in numerous areas. For example, car industry is investigating the potential of some fragrances to generate a more pleasurable driving experience (Martin & Cooper, 2007) or to prevent drowsy drivers from falling asleep (Baron & Kalsher, 1998; Otmani, Pébayle, Rogé, & Muzet, 2005). However, so far, results are mixed; some studies found that human olfaction is not reliably capable of alerting sleepers (Badia, Wesensten, Lammers, Culpepper, & Harsh, 1990; Carskadon & Herz, 2004).

There are some limitations in this study that deserve mentioning. First, in the current study, we included only men between 21 and 30 years old. The advantage is that they form a clearly defined homogeneous group, but future studies should include both women and men, and people from various ages. Second, our sample was relatively small. Consequently, replication of these findings is required with a larger sample. Third, the level of alertness relied on participants' subjective reports —and not objective measures. The application of MRI limited the possibility to administer objective measures. As described above, we did find a correlation between participants' subjective reports concerning their level of alertness and brain changes; however, future studies may consider assessing the level of alertness with objective measures such as pupillometry. Finally, considering our imaging setup (voxels with $3.0 \times 3.0 \times 3.0$ mm $= 27$ mm^3) and the fact that the olfactory bulb is a small region (35–75 mm^3; Herzalla et al., 2013), only two or three voxels will cover that region, which is a limitation. Nonetheless, we wanted to perform a whole-brain analysis. Consequently, as we were using a 3 T scanner, we had to use this voxel size. Future studies are needed, for example using a 7 T fMRI scanner.

9. Conclusion

After lemon fragrance inhalation, we observed a higher level of alertness in our sample of healthy young adult participants as assessed by the self-report questions, suggesting exciting properties of the fragrance. In parallel, we found increased global functional connectivity in the thalamus, paralleled by decreased global connectivity in several cortical regions during the olfaction condition, such as precuneus, postcentral and precentral gyrus, lateral occipital cortex and paracingulate gyrus. Graph theory analysis revealed increased network integration in cortical regions typically involved in olfaction and emotion processing such as olfactory bulb, hypothalamus and thalamus, while decreased network segregation in several regions of the posterior part of the brain during olfaction as compared to resting state. Ultimately, our results may help to understand neural mechanisms of human olfactory, more generally.

Data sharing

The data and the analysis code that support the findings of this study are available from the corresponding author upon request and after meeting the condition established by the local ethical committee (i.e., data sharing agreement).

CRediT author statement

CM, OG and SL secured the funding. CM, ALP, LP, and HC collaborated on data acquisition. All authors contributed to conceptualisation and study design. CM, ML, TP, and AB were involved in the fragrance diffuser preparation. CM, SM, and RP did the analyses. All authors had full access to all the data in the study. CM wrote the original draft. All authors contributed to reviewing and editing the manuscript in detail. All authors had final responsibility for the decision to submit for publication.

Declaration of competing interest

none.

Acknowledgments

The authors thank the whole staff from the MRI department at University Hospital of Liège. This work was supported by Valeo, the BIAL Foundation, the Belgian National Funds for Scientific Research (FRS-FNRS), the University and University Hospital of Liege, the fund Léon Fredericq, the Society for Psychical Research, the Fund Generet, the King Baudouin Foundation, DOCMA project (EU-H2020-MSCA–RISE–778234), the AstraZeneca Foundation, the European Union's Horizon 2020 Framework Programme for Research and Innovation under the Specific Grant Agreement No. 945539 (Human Brain Project SGA3), the European Space Agency (ESA) and the Belgian Federal Science Policy Office (BELSPO) in the framework of the PRODEX Programme, the Center-TBI project (FP7-HEALTH- 602150), the Public Utility Foundation 'Université Européenne du Travail', "Fondazione Europea di Ricerca Biomedica", the Mind-Care Foundation, the Mind Science Foundation and the European Commission. O. G is research associate and S.L. is research director at the F.R.S-FNRS.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cortex.2023.04.012.

REFERENCES

Akerstedt, T., & Gillberg, M. (1990). Subjective and objective sleepiness in the active individual. *International Journal of Neuroscience, 52*, 29–37.

Badia, P., Wesensten, N., Lammers, W., Culpepper, J., & Harsh, J. (1990). Responsiveness to olfactory stimuli presented in sleep. *Physiology & Behavior, 48*, 87–90.

Barnes, L. B. L., Harp, D., & Jung, W. S. (2002). Reliability generalization of scores on the spielberger state–trait anxiety inventory. *Educational and Psychological Measurement, 62*(4), 603–618.

Baron, R. A., & Kalsher, M. J. (1998). Effects of a pleasant ambient fragrance on simulated driving performance. *The sweet smell of safety? Environnement & Behavior, 30*, 535–552.

Bharath, R. D., Panda, R., Saini, J., Sriganesh, K., & Rao, G. S. U. (2017). Dynamic local connectivity uncovers altered brain synchrony during propofol sedation. *Scientific Reports, 7*, 1–10.

Carskadon, M. A., & Herz, R. S. (2004). Minimal olfactory perception during sleep: Why odor alarms will not work for humans. *Sleep, 27*, 402–405.

Cleland, T. A., & Linster, C. (2003). Central olfactory processing. In R. L. Doty (Ed.), *Handbook of olfaction and gustation* (pp. 165–180). New York: Marcel Dekker.

Courtiol, E., & Wilson, D. A. (2015). The olfactory thalamus: Unanswered questions about the role of the mediodorsal thalamic nucleus in olfaction. *Frontiers of Neural Circuits, 9*, 49.

Gane, S. (2010). What we do not know about olfaction. Part 1: From nostril to receptor. *Rhinology, 48*(2), 131–138.

Gusnard, D. A., & Raichle, M. E. (2001). Searching for a baseline: Functional imaging and the resting human brain. *Nature Reviews Neuroscience, 2*, 685–694.

Han, A. Y., Mukdad, L., Long, J. L., & Lopez, I. A. (2020). Anosmia in COVID-19: Mechanisms and significance. *Chemical Senses, 45*, 123–128.

Herz, R. S. (2009). Aromatherapy facts and fictions: A scientific analysis of olfactory effects on mood, physiology and behavior. *International Journal of Neuroscience, 119*, 263–290.

Herzallah, I. R., Askar, S. M., Amer, H. S., Ahmed, A. F., El-Anwar, M. W., & Eesa, M. H. (2013). Olfactory bulb volume changes in patients with sinonasal polyposis: A magnetic resonance imaging study. *Otolaryngoly-Head and Neck Surgery, 148*(4), 689–693.

Hummel, T., Sekinger, B., Wolf, S. R., Pauli, E., & Kobal, G. (1997). Sniffin' sticks': Olfactory performance assessed by the combined testing of odor identification, odor discrimination and olfactory threshold. *Chemical Senses, 22*, 39–52.

Izquierdo-Dominguez, A., Rojas-Lechuga, M., Mullol, J., & Alobid, I. (2020). Olfactory dysfunction in the COVID-19 outbreak. *Journal of Investigational Allergology & Clinical Immunology, 30*(5), 317–326.

Kadohisa, M. (2013). Effects of odor on emotion, with implications. *Frontiers in Systems Neuroscience, 7*, 66.

Kaida, K., Takahashi, M., Åkerstedt, T., Nakata, A., Otsuka, Y., Haratani, T., et al. (2006). Validation of the Karolinska sleepiness scale against performance and EEG variables. *Clinical Neurophysiology, 117*, 1574–1581.

Keville, K., & Green, M. (1995). *Aromatherapy: A complete guide to the healing art*. Freedom, CA: The Crossing Press.

Kiecolt-Glaser, J. K., Graham, J. E., Malarkey, W. B., Porter, K., Lemeshow, S., & Glaser, R. (2008). Olfactory influences on mood and autonomic, endocrine and immune function. *Psychoneuroendocrinology, 33*, 328–339.

Kikuchi, A., Yamagughi, H., Tanida, M., Abe, T., & Uenoyama, S. (1992). Effects of odors on cardiac response patterns and subjective states in a reaction time task. *Tohoku Psychologica Folia, 51*, 74–82.

Kobal, G., Hummel, T., Sekinger, B., Barz, S., Roscher, S., & Wolf, S. R. (1996). "Sniffin' sticks": Screening of olfactory performance. *Rhinology, 34*, 222–226.

Komiya, M., Takeuchi, T., & Harada, E. (2006). Lemon oil vapor causes an anti-stress effect via modulating the 5-HT and DA activities in mice. *Behavioural Brain Research, 172*, 240–249.

Kontaris, I., East, B. S., & Wilson, D. A. (2020). Behavioral and neurobiological convergence of odor, mood and emotion: A review. *Frontiers in Behaviral Neuroscience, 14*, 35.

Lehrner, J., Eckersberger, C., Walla, P., Pötsch, G., & Deecke, L. (2000). Ambient odor of orange in a dental office reduces anxiety and improves mood in female patients. *Physiology & Behavior, 71*, 83–86.

Magson, N. R., Freeman, J. Y., Rapee, R. M., Richardson, C. E., Oar, E. L., & Fardouly, J. (2020). Risk and protective factors for prospective changes in adolescent mental health during the COVID-19 pandemic. *Journal of Youth and Adolescence, 50*, 44–57.

Maquet, P. (2004). A role for sleep in the processing of memory traces. Contribution of functional neuroimaging in humans. *Bulletin et Memoires de l'Academoe Royale de Médecine de Belgique, 159*, 167–170.

Martin, G. N., & Cooper, J. A. (2007). *Odour effects on simulated driving performance: Adding zest to difficult journeys*. York, UK: Poster presented at the British Psychology Society Annual Conference.

Mori, K. (2014). *The olfactory system: From odor molecules to motivational behaviors*. Tokyo, Japan: Springer.

Oh, S. W., Harris, J. A., Ng, L., Winslow, B., Cain, N., Mihalas, S., et al. (2014). A mesoscale connectome of the mouse brain. *Nature, 508*, 207–214.

Oleszkiewicz, A., Schriever, V. A., Croy, I., Hahner, A., & Hummel, T. (2019). Updated Sniffin' Sticks normative data based on an extended sample of 9139 subjects. *European Archives of OtoRhino-Laryngology, 276*(3), 719–728.

Otmani, S., Pébayle, T., Rogé, J., & Muzet, A. (2005). Effect of driving duration and partial sleep deprivation on subsequent alertness and performance of car drivers. *Physiology & Behavior, 84*, 715–724.

Park, J. W., Kwon, D. Y., Choi, J. H., Park, M. H., & Yoon, H. K. (2018). Olfactory dysfunctions in drug-naïve Parkinson's disease with mild cognitive impairment. *Parkinsonism & Related Disorders, 46*, 69–73.

Saraswathi, I., Saikarthik, J., Kumar, K. S., Srinivasan, K. M., Ardhanaari, M., & Gunapriya, R. (2020). Impact of COVID-19 outbreak on the mental health status of undergraduate medical students in a COVID-19 treating medical college: A prospective longitudinal study. *PeerJ, 8*, Article e10164.

Sayorwan, W., Ruangrungsi, N., Piriyapunyporn, T., Hongratanaworakit, T., Kotchabhakdi, N., & Siripornpanich, V. (2013). Effects of inhaled rosemary oil on subjective feelings and activities of the nervous system. *Scientia Pharmaceutica, 81*, 531–542.

Sayorwan, W., Siripornpanich, V., Piriyapunyaporn, T., Hongratanaworakit, T., Kotchabhakdi, N., & Ruangrungsi, N. (2012). The effects of lavender oil inhalation on emotional states, autonomic nervous system, and brain electrical activity. *Journal of the Medical Association of Thailand, 95*(4), 598–606.

Sela, L., Sacher, Y., Serfaty, C., Yeshurun, Y., Soroker, N., & Sobel, N. (2009). Spared and impaired olfactory abilities after thalamic lesions. *Journal of Neuroscience, 29*, 12059–12069.
Sherman, S. M., & Guillery, R. W. (1996). Functional organization of thalamocortical relays. *Journal of Neurophysiology, 76*, 1367–1395.
Spielberger, C. D. (1983). *Manual for the state-trait anxiety inventory: STAI (form Y)*. Palo Alto, CA: Consulting Psychologists Press.
Spielberger, C., Gorsuch, R., & Lushene, R. (1970). *STAI manual for the state-trait anxiety inventory*. Palo Alto: Consulting Psychologists Press.
Tham, W. W., Stevenson, R. J., & Miller, L. A. (2011). The impact of mediodorsal thalamic lesions on olfactory attention and flavor perception. *Brain and Cognition, 77*, 71–79.
Warrenburg, S. (2005). Effects of fragrance on emotions: Moods and physiology. *Chemical Senses, 30*, i248–i249.
Watson, D., Clark, L. A., & Tellegen, A. (1988). Development and validation of brief measures of positive and negative affect: The PANAS Scales. *Journal of Personality and Social Psychology, 54*(6), 1063–1070.
Whitfield-Gabrieli, S., & Nieto-Castanon, A. (2012). Conn: A functional connectivity toolbox for correlated and anticorrelated brain networks. *Brain Connectivity, 2*(3), 125–141.
Wilson, D. A., & Sullivan, R. M. (2011). Cortical processing of odor objects. *Neuron, 72*, 506–519.
Zobel, S., Hummel, T., Ilgner, J., Finkelmeyer, A., Habel, U., Timmann, D., et al. (2010). Involvement of the human ventrolateral thalamus in olfaction. *Journal of Neurology, 257*(12), 2037–2043.
Zucco, G. M., Paolini, M., & Schaal, B. (2009). Unconscious odour conditioning 25 years later: Revisiting and extending 'kirk-smith, van toller and dodd'. *Learning and Motivation, 40*, 364–375.