Introduction

Smell testing is often neglected by the medical community. However, there is an increasing interest in olfactory evaluation since it has been shown that smell deficits occur in many neurological diseases. Olfactory dysfunction is for instance a common feature of neurodegenerative disorders such as Parkinson’s disease (PD) and Alzheimer’s dementia. Moreover, it is now apparent that odour dysfunction is among the earliest signs of these disorders and could help in diagnosis.

Although smell dysfunction is rarely reported by patients, it requires a specific evaluation of their olfactory abilities. Most clinical studies use psychophysical tests such as odour threshold and identification tests, without any investigation of the brain substrate linked to olfactory dysfunction. Since 1990, cerebral imaging techniques have allowed a better understanding of normal olfactory neuronal processes but very few studies have been published to date in patients presenting with olfactory dysfunction. The aim of this paper is to present an overview of this emerging literature and to show the potential of cerebral imaging studies on neurological disorders and presenting olfactory dysfunction. Neurological disorders such as Alzheimer’s disease, Parkinson’s disease, epilepsy, migraine, multiple chemical sensitivity and schizophrenia are examined.

Methods of cerebral imaging

Haemodynamic techniques

Non-invasive functional neuro-imaging techniques are commonly classified into two broad groups: electromagnetic techniques such as EEG, event-related potentials (ERP) and magneto-encephalography (MEG), and haemodynamic techniques such as PET and functional magnetic resonance imaging (fMRI). Briefly, electromagnetic techniques have excellent temporal resolution (a few milliseconds) but poor spatial resolution (several centimetres). Since they are mainly designed for recording superficial brain activity, they are unsuitable for recording olfactory areas located deep in the brain. We can nevertheless note the stereo-EEG (SEEG) technique that consists in recording intracerebral EEG and ERP activity in epileptic patients using deep electrodes prior to surgical treatment for the relief of intractable seizures.
In contrast to EEG or MEG techniques, haemodynamic techniques for cerebral imaging are suitable for investigating deep-brain areas in a way that is necessary for the study of olfactory information processing. Haemodynamic techniques are based on measuring changes in rCBF as an indicator of neural activity. They have good spatial resolution (on a millimetre spatial scale) but poor temporal resolution (several seconds for fMRI and in the order of minutes for PET), which is inherently limited by the blood flow and oxygenation rate changes. The PET technique is based on the detection of positrons emitted from a radioactive tracer isotope injected into the blood stream. fMRI measures rCBF changes through magnetic signal variation related to differences between the blood concentrations of oxyhaemoglobin and deoxyhaemoglobin. Relative to PET scanning, fMRI has several advantages. It is non-invasive, less expensive and more widely available. Additionally, fMRI provides both structural and functional information, whereas PET allows only the recording of functional data.

Analysis of functional data

In the field of cognitive neuroscience devoted to studying the neural bases of cognition, the analysis techniques are primarily based on a principle of subtraction of images to reveal activation patterns; for example subtracting images obtained in the rest condition from those acquired when the subjects perform a cognitive task. Individual or group statistical analyses can then be performed, individual analyses being more suitable for longitudinal studies. Prior to the functional data analysis, several pre-treatment steps are performed on each subject data set, including realignment to a reference image, stereotactic normalisation to a brain template and smoothing with a Gaussian kernel. Realignment corrects for head movement. Normalisation (i.e. plastic transformation) removes positional and morphological topographic variances. Spatial smoothing compensates for normal residual anatomical variability. Statistical models are then computed based on the Generalised Linear Model, generating an image in which the voxel values are statistics, a “statistical parametric map”. The anatomical location of activated regions is often expressed in the form of three-dimensional coordinates defined in the atlas published by Talairach and Tournoux. More recent atlases are now available to identify brain regions. Recently, random-effect group analyses have been developed which allow statistical inferences to be extended to a population. This approach consists of a two-stage analysis that takes account of within- and between-subject variances.

Whole-brain statistical maps are exploratory. In contrast, when a priori hypotheses orientate the search to define brain regions, such as the primary olfactory (piriform) cortex or the amygdala (involved in emotional processing), it is possible to restrict the analysis to anatomical regions of interest (ROI). When drawing ROIs for an individual brain, ROI analysis notably overcomes variability in location and size across subjects. ROI analyses are performed on a limited amount of voxels, and this reduces the multiple-comparison correction, leading to more sensitive maps from a statistical viewpoint than those resulting from whole-brain analysis.

In addition to the strictly hierarchical subtractive method, researchers have attempted to identify the neural bases for neurocognitive functions within the framework of distributed and dynamic neural networks. Two main approaches can be distinguished to study brain connectivity. Functional connectivity is defined as the correlation of activities among remote neurophysiological events. So when the activity of two regions displays a high degree of covariance for a given task, these regions may be defined operationally as functionally connected. Effective connectivity refers to the influence that one set of neurons exerts over another. It therefore embodies how differences in stimuli or brain states modulate the interactions between regions.

Analysis of morphometric data

The size of brain structures can vary for different reasons. Modifications to brain anatomy can be developmental, and they mainly occur during ontogenesis or ageing. It has also been observed in people with specific skills such as musicians or sportsmen in response to intense environmental demands. The reorganisation of brain structure has also been observed in patients suffering from neurological or neuropsychiatric disorders. Size determination is possible by delineating clearly visible structures such as the amygdala, the hippocampus, the striatum, or the ventricles. The size of the olfactory bulb or other areas of the...
olfactory system have also been measured using this method. However, many brain areas are not easy to define structurally. The voxel-based method (VBM) has been described for characterising regional cerebral gray- and white-matter differences in structural magnetic resonance images by the application of methods derived from functional imaging. This approach is not biased to one particular structure and provides an even-handed assessment of anatomical differences throughout the entire brain.

Olfactory cerebral imaging in healthy subjects

The first study of the human olfactory cerebral bases was performed 17 years ago by Zatorre et al. The PET technique was used to delineate the brain regions involved during passive smelling of odours. The authors showed, as previously indicated by physiological investigations of the monkey brain, that the small region located at the junction of the inferior frontal and temporal lobes called the piriform cortex and a larger region called the orbitofrontal cortex are essential in olfaction. The piriform cortex, as the first relay for olfactory information after the olfactory bulb, is considered to be the primary olfactory cortex, and the orbitofrontal cortex to be the secondary olfactory cortex. Subsequent studies have explored several lines of research. We note studies on the neural bases of sniffing focusing on the piriform cortex, those on brain regions activated by strongly emotional odours highlighting the role of the amygdala, and those on the complex neural network called upon during explicit olfactory cognitive tasks such as discrimination, memory, judgment of hedonicity, and identification.

Over recent years, our understanding of the neural bases of olfactory processing in healthy subjects has continued to improve. The use of refined olfactory cognitive tasks, coupled with the development of the event-related fMRI technique which allows complex experimental paradigms, has led to the characterisation of a more precise functional organisation of the medial temporal and orbitofrontal cortices, with identification of the specificities of each olfactory region or sub-region. By manipulating odour valence, Gottfried et al. showed that the posterior piriform cortex and amygdala responded to all odours, while the anterior piriform cortex and posterior orbitofrontal cortex reflected sensitivity to odour valence. Further studies used odours of different valence and intensity to show that amygdala activation was associated with the emotional intensity of odours, since pleasant and unpleasant odours activated the amygdale, but neutral ones did not. Dissociation based on odour valence was also observed in the orbitofrontal cortex, with the medial and lateral parts of the orbitofrontal cortex responding more to pleasant and unpleasant odours respectively. Several recent studies have attempted to unravel the mystery of functional heterogeneity in the primary olfactory cortex. Zelano et al. differentiated the frontal and temporal sub-regions of the piriform cortex, the former being involved in olfactory attention, while the latter responded equally to all attention conditions. Gottfried et al. investigated rostro-caudal axis heterogeneity and revealed that the anterior part of the piriform cortex is associated with the chemical nature (i.e. the functional group) of odorants, while the posterior part processes odour quality. The later result was confirmed recently using more sophisticated techniques; spatially distributed activation patterns in the posterior piriform cortex coincide with the perceptual rating of odour quality.

Olfactory cerebral imaging in patients with neurological disorders

Neurodegenerative diseases

Smell disorders in neurodegenerative disorders have been the subject of an increasing number of studies, especially for Alzheimer’s dementia and PD. The type and/or degree of olfactory dysfunction could differ among neurodegenerative disorders and knowledge of them could help in diagnosis. For instance, patients with progressive supranuclear palsy tend to have a relatively normal sense of smell. It has been reported that about 5% of the population over the age of 64 suffer from dementia. AD is among the most prevalent causes of dementia in the elderly since it accounts for about 50 to 60% of these patients. Neuropathological hallmarks are brain atrophy, senile neuritic plaques, neurofibrillary tangles and neuronal cell loss. Memory loss, the cardinal symptom of this disease, is always associated with impairment of at least one other area of cognitive functioning such as language or visuospatial abilities. Numerous studies have shown that olfactory dysfunction appears to be among
the earliest signs of the disorder.38 Smell disorders reflect both peripheral and central olfactory involvement, as revealed by detection, discrimination, recognition memory and identification tests.39-47

Only one H215O PET study has been performed in patients with AD. Kareken et al.41 demonstrated that odour identification was impaired and that patients had reduced olfactory-evoked rCBF in the right piriform cortex and the right anterior-ventral temporal lobe compared with elderly controls (Figure 1). The neural substrate associated with a decrease of olfactory identification performance in AD has also been studied by Murphy et al.48 Using structural MRI the authors demonstrated that the size of this impairment was correlated to a decrease in the volume of the left hippocampus.

PD is another frequent and disabling disorder. Four clinical signs of Parkinsonism are resting tremor, bradykinesia, rigidity and postural instability. However, olfactory dysfunction is a frequent non-motor symptom in PD and is considered to be an early manifestation of the disease. As with AD, dysfunction is observed for all types of olfactory tasks in these patients.39,49-52 Recently, Westermann et al.53 investigated brain activity related to olfactory processing in PD patients using fMRI. In both the patients and healthy controls, olfactory stimulation activated the brain regions involved in olfactory processing, but the patients had reduced activity in the amygdala and hippocampus. This result could be related to cortical changes observed in the early stages of PD and associated with neuropathological processes that involve primary olfactory regions (e.g. olfactory anterior nucleus, olfactory bulb). Further regions of higher activation in patients were located bilaterally in the inferior frontal gyrus, anterior cingulate gyrus, and the left dorsal and right ventral striatum, suggesting the involvement of compensatory processes via the mesocorticolimbic dopaminergic loop.

Epilepsy
The importance of temporal lobe regions in human olfactory function was first recognised at the end of the 18th century in patients with olfactory epileptic auras. More recently, it was found that patients with mesial temporal lobe epilepsy (MTLE) typically display normal olfactory sensitivity but impaired complex olfactory functions, as assessed by the standard tests of odour discrimination, recognition memory, identification, and naming.54-59 These olfactory deficits are thought to reflect the presence of metabolic abnormalities in the temporal lobe structures and functions that underlie seizure disorders.60 However, very little is known about how these circuits process external stimuli. Ciumás et al.61 tested whether odour activation can help delineate limbic functional disintegration in such patients. They measured rCBF with PET during bithral smelling of familiar and unfamiliar odours, using odourless air as the control stimulus. When compared to controls, patients did not activate the amygdala, piriform and the anterior insular cortex in the epileptogenic hemisphere. Furthermore, patients with left MTLE did not activate the left inferior frontal gyrus with familiar odours, which they perceived as less familiar than the controls. In addition, the authors showed that the piriform cortex in these patients was functionally disconnected from the contralateral region including the amygdala, the piriform cortex and the insula. This study showed that imaging of the olfactory processes could allow the functional delineation of the affected limbic networks in patients with MTLE.

Migraine and Multiple Chemical Sensitivity
Migraine is a common episodic headache characterised by attacks
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consisting of various combinations of headache and neurological, gastrointestinal and autonomic symptoms. During migraine attacks, patients usually complain of hypersensitivity to visual and auditory stimuli (photophobia and phonophobia), and hypersensitivity to olfactory stimuli (olfactory hypersensitivity, OHS) (also called osmophobia). OHS can also be observed during migraine-free periods, and is usually associated with the onset of odour-triggered attacks. Such patients further present altered hedonics, but not intensity, judgments of odours. Demarquay et al.63 aimed to evaluate olfactory processing in migraineurs with OHS and to investigate whether rCBF associated with olfactory stimulation was modified in these patients. During both olfactory and non-olfactory conditions, the authors mainly found higher rCBF in the left piriform cortex and anteroposterior temporal gyrus in migraineurs compared to controls (Figure 2). Apart from the major role played by the piriform cortex in olfaction, data also support its involvement in odour-triggered migraine. It is well known that most odorants possess trigeminal characteristics and therefore activate the trigeminal system, which plays a major role in the pathophysiology of migraine. However, inversely, it has also been shown that trigeminal stimuli such as CO2 can activate the piriform cortex.64 There are therefore several possible sites for the interaction of olfactory and trigeminal information, not only at the peripheral level (olfactory epithelium and bulb), but also at the most central level including the piriform cortex. However, it is not known whether these rCBF changes are the cause or a consequence of odour-triggered migraines and interictal OHS.

Multiple chemical sensitivity (MCS) is characterised by multisystem complaints attributed to exposure to low concentrations of chemicals that are usually well tolerated by the general population. Studies of MCS, a syndrome that encompasses OHS, have shown that patients have normal odour detection thresholds and identification scores, but that odours were rated as less pleasant. This suggests that MCS patients and migraineurs with interictal OHS could share physiopathological features. In a recent PET study of olfactory processing in MCS patients, Hillert et al.65 reported increased olfactory-triggered activation of the anterior cingulate and cuneus, but highlighted the decreased activation in several other odour-processing brain regions. The authors explained hyperactivation in the anterior cingulate and hypoactivation of the olfactory circuits by hypothesising that harm avoidance targeting odours could cause monitoring conflicts in MCS patients related to a desire to reduce odour perception, and to the concomitant instruction to smell. The reduced activation of other olfactory regions was explained as the result of a top-down modulation from the cingulate cortex.

Even though both patients with MCS and some migraineurs report OHS, results found in OHS migraine patients62 and in MCS patients65 do not allow us to state categorically whether the physiopathological bases are common to both diseases because the experimental procedures used were very different. Further investigation will be needed to compare them.

Schizophrenia

Schizophrenia (SZ) is a complex mental disorder that affects approximately 1% of the population worldwide, and causes disruptions in thought processes, perceptions, and emotions.66 The processing of olfactory information is a cognitive function which is disturbed in SZ patients, and behavioural evidence of this dysfunction is found with odour discrimination, recognition memory, identification and naming tasks.58,67-71 Turetsky et al.10 provided evidence that SZ patients have a smaller bilateral olfactory bulb
volume (23%) than control subjects, as measured with MRI scans, and also found a strong correlation between olfactory bulb volume and odour threshold sensitivity (phenyl-ethyl alcohol) in healthy subjects but not in patients. Since the magnitude of this reduction exceeds the 2-10% reduction in total grey matter observed in patients, Turetsky et al. suggested that the olfactory bulb may be particularly vulnerable to those disease processes which produce structural brain changes. Extending their previous work, Turetsky et al. subsequently showed a similar, although severe, impairment in otherwise healthy first-degree relatives.

Three cerebral imaging studies have been performed to study the dysfunction of olfactory processes in patients with SZ. Crespo-Facorro et al. studied the loss of the capacity to experience pleasure in SZ patients and investigated the specific neural systems underlying olfactory emotional disturbances. The authors found that patients failed to activate several limbic and paralimbic regions (e.g. insular cortex, nucleus accumbens, and parahippocampal cortex) during the experience of an unpleasant odour, but recruited a compensatory set of frontal cortical regions.

More recently, Plailly et al. used PET to examine whether activation patterns specifically associated with the odour supra-threshold detection task and the odour familiarity and hedonicity judgment tasks were modified in SZ patients by comparison with healthy subjects. Both groups had an equivalent ability to detect supra-threshold odours, but patients found all odours less familiar, and pleasant odours less pleasant, than healthy comparison subjects. These behavioural results were related to functional abnormalities lateralised in the left hemisphere, leading to a lack of activation in the SZ patients of the posterior part of the piriform cortex and several frontal regions for familiarity judgments (Figure 3A), and of the insular gyrus and the inferior frontal cortex for hedonicity judgments (Figure 3B). The authors suggest that the involvement of the left hemisphere and in particular of the left insular cortex could explain the typical clinical features of schizophrenia such as anhedonia (i.e. the loss of the capacity to subjectively experience pleasure).

Finally, Schneider et al. explored the neural basis of emotional olfactory dysfunction using fMRI by comparing SZ patients with their non-affected siblings and matched healthy controls. rCBF effects and subjective mood changes were assessed using odours of different emotional valence. Subjective ratings were comparable between groups, but less functional activity emerged in regions of the right frontal and temporal cortex in the patients and relatives than in the controls during stimulation with the negative odour. These data therefore revealed a familial influence in the neural substrates of negative olfactory processing, with frontal dysfunction affecting the higher
cognitive processes associated with olfaction.

**Conclusions**

In conclusion, the neuro-imaging of olfaction is an emerging tool in the evaluation of patients with neurological disorders. Whereas psychophysical tests have been used in numerous diseases, only a few functional brain imaging studies using olfactory stimuli have been published to date. This could be explained by the difficulty in accessing suitable olfactometric apparatus, since this is not commercially available, and the limited number of teams specialised in human olfaction imaging techniques. Nevertheless, we are confident that the use of cerebral imaging techniques, and in particular fMRI, could help in the understanding of pathological olfactory processing and so improve our understanding of neurological disorders. It is however important to keep in mind that it is not known at present whether olfactory imaging can help in the ultimate diagnosis of neurological disorders.

**References**

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