UNIVERSITÀ DEGLI STUDI DI PADOVA
DIPARTIMENTO DI PSICOLOGIA GENERALE

SCUOLA DI DOTTORATO DI RICERCA IN SCIENZE PSICOLOGICHE
INDIRIZZO: Psicologia Sperimentale

CICLO XXIV

INSIGHTS INTO THE OLFACTORY ABILITIES OF NEUROLOGICAL POPULATIONS FROM PERCEPTION TO ACTION

DIRETTORE DELLA SCUOLA: Ch.ma Prof.ssa Clara Casco
COORDINATORE DI INDIRIZZO: Ch.ma Prof.ssa Francesca Pazzaglia
SUPERVISORE: Ch.mo Prof. Umberto Castiello

DOTTORANDO: Valentina Parma

DATA DI CONSEGNA TESI
31 Gennaio 2012

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AD	Alzheimer's Disease
ADHD	Attention Deficit Hyperactivity Disorder
ADI-R	Autism Diagnostic Interview Revised
ADOS	Autism Diagnostic Observation Scale
ANOVA	Analysis of Variance
ANCOVA	Analysis of Covariance
ASD	Autism Spectrum Disorders
BAI	Beck Anxiety Inventory
BDI-II	Beck Depression Inventory - II Edition
CCCR	Connecticut Chemosensory Clinical Research
CNS	Central Nervous System
DS	Down Syndrome
EDSS	Expanded Disability Status Scale
EOG	Electrolfactogram
ES	Equivalent Score
FLAIR	Fluid Attenuated Inversion Recovery
GCS	Glasgow Coma Scale
HD	Huntington's Disease
HFA	High Functioning Autism
IFTLs	Inferior Frontal and Temporal Lobes
IPD	idiopathic Parkinson's Disease
LCF	Level of Cognitive Functioning
LL	Congruent Large Condition
LS	Incongruent Large Condition
M	Mean Values
MANCOVA	Multivariate Analysis of Covariance
MIPAV	Medical Images Processing Analysis and Visualization
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
MS	Multiple Sclerosis

NIH	National Institute of Health
NoL	No Odour Large condition
NoS	No Odour Small condition
OAS	Odour Awareness Scale
OB	Olfactory Bulb
OBPs	Odorant-Biding Proteins
OERPs	Olfactory Event Related Potentials
OFC	Orbito-Frontal Cortex
OSNs	Olfactory Sensory Neurons
PD	Parkinson's Disease
PDC	Parkinsonism-Dementia Complex of Guam
PM	Progressive Matrices
PTOL	Post-traumatic Olfactory Loss
RRMS	Relapsing-Remitting Multiple Sclerosis
SD	Standard Deviation values
SL	Incongruent Small condition
STS	Superior Temporal Sulcus
SS	Congruent Small condition
T2LV	Hypertense Lesion Volume
TBI	Traumatic Brain Injury
TD	Typically Developing
TDI	Threshold-Discrimination-Identification (composite) score of the Sniffin' Sticks extended test.
TMT	Trail Making Test
TSE	Turbo-Spin Echo
UPDRS	Unified Parkinson's Disease Rating Scale
UPSIT	University of Pennsylvania Smell Identification Test
URI	Upper Respiratory Infections
VPD	Vascular Parkinsonism
WCST	Wiscosin Card Sorting Test
WMT2	White Matter T2

Abstract

Although smell is involved in a number of survival functions, the comprehension of olfactory processing is still far from being exhaustive.

Following a concise outline of the anatomy and the physiology underlying the olfactory system (Chapter 1), I focused on the description of the quantitative and qualitative smell distortions in patients diagnosed with olfactory disorders as to outcrop the differences between normal and pathological functioning of the sense of smell (Chapter 2). Subsequently, I moved to a higher degree of complexity by considering how odours are perceived and elaborated at a cognitive level, devoting special attention to the way people describe their own olfactory experience (Chapter 3). Chapter 4 provides a brief revision of the methodologies currently utilized to evaluate olfactory performance in humans based on the distinction between explicit (conscious-mediated) and implicit (subliminal) procedures.

The second part of the present thesis concerns the outline of the experimental work I undertook. In the first experiment (Chapter 5), I administered to a carefully selected and homogenous group of relapsing remitting multiple sclerosis patients an explicit olfactory test, the Sniffin' Sticks Extended Test. This measure allows to evaluate specific aspects of olfactory performance, namely odour threshold, discrimination and identification as well as to ascertain participant's general olfactory performance (TDI score). The aim of this study was twofold. First, to understand whether an explicit olfactory test can reliably display either a

global or a specific olfactory loss in multiple sclerosis patients. Second, to ascertain whether alterations of some brain areas of the central nervous system can provide reliable biological markers (e.g., number and volume of white matter T2 lesions within central eminent olfactory regions) which correlate with the olfactory abnormalities in this population. On the basis of the verbalized (conscious) responses provided to the Sniffin' Sticks Test, 34% of our multiple sclerosis sample showed a general olfactory loss compatible with hyposmia. Specifically, odour discrimination and identification (but not odour threshold) allowed to discriminate the patients with multiple sclerosis from the control group. In neural terms, no significant correlation between the number and the volume of the plaques within the central olfactory regions and the scores obtained to the behavioural test was evident. All in all, the results reported in Chapter 5 indicated that explicit olfactory testing, although very useful to screen for the presence of olfactory disturbance, might not have the potency to fully account for human olfactory processing. Further support to this contention is also given by the lack of correlation between explicit test scores and the considered biological markers.

Thus, I questioned whether explicit test procedures have the ability to uncover the presence of different forms of olfactory elaboration. Therefore, I addressed my interest to implicit olfactory testing. In order to minimize the influence of verbal abilities on odour elaboration, I capitalized on paradigms able to reveal, via the analysis of movement kinematics, how odour stimuli are processed and how they modulate motor behaviour. Chapter 6, 7 and 8 were designed in order to elucidate

this issue. In Chapters 6 and 7, I investigated the effects of common odours (e.g., odours produced by inanimate sources) in two groups of patients, whose explicit olfactory abilities, as measured by means of standardized tests, were lacking. Specifically, I tested a group of functionally anosmic traumatic brain injured (Chapter 6) and idiopathic Parkinson's disease patients (Chapter 7) to evaluate the presence of lingering implicit olfactory abilities by indirectly studying the effects odours might have on the kinematics of the moving hand. Participants were asked to perform reach-to-grasp movements towards large or small visual targets (e.g., a plastic apple or strawberry, respectively) following the delivery of olfactory cues. The odour was either 'size' congruent with the visual target (e.g., orange or almond, respectively), incongruent (e.g., almond or orange, respectively) or absent (e.g., fresh air).

Chapter 6 describes the outcomes of the study carried out on a group of anosmic traumatic brain injured patients. Comparing their performance to that shown by matched normosmic/microsmic traumatic brain injured patients and neurologically healthy controls it was found that all the three groups were similarly affected by the exposure to odours. In particular, interference effects were revealed. To elaborate, when participants grasped a large visual target preceded by an odour evoking a small object, a kinematic parameter indicating how hand aperture is shaped towards the visual target (i.e., maximum velocity of grip aperture) was greater than when the same visual target was grasped preceded by an odour evoking a congruent grip or when no odour was presented. This evidence seems to suggest that some sort of implicit

olfactory processing is preserved in traumatic brain injured patients who fail at standardized explicit tests.

The results outlined in Chapter 7 indicate that, as neurologically healthy participants and vascular parkinsonism patients, idiopathic Parkinson's disease patients were facilitated in the execution of their actions when exposed to an odour evoking an object that was similar in size with respect to a visual to-be-grasped target. This kind of olfactory priming resulted in an attenuation of the bradykinesia of hand transport movement and the hypometria of the grip amplitude, which are motor disturbances classically attributed to these patients. However, this facilitation effect was absent when the priming odour evoked an object different in size with respect to the visual to-be-grasped target. Altogether these results speak in favour of an adequate residual implicit olfactory elaboration in idiopathic Parkinson's disease patients.

On the grounds of the findings from the experiments overviewed in Chapter 6 and 7, I advanced some theoretical conjectures. In order to influence behaviour, an odour processed implicitly may not require the conscious recollection of the stimulus. Therefore, implicit olfactory processing might sidestep higher cognitive function involvement and, instead, rely on the integrity of different structures. A proper candidate might be the amygdala, an area which is physically close to the olfactory brain areas and that was not compromised in the traumatic brain injured patients tested and in the early stages of Parkinson's disease. Once confirmed, these findings could be useful when rehabilitation strategies are being hypothesized for both these populations. Indeed, the residual

ability to perceive olfactory stimuli and to respond subconsciously to them could hypothetically be utilized to design trainings as to improve upper limb motor control.

In the experiments described above, patients diagnosed with olfactory loss displayed implicit olfactory processing following the exposure to common odours, as evident from the alteration of the kinematic variables of the reach-to-grasp movement. Given the differences in the neural circuits undelying the elaboration of odours characterized by different properties (e.g., a diverse degree of biological relevance), would the exposure to body odours elicit implicit odour processing in patients suffering from reduced olfactory abilities, as common odours do? The study reported in Chapter 8 was specifically tailored to answer this question.

A group of high functioning autistic children and a group of typically developing children was recruited. In order to expose them to a biologically relevant olfactory stimulus, we collected the body odours from the children's mother axillæ. As to indirectly test implicit body odour processing, I underwent a modified version of the visuomotor priming paradigm, enriched with an olfactory cue. Classically, this paradigm reveals a motor facilitation effect induced by the pure observation of a movement on the execution of a similar action (Craighero, Fadiga, Umiltà, & Rizzolatti, 1996). Children were exposed to either their own mother's odour, the odour of the mother of another participant or no odour. Then, they were asked to observe a model (either their mother or the mother of another participant) executing a reach-to-

grasp action towards a visual target and perform the observed action, in the absence of specific instructions to imitate. As reported in Chapter 8, the analyses revealed that familiar body odours succeeded in modulating visuomotor priming effects. Although typically developing children showed visuomotor facilitation in all conditions, in high functioning autism participants such facilitation was evident only when they acted in the presence of their own mother's odour. Taken together these results suggest that familiar body odour might have the ability to transmit some social significance to the visual target. This would lead to the proposal that implicit olfactory processing is preserved in high functioning autism participants, even though explicit olfactory testing places them within the hyposmia range. It might well be that, olfaction has the potency to help autistic people in forging social interactions.

Overall the findings of the experiments described here suggest that explicit olfactory testing might produce non-conclusive results in some neurological populations (Chapter 5) and might hide residual implicit odour processing in others (Chapters 6, 7 and 8). Moreover, the evaluation of odours' biological relevance raise the possibility of an implicit olfactory-mediated communication.

With this in mind the advances of the present thesis are several and multifaceted. First, I applied a technique apt to investigate human sensorimotor control to the clinical domain. This methodology showed its potential to 'catch' facets of olfactory processing that otherwise would remain uncovered if simply considering the explicit aspects of olfactory performance (Chapter 5). It allowed to disclose residual implicit olfactory

abilities in patients diagnosed with syndromes characterized by hyposmia or anosmia (Chapters 6, 7 and 8). The preservation of implicit olfactory skills in different neurological populations seems to indicate that it is a non-specific ability. The high-degree of interconnections among olfactory brain areas might provide a good explanation for the permanence of this form of olfactory elaboration in association with different patterns of brain regions malfunctioning.

Second, the present thesis extends previous literature on human olfactory processing by pointing out the existence of a dissociation between explicit and implicit olfactory processing (Chapter 6, 7 and 8), which might be reflected, in neural terms, by the involvement of different brain circuits.

Third, considering the level of biological relevance of odour stimuli in neurological populations depicts a novel aspect of the present work (Chapter 8).

Finally, on a clinical perspective, the findings reported within the present thesis (Chapter 6, 7 and 8) might be taken into account when developing new rehabilitation strategies at least for the neurological populations considered here.

RIASSUNTO

Sebbene gli stimoli olfattivi forniscano informazioni utili alla sopravvivenza, la comprensione dei meccanismi responsabili dell'elaborazione olfattiva è ancora lontana dall'aver prodotto conclusioni definitive.

Dopo una breve introduzione relativa alle caratteristiche anatomiche e fisiologiche del sistema olfattiva (Capitolo 1), ho focalizzato la mia attenzione sulla descrizione dei disturbi olfattivi quantitativi e qualitativi presentati da pazienti cui sono state diagnosticate alterazioni del senso dell'olfatto (Capitolo 2). Questo con lo scopo di evidenziare le differenze tra elaborazione olfattiva normale e patologica. Successivamente, ho spostato il livello di analisi ad un grado di complessità maggiore considerando il modo in cui gli odori sono percepiti ed elaborati a livello cognitivo, con particolare riferimento alle modalità con cui le persone descrivono la propria esperienza olfattiva (Capitolo 3). Il Capitolo 4 fornisce una concisa revisione delle metodologie attualmente utilizzate per valutare la prestazione olfattiva nell'uomo, distinguendole in procedure esplicite (che richiedono una mediazione consapevole) e implicite (che richiedono un'elaborazione a livello subliminale).

La seconda parte di questa tesi concerne la presentazione del lavoro sperimentale da me svolto. Nel primo esperimento (Capitolo 5) ho somministrato ad un gruppo omogeneo e altamente selezionato di pazienti con sclerosi multipla recidiva-remittente un test olfattivo esplicito, chiamato Sniffin' Sticks Extended Test. Questa misura permette sia di

valutare aspetti specifici della prestazione olfattiva, quali soglia, discriminazione e identificazione olfattiva, sia di ottenere un punteggio (TDI) che informa relativamente alla capacità olfattiva globale. Due sono gli scopi principali di questo studio. Comprendere se un test olfattivo esplicito è in grado di rilevare affidabilmente una disfunzione olfattiva globale o specifica in pazienti con sclerosi multipla. Verificare se alcune specifiche aree del sistema nervoso centrale possono fornire marcatori biologici attendibili (es., numero e volume delle placche all'interno delle principali aree olfattive centrali) che correlano con la prestazione olfattiva di questi pazienti. Sulla base di risposte verbalizzate (che raggiungono il livello di consapevolezza manifesta) al test Sniffin' Sticks, il 34% del nostro campione di pazienti con sclerosi multipla presenta una riduzione olfattiva compatibile con l'iposmia. In particolare, le componenti di discriminazione e identificazione olfattiva (ma non quella di soglia) hanno efficacemente discriminato il gruppo di pazienti con sclerosi multipla dal gruppo di controllo. Dal punto di vista neurale, non sono state rilevate correlazioni significative tra il numero e il volume delle placche nelle regioni centrali del sistema olfattivo e i punteggi al test comportamentale. In conclusione, i risultati presentati nel Capitolo 5 indicano che l'utilizzo di test olfattivi espliciti, sebbene molto utile dal punto di vista clinico per una rapida valutazione dei disturbi olfattivi, non riesce a spiegare in modo conclusivo diversi aspetti della capacità umana di elaborare gli odori. Questa affermazione è ulteriormente supportata dalla mancata correlazione tra i punteggi al test e i marcatori biologici considerati.

Mi sono, quindi, chiesta se le procedure di valutazione esplicite potessero celare altre forme di elaborazione degli odori. Per questo ho diretto il mio interesse verso forme di valutazione olfattiva implicita. Per minimizzare l'influenza che le abilità verbali posso avere sull'elaborazione olfattiva, ho utilizzato dei paradigmi che rivelano, attraverso l'analisi della cinematica del movimento, come gli stimoli olfattivi vengono elaborati e come influenzano il comportamento motorio. Gli esperimenti inclusi nei Capitoli 6, 7 e 8 sono stati specificatamente costruiti per valutare questo aspetto. Negli esperimenti riportati nei Capitoli 6 e 7 ho studiato gli effetti di odori comuni (ovvero, prodotti da fonti inanimate) in due gruppi di pazienti che, ai test olfattivi espliciti, falliscono. In particolare, ho testato un gruppo di pazienti con esiti da trauma cranico (Capitolo 6) e con morbo di Parkinson (Capitolo 7) - considerati funzionalmente anosmici - per valutare la presenza di residue abilità olfattive implicite attraverso lo studio indiretto dell'influenza prodotta da un odore sulla cinematica dei movimenti della mano. Ai partecipanti è stato chiesto di compiere movimenti di raggiungimento-prensione verso un oggetto grande o piccolo (es., rispettivamente, una mela o una fragola di plastica) dopo aver presentano degli stimoli olfattivi. L'odore poteva richiamare (condizione congruente) o meno (condizione incongruente) la dimensione dell'oggetto da afferrare oppure poteva non essere presente (condizione di controllo). La rilevazione di effetti di facilitazione era attesa per alcune alcune variabili cinematiche quando veniva presentato un odore congruente. Ipotizzavo, invece, che la presentazione di un odore incongruente fosse legata a effetti di interferenza.

Il Capitolo 6 descrive i risultati dello studio condotto su un gruppo di pazienti anosmici con esiti di trauma cranico. Confrontando la prestazione di questi pazienti con un gruppo di pazienti con trauma cranico normosmici o lievemente microsmici e un gruppo di controlli neurologicamente sani, emerge che i tre gruppi erano influenzati in maniera analoga dalla presentazione degli stimoli olfattivi. Sebbene nei presenti gruppi non siano stati rilevati effetti di facilitazione, si evidenzia un effetto di interferenza. In particolare, quando un partecipante afferrava un oggetto grande preceduto dalla presentazione di un odore che evocava un oggetto di piccole dimensioni, uno dei parametri cinematici che indicano come la mano si conforma attorno all'oggetto (massima velocità di apertura della mano) era maggiore rispetto a quando lo stesso oggetto veniva preceduto da un odore che evocava una presa congruente alla dimensione dell'oggetto visivo o quando nessun odore veniva presentato. La presente evidenza suggerisce che una qualche forma di elaborazione olfattiva implicita permane in pazienti che falliscono ai test olfattivi espliciti.

I risultati presentati nel Capitolo 7 indicano che, analogamente ai controlli neurologicamente sani e a pazienti con parkinsonismo vascolare - che non presentano disturbi olfattivi - pazienti con diagnosi di morbo di Parkinson idiopatico erano facilitati nell'esecuzione dei azioni quando esposti a odori che evocano un oggetto di dimensioni simili a quelle dell'oggetto da afferrare. Questo tipo di preparazione basata sullo stimolo olfattivo produce una riduzione della bradicinesia del movimento di raggiungimento e dell'ipometria dell'apertura della mano, che sono

identificati tra i classici disturbi motori presentati da questi pazienti. Tuttavia, tale effetto di facilitazione non si manifestava quando l'odore evocava un oggetto di dimensioni diverse rispetto a quello da afferrare. Nel complesso questi risultati sembrano supportare la presenza di un'adeguata residua elaborazione olfattiva implicita in pazienti con morbo di Parkinson idiopatico.

Sulla base dei dati prodotti dagli esperimenti riportati nei Capitoli 6 e 7, ho avanzato alcune possibili congetture teoriche. Per avere un effetto sul comportamento, l'elaborazione implicita di un odore non richiede il riconoscimento consapevole dello stimolo. Perciò, l'elaborazione olfattiva implicita sembra eludere il coinvolgimento delle funzioni cognitive superiori e sfruttare, piuttosto, l'integrità di altre aree cerebrali. Ipotizzo che l'amigdala, un'area fisicamente vicina alle aree olfattive centrali e preservata nei pazienti testati, sia un buon candidato per giocare questo ruolo. Una volta confermati, questi dati potrebbero essere utilizzati per lo sviluppo di strategie di riabilitazione per pazienti con trauma cranico e morbo di Parkinson idiopatico. Infatti, la capacità residua di percepire gli stimoli olfattivi e di rispondere inconsapevolmente in modo adeguato a tali stimoli può essere utilizzata per progettare esercizi per migliorare il controllo motorio degli arti superiori.

Negli esperimenti appena descritti, pazienti identificati come anosmici presentano preservate capacità di elaborazione implicita di odori comuni, come dimostrato dalle alterazioni cinematiche rilevate sul movimento di raggiungimento-prensione. Dato che l'elaborazione di odori

caratterizzati da diverse proprietà (es., un diverso grado di rilevanza biologica) richiede l'implementazione in circuiti neurali differenti, l'esposizione a odori corporei potrebbe far emergere un'elaborazione olfattiva di carattere implicito in pazienti con ridotte abilità olfattive? Lo studio presentato nel Capitolo 8 è stato specificatamente disegnato per rispondere a questa domanda.

Un gruppo di pazienti con autismo ad alto funzionamento e un gruppo di controlli di pari età e sesso sono stati reclutati ed esposti a stimoli olfattivi biologicamente rilevanti. Gli odori sono stati raccolti da dischetti di cotone indossati sotto le ascelle dalle madri dei partecipanti. Per valutare in modo indiretto l'elaborazione implicita di questi odori, ho applicato una versione modificata del paradigma di *priming* visuomotorio, cui ho aggiunto una stimolazione olfattiva. Classicamente, questo paradigma rivela un effetto di facilitazione motoria indotto dalla semplice osservazione di un movimento sull'esecuzione di un'azione simile (Craighero, Fadiga, Umiltà, & Rizzolatti, 1996). I partecipanti sono stati esposti sia all'odore della loro stessa madre, all'odore della madre di un altro partecipante o a nessun odore. Successivamente, è stato chiesto loro di osservare un modello (la loro madre o la madre di un altro partecipante) mentre eseguiva un'azione di raggiungimento-prensione verso un oggetto e sono stati osservati eseguire l'azione in assenza di specifiche istruzioni relative all'imitazione del gesto. Come riportato nel Capitolo 8, le analisi rivelano che l'odore corporeo familiare è efficace nel modulare gli effetti di *priming* visuomotorio. Sebbene i partecipanti a sviluppo tipico presentino un effetto di facilitazione visuomotoria in tutte

le condizioni, i partecipanti con autismo ad alto funzionamento presentano tale facilitazione motoria selettivamente quando esposti all'odore della propria madre. L'insieme di questi risultati suggerisce che gli odori corporei familiari possono avere la capacità di trasmettere significati sociali all'oggetto su cui viene eseguita l'azione. Questo implicherebbe che l'elaborazione implicita degli stimoli olfattivi sia preservata in pazienti con autismo ad alto funzionamento, sebbene l'applicazione di test espliciti non lo rilevi. Inoltre, il senso dell'olfatto potrebbe avere la rilevanza necessaria per aiutare le persone con autismo ad alto funzionamento a creare interazioni sociali.

Complessivamente i risultati degli esperimenti presentati nella presente tesi suggeriscono che le modalità di valutazione esplicita della percezione olfattiva possono produrre conclusioni non definitive in alcune popolazioni neurologiche (Capitolo 5) e possono nascondere residue abilità olfattive implicite in altre (Capitolo 6, 7 e 8). Inoltre, la valutazione di odori con diversi gradi di rilevanza biologica fa emergere la possibilità che una comunicazione implicita mediata da stimoli olfattivi sia possibile.

Alla luce di quanto detto sopra, gli aspetti innovativi della presente tesi sono molti e poliedrici. In primo luogo, ho applicato una tecnica per lo studio del controllo sensorimotorio nell'uomo al contesto clinico. Questa metodologia ha dimostrato il suo potenziale nel cogliere aspetti dell'elaborazione olfattiva che, altrimenti, sarebbero rimasti nascosti, se si fossero considerati solamente gli aspetti olfattivi espliciti (Capitolo 5) – come accade nella maggior parte degli studi presenti nella letteratura

sull'argomento. Ha, inoltre, permesso di svelare la presenza di un tipo di elaborazione olfattiva implicita in diverse popolazioni neurologiche che presentano sindromi caratterizzate da iposmia o anosmia (Capitoli 6, 7 e 8). Il grado di interconnessione tra le aree cerebrali olfattive può fornire una spiegazione alla permanenza di questa forma di elaborazione olfattiva in relazione al malfunzionamento di diverse regioni cerebrali.

In secondo luogo, questa tesi estende la precedente letteratura sul tema dell'elaborazione olfattiva umana evidenziando l'esistenza di una dissociazione tra elaborazione olfattiva implicita ed esplicita (Capitoli 6, 7, e 8), che a livello neurale, può riflettersi nel coinvolgimento di differenti aree cerebrali.

In terzo luogo, da un punto di vista clinico, considerare il livello di rilevanza biologica degli odori in popolazioni neurologiche costituisce un ulteriore aspetto di novità del presente lavoro (Capitolo 8)

Per concludere, in una prospettiva clinica, i risultati qui riportati (Capitoli 6, 7 e 8) possono essere utilizzati in fase di sviluppo di nuove strategie riabilitative per le popolazioni neurologiche considerate.

RATIONALE OF THE PRESENT THESIS

Scientific articles on perception sometimes begin by listing the five senses. But, forthwith, they usually continue by specifying that, for the sake of clarity, the discussion will be confined to the visual experience, only. The other senses-especially the chemical senses - smell and taste - are most of the time neglected.

With respect to smell, the community of psychologists and neuroscientists demonstrated to be only slighted interested into its investigation. Rather, the vast majority of scientists focused on the comprehension of visual mechanisms, possibly because of the great importance humans attribute to such modality to navigate the world. Then, it is not surprising that a quick search in the PubMed database reveals that the scientific production regarding olfaction is around 10 times smaller than the publication of studies concerning vision. And, as if that were not enough, scientists working on olfaction, usually do not come to smell as a primary topic but in pursuit of research on different issues, such as memory or emotions (e.g., Koch et al., 2007).

This persistent misappreciation for olfaction, although hard to be explained from an epistemological perspective, has been suggested to be rooted in the western philosophical principles and psychoanalytical theories which deny this sense as compared with the other sensory modalities (Freud, 1978; LeGuérer, 2002).

Irrespective of the reasons why olfaction has been poorly investigated, it remains a fact that we are well equipped for perceiving

odours and that smell evolved over millions of years in a wide number of animal species. Given that evolution promotes abilities and their biological substrates as to favour survival, it is a foregone conclusion that we are provided with olfactory abilities because of their essential life-supporting functions (Herz, 2002; Schaal & Porter, 1991; Shepherd, 2004). In the first instance, smell allows organisms to sense relevant objects from a distance, before coming into more direct contact with them. This provides the invaluable opportunity to escape from dangerous objects/events before they become irreparably lethal. In the second instance, many animal species still assign olfactory information a primary role in detecting food, predators and conspecifics. With respect to humans, although they mostly underestimate the importance of smell, they do rely upon odour stimuli to guide their own behaviour more often (and unexpectedly) than imagined. In the third instance, smell stimuli are invaluable cues prompting the access to intimate past events. With this respect, the famous passage of Proust's experience with the madeleine paradigmatically resume the issue (Proust, trans. 1996):

> "*Whence could it have come to me, this all-powerful joy? I sensed that it was connected with the taste of the tea and the cake, but that it infinitely transcended those savours, could, no, indeed, be of the same nature. Whence did it come? What did it mean? How could I seize and apprehend it?* "

Taken together, these pieces of evidence acknowledge that, although smell is involved in a number of survival functions, the comprehension of olfactory mechanisms is still far from being exhaustive. With the specific

aim of investigating how olfactory perception is shaped, I capitalized on selective deficits presented by neurological patients suffering from different pathologies in order to elucidate in more detail the issue.

In the first part, I began by outlining the anatomical structures and physiological mechanisms underlying the sense of smell to provide the biological basis upon which olfactory experience is grounded (Chapter 1). Second, I described the quantitative and qualitative distortions present in patients suffering from different olfactory disorders in order to highlight differences between normal and pathological functioning of the sense of smell (Chapter 2). Moving away from the biological aspects of smells, in Chapter 3 I translated these notions within the cognitive domain considering how odour objects are

perceived, processed and how people report about their olfactory experience. In Chapter 4, I briefly reviewed the current procedures to assess olfactory abilities in humans, differentiating the testing methods involving explicit (conscious) and implicit (subliminal) olfactory experience.

The second part of the present thesis is dedicated to the report of the experimental work. In Chapter 5, I applied an explicit olfactory testing method to a group of patients diagnosed with multiple sclerosis in order to specify the olfactory deficits reported by this population. This was particularly relevant given that the standardized tests applied could not reliably reveal the presence of olfactory disturbance. In the light of this controversial issue, I asked whether the explicit test procedure might hide some residual implicit olfactory abilities. Therefore, I draw my

attention to implicit olfactory testing capitalizing on a paradigm able to reveal how task-irrelevant odour stimuli are processed and modulate motor behaviour. In Chapters 6 and 7, I applied this paradigm to a group of anosmic traumatic brain injured (Chapter 6) and a group of idiopathic Parkinson's disease patients (Chapter 7) - populations well known for their reduced olfactory performance - to evaluate the presence of residual implicit olfactory abilities via the kinematics of the hand. Finally, in order to ascertain whether biologically relevant odours can modulate the control of action , I evaluated a group of children diagnosed with autism spectrum disorder when performing an olfactory triggered action observation test (Chapter 8). The obtained results have been discussed in light of current theories proposed to explain how olfactory stimuli are processed by the human nervous system and to which extent they are able to influence human behaviour (see 'Discussion' sections for each experimental chapter and Chapter 9).

PART I

THEORETICAL BACKGROUND

CHAPTER 1

MAKING SENSE OF SCENTS

The organization of the olfactory systems across phyla, from flies to mammals, reveals an extraordinary evolutionary convergence in terms of neural mechanisms for detecting and discriminating among olfactory stimuli (Hildebrand & Shepherd, 1997). In most animal species, two olfactory systems have developed. The main olfactory system has the primary aim to explore the environment in order to search for food, detect predators or preys, and mark territory. It is an open system that elaborates a large amount of unpredictable information coming from the external environment. In other words, it detects any environmental odorants that can enter the nasal cavities in a certain environment, without any a priori selection (Firestein, 2001). Therefore, olfactory perception is tightly linked to the mantainance of an indeterminate, but nonetheless precise, sensory organization. The accessory olfactory system, also known as the vomeronasal system, has mainly developed for recognizing species-specific olfactory signals mediating social behaviours and therefore, the mating process. Due to the importance and complexity of these reproductive functions, the accessory olfactory system evolved as an independent and dedicated structure (Firestein, 2001). Although identified in many animal species, the accessory olfactory systems are not present in humans (Tirindelli, Dibattista, Pifferi, & Menini, 2009). However, the striking evidence on the complementary role of the two

subsystems raises the possibility that in humans the main olfactory system can regulate some type of species-specific chemosignal communication (Tirindelli et al, 2009).

Over the past decades, the organization of the olfactory systems has been investigated with a multidisciplinary perspective, clearly revealing a set of principles of functional organization encompassing the peripheral (lower-level) and central (higher-level) processing of odours. In the following sections the most relevant anatomical and physiological findings in both animals and humans will be summarized.

1.1 Anatomy

1.1.1 Peripheral structures

In vertebrates, the olfactory epithelium (Figure 1.1) constitutes the receptor structure for odorous molecules. The sensory cells transducing the olfactory information, differently from other sensory systems, are veritable neurons projecting toward the central regions of the brain (Mombaerts, 2006). Moreover, they have the extraordinary capability to regenerate throughout life (Graziadei, Levine, & Graziadei, 1978).

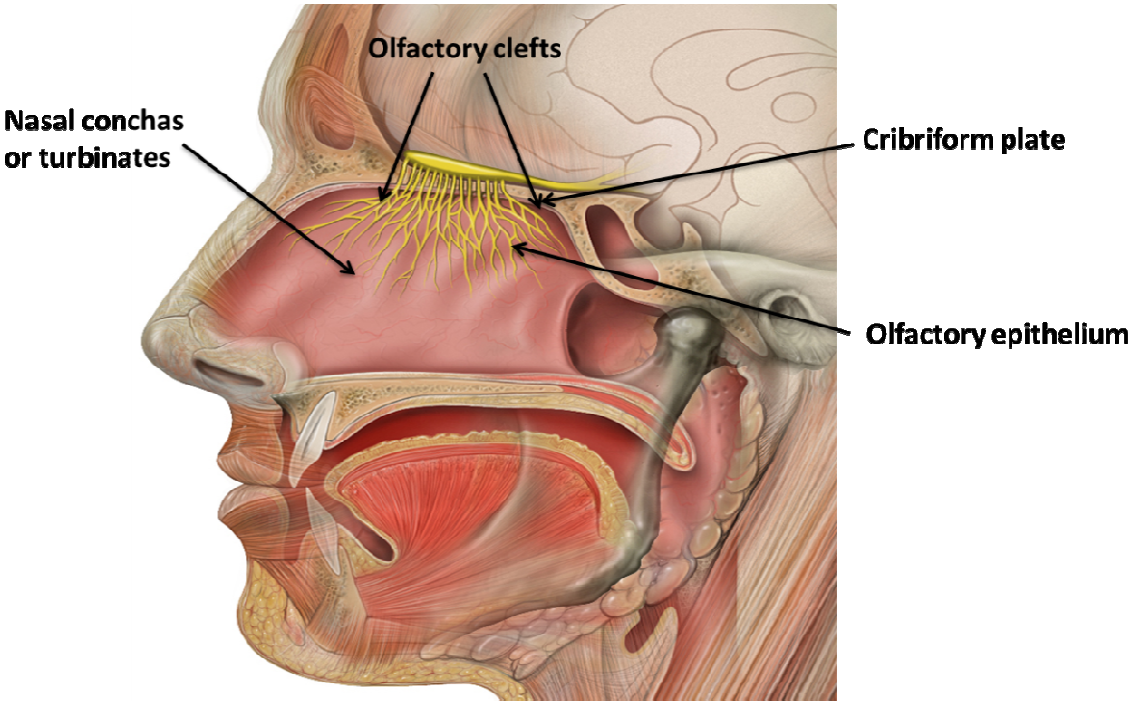


Figure 1.1. *Pictorial representation of the macroanatomy of the olfactory system (adapted from Lynch, medical illustrator).*

In humans, the olfactory epithelium is located in two narrow passages, namely the olfactory clefts, present in the upper part of the nasal cavities (Figure 1.1). In humans, the olfactory clefts lay dorso-caudally on the roof of the nasal cavities (Moran, Rowley, Jafek, & Lovell, 1982). The olfactory epithelium consists of four principal classes of cells: olfactory sensory neurons, supporting cell, basal cells and microvillar cells (Figure 1.2).

The olfactory sensory neurons (OSNs) are the primary sensory cells. Approximately 6–10 million olfactory neurons form a neuroepithelium that lines a series of cartilaginous protrusions, called nasal conchas or turbinates. The OSNs are single-dendrite bipolar neurons medially located in the epithelium. In their apical portion, the OSNs end with olfactory knobs, each equipped with some 20–30 cilia. Cilia and olfactory knobs, which are embedded in the mucus layer, are the site responsible for the

olfactory transduction (Elsaesser & Paysan, 2005). Mucus is produced by Bowman's olfactory glands and constitutes the ideal ionic environment to maximize the interaction between odorant molecules and receptors. These glands also produce large quantities of odorant-binding proteins (OBPs) which are small extracellular proteins possibly participating in the perireceptor events of odour detection by carrying, deactivating, and/or selecting odorant molecules (Briand et al., 2002). In the basal side, the OSNs axons converge in olfactory bundles that, in turn, form the olfactory nerve following the crossing of the cribriform plate of the ethmoid bone (Bozza, Feinstein, Zheng, & Mombaerts, 2002; Elsaesser & Paysan, 2005).

The supporting cells are tall columnar cells located in the superficial region of the epithelium, above the OSN layer. Their function is to provide metabolic and physical/protective support to the OSNs (Menco & Morrison, 2003).

The basal cells, which are found in the basal lamina of the epithelium, owns multipotent stem-cell properties (Beites, Kawauchi, Crocker, & Calof, 2005; Mackay-Sim & Kittel, 1991). Therefore, their function is to generate new OSNs to contrast their rapid turnover throughout life (Huard, Youngentob, Goldstein, Luskin, & Schwob, 1998).

The microvillar cells are columnar cells whose density reaches about one in 20 ciliated OSNs. Although the function of these cells has not yet been fully clarified, there is evidence that they function as a regulatory link between degenerating OSNs and olfactory basal cells, cooperating in the control of neuronal proliferation in the postnatal olfactory epithelium (Montani, Tonelli, Elsaesser, Paysan, & Tirindelli, 2006).

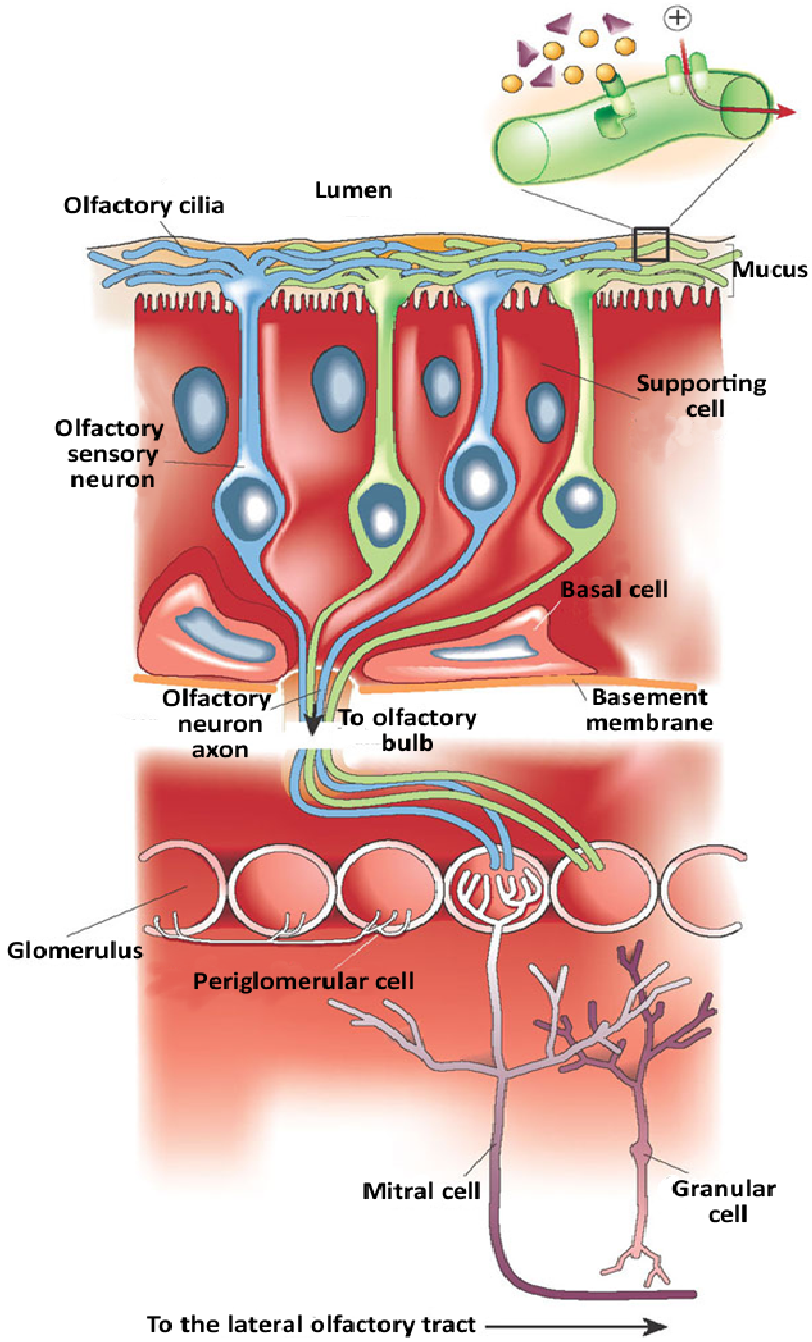


Figure 1.2. *Pictorial representation of the olfactory neuroepithelium (adapted from Firestein, 2001).*

1.1.2 Central structures

The olfactory bulb (OB; Figure 1.3) is the first central relay station of the olfactory system and protrudes bilaterally from the basal portion of the frontal lobe. The olfactory bulb has one source of sensory input, namely the axons from the OSNs of the olfactory epithelium, and one output, namely the mitral cell axons. OSN and mitral cell synapses form spherical structures called glomeruli. The number of glomeruli varies across species from 300-700 in the human olfactory bulb to around 2000 in the mouse. Therefore, approximately 10,000 OSN axons converges in each glomerulus.

In the OB the first spatial representation of odorants is established. The OB also receives 'top-down' information from cortical and subcortical structures such as the amygdala, neocortex, hippocampus, locus coeruleus, and substantia nigra. Therefore, it can potentially mediate the sensitivity of odour detection and the selection of relevant and irrelevant odours. This allows higher brain areas involved in arousal and attention to modify the detection or the discrimination of odorants.

The axons of the mitral cells bundle together in the postero-ventral OB, forming the olfactory tract. This structure projects widely and bilaterally to other brain regions – first of all, the primary olfactory areas - where further olfactory information processing occurs.

The piriform cortex is considered as "the largest and most distinctive [primary] olfactory cortical area in most mammals" (Price, 1990; Figure 1.3). It is localized at the end of the lateral olfactory tract, inhabiting a small portion of both frontal and temporal lobes at the

ventral junction of the two. The piriform cortex receives direct input from the olfactory tract and has extensive output interconnections with the amygdala, hypothalamus, entorhinal cortex, and orbitofrontal cortex (Carmicheal, Clugnet, & Price, 1994). This privileged access to sensory, affective, neuroendocrine, and motivational features of an olfactory stimulus raises the piriform cortex as the ideal candidate for shaping odour representations with direct relevance for perception and behaviour (Howard, Plailly, Grueschow, Haynes, & Gottfried. 2009).

The anterior cortical nucleus of the amygdala and the periamygdaloid cortex are positioned in the inner portion of the infero-temporal lobe (Figure 1.3). They receive direct afferences from the OB and project to the hypothalamus and the thalamus. Importantly, they contribute to the processing of the emotional tone of the olfactory stimuli (Winston, Gottfried, Kilner, & Dolan, 2005).

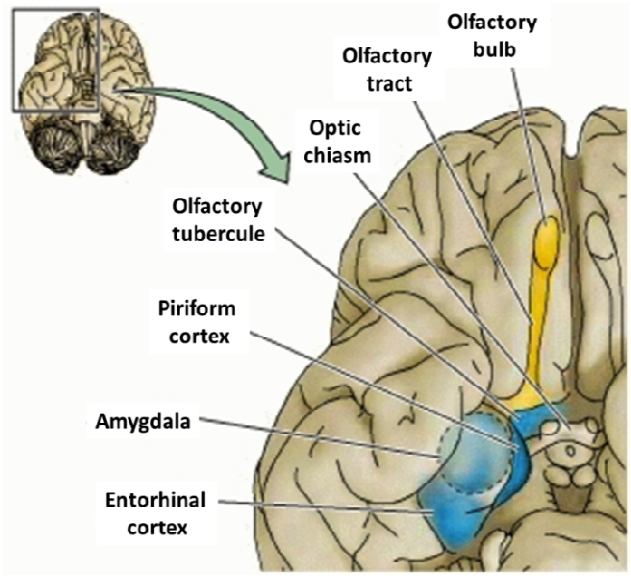


Figure 1.3. *Pictorial representation of the olfactory central structures (adapted from Purves et al., 2004).*

As shown in Figure 1.3, the entorhinal cortex is positioned close to the amygdala region in the medial temporal lobe. It directly receives information from the OB and its output connections constitute the main interface between the hippocampus and the secondary olfactory cortices. Therefore, it is recognized to play a role in odour memory (Ferry, Ferreira, Traissard, & Majchrzak, 2006).

From the primary cortices, the olfactory information is submitted to secondary olfactory cortices for further processing. The hippocampus belongs to the limbic system and it is bilaterally situated in the medial temporal lobe. It is tightly interconnected with the entorhinal cortex and contributes to the formation of memory of odours (Price, 1990).

The hypothalamus is located at the central bottom portion of the brain. It receives most prominently afferences from the piriform cortex and the amygdala (Price, 1990). The hypothalamus is involved in the regulation of the neuroendocrine cascade induced by the exposure to an odorant.

The thalamus is a midline paired symmetrical structure localized within the medial subcortical region of the brain. Differently from other sensory systems, it receives indirect projections from the piriform cortex, periamygdaloid cortex and entorhinal cortex (Price, 1985). Its output fibres target the insular and the orbitofrontal cortex, placing the thalamus at the centre of the cortico-cortical connections.

The insula is a structure deeply folded within the lateral sulcus between the temporal lobe and the frontal lobe. The insula receives input from the piriform cortex and sends projections to the limbic structures

(Price, 1985). It is believed to be involved in the elaboration of the emotional content of odours.

The orbitofrontal cortex (OFC) is considered the main site of secondary olfactory processing in humans. This cortical region, located on the ventral portion of the frontal lobe, receives both direct and indirect (via the thalamus) inputs from the primary olfactory cortices (Price, 1990; Yarita, Iino, Tanabe, Kogure, & Takagi, 1980; Figure 1.4). It is connected with a wide constellation of other prefrontal, limbic, sensory and premotor areas (Cavada, Company, Tejedor, Cruz-Rizzolo, & Reinoso-Suarez, 2000).

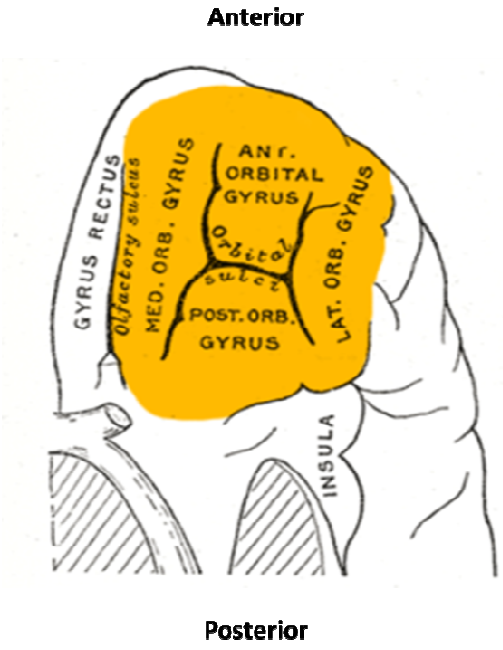


Figure 1.4. *Basal view of the orbitofrontal cortex and its gyri (adapted from Gray, 1918).*

Evidence from studies on monkeys demonstrated that neurons in the OFC respond to odorant-specific stimuli (e.g. Critchley & Rolls, 1996b; Rolls, Yaxley, & Ienkiewcz, 1990). In humans, OFC lesions lead to impairments

in higher level cognitive processes, such as odour discrimination and identification, indicating that it is involved in the conscious perception of odours (Jones-Gotman & Zatorre, 1988; Zatorre & Jones-Gotman, 1991), odour discrimination learning (Critchley & Rolls, 1996a; Schoenbaum & Eichenbaum, 1995), encoding of food-based reward value (Critchley & Rolls, 1996b) and multisensory integration (Rolls & Baylis, 1994). This suggests that OFC is the structure responsible for the ability of decoupling odour stimuli from stereotyped responses conferring to mammals and humans behavioural flexibility (Gottfried, 2010).

A schematic version of the connections among the areas of the central nervous system (CNS) involved in olfactory perception has been reported in Figure 1.5.

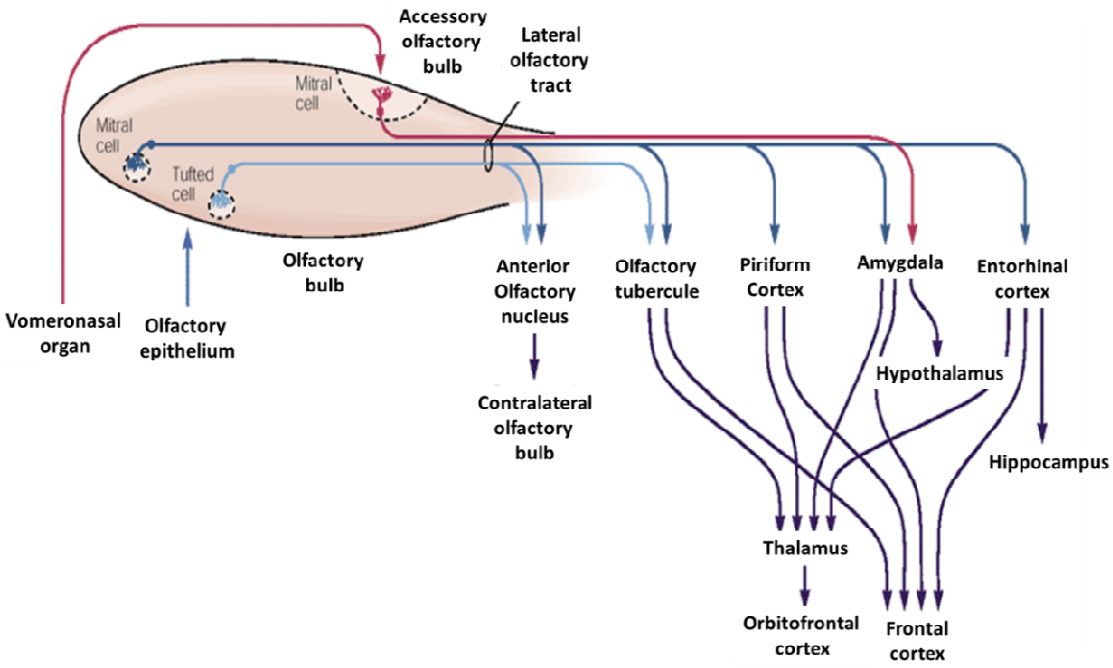


Figure 1.5. *Schematic representation of the connections within the main olfactory system (blue lines) and the accessory olfactory system (purple lines; adapted from Buck, 2000).*

1.2 PHYSIOLOGY

As graphically summarized in Figure 1.6, the transduction of olfactory information begins with the binding of a chemical to receptor proteins located in the cilia of the OSNs. This association constitutes an odotope which can be considered the elemental odour stimulus (Cleland, 2008).

Following the principle expressed by the 'one neuron-one receptor rule', a single OSN expresses only one odorant receptor (Lancet, 1994; Mombaerts, 2004). Thus, in humans an individual receptor expresses only one among the approximately 350 receptors identified (Malnic, Godfrey, & Buck, 2004). In macrosmatic organisms, such as mice, the total number of receptors expressed increases approximately to 1500 (Buck & Axel, 1991). The neurons expressing a given receptor appear randomly distributed within four zones of the epithelium, resulting in a dispersed pattern of activation elicited by each odorant (Figure 1.6). A single odorant can interact with multiple distinct receptors, as well as a single receptor type can recognize many odorants with different affinities (Oka, Katada, Omura, Suwa, Yoshihara, & Touhara, 2006). Therefore, any pattern of activation is subjected to the concentration of the delivered odour (Malnic, Hirono, Sato, & Buck, 1999). Independently on the number of OSN activated, each receptor projects to two spatially invariant glomeruli in the OB (Mombaerts, 2006; Figure 1.6) and, as a consequence, the random distribution of the active OSNs is consolidated into segregated 2D spatial maps of glomerular activity (primary olfactory representation; Cleland, 2008; Soucy, Albeanu, Fantana, Murthy, &

Meister, 2009). This type of organization combines the robustness of a redundant, spatially unbiased sampling system in the nose with the economy of a condensed spatial representation in the olfactory bulb. Nevertheless, the chemotopical organization of the OB seems not to be maintained in the subsequent relay stations.

It has been suggested that a single odorant activates a subpopulation of cortical neurons distributed across the piriform cortex (Isaacson, 2010; Stettler & Axel, 2009). Therefore, the piriform cortex discards spatial segregation in favour of a highly distributed organization in which single odorants activate unique - but dispersed - ensemble of cortical neurons (Figure 1.6). In addition, extensive feedback interactions between the olfactory bulb and cortical neurons synchronize the activity of related circuits, providing a timebase for the representation and processing of olfactory information on short timescale (Clelland, 2008). Hence, the information contained in the primary olfactory representation must be transformed to become physically compatible with the processing rules and architecture of the cerebral cortex (Cleland, 2008).

This brief review of the existing literature reveals that the organization of this neurobiological system is highly complex. From an evolutionary perspective, it might be said that the actual features of the olfactory system evolved in order to facilitate the detection and discrimination of millions of odorants, with the final aim of creating coherent, perceptual representations of the external environment (Howard et al., 2009; Mori, Takahashi, Igarashi, & Yamaguchi, 2006). The

scatter distribution of the olfactory areas within the cerebral cortex and their tight bilateral interconnections may provide sufficient redundancy – but also specificity (see Chapter 3 of the present thesis) - to the system as to preserve olfactory information from being lost. Nevertheless, lesions or malfunctioning occurring at different levels of the olfactory processing may result in a variety of clinical disturbances. In the following chapter I shall outline in the detail the features and the possible etiopathogenetic mechanisms underlying olfactory disorders.

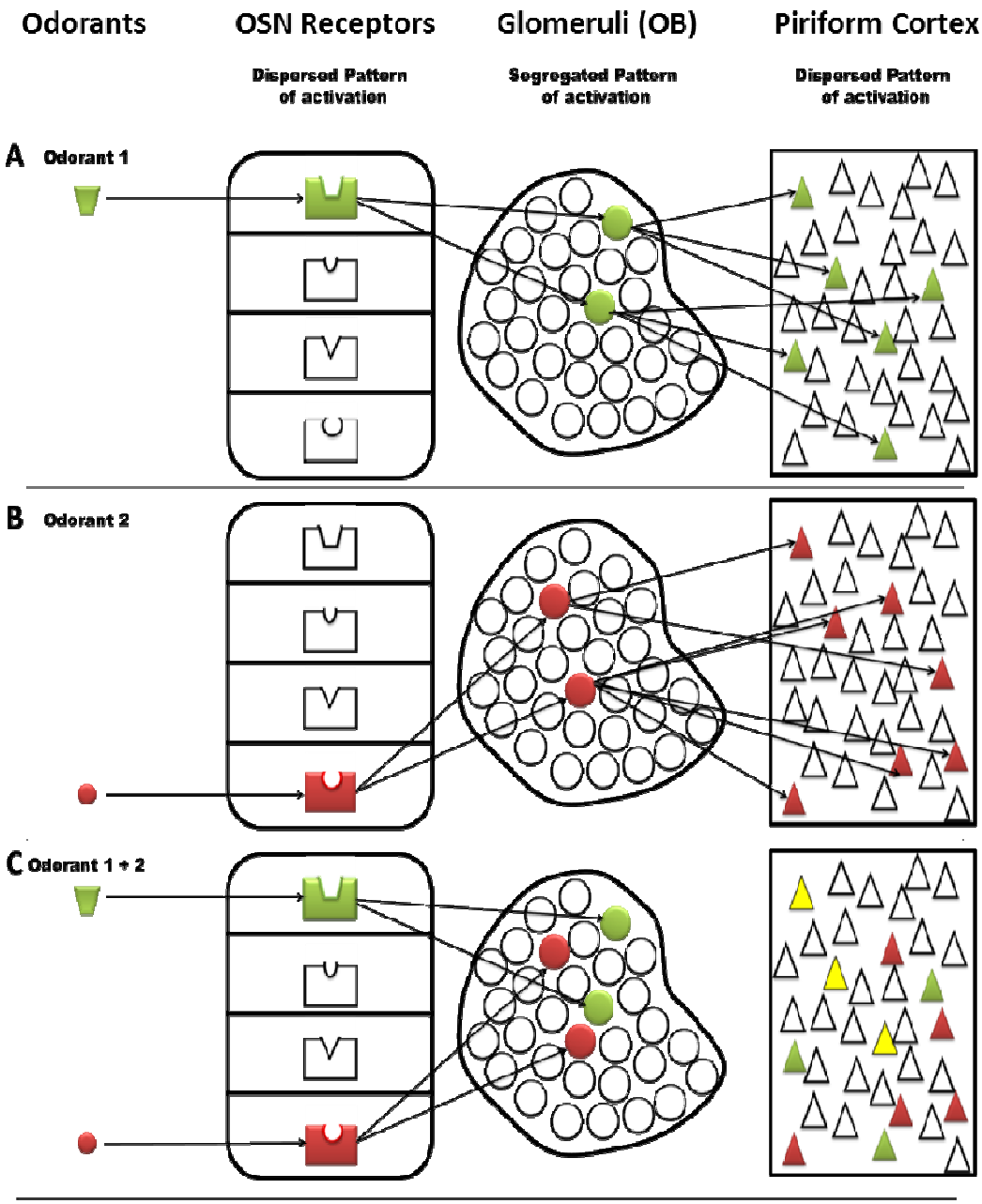


Figure 1.6. *Odorant induced activation at different levels of the peripheral and central olfactory system. Panel A, B: Activation patterns following the exposure to a single odorant. Panel C: Activation patterns following the exposure to two odorants. Yellow: overlapping activation odorant 1 + 2.*

CHAPTER 2

NEUROPSYCHOLOGY OF OLFACTORY DISORDERS

Olfactory disorders can be reliably classified in relation to the site at which the olfactory information pathway is interrupted, resulting in three general classes: (i) conductive or transport impairments from obstruction of the nasal passages; (ii) sensorineural impairment from damage to the olfactory epithelium; and (iii) central olfactory neural impairment from CNS damage. However, definitive classification of a patient's disorder into a given class is often not feasible, and these categories are not always mutually exclusive (Murphy, Doty, & Duncan, 2005).

To circumvent this issue, alternative classifications have been proposed. A very common approach is to consider the type of olfactory perception compromised. Some of the patients may experience a quantitative variation of their olfactory ability, whereas some others may report a change in olfactory stimuli quality (Table 2.1).

Table 2.1. Brief taxonomy of quantitative and qualitative olfactory disorders.

Quantitative disorders	*Anosmia*	Inability to detect qualitative olfactory sensations (i.e., absence of smell function)
	Specific anosmia	Inability to perceive some particular odorants
	Hyposmia (or microsmia)	Decreased sensitivity to odorants
	Hyperosmia	Abnormally acute smell function
Qualitative disorders	*Dysosmia (or cacosmia or parosmia)*	Distorted or perverted smell perception to odorant stimulation
	Phantosmia (or olfactory allucination)	A distorted sensation perceived in the absence of an odour stimulus
	Olfactory agnosia	Inability to recognize an odour sensation, even though olfactory processing, language, and general intellectual functions are essentially intact

With regards to the quantitative aspect of olfactory perception, it has been estimated that a complete loss of the sense of smell is found in at least 1 or 2% of the general population (Gilbert & Wysocki, 1987; Olsson, Berglind, Bellander, & Stjärne, 2003; Panel on Communicative Disorders to the National Advisory Neurological and Communicative Disorders and Stroke Council, 1979). More recently, the percentage of patients who exhibit a significant loss of olfaction - corresponding to a diagnosis of functional anosmia - has been approximated to 5% (Brämerson, Johansson, Ek, Nordin, & Bende, 2004; Landis, Konnerth, & Hummel, 2004). To further trace the argument, in Germany it has been counted that approximately 70,000 individuals refers to clinics to cope with some kind of olfactory loss problems (Damm et al., 2004). Aging has been identified as the main factor related to olfactory loss given that 24% of individuals aged 53–97 years were found to present some form of olfactory disorder (Murphy, Schubert, Cruickshanks, Klein, Klein, & Nondahl, 2002). In addition, data from a worldwide National Geographic Magazine survey

suggest that, although unfamiliar odours affects olfactory abilities, age-related losses are culture-independent (Doty, Bartoshuk, & Snow, 1991).

Along with these quantitative olfactory disorders, patients may also show qualitative olfactory disturbance (Table 2.1). Around 5–10% of the patients showing anosmia or hyposmia also complain spontaneously about disosmia or parosmia (e.g., Deems et al., 1991; Miwa, Furukawa, Tsukatani, Costanzo, DiNardo, & Reiter, 2001; Quint, Temmel, Schickinger, Pabinger, Ramberger, & Hummel, 2001). Nevertheless, more focused investigations revealed an even higher incidence showing that up to 60% of patients with olfactory dysfunctions has some parosmic experience (Faulcon, Portier, Biacabe, & Bonfils, 1999; Nordin, Murphy, Davidson, Quinonez, Jalowayski, & Ellison, 1996). This discrepancy might be likely due to the missing awareness of qualitative olfactory dysfunction, and, consequently, to the lack of active questioning of patients with regard to qualitatively distorted sensations (Figure 2.1).

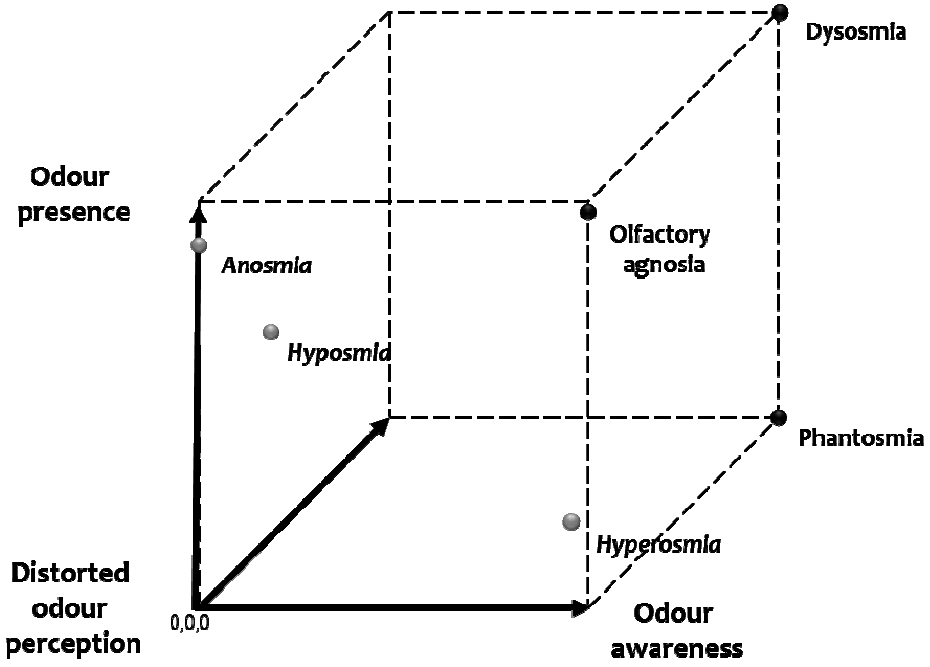


Figure 2.1. *Graphical 3d representation of three dimensions (Odour presence, distorted odour presence, odour awareness) which can describe olfactory disorders. Grey dots represent quantitative olfactory disorders. Black dots represent qualitative olfactory disorders. 0,0,0: origin of the axes.*

However, this lack of awareness and attention to one's own olfactory ability is not only confined to qualitative aspects of olfactory perception, but it is rather generalized to all the aspects of the smell experience (Figure 2.1). To date, there is much evidence suggesting that olfaction is a sense that frequently goes unnoticed (Deems et al., 1991; Nordin, Monsch, & Murphy, 1995; Shu, Hummel, Lee, Chiu, Lin, & Yuan, 2009; Temmel, Quint, Schickinger-Fischer, Klimek, Stoller, & Hummel, 2002).

2.1 CAUSES OF OLFACTORY LOSS

Despite the importance of a normal smell function in everyday life, the practical utility of olfaction is best highlighted by the consequences of its modifications. Thus, much research in this domain has highlighted the differential aetiologies associated with olfactory loss. A schematic extract is reported in Table 2.2.

The most frequent cause of smell loss in the adult population is due to upper respiratory infections (URI), such as common cold, influenza, pneumonia, or human immunodeficiency virus (Akerlund, Bende, & Murphy, 1995; Deems et al., 1991; Hummel, Rothbauer, Barz, Grosser, Pauli, & Kobal, 1998; Murphy et al., 2000). In such cases there is a close temporal association between the infection and the subsequent olfactory loss report (Welge-Lüssen & Wolfensberger, 2006). The exact location of the damage is not yet known. On the one hand, biopsies lean towards a direct damage of receptor cells; on the other hand, current knowledge does not allow to completely ruling out any central damage (Welge-Lüssen & Wolfensberger, 2006). Spontaneous recovery of olfactory performance occurs in about one third of the patients within the first two years (Hummel, 2000; Reden et al., 2006). Though, the longer the disorder has been persisting, the less likely a recovery is (Reden et al., 2006).

A great number of patients presenting smell loss suffers from nasal or sinus disease. This olfactory disturbance has been traditionally viewed as being solely conductive. More recent perspectives suggest, that although marked airflow blockage undoubtedly alters olfactory sensitivity

in some patients, it cannot completely explain the olfactory loss (for review, see Doty & Mishra, 2001), hypothesizing that the severity of histopathological changes within the olfactory mucosa concurrently plays a role (Jafek, Moran, Eller, Rowley, & Jafek, 1987). Nasal surgery, in most cases, has proven to be helpful in recuperating olfactory function (Delank & Stoll, 1998; Downey, Jacobs, & Lebowitz, 1996; Hoseman, Goertzen, Wohlleben, Wolf, & Wigand, 2000; Kimmelman, 1994; Leonard, Cain, & Clavet, 1988; Seiden & Smith, 1988), even though in rare cases, it might produce iatrogenic outcomes (Briner, Simmen, & Jones, 2003).

Traumatic events, such as head or nasal trauma, are among the most common causes leading to olfactory disorders (Nordin & Brämerson, 2008). The literature favours the hypothesis that shearing or tearing of the OSN axons is the most likely cause of olfactory loss, although little research has been done in this area. Investigations by Delank and Fechner (1996) indicate that the vulnerability of the OSN axons varies. This seems to confirm reports by Sumner (1964), who already observed that even minor trauma can lead to severe olfactory impairment. In some patients a with posttraumatic olfactory loss, fractures or edema in the area of the olfactory cleft can be detected. Furthermore, acute head trauma may also cause cerebral hemorrhage or contusion, which may lead to olfactory loss (Reden et al., 2006). With regards to the prognosis, recovery is most likely to occur within the first 6-12 months from the trauma. Two years following injury, the chances of improvement decrease to less than 10% (Costanzo & Becker, 1986; Sumner, 1964), even though the regenerative ability of the olfactory system might bring to recovery without any

intervention and following longer periods (Graziadei & Monti-Graziadei, 1985; Mueller & Hummel, 2009; Schwob, 2002).

Olfactory dysfunctions may also depend on intranasal and intracranial tumours (Murphy et al., 2005). Although signs other than olfactory ones are typically present in such cases, olfactory dysfunction can, in fact, be the sole and early sign to base the diagnosis upon (e.g., Fitzsimon, Waring, Kokmen, McLaren, & Brubaker, 1997; McCormack & Harris, 1955). Prognosis highly depends on the size of the tumour and the surgery duration. Although postoperative olfactory function contralaterally to the tumour can be preserved, it is extremely difficult to maintain ipsilateral olfactory functions (Welge-Lüssen, Temmel, Quint, Moll, Wolf, & Hummel, 2001).

Further, genetic factors may be responsible for some kind of olfactory loss, such as congenital anosmia, resulting from the agenesis or dysgenesis of the olfactory bulbs and stalks without any association to other anomalies (Yousem, Geckle, Bilker, McKeown, & Doty, 1996). Congenital cases are infrequently reported because patients affected since birth show no comprehension of 'smell' and are unaware of their deficit (Vowles, Bleach, & Rowe-Jones, 1997).

Unlike more peripheral disorders leading to olfactory impairment many pathologies play a major role in central olfactory dysfunctions. As an example, this is the case of smell dysfunction associated with endocrine disorders, such as Addison's disease (Henkin & Bartter, 1966), Turner's syndrome (Henkin, 1967), Cushing's syndrome (Henkin, 1975),

hypothyroidism (Deems et al., 1991), pseudohypoparathyroidism (Henkin, 1968), and Kallman's syndrome (Males & Schneider, 1972).

As another example, it is worth considering a number of psychiatric disorders which are reportedly associated with altered smell function. Among them, panic disorder (Kopala & Good, 1996), mood disorders (Gross-Isseroff, Luca-Haimovici, Sasson, Kindler, Kotler, & Zohar, 1994; Lombion-Pouthier, Vandel, Nezelof, Haffen, & Millot, 2006; Pause, Miranda, Göder, Aldenhoff, & Ferstl, 2001), seasonal affective disorder (Postolache, Doty, Wehr, Jimma, Han et al., 1999; Postolache, Wehr, Doty, Sher, Turner et al., 2002), severe stage anorexia nervosa (Fedoroff, Stoner, Andersen, Doty, & Rolls, 1995; Kopala, Good, Goldner, & Birmingham, 1995; Roessner, Bleich, Banaschewski, & Rothenberger, 2005), and schizophrenia (Corcoran et al., 2005; Hudry, Saoud, Amato, Daléry, & Royet, 2002; Moberg et al., 2003).

Recently, it has also been reported that patients diagnosed with some neurodevelopmental disorders, such as autism spectrum disorder (ASD; Bennetto, Kuschner, & Hyman, 2007) or attention-deficit hyperactivity disorder (ADHD; Gansler, Fucetola, Krengel, Stetson, Zimering, & Makary, 1998), present some kind of olfactory disturbance.

Moreover, patients diagnosed with neurodegenerative diseases, including Alzheimer's disease (AD), Down's syndrome (DS), Huntington's disease (HD), idiopathic Parkinson's disease (PD), multiple sclerosis (MS), and the parkinsonism-dementia complex of Guam (PDC), are affected to different extent by olfactory disturbances (e.g., Doty, 1991, 2001; Ferreyra-Moyano & Barragan, 1989; Hawkes, Shephard, & Daniel,

1999; Mesholam, Moberg, Mahr, & Doty, 1998; Moberg & Doty, 1999; Murphy, 1999; Serby, 1987).

As evident from this review, alterations in olfaction are present in a wide range of disorders. This might raise the possibility that such olfactory anomalies simply reflect a nonspecific general disruption of CNS pathways. However, this is unlikely for several reasons. First, in some diseases, such as PD, the olfactory deficit presents very early in the disease process, long before the typical motor-related brain deterioration becomes evident (Braak, Ghebremedhin, Rüb, Bratzke, & Del Tredici, 2004). Second, the degree of olfactory dysfunction differs, on average, among most of these disorders. For example, PD is accompanied by marked alterations in the ability to smell, whereas other motor disorders - such as vascular parkinsonism - are accompanied by no alterations (Doty, 2003). Third, scores on most olfactory tests may be unrelated to disease stage or progression such as in PD, whereas in other disorders, such as head trauma, this appears not to be the case (Doty, Yousem, Pham, Kreshak, Geckle, & Lee, 1997). Fourth, it has been suggested that in MS the number of plaques within olfactory-related CNS structures, but not in other brain regions, is correlated with the degree of olfactory dysfunction (Doty, Li, Mannon, & Yousem, 1999). Finally, the agenesis or dysgenesis of the olfactory bulb might trigger an endocrine cascade contributing in the development of autistic phenotypes (Brang & Ramachandran, 2010). In conclusion, the abovementioned results suggest the possibility that olfaction might be considered as a potential marker for different

psychiatric and neurodegenerative disorders (Atanasova, Graux, El-Hage, Hommet, Camus, & Belzung, 2008; Doty, 2009).

*Table 2.2. Reported etiological categories associated with olfactory dysfunction**

Industrial Dusts, Metals, Volatiles
- Acetone
- Acids (e.g., sulfuric)
- Ashes
- Benzene
- Benzol
- Butyl acetate
- Cadmium
- Carbon disulphide
- Cement
- Chalk
- Chlorine
- Chromium
- Coke/coal
- Cotton
- Cresol
- Ethyl acetate
- Ethyl and methyl acrylate
- Flour
- Formaldehyde
- Grain
- Hydrazine
- Hydrogen selenide
- Hydrogen sulfide
- Iron carboxyl
- Lead
- Mercury
- Nickel
- Nitrous gases
- Paint solvents
- Paper
- Pepper
- Peppermint oil
- Phosphorus oxychloride
- Potash
- Silicone dioxide
- Spices
- Trichloroethylene

Infections — Viral/Bacterial
- Acquired immunodeficiency syndrome (AIDS)
- Acute viral rhinitis
- Bacterial rhinosinusitis
- Bronchiectasis
- Fungal
- Influenza
- Rickettsial
- Microfilarial

Pulmonary
- Chronic obstructive pulmonary disease

Medical Interventions
- Adrenalectomy
- Anesthesia
- Anterior craniotomy
- Arteriography
- Chemotherapy
- Frontal lobe resection
- Gastrectomy
- Hemodialysis
- Hypophysectomy
- Influenza vaccination

Medical Interventions *(continued)*
- Laryngectomy
- Oophorectomy
- Paranasal sinus exenteration
- Radiation therapy
- Rhinoplasty
- Temporal lobe resection
- Thyroidectomy

Drugs

Adrenal steroids (chronic use)

Amino acids (excess)
- Cysteine
- Histidine

Analgesics
- Antipyrine

Anesthetics, local
- Cocaine HCl
- Procaine HCl
- Tetracaine HCl

Anticancer agents (e.g., methotrexate)

Antihistamines (e.g., chlorpheniramine malate)

Antimicrobials
- Griseofulvin
- Lincomycin
- Macrolides
- Neomycin
- Pencillins
- Streptomycin
- Tetracyclines
- Tyrothricin

Antirheumatics
- Mercury/gold salts
- D-Penicillamine

Antithyroids
- Methimazole
- Propylthiouracil
- Thiouracil

Antivirals

Cardiovascular/hypertensives

Gastric medications
- Cimetidine

Hyperlipoproteinemia medications
- Artovastatin calcium (Lipitor)
- Cholestyramine
- Clofibrate

Intranasal saline solutions with:
- Acetylcholine
- Acetyl, -methylcholine
- Menthol
- Strychnine
- Zinc sulphate

Local vasoconstrictors

Opiates
- Codeine
- Hydromophone HCl
- Morphine

Psychopharmaceuticals (e.g., LSD, psilocybin)

Sympathomimetics
- Amphetamine sulphate
- Fenbutrazate HCl
- Phenmetrazine theoclate

*Categories are not mutually exclusive

Table 2.2. (continued)

Lesions of the nose/Airway blockage
- Adenoid hypertrophy
- Allergic rhinitis
  - Perennial
  - Seasonal
- Atrophic rhinitis
- Chronic inflammatory rhinitis
- Hypertrophic rhinitis
- Nasal polyposis
- Rhinitis medicamentosa
- Structural abnormality
  - Deviated septum
  - Weakness of alae nasi
- Vasomotor rhinitis

Neoplasms—Intracranial
- Frontal lobe gliomas and other tumours
- Midline cranial tumors
  - Parasagittal meningiomas
  - Tumors of the corpus callosum
- Olfactory groove/ Cribriform plate Meningiomas
- Osteomas
- Paraoptic chiasma tumors
  - Aneurysms
  - Craniopharyngioma
  - Pituitary tumors (esp. adenomas)
  - Suprasellar cholesteatoma
  - Suprasellar meningioma
  - Temporal lobe tumours

Neoplasms—Intranasal
- Neuro-olfactory tumors
  - Esthesioepithelioma
  - Esthesioneuroblastoma
  - Esthesioneurocytoma
  - Esthesioneuroepithelioma
- Other benign or malignant nasal tumors
  - Adenocarcinoma
  - Leukemic infiltration
  - Nasopharyngeal tumors with extension
  - Neurofibroma
  - Paranasal tumors with extension
  - Schwannoma

Neoplasms—Extranasal and Extracranial
- Breast
- Gastrointestinal tract
- Laryngeal
- Lung
- Ovary
- Testicular

Genetic
- BBS proteins
- CEP290

Endocrine/Metabolic
- Addison's disease
- Congenital adrenal hyperplasia
- Cushing's syndrome
- Diabetes mellitus
- Froelich's syndrome
- Gigantism
- Hypergonadotropic hypogonadism
- Hypothyroidism
- Kallmann's syndrome
- Pregnancy

Endocrine/Metabolic *(continued)*
- Panhypopituitarism
- Pseudohypoparathyroidism
- Sjögren's syndrome
- Turner's syndrome

Nutritional/metabolic
- Abetalipoproteinemia
- Chronic alcoholism
- Chronic renal failure
- Cirrhosis of liver
- Gout
- Protein-calorie malnutrition
- Total parenteral nutrition w/o adequate Replacement
- Trace metal deficiencies
- Copper
- Zinc
- Whipple's disease
- Vitamin deficiency
- Vitamin A
- Vitamin B 6
- Vitamin B 12
- Cirrhosis of liver

Psychiatric
- Anorexia nervosa (severe stage)
- Attention deficit disorder
- Depressive disorders
- Hysteria
- Malingering
- Olfactory reference syndrome
- Schizophrenia
- Schizotypy
- Seasonal affective disorder

Neurological
- Amyotrophic lateral sclerosis
- Alzheimer's disease
- Cerebral abscess (esp. frontal or ethmoidal regions)
- Down syndrome
- Familial dysautonomia
- Guam ALS/PD/dementia
- Head trauma
- Huntington's disease
- Hydrocephalus
- Korsakoff's psychosis
- Migraine
- Meningitis
- Multiple sclerosis
- Myesthenia gravis
- Paget's disease
- Parkinson's disease
- Refsum's syndrome
- Restless leg syndrome
- Syphilis
- Syringomyelia
- Temporal lobe epilepsy
  - Hamartomas
  - Mesial temporal sclerosis
  - Scars/previous infarcts
- Vascular insufficiency/anoxia
  - Small multiple cerebrovascular accidents
  - Subclavian steal syndrome
  - Transient ischemic attacks

Adapted from Murphy et al., 2005.

2.2 Consequences of olfactory loss

It has been demonstrated that the ability to perceive odours profoundly influence daily life experiences, especially for people whose work require an intact sense of smell (Deems et al., 1991; Miwa et al., 2001; Callahan & Hinkebein, 1999; Temmel et al., 2002). As an example, patients with olfactory problems experience altered quality of life, changes in appetite or body weight, and a decrement in psychological well-being, also expressed in terms of reduced libido (Costanzo & Zasler, 1991; Deems et al., 1991; Frasnelli et al., 2004; Hummel & Nordin, 2005; Van Toller, 1999). As another example, olfactory loss may bring to bodily insecurity, inducing patients who do not perceive their own body odours to disproportionally use hygiene measures or perfumes (Callahan & Hinkebein, 1999; Costanzo & Zasler, 1991). Finally, and more importantly, patients presenting chemosensory dysfunctions are subjected to obvious safety consequences such as the inability to detect leaking gas, spoiled food, smoke, or hazards as burning electrical wires or cooking food (Mann, 2002; Van Toller, 1999).

Examining the effects produced by the lack of olfactory ability is a very useful manner to assess how the olfactory system functions. In the first instance, olfaction plays a role in ingestive behaviours. It regulates the decision of intaking foods and drinks on the basis of a sensory experience characterized by odour intensity, hedonics and quality (e.g., Carrasco & Ridout, 1993). In the second instance, olfactory information is involved in disease avoidance decisions. Some odours, such as fecal and

organic decomposition odours, generally trigger in adults disgust experiences (Rozin, Haidt, & McCauley, 2000). Although, some variables - such as context familiarity - seems to mitigate avoidance reactions (e.g., babies or pets odours; Stevenson & Repacholi, 2005), it is contemporary agreement that repulsion to such odours is acquired during childhood (Stevenson & Repacholi, 2003) and it is connected to potential 'objects' considered vectors of disease (Curtis, Aunger & Rabie, 2004; Curtis & Biran, 2001). In the third instance, olfaction contributes to some extent to kin recognition, infant attachment, and mate selection. Actually, the recognition of family members can occur purely on the basis of smell body odour information (Porter, 1999; Porter & Schaal, 2003). Moreover, breastfed children appear to develop preferences for their own mother odour rather than another lactating mother (Russel, 1976) and olfactory cues have been reported to be the most important determinant of attraction in women (Herz & Inzlicht, 2002). In the fourth instance, the olfactory cues serve as warning system. Particular smells, such as burning or gas odours, might alert on the presence of potentially hazardous situations (e.g., Russel, Cummings, Profitt, Wysocki, Gilbert, & Cotman, 1993).

It is evident that the olfactory system is involved in a variety of different functions, which refer to diverse domains of life. Given that all these functions are mediated by the same sensory system, they should share some commonalities. Primarily, the entire set of stimuli evoking the abovementioned responses are composed by an array of chemicals, each

contributing to the overall olfactory sensation. It follows that the fundamental and general task faced by the olfactory system concerns the identification of a complex mixture of chemicals, or odour object. From a behavioural perspective, consequences - either pleasant or not - are related to specific odour objects (e.g., getting sick following the consumption of a specific food). Therefore, the object level description of an odour seems to be the most useful form of representation (Wilson & Stevenson, 2006). In the ensuing chapter I shall review the current literature about 'odour objects' and their inclusion within the most important theorethical explanation models of the olfactory system.

CHAPTER 3

OLFACTORY REPRESENTATIONS: FROM PERCEPTION TO COGNITION

Ever present never twice the same,
ever changing never less than whole.

Robert Irwin
The Central Gardens at the Getty, Los Angeles

A key property of the brain is to create coherent, meaningful representations of the outside world. Although the formulation of the concept of representation is not generally accepted (Maturana & Varela,1987; O'Regan & Noe, 2001; Thompson & Varela, 2001), in the field of neuroscience the notion of 'neural representation' has come into common use (Andersen, Snyder, Bradley, & Xing, 1997; Knudsen & Brainard, 1995; Singer, 1998). Albeit naïve, it is traditionally considered that the brain elaborates features of the external world and integrates them as to build internal representations with the final aim of creating a model of the world enabling complex sensory-motor interactions and cognitive functions.

As to make sense of the external world many animal species primarily rely on olfactory information. Think, for example, of macrosmatic organisms such as mice and dogs which are able to navigate the environment by defining and locating individual odour sources, such as food or possible mates. Comparatively, in humans the biological role of smell has decreased in favour of more sophisticated and more accurate

sources of information - such as visual information. Nevertheless, olfactory information is not totally neglected. It is now established that objects are defined by our brain as rich multisensory representations, formed by the integration of the unimodal information codified in each sensory modality (Newell, 2004; Taylor, Moss, Stamatakis, & Tyler, 2006). Thus, olfactory information is processed and integrated as part of the unitary percept of objects. Although much of the information coming from the different sensory pathways may be complimentary and redundant, some objects might preferentially be encoded by just one sensory modality. As an example, methane, due to its gaseous nature, would not be detected via sight, hearing or touch but only via olfaction.

Hence, understanding how olfactory stimuli are interpreted at a neural level and perceptually codified is a prerequisite for investigating how olfactory representations contribute to fundamental processes such as object recognition, identification, and categorization, which are cognitive skills providing a critical survival advantage to the living organisms.

3.1 Odour objects perception

The nature of human sensory experience is preferentially visuo-centric (Kubovy & Van Valkenburg, 2001). And, as a consequence, it is common sense that objects should own visual-like features to be properly deemed 'objects' (Gottfried, 2010). Objects have shapes, colours and their spatial position is delimitated by edges. However, none of these attributes can be

translated tout court to odours, which are neither visible nor easily localizable. Therefore, thinking of the phenomenology of olfaction raises a natural question: may olfactory stimuli be considered objects?

In order to address this issue, in the following sections I will provide a description of the unique nature of olfactory stimuli and the rules regulating the perception of odours.

3.1.1 Properties of an odour

The vast majority of real-world odours are complex mixtures ranging from a few to a numerous amount of different molecules. For instance, when smelling a ripe strawberry we are not able - but neither expert panelists do - to distinguish more than three or four of the molecules which contribute to that specific odour (Laing & Francis, 1986; Livermore & Laing, 1996). This suggests that the olfactory system needs a minimal amount of information to correctly encode a single smell without misinterpreting it as a similar non-identical odour, that is an odour which shares some - but not all - compounds with the former.

Another key feature of real-world smells is inconstancy. The perception of olfactory stimuli can be substantially modulated by air temperature, humidity or wind direction (Gottfried, 2010). Nevertheless, the olfactory system is challenged in maintaining the regularity of the chemosensory pattern, as to attribute object properties to the stimulation. Moreover, the fact that different chemicals are freely available within the environment, requires that the olfactory system is able to discriminate among different odour objects (Gottfried, 2010).

Taken together, the properties of real-world odours represent some constraints that contributed to evolutionarily shape - both anatomically and functionally - the olfactory system in the way we actually know it (Eisthen, 2002). In the ensuing paragraph I will delve into the functional mechanisms regulating odour object perception by using a psychological framework.

3.1.2 Formation of an odour object

Given that real-world odours are complex blends of many chemicals, a necessary step forward to the perception of an odour object is the synthesis of single components into a unified whole (Figure 3.1, Panel A). As an example, the characteristic smell of a rose is composed by up to 260 mixed molecules (Keller & Vosshall, 2004).

The subsequent step in the perception of odour objects is the segmentation of an ensemble of features (i.e., molecules) from an irrelevant background (i.e., background molecules), which is filtered or tuned out (Kubovy & Van Valkenburg, 2001; Figure 3.1, Panel B). This principle, well known as the figure-ground segmentation, has been pioneristically studied by Gestalt scientists in the visual domain (Koffka, 1935) and then transposed to the other sensory modalities, including olfaction. Imagine, for example, to smell a Melody perfumée rose embedded in a nosegay. The olfactory features which arise from the other flowers and leaves constitute the background stimulus that has to be inhibited as to perceive the rose odour object.

The third step is the maintenance of object constancy, that is the ability for perceived odours to remain constant despite any fluctuation. This mechanism allows for the extraction of the perceptual sameness across different stimuli. In other words, it allows object categorization (Figure 3.1, Panel C). Referring to the *Melody perfumée* rose example, the idea is that if we smell the rose in different environmental conditions (such as in different air humidity conditions) we will recognize those different olfactory experiences as linked to the same odour object (Gottfried, 2010).

A further step refers to the ability of the olfactory system to discriminate across multiple versions of the same object or across multiple exemplars of the same object category (Figure 3.1, Panel D). This ability, which promotes a flexible adaptation within the environment, is facilitated by perceptual learning and experience (Goldstone, 1998). Thus, the olfactory system is able to discriminate between different blossoms of Melody perfumée roses as well as to differentiate among diverse species of smelling roses, such as the *Melody perfumée*, the *Double delight* or the *Honey perfume*.

Finally, real-world odours are rarely encountered in isolation. More often, many different odours are present within an environment in a precise temporal framework. Given human limited ability to process simultaneous information, it is crucial to activate a selection procedure only towards the odours that show behavioural relevance (Gottfried, 2010; Figure 3.1, Panel E). Think of being a perfumer smelling a number of different flowery odorants in search of the Melody perfumée rose odour

with the aim of including it in a new preparation. The exposure to the samples will result in the inhibition of all the odour object others than the Melody perfumée rose and the final selection of the recognized odour.

Insofar, an odour object might be considered an 'olfactory form', a synthetically-built perceptual structure undergoing a spatiotemporal development (Roudnitska, 1983) towards which the olfactory system applies an heuristic based on a global resemblance principle which allows for odour object categorization, discrimination and selection (Kay, Crk, & Thorngate, 2005; Wilson & Stevenson, 2006).

Speaking about forms, it appears evident that - differently from visual forms - there is still no objective criterion for determining if two odours have similar forms. This might claim for a new definition of odour objects based on issues other than pure perception. In this respect, Yeshurun and Sobel (2010) suggested that an odour object derives from the integration of the odour molecules (i.e., perceptual features) with the pleasantness generated by the odour and the subjective state at which the perception of the odour takes place (i.e, affective features). However, considering that the steps bringing to odour object perception are equally pertinent for approaching smells of different valence, these two alternative definitions of odour objects can be viewed as compatible and should be applied in chorus (Gottfried, 2010).

In conclusion, odour object perception might be considered a bottom-up process in which sensory olfactory information shapes and modulates the formation of cognitive olfactory representation. Nevertheless, behavioural reactions to odours frequently require a higher

level of cognitive elaboration. The aim of the following section will be to account for higher-level cognitive functions involved in olfactory behaviours.

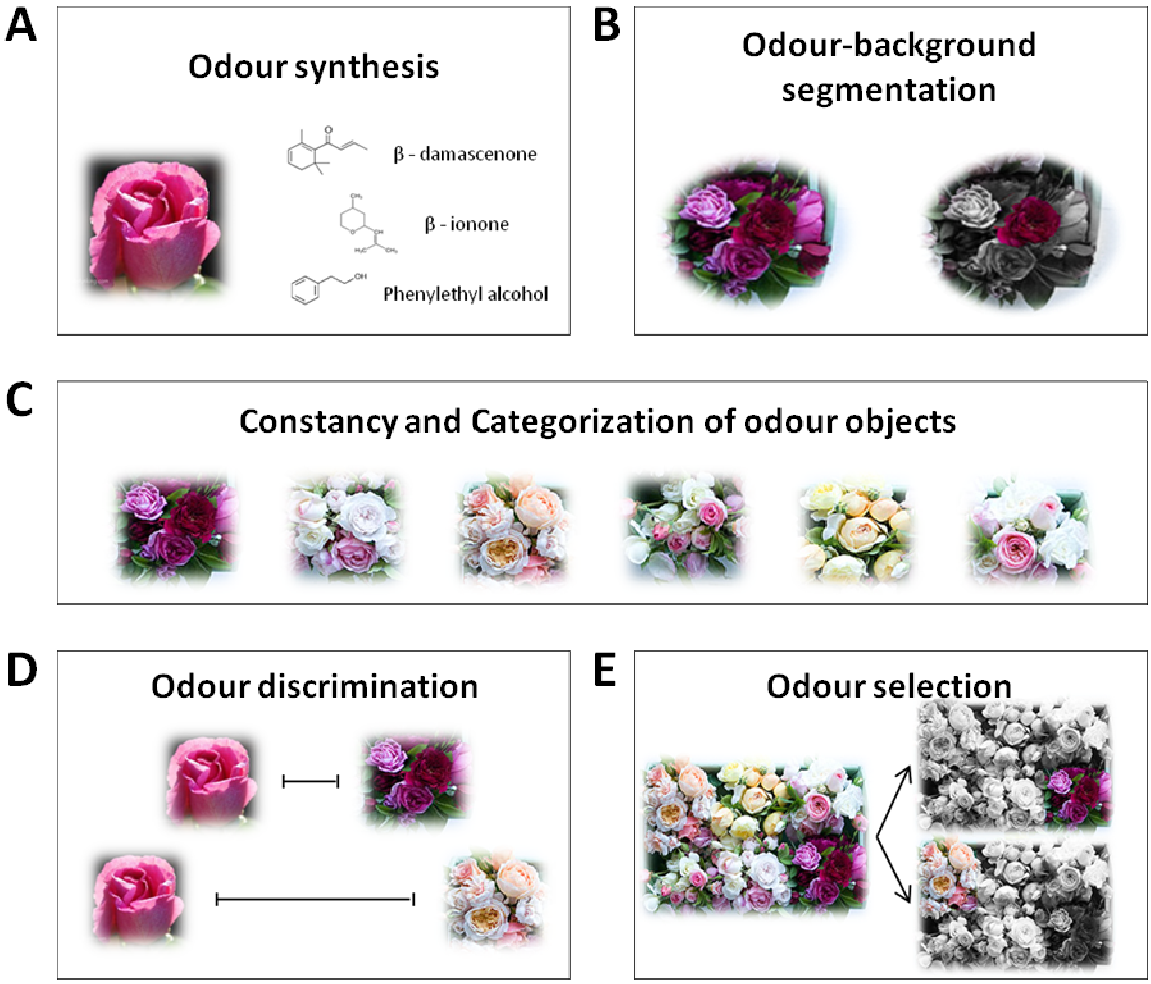


Figure 3.1. *Panel A:the Melody perfumée rose produces around 260 volatile components, 3 of which are depicted here: β– damascenone, β – ionone and phenylethyl alcohol. Panel B:When considering a Melody perfumée rose embedded within a bouquet as an odour object, the olfactory features that arise from the other flowers and the leaves of the Melody perfumée rose constitute the background stimulus. Panel C:Despite differences in odour, colour, size, shape and texture, all of the objects represented belong to the category 'rose'. Panel D:The perceptual similarity between two exemplars belonging to the same category make odour discrimination harder in the top case whereas easier in the bottom case. Panel E: The olfactory system may be confronted with many different odourous objects simultaneously (left part). Attentional mechanisms provide a dynamic way of selecting among these competing alternatives, bringing one odour object to the perceptual foreground (right part) in accordance with physiological needs and motivational states.*

3.2 High-level cognitive processing of odours

It is well known that higher cognitive processes - such as awareness and memory - clearly influence perception (Goldstein, 2011). With respect to the olfactory realm, although olfactory experience is difficult to define, there is evidence that top-down processing contributes to shape the odour object representations used to respond to olfactory demands in the real-world. The main thrust of the upcoming section will be to analyse the odour awareness and memory which contribute to the regulation of olfactory representations.

3.2.1 Odour awareness

A wide number of olfactory stimuli are simultaneously omnipresent in our environment and they are mostly processed without people consciously noticing them (Sela & Sobel, 2010). From a physiological perspective, this may occur because the olfactory system is particularly sensible to the phenomenon of desensitization due to habituation. It consists in a progressively reduced perception of an odour in the presence of stimulus constancy, as a consequence of gradual decrease in the sensitivity of neurons in the principal olfactory areas (e.g., Hummel, Knecht, & Kobal, 1996; Wang, Walker, Sardi, Fraser, & Jacob, 2002). Although this process is not a unique prerogative of the olfactory system, it is worth noting that different sensory modalities behave in different manners. In fact, referring to our everyday life experience, it is far harder to re-smell an odour (a lavender ambient fragrance) to which we have habituated rather than re-

hear a sound (a ticking clock) to which we have desensitized (Stevenson, 2009).

From a cognitive viewpoint, the progressive loss of odour awareness throughout time has been explained in terms of both attentional mechanisms and access to consciousness. With regards to attention, there is evidence that the way we attend to odours can affect how information is encoded by the brain and how we react to external stimuli (Dijksterhuis & Aarts, 2010; Spence, Kettenmann, Kobal, & McGlone, 2001). Humans are able to selectively pay attention (Spence, McGlone, Kettenmann, & Kobal, 2001) and to adopt different attentional strategies as to process odour stimuli (Prescott, Johnstone, & Francis, 2004). With respect to consciousness, it has been demonstrated that higher order processes do not need any conscious guidance to be implemented (e.g., Bargh, Chen, & Burrows, 1996; Bargh, Gollwitzer, Lee-Chai, Barndollar, & Trotschel, 2001; Dijksterhuis & Nordgren 2006; Libet, Gleason, Wright, & Pearl. 1983) and this can be also transposed to odour processing (olfactory priming; Larsson, 2002).

Notwithstanding, attention and consciousness are not unrelated functions (Dijksterhuis & Aarts, 2010; Posner, 1994). To date, at a neural level, it has been demonstrated that the manipulation of the orientation of attention modulates the brain activity between cortical olfactory areas (i.e., OFC) and the thalamus (i.e., mediodoursal nucleus; Plailly, Howard, Gitelman, & Gottfried, 2008), which is considered a structure involved in conscious attention but it is not required to consciously discriminate between odorants. Thus, it is reasonable to think that they contribute in

concert to the experience of odour awareness. Actually, when a person pays more attention to an incoming odour, the probability that that person becomes consciously aware of it increases. This hypothesis has been confirmed by a series of studies contrasting the performances of chemosensory experts (e.g., perfumers, wine testers) and novice participants. Experts outperformed laypersons in virtue of the higher level of cognitive skills rather than to perceptual advantages per se (Bende & Nordin, 1997; Melcher & Schooler, 1996; Solomon, 1990).

Overall, these results led to the idea that individual differences in perception and reactions to odours may depend on initial differences in awareness of such odours. To assess this issue Smeets and co-workers (Smeets, Hendrik, Schifferstein, Boelema, & Lensvelt-Mulders, 2008) developed the Odour Awareness Scale (OAS) with the aim of measuring how much individuals consciously pay attention to chemosensory stimuli available in the surrounding environment. The results showed that it is possible to distinguish between people with high and low odour awareness (Smeets et al., 2008) and that this distinction is useful as to interpret general olfactory behaviour. It has been established that people highly interested to olfactory stimuli as compared with a group of individuals who were less aware of odours (i) have better chances to correctly identify odours (Arshamian, Willander & Larsson, 2011; Dematté, Endrizzi, Biasoli, Corollaro, Zampini & Gasperi, 2011; Smeets et al, 2008; Stevenson & Case, 2005); (ii) perceive real odours as more familiar (Bensafi & Rouby, 2007); (iii) report more affective experiences when smelling odours (Wrzesniewski, McCauley, & Rozin, 1999); (iv)

refer a higher rate of odour-related memories (Wrzesniewski et al., 1999); (v) generate odour images easier (Gilbert, Crouch, & Kemp, 1998); (vi) do not show better odour naming abilities (Arshamian et al., 2011).

Considering all these pieces of evidence it is clear that odour awareness can be tightly related to odour memory, which is a fundamental process involved in the establishment and recollection of olfactory representations.

3.2.2 Odour memory

In the real world, odour objects are in most cases learned unintentionally following repeated exposure to stimuli and in the absence of personal awareness (Issanchou, Valentin, Sulmont, Degel, & Köster, 2002; Wilson & Stevenson, 2006). As a result, odours are difficult to be precisely described in terms of specific constituents and names (Gibson, 1969). According to this description, olfactory learning can be numbered among the non-declarative or implicit forms of memory (Tulving, 1995). Nevertheless, one might say that this is limited view of the topic. Recently, Larsson (2002) proposed to conceptualize the different expression of olfactory memory in the framework of memory systems. As a result, olfactory memory can be conceived as a 5-interrelated-modules system (e.g., Nyberg & Tulving, 1996; Roediger, Buckner, & McDermott, 1999): procedural memory, perceptual representation system, semantic memory, short-term memory and episodic memory. The first and the second systems are typically described as non-declarative forms of memory whereas the latter three are declarative expressions of memory

(Larsson, 2002). A brief description of each system will be provided in detail below. Procedural memory is a form of memory underlying the acquisition of procedures (actions). In the olfactory domain, it is responsible for odour conditioning, which are chiefly responsible for chemosensory aversions (Larsson, 2002). The perceptual representation system is thought to be in charge of odour priming effects, which refer to the unintentional facilitation of a performance following a preceding exposure to a related odour (Tulving & Schacter, 1990). However, the sparse evidence available on this topic reveal a panorama of mixed results (Olsson, Jonsson, & Faxbrink, 2002). Semantic memory identifies remembrance of concept-based knowledge unrelated to specific experiences. The short-term memory system can be split into two subsystems: odour primary and odour working memory. In both cases, the items are accessible to conscious experience, but odour primary memory do not require any additional active operation on the information stored whereas odour working memory does (Baddeley, 1992). Odour discrimination is a typical task used to test odour working memory (Larsson, 2002). Episodic memory deals with autobiographical events that can be correctly placed in space and time (Tulving, 1993). With regards to olfaction, it is responsible for odour recognition memory performance (Larsson, 2002).

The order of presentation of the five systems reflects the order of evolution both in phylogenetic and ontogenetic terms (Schacter & Tulving, 1994). Interestingly, the system that evolved last, episodic memory, is the form of memory that has been proved to be the most

affected by disturbances (e.g., aging, depression, dementia, health status,...), whereas the systems that appeared earlier in evolution (non-declarative systems) are considerably more preserved (Larsson, 2002).

Addressing the interdependence between the memory systems, it is of interest to highlight some of the interactions observed among working memory, semantic memory, and proficiency in episodic-memory odour recognition. In this perspective, it has been recently proposed that olfactory perception is regulated via a mnemonic-based object recognition system (Wilson & Stevenson, 2006).

This recognition system is particularly usefull when considering the elaboration of odours relevant for the fulfilment of survival functions.

3.3 BIOLOGICAL RELEVANCE OF ODOURS

In order to survive, the animals of all species - humans included - have to be able to correctly codifying the message embedded within the signals carried by (sensory) stimuli. Signals can be produced by inanimate sources (e.g., common odours) or by living beings. The former category includes stimuli which may - or may not - convey biologically relevant information. As an example, common odours might help in localizing food sources, which is a survival significant function, but can also indicate the presence of a objects irrelevant from a biological perspective (e.g., chalk odour). The latter category, instead, consists of stimuli - such as human body odours - which allow for a social communication among conspecifics. For this reason, they are always carrying a message

conveying specific (relevant) information between two individuals. So far, four are the areas described as examples of human chemosensory communication, each demonstrating physiological and/ or behavioral consequences of signal perception in the receiver (Pause, 2011). They are related to kin recognition (Porter, 1999; Porter & Schaal, 2003), mate selection (Havlicek & Roberts, 2009), menstrual cycle synchronicity (Stern & McClintock, 1998), and emotional contagion (Prehn-Kristensen et al., 2009).

Support to the contention that common odours are functionally different from social biologically relevant odours comes from the investigation of the substrates underlying the neural elaboration of those stimuli (Lundström, Boyle, Zatorre, & Jones-Gotman, 2008, 2009; Pause, 2011). Common odours typically activate areas within the olfactory system (see Chapter 1 of the present thesis for further details) whereas biologically relevant odours recruit areas outside the olfactory crcuits, preferentially deputated to the elaboration of social stimuli. It is the case of the fusiform, the cingulate and the insular cortex (e.g., Lundström et al., 2008, 2009; Prehn-Kristensen et al., 2009). Altogether, these evidences instill the need of experiments considering the degree of biological relevance when selecting the experimental stimuli.

In conclusion, olfactory processing begins with the perception of a complex mixture of chemicals which is recognized as a unitary object, with different degrees of biological/social relevance. This generates a neural and a cognitive representation of the odour object which is

elaborated most of the times unintentionally and is commonly retrieved via implicit procedures. Taken together these features have set the basis for the development of test tools and procedures to evaluate olfactory abilities in the general population and in patients. In the next chapter I shall provide an overview of the assessment techniques currently used in the clinical and scientific fields with reference to both explicit and implicit forms of olfactory testing.

CHAPTER 4

OLFACTORY ASSESSMENT

The unique features of the olfactory system structural and functional architecture have driven the development of numerous tests and procedures to evaluate human olfactory abilities. This, with the general aim of increasing knowledge regarding the normal appearance of the system. And, the specific scope of evaluating the patients suffering from olfactory deficits. The goal is to provide a more precise diagnosis and, therefore, a more appropriate treatment (Hummel & Welge-Lüssen, 2006).

For research as well as for clinical purposes, it will be of help to dispose of the medical history of the patient, a physical examination performed by a specialized otolaryngologist and an objective olfactory assessment as to verify the existence or the level of a chemosensory problem invalidating the functioning of the sense of smell (Doty, 2009).

First, the assessment of olfactory functions require the collection of the medical history regarding odour concerns as to determine whether possible events might have influenced olfactory abilities (see Chapter 2 of the present thesis for further details). Further, it will be of help to ask for information regarding the food usage and perception of proper body odours. Finally, when a chemosensory disorder is ascertained, it is

mandatory to question the patient as to define the olfactory abilities prior to the chemosensory loss has been acknowledged (Murphy et al., 2005)

Even though interviewing the patient provides plenty of information about the possible causes and consequences underlying an olfactory deficit, this cannot be considered sufficient material to produce a diagnosis. The clinician has to take into account the subjective point of view of the patient and her own qualitative impressions, but she must rely upon objectively based information. Quantitative testing had proven to be useful in (i) accurately characterising the nature and extension of the disturbance; (ii) ascertaining the validity of the patient's complaint, including the detection of malingering; (c) monitoring changes over time; (d) establishing efficacy of treatment and management programmes; and (e) providing objective data for grounding disability compensation (Doty, 2006).

Therefore, the clinical assessment of olfactory function should include a complete rhinologic examination (Grevers, 2006). This will be of help in achieving information about structural changes in the olfactory system resulting in a disturbed olfactory perception. The ENT evaluation may start with the visual inspection of the nasal cavities and the palpation as to reveal bone discontinuities. To objectively evaluate the appearance of the inner part of the nose (nasal and sinus passages), nasal endoscopy is frequently performed. Apart from these routinely performed tests, special rhinologic procedures might be carried out as to diagnose particular disorders (e.g., nasal patency, allergy testing...). Further, imaging techniques (e.g., conventional radiographs, computerized

tomography and magnetic resonance imaging) might be performed as to ascertain the presence of anatomical malformations or inflammatory processes within the nasal cavities or in the cerebral parenchima. Recently, electroolfactograms (EOGs) and olfactory-evoked related potentials (OERPs) have been included in the routine investigation of patients with olfactory loss to ascertain the presence of peripheral and central odour signalling, respectively (Hummel & Kobal, 2001)

As to implement an objective, but fast manner to test human's olfactory performances many psychophysical tests have been devised and standardized (Table 4.1).

Table 4.1. *Psychophysical tests to measure olfactory function.*

Test	Olfactory function tested
Smell Threshold Test	Threshold
T&T Olfactometer	Threshold
Olfactory Perception Threshold Test (OPTT)	Threshold
University of Pennsylvania Smell Identification Test (UPSIT)	Identification
Biolfa olfactory test	Identification
Cross-Cultural Smell Identification Test (CC-SIT)	Identification
Pocket Smell Test (PST)	Identification
Odour Stick Identification test (OSIT)	Identification
Combined olfactory test	Identification
Odorant confusion matrix	Identification
Connecticut Chemosensory Clinical Research Center (CCCRC) Test	Identification, Threshold
European Test of Olfactory capability (ETOC)	Identification, Threshold
Sniffin' Sticks (SS)	Identification, Discrimination, Threshold
12-item Odour Memory Test	Odour memory, Discrimination
Sniff Magnitude Test	Pleasantness

At present, the most widely used are the University of Pennsylvania Smell Identification test (UPSIT; Doty, Shaman, & Dann, 1984), the Connecticut Chemosensory Clinical Research test (CCCR; Cain, Gent, Goodspeed, & Leonard, 1988) and the Sniffin' Sticks (Kobal, Hummel, Sekinger, Barz, Roscher, & Wolf, 1996). All these tests require the participant/patient to be confronted with odours and to provide a response, which is monitored and recorded. The available procedures measure different aspects of olfactory performance such as olfactory threshold, discrimination and identification abilities. The odour threshold tests measure the lowest concentration of a stimulus that can be detected (the detection threshold), recognised (the identification threshold) or discerned from another concentration of the same stimulus (the differential threshold; Amoore & Ollman, 1983; Kobal et al., 2000; Stevens, Cain, & Burke, 1988). The odour discrimination tests measure the ability to differentiate between odorants and requires the participant to decide whether two stimuli are similar or different (Kobal et al., 2000). The odour identification tests evaluate the ability to recognize an odorant presented at the suprathreshold level. Identification of odour name can be free (recall) or based on a multiple-choice (recognition). This latter case is preferred since it has been demonstrated that it prevents participants from being biased. Therefore, it is more sensitive to evaluate the identification abilities of participants (Doty, Shaman, & Dann, 1984; Cain et al., 1988; Hummel, Konnerth, Rosenheim, & Kobal, 2001; Kobal et al., 1996). Most identification tests are based on the recognition of 10 to 40 odours: the higher the number of odorants administered, the more reliable

the information regarding olfactory abilities. Although widely used as screening procedures, odour identification tests present two major limitation: (i) they strongly tap onto the verbal abilities of participants (Larsson, Nilsson, Olofsson, & Nordin, 2004) and, (ii) they have a strong cultural connotation, making odour judgement be subjected to familiarity (Ho, Kwong, Wei, & Sham, 2002). For these reasons the so-structured identification tasks can be considered examples of classic explicit memory tasks. Given the abovementioned limitations, the creation of implicit memory tasks has become necessary. In the ensuing sections explicit and implicit olfactory memory testing will be reviewed and compared.

4.1 Explicit olfactory testing

Independently of the sensory modality involved, explicit memory test require participants to think back and try to recall information about some specific event (Schacter, 1987). Recalling olfactory material do not represent an exception to this general rule (Issanchou et al., 2002).

There is now compelling evidence of the fact that the structures involved in the explicit memory are localized close to the olfactory areas (e.g., temporal lobe; Schott et al., 2005; Zald & Pardo, 2000). The study of amnesic patients presenting medio-temporal and/or diencephalic lesions showed that they clearly fail at explicit retrieval tasks (e.g., Brooks & Baddeley, 1976; Cermak, Talbot, Chandler, & Wolbarst, 1985; Cohen & Squire, 1980; Graf & Schacter, 1985; Graf, Shimamura, & Squire, 1985;

Graf, Squire, & Mandler, 1984; Haist, Musen, & Squire, 1991; Shimamura & Squire, 1984; Smith & Oscar-Berman, 1990; Verfaellie, Cermak, Letourneau, & Zuffante, 1991; Warrington & Weiskrantz, 1968; 1970). Then, it is not surprising that patients with hippocampal lesion fail to an explicit odour memory tasks (Levy, Manns, Hopkins, Gold, Broadbent, & Squire, 2003). The present evidence suggest that failure to an explicit odour memory task might not be unquestionably dependent on poor olfactory performance. Actually, a memory problem might be responsible for the lack of olfactory abilities as measured by means of explicit identification tests.

A point worth noting is that the majority of investigations capitalized on olfactory tests which require an explicit report of odour features (Doty, Deems, & Stellar, 1988). Such explicit report implies intact forms of odour memory involving the generation of a name or the recognition of verbal material. But what to do if patients are not able to remember the correct answer, or they could not access to the correct verbal label?

Studies on amnesic patients might help this endeavour. If, on the one hand, amnesic patients are not able to solve explicit tasks involving a wide range of materials, on the other hand, their performance to implicit memory testing have been proven to be mostly unaffected (Roediger & McDermott, 1993). Therefore, olfactory tasks relying on implicit memory should be considered.

4.2 Implicit olfactory testing

Indirect memory tests have been developed to assess the retention of data without direct reference to the source of information (Schacter, 1987). Thus one of the main advantages of implicit retrieval tasks is that they do not require intentional access; rather, memory is inferred from a change in behaviour attributed to the previous episode (Rajaram & Roediger, 1993). This concept can be extended to the olfactory domain. As an example, one study demonstrated that participants exposed to odours, without being aware of such exposure, exhibited behaviours that were modulated by their exposure to those specific odours (Degel & Köster, 1999).

In a typical implicit experiment, a participant is presented with a set of target odours and, in a second time, she chooses between targets (i.e. the 'old' odours) and distractors (i.e. the 'new' odours). Importantly, the similarity between the target and the distractor odour (e.g., grape vs. watermelon, or grape vs. turpentine) and the size of the distractors set in which the target is embedded substantially influence memory success. The more similar the distractors and the larger the distractor set, the more difficult the odour recognition memory test (Herz & Engen, 1996).

Moreover, Degel and colleagues (Degel, Piper, & Köster, 2001) found that being able to identify an odour by its correct name interferes with the establishment, the retention or the retrieval of an implicitly acquired and phenomenally unconscious memory for that odour. In this study, participants were exposed to a room with low concentration of an odour, or an odourless room. Neither the participants, nor the

experimenters, were made aware of the presence of the odour. Later they were asked to indicate how well each 12 odours stimuli presented befitted 12 visual contexts, including the exposure room. At the end of the session they rated the pleasantness and the familiarity of odours, and identified them by name. The results confirmed that those participants who did not perceived the odour in the room, implicitly linked the odour with the exposure room, whereas participants that did not identify the odour in the room, did not show such a link. Again, the problem of name generation occurs and might prevent impaired patients to accurately respond, irrespectively of their olfactory residual abilities (Olsson & Fridén, 1999).

At this stage, one might question whether an implicit test of olfactory abilities grounded on the memory skills of the participants, which better resist to the assault of disturbances, might be conceived. In this connection, recent developments in the investigation of the role played by olfactory stimuli in shaping motor behaviour might help to address this issue (Castiello, Zucco, Parma, Ansuini, & Tirindelli, 2006; Tubaldi, Ansuini, Tirindelli, & Castiello, 2008).

4.2.1 THE CASE OF MOVEMENT KINEMATIC ANALYSIS

Like all sensory modalities, olfactory information contributes to the representation objects (Newell, 2004). Unavoidably, it exerts some crossmodal influence in a variety of tasks involving attention (e.g., Spence, McGlone, et al., 2001), memory (e.g., Herz, 1998; Zucco, 2003),

emotion (e.g., Herz, Eliassen, Beland, & Souza, 2004), airflow motor control (e.g., Bensafi et al., 2003) and scent tracking (e.g. Porter et al., 2006).

Additionally, different sensory modalities are used in concert to represent actions, with the final goal of facilitating the planning and execution of movements (Fogassi & Gallese, 2004). The investigation of multisensory coding during natural actions is still in its infancy, but there is evidence suggesting that cross-modal links in motor control are potentially numerous and substantial (Gentilucci, Daprati, & Gangitano, 1998; Patchay, Castiello, & Haggard, 2003; Patchay, Haggard, & Castiello, 2005). Although these studies have initially restrained their locus of investigation to the relationships between vision and proprioception (e.g., Patchay et al., 2003, 2005) and vision and audition (e.g., McGurk & MacDonald, 1976), more recent research has investigated the effects of coupling vision and olfaction (Castiello et al., 2006; Tubaldi, Ansuini, Tirindelli, et al., 2008) and vision and flavour on motor control (Parma, Ghirardello, Tirindelli, & Castiello, 2010; Parma, Roverato, Ghirardello, Tirindelli, Bulgheroni, & Castiello, 2011).

The common denominator underlying this bulk of experiments is that participants reached and grasped with one hand a visual target of different sizes while receiving an irrelevant stimulation (distractor) in a different sensory modality. Given that the appropriateness of hand shaping is directly proportional to the object dimension, with a slope estimated around 0.8 (Jeannerod, 1981), the differences in the parameterization of hand aperture largely depend upon the first-coming

sensory modality. For instance, when a preceding orthonasally delivered olfactory information evokes the representation of an object similar in size to the visual target, then the aperture of the hand during reaching is more accurately sized than when the target is grasped in the absence of any preceding olfactory information. If the administered odour evokes an object of a different size than that evoked by the visual target, then hand coreography is less precise (Tubaldi, Ansuini, Tirindelli, et al., 2008). Having two modalities signalling target-motor-related properties determines either facilitation or interference effects depending on the congruency between preceding sensorial information and visual target information. It is worth noting that both the facilitation and the interference effects reported in the abovementioned experiments were not voluntarily produced by the participants, who were not even aware of the differences in their hand movements between conditions.

Given the fact that the reach to grasp movement (i) cannot be voluntarily controlled in its parameterization, (ii) it is influenced by the exposure to an odour stimulus and (iii) it is an ecological and extensively rehearsed action, one might think that this movement particularly fits the purpose of studying the implicit olfactory performance and succeeds in avoid participants' verbal memory bias.

In the second part of the present thesis, the experimental results obtained by administering either explicit (Chapter 5) or implicit (Chapters 6, 7 and 8) testing methods of olfactory abilities in different populations of patients will be outlined.

PART II

THE EXPERIMENTS

CHAPTER 5

SPECIFIC SMELL DYSFUNCTIONS IN MULTIPLE SCLEROSIS[1]

5.1 ABSTRACT

The present study assessed odour threshold, discrimination and identification in relapsing-remitting MS (RRMS) patients. We administered the Sniffin' Sticks Extended Test (Burghart Messtechnik GmbH, Wedel, Germany) to 50 RRMS female patients and to 50 matched control participants. Also, the number and the volume of MRI-visible demyelinating plaques within the inferior frontal and temporal lobes (IFTL complex) were quantified in a patient subgroup. The results indicated that up to 34% of the RRMS sample exhibited hyposmia, but none of the MS patients could be considered functionally anosmic. Odour identification and general olfactory performance significantly decreased with respect to the patients' age. No significant correlations between olfactory scores and the number and the volume of plaques within the IFTL complex were found. These findings suggest that some specific forms of olfactory dysfunction do exist in MS patients and that the correlation between olfactory dysfunctions and structural brain damage might not be as strict as previously suggested.

[1] *Published*: Parma, V., Tornasi, C., Grossi, P., Atzori, M., Calabrese, M., Tirindelli, R., Gallo, P., & Castiello, U. (2010). Specific smell dysfunctions in relapsing-remitting multiple sclerosis. *Journal of Neurology and Neurophysiology*, doi:10.4172/2155-9562.1000101.

5.2 Introduction

A controversy within the neurological sciences concerns the presence of olfactory dysfunction in MS (Confavreux, Vukusic, Moreau, & Adeleine, 2000; Sørensen, Frederiksen, Brønnum-Hansen, & Petersen, 1999), the most common chronic disabling neurological disease in young adults, with particular reference to the female population (Compston, 1994). Whereas some investigators reported that a decrease in odour perception can be one of the symptoms experienced by MS patients (Constantinescu, Raps, Cohen, West, & Doty, 1994; Doty, Li, Mannon, & Yousem, 1997; Doty, Li, Mannon, & Yousem, 1998; Doty et al., 1999; Hawkes, Shephard, & Kobal, 1997; Pinching, 1977; Wender & Szmeja, 1971), others have failed to demonstrate any olfactory alterations in MS at all (Kesslak et al., 1988; Lumsden, 1970; Samkoff, 1996).

Preliminary evidence in favour of an olfactory dysfunction in MS patients comes from Wender and Szmeja (1971), who reported an odour identification dysfunction in 18 out of 52 patients, i.e., 35% of the examined population. Subsequently, Pinching (1977) administered a suprathreshold odour identification task and noticed the presence of anosmia or hyposmia in 10 out of 22 MS patients (i.e., 45.4%). More recent and compelling demonstrations of altered olfactory perception in MS have been reported in a series of studies using an objective and highly reliable standardized psychophysical test of olfactory functions, the UPSIT (Doty & Frye, 1989; Doty, McKeown, Lee, & Shaman, 1995; Doty, Shaman, Applebaum, Gibersen, Sirkowski, & Rosenberg, 1984) or a

modified version of the same test (Kostic, Stefanova, Pekmezovic, & Drulovic, 2009; Zivadinov, Zorzon, Monti Bragadin, Pagliaro, & Cazzato, 1999; Zorzon et al., 2000). In particular, Doty and colleagues (Doty, Shaman, Kimmelmann, & Dann, 1984) found that 23% of 31 patients obtained UPSIT scores significantly lower than the controls, a result which was confirmed in further studies by the same group which revealed an even higher percentage (38.5%) of microsmic MS patients (Doty et al., 1998, 1999).

Other studies, however, failed to observe olfactory deficits in MS patients. For instance, Ansari (1976) using serial binary dilutions of amyl acetate and nitrobenzene to evaluate odour detection thresholds, did not find any difference in the olfactory performance of MS patients compared with age- and gender-matched healthy controls. Similarly, MS patients scored as high as healthy controls in two further studies using the UPSIT (Kesslak et al., 1989; Lumsden, 1970; Samkoff, 1996).

Previous research has also considered the MRI approach, which implies the correlation of olfactory test scores with the in vivo quantitative assessment of focal demyelinating areas (i.e., plaques) within primary olfactory regions by means of high-resolution magnetic resonance imaging (MRI). A strong negative correlation between the olfactory test scores (i.e., UPSIT) and the number of demyelinating plaques within the inferior frontal and temporal lobe (IFTLs) regions, which are involved in olfaction, has been reported (Doty, Li, et al., 1997; Doty et al., 1998). Such correlations were not evident in brain regions unrelated to olfaction (Doty et al., 1998). Similar evidence was reported in a study in which a robust

correlation between a T2 lesion load within the IFTLs complex and smell loss was found (Zorzon et al., 2000). From the above brief literature review, it emerged that a clear consensus on this matter has yet to be reached. One of the factors which might have contributed to such controversial results is the nature of the olfactory test that was administered. Indeed, different - sometimes non-standardized tests - were used (Pinching, 1977; Wender & Szmeja, 1971; Zorzon et al., 2000) and only odour identification was chiefly considered (Ansari, 1976; Costantinescu et al., 1994; Doty et al., 1997, 1999; Hawkes, Shephard, & Kobal, 1997; Kostic et al., 2009; Samkoff, 1996; Zivadinov et al., 1999; Zorzon et al., 2000). Also, even when the same test for the evaluation of odour identification was utilized (i.e., the UPSIT), opposite results were found (Doty et al., 1998; Samkoff, 1996). To date, the effect of MS on other measures of olfactory performance, such as odour threshold and discrimination, remain largely unknown.

Therefore the main aim of the present study was to elucidate whether RRMS patients are affected by a loss of smell, either global or task specific. A secondary aim was to evaluate the possible correlation between olfactory dysfunctions and the presence of demyelinating plaques within the inferior frontal and temporal lobes, the central brain structures associated with olfactory processing

5.3 METHODS

5.3.1 PARTICIPANTS

Seventy-five female RRMS patients (age range 19 to 55 years; mean age = 37.5 years; SD = 8.7) and 64 controls (age range 16 to 55 years; mean age = 35.5 years; SD = 8.3) were enrolled in the experiment (Table 5.1). For the MS group the disease duration ranged from 1-28 years (mean 9.22 years). All patients were selected by three board certified neurologists after having their diagnosis verified on the basis of the revised McDonald criteria (McDonald, Compston, Edan, Goodkin, Hartung, Lublin, et al., 2001) and who were regularly followed at the MS Centre of the Veneto Region. In order to evaluate the disability of the RRMS patients the Expanded Disability Status Scale (EDSS; Kurtzke, 1983) was administered (EDSS mean = 1.80; SD = 1.21). Both patients and controls were tested with the Mini Mental State Examination (MMSE) test (Folstein, Folstein, & Brown, 1996) (patients: MMSE mean = 29.28; SD = 0.64; controls: MMSE mean = 29.73; SD = 0.52), the Beck Depression Inventory – II (BDI-II; Beck, Steer, & Brown, 1996) and the Beck Anxiety Inventory (BAI; Beck & Steer, 1990) to exclude participants with cognitive impairment, depression and/or anxiety at the time of olfactory testing. Patients treated with immunosuppressants, prone to epilepsy or undergoing steroid therapy were excluded from the sample (Stenner, Vent, Huttenbrink, Hummel, & Damm, 2008). Finally, a questionnaire was administered to all participants to ascertain previous history of nasal disease, smoking habits and current status of olfactory functions (adapted from Zucco, Amodio, &

Gatta, 2006; Appendix A). All patients and controls affirmed they were non-smokers. On the basis of these exclusion criteria, 25 patients and 14 controls were excluded. The final cohort consisted of 50 female RRMS patients (mean age = 37.4 years; SD = 8.7; Table 5.1) and 50 female age-matched controls (mean age = 35.5 years; SD = 14.2). All participants were naive as to the purpose of the investigation and gave informed written consent to participate in the study. The experimental procedures were approved by the Institutional Review Board at the University of Padova and were in accordance with the Declaration of Helsinki.

Table 5.1. *Demographical, pharmacological and cognitive preservation data for the 50 females RRMS patients participating in the study.*

ID	Age	Onset yrs	DMA	MMSE
1	29	7	IFNβ i.m.	28
2	28	6	IFNβ i.m.	28
3	42	9	IFNβ s.c.	30
4	41	5	IFNβ s.c.	29
5	28	5	-	29
6	29	3	IFNβ i.m.	29
7	44	22	-	29
8	42	14	IFNβ s.c.	29
9	49	6	IFNβ i.m.	29
10	39	8	IFNβ i.m.	29
11	33	15	-	29
12	40	5	IFNβ i.m.	29
13	46	2	-	30
14	30	5	glatiramer acetate	30
15	44	8	IFNβ i.m.	30
16	43	4	IFNβ s.c.	29
17	52	15	IFNβ s.c.	29
18	37	13	-	29
19	55	13	glatiramer acetate	30
20	33	7	-	30
21	44	7	IFNβ i.m.	29
22	39	14	IFNβ s.c.	30
23	27	9	IFNβ i.m.	28
24	42	17	glatiramer acetate	29
25	43	22	IFNβ i.m.	30
26	20	2	IFNβ i.m.	29
27	43	28	-	29
28	29	11	IFNβ s.c.	30
29	36	12	IFNβ i.m.	30
30	46	3	IFNβ s.c.	30
31	32	9	-	29
32	36	2	glatiramer acetate	29
33	33	10	IFNβ i.m.	29
34	34	9	IFNβ s.c.	29
35	30	3	IFNβ i.m.	30
36	38	13	IFNβ s.c.	29
37	37	7	IFNβ i.m.	28
38	35	7	IFNβ s.c.	30
39	52	16	IFNβ i.m.	28
40	51	22	IFNβ i.m.	29
41	37	20	IFNβ s.c.	30
42	30	1	IFNβ i.m.	30
43	27	1	IFNβ s.c.	30
44	55	9	glatiramer acetate	29
45	19	2	IFNβ i.m.	30
46	42	6	glatiramer acetate	29
47	23	6	IFNβ s.c.	30
48	42	10	IFNβ s.c.	29
49	31	2	IFNβ i.m.	30
50	37	9	IFNβ i.m.	29

DMA = Disease Modifyng Agent; IFNβ i.m = interferon beta into the muscle; IFNβ s.c = interferon beta subcutaneous.

5.3.2 *Stimuli and apparatus*

To test the olfactory functions we used the Sniffin' Sticks Extended Test (Burghart Messtechnik GmbH, Wedel, Germany), a test devised to examine the three main aspects of olfactory functions, namely threshold, discrimination and identification, by means of pen-like odour dispensing devices presented alone (identification subtest) or in triplets (threshold and discrimination subtests; Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997; Kobalet al., 1996). Normative data by age and gender for this test were used to determine the relative degree of general and specific olfactory loss which permitted a categorization of olfactory function in normal people and those with hyposmia and functional anosmia (Hummel, Kobal, Gudziol, & Mackay-Sim, 2007; Appendix B).

The MR images for a subgroup of the tested patients (N = 13) were acquired within one month of administration of the olfactory test by means of a 1.5 T Philips Achieva (Philips Medical Systems, Best, Netherlands). The MR images were acquired on the same day as the Sniffin' Sticks Test was performed for five of the patients. No major hardware upgrades were applied to the scanner during the study and quality evaluation sessions took place weekly to guarantee measurement stability. The following sets of images were acquired: (i) Fluid attenuated inversion recovery (FLAIR), a 2D sequence with 50 contiguous axial slices (TE 120 ms, TR 10000 ms, inversion time 2500 ms, slice thickness 3.0 mm, matrix 256 × 256, gap 0); (ii) Turbo-spin echo Dp/T2 (TSE) sequences. By using a semiautomatic thresholding technique, implemented in the

software called Medical Images Processing, Analysis and Visualization (MIPAV) (http://mipav.cit.nih.gov) developed at the National Institute of Health (NIH), lesions were selected and segmented on the FLAIR images providing a white matter T2 (WMT2) hyperintense lesion volume (T2LV). The presence of possible FLAIR-related artifacts was controlled on proton density and T2 images. In addition, we used a digital version of the Talairach and Tournoux atlas (Talairach & Tournoux, 1988) to perform regional volumetric analyses. The number and the volume (expressed in mm^3) of demyelinating plaques within the inferior frontal and inferior temporal lobes and within the whole brain, excluding the IFTL complex, were calculated by means of MR examination. These brain regions contain the major zones of known central olfactory connections and include, respectively, (i) the olfactory striae, subcallosal medial frontal lobe, paraterminal gyrus, orbitofrontal zone and gyrus rectus, and (ii) the prepiriform, enthorinal, amygdaloidal, hippocampal, and parahippocampal regions of the brain. The inferior frontal lobes were designated as being inferior and anterior to the body and genu of the corpus callosum, respectively. The inferior temporal lobes were considered as being superiorly bound by the plane of the Sylvian fissure. Plaques were counted and measured without knowledge of the scores obtained with the Sniffin' Sticks Extended Test by two independent expert neuroradiologists. A reliability test revealed no significant differences between the two neuroradiologists with respect to plaque counting.

5.3.3 *Procedures*

The experimental session began with the collection of anamnestic data concerned with age, possible epilepsy and pharmacological history. Then the experimenter administered the questionnaire for testing the status of olfactory functions (adapted from Zucco et al., 2006; Appendix A). During this phase participants were not allowed to eat and/or drink. Subsequently, the Sniffin' Sticks Extended Test was administered. Please refer to Kobal and colleagues (Kobal et al., 1996) for more details regarding the procedure administration. The experimental session lasted approximately 60 minutes.

5.3.4 *Data Analysis*

The following descriptive measures were calculated: mean, standard deviation and the minimum and maximum values together with the percentiles (Table 5.2). A Chi-square test was used to compare global olfactory performance in the RRMS group and the control group. A between-subjects MANCOVA together with Spearman's and Pearson's correlations were used when appropriate. The MANCOVA was applied to compare olfactory performance in the RRMS and control group, with 'Group' as the independent variable and each of the olfactory indexes as dependent variables. 'Age' was used as the covariate to statistically control for variance in olfactory performance. Spearman's rank correlation coefficients were used to examine the relationship between olfactory Sniffin' Sticks scores, MRI data (plaque numbers and volume within and

outside the IFTLs) and clinical variables (i.e., MS onset). Partial correlation analysis was performed to remove the effect of potential confounding factors.

5.4 RESULTS

5.4.1 HYPOSMIA AND FUNCTIONAL ANOSMIA

The Sniffin' Sticks Extended Test ascertains the presence of both hyposmia and functional anosmia. Hummel and colleagues (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997) define the TDI cut-off point between normosmic patients and hyposmic patients as 30.3. Following this criterion, we found a percentage of hyposmic participants of 34% (17 out of 50) within our RRMS group and of 8% (4 out of 50) within our control group. A Chi-square test revealed that hyposmic participants were statistically more frequent in the patient than in the control group (χ^2 = 8.04, $p < 0.005$). With respect to functional anosmia, which refers to a TDI score less than 16.5 (Hummel et al., 2007; Kobal et al., 2000), none of the RRMS or control participants were found to be totally functionally anosmic (Figure 5.1).

Table 5.2. Sniffin' Sticks scores for the MS patients and the considered female normative population.

		MS Patients				Normative Data			
		THR	DIS	ID	TDI	THR	DIS	ID	TDI
AGE GROUP	16-35 yrs								
N		25	24	25	24	760	741	827	704
Mean		8,44	12,17	13,33	33,94	9,39	12,91	13,68	36,06
SD		3,00	1,53	1,44	4,10	2,56	1,92	1,62	4,17
Minimum		3,50	10,00	10,00	27,30	1,75	5,00	8,00	23,00
Maximum		15,00	15,00	16,00	41,00	16,00	16,00	16,00	46,75
Percentiles	5	3,69	10,00	10,25	27,34	5,51	9,00	11,00	29,50
	10	4,75	10,00	11,50	28,22	6,50	10,00	11,00	30,50
	25	6,38	11,00	12,00	30,30	7,50	12,00	13,00	33,50
	50	7,63	12,00	13,50	33,80	9,00	13,00	14,00	36,00
	75	10,75	14,00	14,00	37,00	11,25	14,00	15,00	39,00
	90	13,00	14,00	15,00	40,30	12,50	15,00	16,00	41,50
	95	14,50	14,80	15,75	40,90	14,00	16,00	16,00	43,00
AGE GROUP	36-55 yrs								
N		25	25	25	25	295	291	586	288
Mean		8,89	11,48	12,23	32,59	9,08	12,46	13,49	35,16
SD		2,54	1,85	1,56	3,65	3,09	1,96	1,56	4,52
Minimum		5	8,00	9,00	24,80	1,00	6,00	4,00	22,50
Maximum		15,00	15,00	15,00	39,00	16,00	16,00	16,00	45,75
Percentiles	5	5,45	8,30	9,35	25,61	4,25	9,00	11,00	26,86
	10	6,50	9,00	10,00	28,10	5,50	10,00	12,00	28,75
	25	6,88	10,00	11,00	29,75	6,75	11,00	13,00	32,50
	50	8,00	12,00	12,00	32,80	8,75	13,00	14,00	35,50
	75	10,88	13,00	13,00	35,15	11,00	14,00	15,00	38,00
	90	12,80	14,00	15,00	37,90	13,60	15,00	15,00	40,50
	95	14,48	14,70	15,00	38,85	15,30	15,00	16,00	42,89

THR = Threshold; DIS = Discrimination; ID = Identification; TDI = The sum of THR, DIS and ID.

MANCOVA Wilks' lambda revealed a significant main effect for 'Group', $F(4, 91) = 239.3$, $p < 0.0001$, $\eta^2 = 0.26$. The covariate analyses for 'Age' were significant, $F(4, 91) = 4.29$, $p < 0.05$, $\eta^2 = 0.23$). Because the MANCOVA revealed a significant main effect of group, examination of univariate ANCOVA analyses of the dependent variables was performed to identify which dependent variable contributed to the overall effect. Significant group effects emerged for the discrimination and identification scores ($p = 0.01$, $\eta^2 = 0.10$ and $p = 0.0001$, $\eta^2 = 0.20$, respectively). No significant 'Group' effect was found when the threshold and TDI scores were considered ($p > 0.05$, $\eta^2 = 0.05$). Significant covariate relationships were

also observed. 'Age' was significant for both the identification and the TDI scores ($p < 0.05$, $\eta^2 = 0.07$). Pearson's correlations indicated that as age increased identification and TDI scores significantly decreased in both the RRMS and the control group ($r = -0.27$, $p < 0.01$).

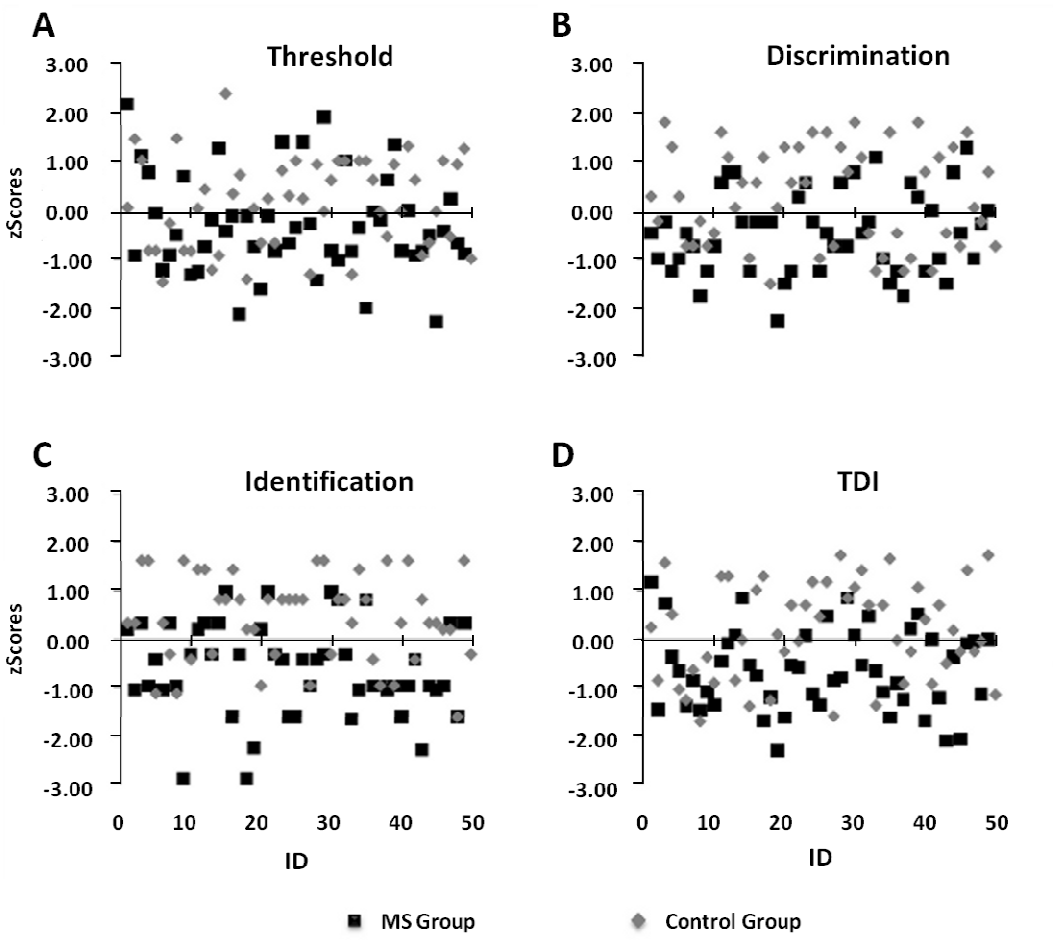


Figure 5.1. *MS and control group Sniffin' Sticks scores expressed with standard deviations from the age- and gender-matched normative population means. Panel A represents threshold scores, Panel B represents discrimination scores, Panel C represents identification scores and Panel D represents TDI scores.*

5.4.2 CORRELATING THE DURATION OF MS WITH OLFACTORY FUNCTIONS AND NEUROPATHOLOGICAL MARKERS

As shown in Table 5.3, no significant correlation was found between the duration of MS and the scores obtained with the Sniffin' Sticks Extended Test, the number and the volume of the plaques within the IFTL complex (Table 5.3) and the areas outside the IFTL complex in terms of plaque numbers and plaque volumes (Table 5.3).

Table 5.3. *Pearson's correlation between the years from the onset of MS, the Sniffin' Sticks scores and the neuropathological markers within and outside the IFTL complex.*

	Test olfactory scores				Plaques Number		Plaques Volume	
	THR	DIS	ID	TDI	Within IFTL	Outside IFTL	Within IFTL	Outside IFTL
Years onset	-0.10	-0.14	-0.22	-0.22	0.04	0.19	0.25	0.54

THR = Threshold; DIS = Discrimination; ID = Identification; TDI = The sum of THR, DIS and ID, IFLT = Inferior frontal and temporal lobes.

5.4.3 CORRELATING THE NUMBER AND THE VOLUME OF THE PLAQUES WITHIN AND OUTSIDE THE IFTL COMPLEX WITH OLFACTORY INDEXES

Correlations between the number and the volume of the plaques within the IFTL complex, in the whole brain excluding the IFTL complex (e.g., outside IFTLs) and the olfactory indexes were performed. The number and volume of plaque within the IFTL complex did not negatively correlate either with any of the olfactory indexes considered or with the areas outside the IFTL complex in terms of plaque number and plaque volume. To remove the effect of potential confounders such as age and disease duration, partial correlations were also performed (Table 5.4). Thus, no association between Sniffin' Sticks scores and neuropathological markers

within and outside the IFTL complex was demonstrated ($p > 0.05$). As an example of how the correlation between the number of plaques within the IFTL complex and the olfactory scores (i.e., TDI) might not be an optimal index, Figure 5.2 shows the MR images for two representative patients with the same sub-threshold performance (TDI = 29) on the olfactory tests, one with a small number of plaques (Figure 5.2, Panel A) and the other with a larger number (Figure 5.2, Panel B) of plaques.

Table 5.4. *Partial Correlation between the neuropathological marker and the Sniffin' Sticks scores controlling for age and years from MS onset.*

	Plaques Number		Plaques Volume	
	Within IFTL	Outside IFTL	Within IFTL	Outside IFTL
Threshold	-0.48	0.04	-0.36	-0.09
Discrimination	-0.01	-0.08	-0.10	-0.30
Identification	0.37	0.22	0.15	0.07
TDI	-0.20	0.08	-0.23	-0.15

IFTL = Inferior frontal and temporal lobes.

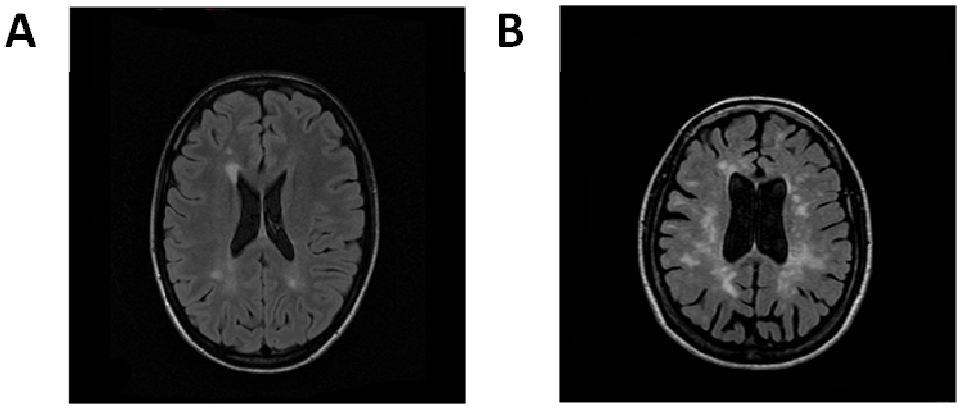


Figure 5.2. *Panel A represents an axial T2-weighted MRI scan for a hyposmic patient (TDI =29), a 30-year-old woman with a three-year history of MS. Panel B represents an axial T2-weighted MRI scan for a hyposmic patient (TDI = 29), a 39-year-old woman with an eight-year history of MS.*

5.5 Discussion

The main aim of the present study was to evaluate olfactory functions in a carefully selected group of female RRMS patients. Furthermore, we ascertained whether olfactory scores correlated with the number and volume of WMT2 lesions within (and outside) central eminent olfactory regions (IFTL complex).

Estimates of the prevalence of olfactory dysfunctions in MS vary widely (Ansari, 1976; Doty et al., 1998, 1999; Hawkes, Shephard, & Kobal, 1997; Kesslak et al., 1988). Previous studies only testing odour identification on unspecified MS-subtypes samples reported olfactory dysfunctions affecting 15% (Hawkes, Shephard, & Kobal, 1997), 35% and 38% (Doty et al., 1998, 1999; Wender & Szmeja, 1971) and 45% (Pinching, 1977) of the considered MS populations. Various factors, such as the administered olfactory test, the patients' selection criteria (i.e., age, gender, clinical form of MS), the interval of time since the last relapse/high-dose steroid therapy and concomitant medication affecting olfactory function (i.e., calcium-channel inhibitors, chemotherapeutics), might have contributed to these discrepancies. As an example, a study by Hawkes and colleagues (Hawkes, Shephard, & Kobal, 1997) included patients who had experienced a recent relapse and received a course of high-dose steroids, which could have modified their performance either at the nose level or within the central olfactory pathways. In our study, 34% of the RRMS participants tested in our clinical setting exhibited a decreased olfactory ability on smell testing, which is in line with previous

reports (Doty et al., 1998, 1999; Wender & Szmeja, 1971) but, importantly, was obtained from a carefully selected group of RRMS patients: they were all females, had not received drugs known to affect the olfactory system, did not smoke and they were far from the last relapse/steroid course.

To the best of our knowledge, no previous research has evaluated specific aspects of olfactory functions in order to obtain detailed information on the smelling ability of MS patients. In this perspective, our findings not only support a general loss in the sense of smell of MS patients, but also suggest the presence of specific olfactory dysfunctions. When comparing the scores for the odour discrimination and identification tasks between the RRMS and the control group, olfactory deficits within the RRMS sample did emerge. The odour threshold scores did not significantly discriminate between the RRMS and the control group. Taken altogether these findings are in collusion with previous research reporting that odour identification ability was, to a certain extent, compromised in MS patients (Doty, Li, et al., 1997; Doty et al., 1998, 1999; Hawkes, Shephard, & Kobal, 1997; Pinching, 1977; Wender & Szmeja, 1971).

Another aspect of the present findings is that both the identification and the TDI scores seemed to be significantly affected by age-related effects. In other words, part of the amount of variance for the global and identification components of olfactory performance was accounted for by age, in the older participants, for both the MS and the control group, who performed worse than younger participants. Nevertheless, the significant MANCOVA results, considering 'Age' as a

covariate, suggested that the specific impairment in odour identification and general olfactory loss is a disease-related progression of the olfactory deficit.

In neural terms, we expected a strong negative correlation between the number of WMT2 lesions detected within the regions of the frontal and temporal lobes involved in olfaction and the scores obtained for the different components of the olfactory test (Doty, Li, et al., 1997; Doty et al., 1999; Zorzon et al., 2000). Although we adopted a methodological approach similar to that of previous studies (Doty, Li, et al., 1997; Doty et al., 1998, 1999; Zorzon et al., 2000), we failed to demonstrate such a relationship. This result was also found for the patients who had the olfactory assessment and the MR scan on the same day. This is important because it might well be that from the time the MR assessment was conducted to the time the olfactory test was administered the number and volume of the WMT2 lesions might have changed. We acknowledge that, due to the limited number of patients who underwent MRI scanning, we might not be able to state definite conclusions, which may require further investigation in highly homogeneous samples of patients. However, it is worth mentioning that the studies which reported a strong correlation between the number of plaques and the UPSIT score considered an even smaller sample (Doty, Li, et al., 1997).

The finding of a lack of correlation between the decrease in olfactory performance and structural changes in central olfactory areas may suggest two alternative explanations. On the one hand, the olfactory dysfunctions in RRMS might be linked to a central functional rather than

a structural impairment. To this end, recent research has outlined a diffuse brain network activating task-specific regions while performing different olfactory tasks. Specifically, odour threshold is linked to the activation of right thalamus, amygdala-pyriform, cingulate, orbito-frontal and insular cortex whereas qualitative odour discrimination is known to engage thalamus, right caudate, subiculum, cingulate, orbito-frontal, pre-frontal, left insular and right cerebellar cortex (Savic, 2002). Odour identification, though sharing part of the odour discrimination activation (thalamus, cingulate, orbito-frontal, pre-frontal, left insular and right cerebellar cortex), recruits in addition pyriform cortex and parts of the temporal and parietal cortex (Savic, 2002). Given that an odour discrimination and identification loss is reported in our RRMS sample, it is tempting to speculate that the results of the present study might reflect the impairment of brain circuits engaged both in the odour discrimination and identification processes. Such an hypothesis seems to be supported by evidence from neurophysiological data considering the orbito- frontal cortex (OFC). As an example, Critchley and Rolls (1996b) demonstrated that the primate OFC responds in a highly selective way to olfactory stimuli. In a similar vein, neuroimaging studies report that OFC lesions in the human brain account for specific smell disturbances, such as odour discrimination and identification impairment (Jones-Gotman & Zatorre, 1988; Zatorre & Jones-Gotman, 1991).

On the other hand, olfactory dysfunctions in RRMS might reflect a peripheral rather than a central deficit. The possibility that the odour spatial map is primarily affected by the disease subsists. In the olfactory

system a spatial map for odour detection already exists in the periphery (Mombaerts et al., 1996) in which each olfactory neuron expresses just one of the 350 (in human) odorant receptors (Buck, 2004) and olfactory receptors also play an instructive role in determining the central projections of the olfactory neurons in which they are expressed (Wang, Nemes, Mendelsohn, & Axel, 1998). Since each receptor responds to several odour molecules as well as each odour molecule is capable to stimulate a variety of receptors, the mammalian olfactory system uses a combinatorial receptor coding scheme to identify and discriminate odours (Malnic et al., 1999). Thus, an impairment of such combinatorial coding may explain the deficits in identification and discrimination of odours in MS. In other words, slight alterations of this wiring diagram might be responsible for an impairment of olfactory performance in MS.

With respect to the plaques indexes considered, here we not only took into account the number of plaques, but we extended this literature by performing analysis of the volume of plaques within the IFTLs. At present, this measurement is considered to be less prone to the subjective judgment of the operator than the counting of plaques. The plaque volume analysis did not find any significant correlations with the olfactory scores.

In this respect, it is of interest to draw a parallel between the present findings on RRMS patients and those obtained for people affected either by Parkinson's disease (PD) or degenerative ataxias. The extensive literature on olfactory disturbances in PD (Müller, Müngersdorf, Reichmann, Strehle, & Hummel, 2002) also suggests that the olfactory

disorder is a sensitive sign of pathology. However, as found here, the olfactory loss in PD is unspecific and it does not correlate with disease severity (Doty, 2007). Furthermore, the present findings remind of those reported for degenerative ataxias patients, in which smell test scores do correlate neither with a genetic severity marker (GAA trinuclotide repeats) nor with disease duration (Connelly, Farmer, Lynch, & Doty, 2003).

Finally, the limitations of conventional MRI in depicting MS pathology may explain the discrepancies between the findings of the present study and previous ones. Several reviews and consensus statements over the past few years have questioned the value of current MR measures (e.g., T2 lesion burden, number of T2 lesions) as surrogate markers by noting that the correlation between these MRI measures and disability has been relatively poor (Nyul & Udupa, 1999; Udupa & Nyul, 2001). Indeed, WMT2 lesion burden does not reflect the complexity of MS pathology, which includes cortical demyelinization and atrophy, spinal cord involvement and subtle biochemical alterations in the normally-appearing white matter (Barkhof, 2002; Miller, Grossman, Reingold, & McFarland, 1998).

Our data confirms and extends previous findings on the evaluation of olfactory functions in MS patients. They outline the importance of using highly reliable tests able to capture more finely-grained aspects of olfactory performance together with the use of more carefully selected population samples. Although no correlations were identified between the olfactory scores and the neuropathological markers, we suggest that

future research on this issue should consider less operator-dependent measures such as plaques volume. Moreover, it will be of great interest to explore if (and to which extent) MS patients share the same functional activations in brain circuits engaged in olfactory performance when compared to control participants. Furthermore, having insights on the molecular basis of the peripheral functioning in MS patients may help in clarifying the causal relationship of the olfactory impairment found in this population. Future research should also consider testing of the olfactory ability in pre-clinical, primary and secondary progressive homogeneous MS samples in order to elucidate the features of olfactory loss throughout the natural course of MS. This might open the possibility to identify some markers, either functional or molecular, as to improve patients' outcomes. Since the sense of smell is fundamental for the quality of life, intended both in terms of survival mechanisms (e.g. detection and identification of potentially dangerous events signalled through smoke, the leaking of natural gas and spoiled foods) and in terms of daily well-being (e.g. appreciation of food, nutritional status, mood rate and social interactions), we suggest that a precise assessment of olfactory functions might be routinely performed in MS patients.

CHAPTER 6

IMPLICIT OLFACTORY PROCESSING IN TRAUMATIC BRAIN INJURED PATIENTS [2]

6.1 ABSTRACT

It is now well established that olfactory loss is a common outcome within traumatic brain injured (TBI) patients. Nevertheless, the issue of implicit olfactory processing has never been tested in this population. To investigate this issue an olfacto-motor priming paradigm has been administered to a group of anosmic TBI patients. A group of age- and gender-matched normosmic/mildly microsmic TBI patients and a group of neurologically healthy participants served as controls. Olfacto-motor priming allows to reveal whether implicit olfactory processing is preserved by indirectly investigating the effect of subliminally perceived odours on the motor control of the hand. In detail, participants were asked to perform reach-to-grasp movements towards large or small visual targets following the presentation of olfactory cues - which anticipate or not the size of the visual target. Odours were delivered via a computer-controlled olfactometer and hand kinematics were recorded by means of a three-dimensional motion analysis system (SMART-D). For all the groups, an interference effect was revealed when participants grasped a large

[2] *Submitted*: Parma, V., Straulino, E., Zanatto, D., Cantagallo, A., Tirindelli, R., & Castiello, U. (2011). Implicit olfactory processing in traumatic injured patients. *Journal of Clinical and Experimental Neuropsychology*.

visual target preceded by a 'small' odour. Maximum velocity of grip aperture was greater than when the same target was grasped preceded by a 'large' odour or no odour. The present results suggest that some form of implicit olfactory processing is preserved in TBI patients even when diagnosed as anosmic on the basis of explicit olfactory testing. Future studies would seem warranted in view of the hypothesis of new rehabilitation strategies for these patients.

6.2 Introduction

Head trauma (or traumatic brain injury, TBI) is a diffuse cause of disability (and death) in the adult population (Bruns & Hauser, 2003; Costanzo & Zasler, 1991). TBI is a multifaceted pathological phenomenon, which cannot be ascribed within a unidimensional classification. From a physical perspective, it results from the effect of mechanical forces occurring at the moment of trauma - such as laceration of brain tissue, diffuse white matter damage, intracerebral haemorrhage, or hematoma (primary mechanisms, Adams, Doyle, Ford, Gennarelli, Graham, & McLellan, 1989) - or in a second moment - as in the case of hypoxia, intracranial hypertension, or cerebral edema (secondary mechanisms, Pitts & McIntosh, 1990). From a clinical perspective, TBI patients incur in deficits both in the cognitive (memory, attention, language, executive functions; National Institute of Health, 1999), psychosocial (emotion regulation; Cunningham, Chan, Jones, Kramnetz, Stoll, & Calabresa, 1999) and in the sensorimotor domains (visual, auditory, proprioceptive,

olfactory, and gustatory; Lynch, 1986). Of relevance, the investigation of sensory impairement following head trauma has not been confined to the most studied sensory modalities (e.g., vision, audition, and touch), but it has also been extended to the chemical senses. As a result, head trauma is now paradigmatically remembered as an example of pathology presenting moderate or severe olfactory disturbance.

Post-traumatic olfactory loss (PTOL) is the third most common aetiology for olfactory disorders (Collet Grulois, Bertrand, & Rombaux, 2009) and it accounts for 4-15% of the chemosensory disturbance in the general population (Doty, Yousem, et al., 1997). PTOL has usually been reported following frontal basal injuries as well as occipital blows (Doty, Yousem, et al., 1997; Fujii, Fukazawa, Takayasu, & Sakagami, 2002; Sumner, 1964). Differently from other pathologies, such as MS and PD (see Chapter 5 of the present thesis, Mesholam et al., 1998), the likelihood of completely losing the ability to smell is directly correlated to the severity of the trauma and to the mechanical characteristics of the impact (e.g., strong acceleration/ deceleration of the head). The most extremes forms of PTOL are presumably due to a coup-contrecoup mechanism responsible for the shearing of olfactory nerves penetrating the cribriform plate (Zusho, 1982) or to contusions or secondary hemorrhages within the central olfactory areas (Reden et al., 2006).

The number of patients complaining olfactory loss is higher in those presenting frontal and occipital lobes insults, when compared to patients presenting traumatic lesions outside these areas (Doty, Yousem, et al., 1997). However, as a general rule, patients have poor awareness of their

olfactory dysfunctions, especially when it is associated with other neurological deficits (Callahan & Hinkebein, 2002).

PTOL prognosis may vary widely. Overall, it results in a distorted perception of flavours and its iatrogenic effect has been documented in terms of decreased quality of life, safety, social relationships, and dietary intake (Corydon Hammond, 2009). When possible, recovery occurs, on average, within the first year from the injury even though, recent research suggests that belated improvement of olfactory function might occur (London, Nabet, Fisher, White, Sammel, & Doty, 2008). Nevertheless, the likelihood of recovery to functional smell abilities hinges upon the integrity of the brain regions involved in olfactory processing.

At a neural level, head trauma presents multiple and various landscapes, which cannot be traced back to regular patterns, as it has been done for other pathologies (e.g., Braak, Del Tredici, Rüb, de Vos, Janse Steur, & Braak, 2003; Braak et al., 2004). Nevertheless, the dispersed nature of the olfactory system within cortical and subcortical regions facilitates the fact that traumatic lesions involve, at least in part, the areas concerned with olfactory processing. Evidence from neuroimaging studies indicate that a damage at the level of eminent olfactory regions, such as enthorhinal cortex or orbitofrontal cortex, is associated with poor performances at olfactory behavioural tasks (Atigechi, Salari, Baradarantar, Jafari, Karimi, & Mirjali, et al., 2009; Bonanni et al., 2006; Fujiwara, Schwartz, Gao, Black, & Levine, 2008; Geisler, Schlotfeldt, Middleton, Dulay, & Murphy, 1999; Haxel, Grant, & Mackay-Sim, 2008; Mann & Vento, 2008; Roberts, Sheehan, Thurber, &

Roberts, 2010; Sandford et al., 2006; Yousem et al., 1996). Nevertheless, the present bulk of studies, as well as those only considering patients performance at olfactory psychophysical tests (Callahan & Hinkebein, 1999, 2002; De Kruiijk, Leffers, Menheere, Meerhoff, Rutten, & Twijnstra, 2003; Fortin, Lefebvre, & Ptito, 2010; Green & Iverson, 2001; Green, Rohling, Iverson, & Gervais, 2003; Landis et al., 2010; Sigurdardottir, Jerstad, Andelic, Roe, & Schanke, 2010; Swann, Bauza-Rodriguez, Currans, Riley, & Shukla, 2006) applied testing methods which require some specific cognitive functions to be intact. To date, as to succeed in the completion of tests such as the UPSIT (Doty, Shaman, & Dann, 1984) and the Sniffin' Sticks Extended Test (Kobal et al., 1996), unharmed verbal and memory skills are needed (Olsson et al., 2002). But, TBI patients are frequently diagnosed with language and memory disturbance (Jennet & Teasdale, 1981; Teasdale & Mendelow, 1984), indicating that the conclusions descending from the abovementioned studies should be taken into account with a certain degree of caution.

A further point worth noting is that, still in everyday life, it is a hard task to correctly label the name of an odour (de Wijk & Cain, 1994; Engen, 1987). Our daily experience suggests that odours are mingle within each other, making difficult even to discriminate - without naming them - the odours we simoultaneously encounter. These are examples of the fact that the learning experience of dealing with odours primarily occurs unintentionally and subliminally (Issanchou et al., 2002; Wilson & Stevenson, 2006). Together with the scattered nature of olfactory circuits, this might indicate that different, and partially independent, mechanisms

of odour processing might exist. Then, it is reasonable to think that explicit (language-mediated) and implicit (non-linguistic) forms of olfactory processing coexist in order to cover all the aspects of the multifaceted world of odours.

To the best of our knowledge, no studies have yet investigated whether (and possibly, how) implicit forms of olfactory processing takes place in TBI patients. However, recent research concerning the role of olfaction in sensorimotor control might help to handle this endeavour (Castiello et al., 2006; Tubaldi, Ansuini, Dematté, et al., 2008; Tubaldi, Ansuini, Tirindelli, et al., 2008). In these studies, visually guided reach-to-grasp movements performed in the presence of olfactory task-irrelevant stimuli were investigated. The olfactory cue could evoke an object similar or dissimilar in size when compared to the visual to-be-grasped target. The 'size' incongruency between the odour and the visual target determined interference effects evident on the kinematic variables of the manipulation phase of the reach-to-grasp movement. As an example, if the olfactory stimulus evoked an object smaller than the visual target, then the maximum hand aperture was smaller than when no-odour was presented. Similarly, if the olfactory stimulus evoked an object larger than the visual target, then maximum hand aperture was larger than when grasping the same visual target occurred in the absence of any olfactory information. The 'size' congruency between the odour and the visual to-be-grasped target showed facilitation effects in the very same kinematic parameters. For instance, maximum hand aperture was smaller than when

the visual stimulus did not correspond to the olfactory stimulus or the olfactory stimulus was not present.

Altogether such evidence indicates that, although the olfactory stimulus was irrelevant for fulfilling the task, it was nevertheless implicitly elaborated in motor terms such as to interfere with, or facilitate, the motor plan established for the to-be-grasped target.

On the basis of previous evidence (Castiello et al., 2006; Tubaldi, Ansuini, Tirindelli, et al., 2008; Chapter 7 of the present thesis), we hypothesized that, if some sort of implicit processing of olfactory stimuli is preserved in TBI patients, then this might be reflected in terms of either motor behaviour facilitation or interference. Thus, we asked a group of anosmic TBI (aTBI) patients to execute reach-to-grasp movements in the direction of visual targets different in size following the presentation of olfactory cues, which could be size-congruent, incongruent or non-existent. For comparison purposes, the performance of this group was matched with the performance of two control groups. In the first, TBI patients showing similar cognitive and psychosocial abilities as the aTBI patients, but without severe olfactory deficits, were recruited. In the second, neurologically healthy participants were enlisted.

Focusing on the kinematic variables belonging to the reach-to-grasp movement phase recognized as the elective for such an approach (Castiello et al., 2006; Tubaldi, Ansuini, Tirindelli, et al., 2008), we expect that, when the object evoked by the odour has similar structural features as the visual target, then facilitation effects should be evident.

Conversely, when the object evoked by the odour has incongruent structural features as the visual target, then interference effects should be observed. In line with this prediction, we foresee that the kinematic variables of the manipulation phase would be affected by the size conveyed by the odour cue. If implicit olfactory processing is preserved in aTBI patients, we expect that this group would be affected by task-irrelevant odours as well as normosmic/mildly microsmic TBI (nTBI) patients and healthy controls groups.

6.3 METHODS

6.3.1 PARTICIPANTS

The study included 12 patients diagnosed with severe head trauma on the basis of the Glasgw Coma Scale (GCS = 3 to 8; Teasdale & Jennett, 1974) and the Level of Cognitive Functioning Scale scores (LCF > 5; Gouvier, Blanton, LaPorte, & Nepomuceno, 1987). 12 age- and gender-matched controls were recruited for comparison purposes. The sample was composed by 83% males. Participants were divided into three groups (Table 6.1) considering their olfactory abilities as determined by the scores obtained at the UPSIT (Doty, Shaman, & Dann, 1984; Appendix C). The group of aTBI patients included 6 participants (mean age = 38.68 years, sd = 9.12 years), 5 patients formed the nTBI group (mean age = 39.46 years, sd = 8.38 years). Both patients and controls were tested with the: (i) BDI-II (Beck et al., 1996), (ii) BAI (Beck et al., 1990), (iii) Raven's Progressive Matrices (PM, Raven, 1954), (iv) Trial Making Test (TMT,

Reitan, 1955), (v) Wisconsin Card Sorting Test (WCST, Heaton, Chelune, Talley, Kay, & Curtiss, 1999), (vi) Verbal span (Spinnler & Tognoni, 1987) to check for depression, anxiety and/or cognitive impairment at the time of olfactory testing. When compared to neurologically healthy participants, aTBI patients reported a significantly higher depressive symptoms (BDI-II) and poorer performance at the TMT, which gives indications on the attentional/executive abilities of participants ($p < 0.05$). No significant difference was found when comparing the anosmic with the normosmic/mildly microsmic groups ($p > 0.05$). Moreover, participants presenting aphasia, apraxia, ataxia, drugs abuse, and previous neurological diseases were excluded from the present sample. The Edinburgh Handedness Inventory (Oldfield, 1971; Appendix D) was used in order to determine hand preference. Finally, a questionnaire was administered to all participants to evaluate the previous history of nasal disease, smoking habits and the current subjective status of olfactory functions (adapted from Zucco et al., 2006; Appendix A). All participants were naïve as to the purpose of the investigation and gave informed written consent to participate in the study. The experimental procedures were approved by the Institutional Review Board at the University of Padova in accordance with the Declaration of Helsinki.

Table 6.1. Demographic data and clinical features of the Traumatic Brain Injured patients and the control participants.

Group	Age (years)	Gender	Education (years)	UPSIT score (raw score)	BDI-II (ES)	B-A TMT (ES)	CPM Raven (ES)	Verbal span (ES)
aTBI	46	M	10	7	> 99	3	2	4
aTBI	30	M	13	8	< 85	0	4	5
aTBI	33	M	13	13	> 99	1	4	4
aTBI	51	M	17	17	> 95	4	4	5
aTBI	30	F	18	20	< 85	3	4	6
aTBI	32	M	8	20	85-90	3	4	5
nTBI	29	M	13	25	< 85	4	4	4
nTBI	39	M	13	25	85-90	3	4	6
nTBI	38	F	18	27	< 85	1	4	5
nTBI	38	M	18	29	< 85	2	4	5
nTBI	40	M	18	28	< 85	1	4	5
nTBI	49	M	7	35	< 85	4	4	5
Control	29	M	13	26	< 85	4	4	4
Control	34	M	18	28	< 85	3	4	5
Control	52	M	18	30	< 85	4	4	5
Control	56	M	8	30	< 85	4	4	6
Control	32	M	18	31	< 85	3	3	3
Control	51	M	8	32	< 85	4	4	5
Control	39	M	18	33	< 85	3	6	5
Control	35	F	18	34	< 85	3	4	5
Control	40	M	18	36	85-90	3	5	6
Control	37	M	18	37	< 85	3	4	7
Control	29	F	13	38	< 85	3	6	6

aTBI: anosmic traumatic brain injured patients; nTBI: normosmic/mildly microsmic traumatic brain injured patients, control: neurologically healthy control participants; M = males; F ? females; BDI-II = Beck depression inventory; ES = equivalent score; B-A TMT = Trail making test version B-A; CPM = Color progressive matrices.

6.3.2 *STIMULI AND APPARATUS*

The visual stimuli consisted of four plastic objects grouped on the basis of their natural size: large (apple, orange) and small (almond, strawberry). Plastic objects were used in order to maintain consistent visual attributes and sizes similar throughout the period of experimentation. The odour stimuli corresponded to the target stimuli described above. Odour solutions of strawberry, almond, orange, and apple were obtained mixing 6000 µl of prophylene glycol and 180 µl (3%), 60 µl (1%), 420 µl (7%), and 45 µl (0.75%) of the specific odorant compound, respectively. The fruit odours were rated as isointense to each other ($p > 0.05$) - but significantly more intense than propylene glycol ($p < 0.05$) - by 43 participants, who smelled the odours for 3 seconds and judged the perceived intensity of

each stimulus on a visual analogous scale anchored to 'Not intense at all' to 'Extremely intense' polarities.

As depicted in Figure 6.1, the visual/olfactory stimuli combinations produced six experimental conditions: (i) congruent large (LL) in which both the odour and the visual target evoked a large object (e.g., orange–apple); (ii) congruent small (SS) in which both the odour and the visual target evoked a small object (e.g., strawberry–almond); (iii) incongruent large (LS) in which the odour evoked a large object but the visual target evoked a small object (e.g., orange–almond); (iv) incongruent small (SL) in which the odour evoked a small object and the visual target evoked a large object (e.g., strawberry–apple); (v) control large (NoL) in which the odour stimulus was odourless air and the visual target evoked a large object (e.g., air–apple); and (vi) control small (NoS) in which the odour stimulus was odourless air and the visual target evoked a small object (e.g., air–almond).

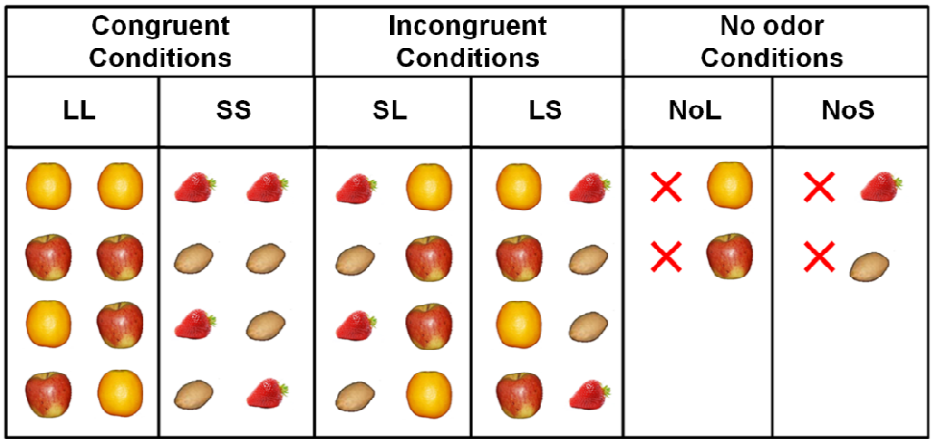


Figure 6.1. *From left to right columns report the congruent, incongruent and no odour experimental conditions resulting from the combination of olfactory (first drawing of each couple within a column) and visual (second drawing of each couple within a column) stimulations. LL: congruent large condition; SS: congruent small condition; SL: incongruent large condition; LS: incongruent small condition; NoL: no odour large condition; NoS: no odour small condition.*

A custom-built computer-controlled olfactometer was used to deliver the odour stimuli or odourless air. Each of the 4 to-be-delivered olfactory stimuli was contained in one glass boat. As to deliver odourless air, a fifth glass boat contained propylene glycol. The air entered the glass boats at a flow rate of 8 l/min and the resulting odourous and odourless air were delivered to participants via Teflon tubing to a facial mask.

Movements were recorded by means of a three-dimensional motion analysis system (SMART-D; BTS, Garbagnate Milanese, Italy) equipped with six- infrared-cameras (frequency: 140Hz) recording the position of three passive markers (diameter=0.25cm). Markers were fastened using double-sided tape to (i) the wrist, (ii) the tip of the index finger, (iii) the tip of the thumb of the participants' right hand, (iv) and to the plastic object. Co-ordinates of the markers were reconstructed with an accuracy of 0.2 mm over the field of view. The standard deviation of the

reconstruction error was 0.2 mm for the vertical (Y) and horizontal (X and Z) axes. Data were reconstructed, filtered (10Hz) and analyzed with the SMART- D analyzer software.

Vision was controlled using spectacles fitted with liquid crystal lenses that rendered the target visually accessible by changing from opaque to clear (Plato Technologies, Toronto, Canada). At the beginning of each trial, participants placed their right hand on a starting platform within which a pressure sensitive switch was embedded. Relevant kinematic parameters of the manipulation phase of the reach to grasp movement, such as the velocity of maximum grip aperture, were analysed.

6.3.3 PROCEDURES

The target was aligned with the participant's body midline and located at 33 cm distance from the hand starting position. The right hand of each participant rested on a starting pad with the index finger and the thumb gently opposed (Figure 6.2). The sequence of events for each trial was as follows: (i) vision was occluded before (500 ms) the target was positioned on the working surface; (ii) an auditory tone indicated odour delivery; (iii) after 3 s, a similar tone indicated the offset of odour delivery; (iv) following a 500 ms interval the tone was presented again; (v) upon hearing the tone, participants were instructed to reach towards, grasp and lift the visual target. We instructed the participants to reach at a natural speed and not to grasp the object by the stem. The experimenter visually monitored each trial to ensure participant's compliance to these

requirements. Participants naturally grasped the small visual targets between the thumb and the index and, occasionally, the middle fingers and the large visual targets opposing the thumb with all the other fingers. In order to evaluate how participants grasped the targets a pre-test session was executed. Participants performed a total of 48 trials (12 for each experimental condition) which were presented in randomized order within four blocks.

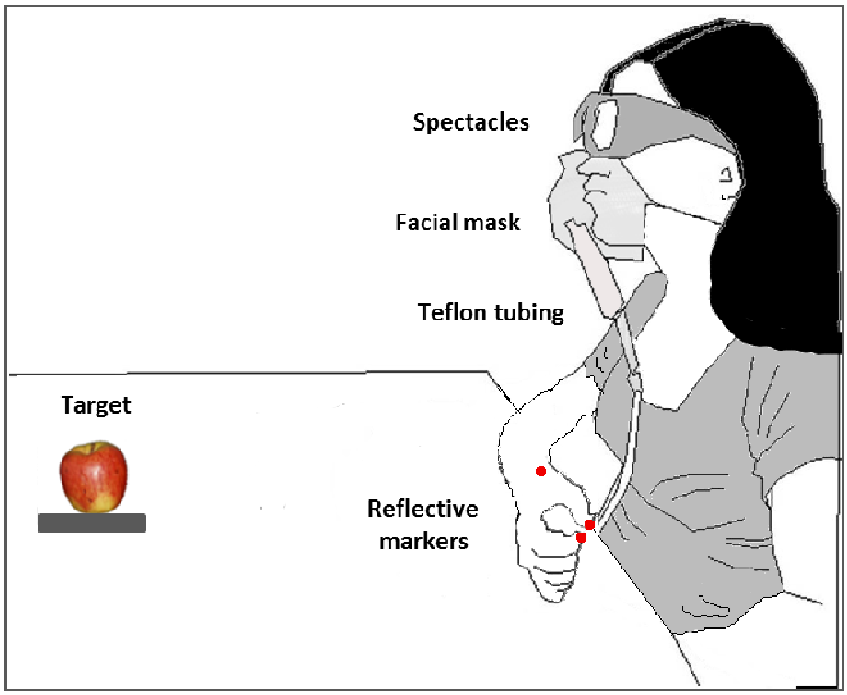


Figure 6.2. *Graphical representation of the experimental set-up. Legends indicate the relevant details.*

6.3.4 DATA ANALYSIS

To test for possible differences in the parameters of the manipulation phase as a function of experimental conditions a mixed Analysis of

Variance (ANOVA) with 'Olfactory condition' (congruent, incongruent, control) and 'Target size' (small, large) as within-participant factors and 'Group' (aTBI, nTBI, control) as between-participant factor was performed. Simple effects were used to explore the means of interest. Bonferroni corrections (α-level: $p < 0.05$) were applied, when required.

6.4 Results

Among the kinematic parameters of the manipulation phase of the reach-to-grasp movement, the variable that brought to significant results was the maximum velocity of grip aperture.

6.4.1 Maximum velocity of grip aperture

The ANOVA revealed a significant two-way interaction 'Olfactory condition by Target size', $F(2, 40) = 11{,}94$, $p < 0.001$, $\eta_p^2 = 0.37$. No effect of 'Group' was shown, $F(4, 40) = 0{,}87$, $p > 0.05$, $\eta_p^2 = 0.03$. As represented in Figure 6.3, aTBI patients speeded up grip aperture when a 'small' odour, rather than a 'large' olfactory cue or no odour, preceded the presentation of a large to-be-grasped object ($p < 0.05$). Similarly, aTBI patients exposed to a 'large' odour, rather than a 'small' olfactory cue or no odour, slowed down the velocity of grip aperture when grasping for a small visual target ($p < 0.05$). A similar pattern of results emerged also for the nTBI and the control groups (Figure 6.3). Based on the odour anticipating the reach-to-grasp movement, all of the three groups showed an interference effect on the maximum velocity of grip aperture. The comparison between

congruent and no odour conditions for both the large and the small object did not reveal any facilitation effect ($p > 0.05$), exception made for the nTBI group when grasping large objects ($p < 0.05$).

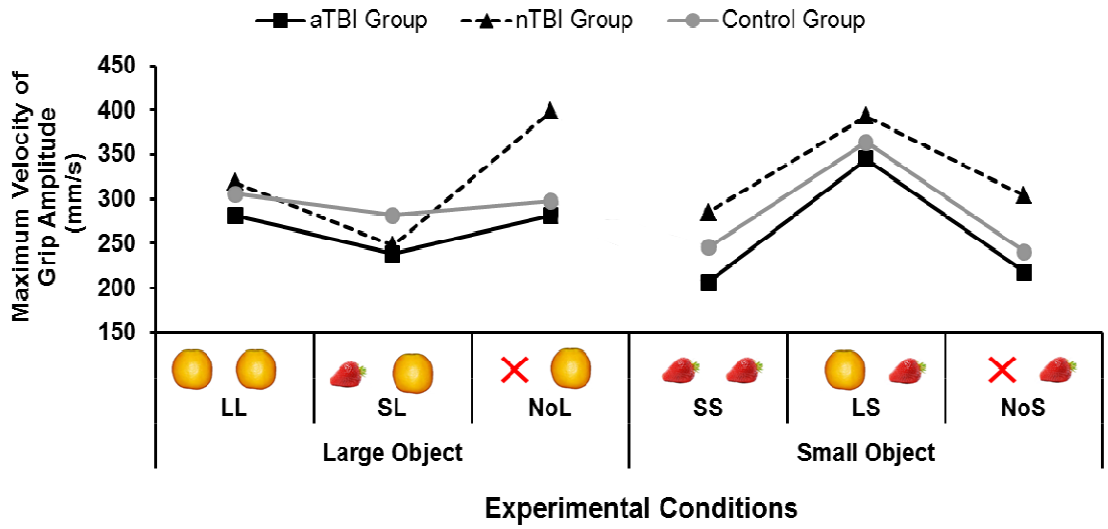


Figure 6.3. *Lines represent the maximum velocity of grip aperture expressed in mm/s for the anosmic TBI group (black solid line), normosmic/microsmic TBI group (grey dotted line) and healthy participants (black dashed line) for the six experimental conditions tested (from left to right: congruent, incongruent, no odour condition for the large and for the small targets, respectively). LL: congruent large condition; SS: congruent small condition; SL: incongruent large condition; LS: incongruent small condition; NoL: no odour large condition; NoS: no odour small condition.*

6.5 Discussion

The purpose of the present study was to evaluate the possible existence of lingering implicit odour processing in anosmic TBI patients. The present findings indicate that aTBI patients do show some form of residual subliminal processing of olfactory stimuli. As for normosmic/microsmic TBI patients and neurologically healthy participants, for the aTBI group the presentation of an odour incongruent with the size of the visual target interfered with the execution of the reach-to-grasp movement. This occurred independently of the size of the to-be-grasped target. However,

no facilitation effect emerged from the comparison of the congruent and the no odour conditions. Altogether, the present findings seems to suggest that the anticipation of target size information via an odour cue has the potency to affect the motor control of the hand in these patients, revealing an actual implicit olfactory elaboration. This is a relevant finding given that TBI patients diagnosed with anosmia are thought to be completely unable to adequately react to odours.

Running parallel to the olfactory issue, the present work also provides novel insights into the parameterization of the reach-to-grasp movement in TBI patients, a population which have never been tested with this experimental window. The outcomes obtained from the analyses of the no odour conditions indicate that TBI patients attempt to shape grip aperture in accordance with the size of the object.

With specific reference to olfaction, the findings reported here confirm previous evidence on healthy participants (Castiello et al., 2006; Tubaldi, Ansuini, Tirindelli, et al., 2008) and extend it to both TBI patients, either anosmic or not. Specifically, the occurrence of an interference effect reveal that the planning of the reach-to-grasp action is rooted on the irrelevant olfactory information preceding the sight of the to-be-grasped target. Put differently, the motor plan subliminally activated by the 'size' of the incongruent odour leak into the motor plan specifically tailored to grasp the visual target. This parallel activation of two motor representations based on different structural properties, elicited by an olfactory cue incongruent to the visual target, well explains the differences evident at the kinematic level.

When the 'size' of the odour did match the size of the visual target, facilitation effects were expected. But, they did not emerge for any of the groups considered here. A possible explanation for this negative outcome might be that the maximum velocity of grip aperture is not sufficiently fine-grined as to discriminate between the contribution of different sensory modalities conveying the equivalent information as to accomplish the task goal. Alternatively, having two sensory systems signalling the very same structural information might contribute to the execution of a more stable action.

Further, the present findings suggest the existence of different and dissociable mechanisms responsible for olfactory processing. Olfactory deficits in TBI patients have been moslty described in behavioural terms on the basis of odour recognition tests (Bonanni et al., 2006; De Kruiijk et al., 2003; Fortin et al., 2010; Fujiwara et al., 2008; Geisler et al., 1999; Green et al., 2003; Roberts et al., 2010; Sandford et al., 2006; Sigurdardottir et al., 2010; Swann et al., 2006; Yousem et al., 1996). Only occasionally, attempts have been made to extend the evaluation of TBI olfactory abilities to odour discrimination and threshold (Haxel et al., 2008; Landis et al., 2010). Nevertheless, this kind of psychophysical tests require the integrity of some cognitive functions in order to efficiently complete the task (Olsson et al., 2002). That is, efficient verbal and memory skills are compulsory. However, these functions are usually compromised in people presenting head trauma outcomes (e.g., Jennet & Teasdale, 1981). Thus, it is not surprising that these patients fail when tested with classical explicit olfactory methods. Neverthless, the present

results suggest that TBI patients might not require conscious recollection of olfactory stimuli, and therefore the integrity of structures deputated to explicit memory functions, supporting the evidence that both storage of and access to olfactory information might be automatic and implicit (e.g., Zucco, 2003).

Moving to a neural level, which might be the cerebral substrates regulating implicit odour processing? Whenever undamaged, the amygdala might be suggested as an appropriate contributor. Amygdala is a region embedded within the rhinencephalon (Bargmann & Schadé, 1963), it is physically close to and widely interconnected with the primary olfactory brain areas (Price, 1990), and it is has an active role in emotional regulation (Le Doux, 2000). For these reasons, it might mediate olfactory information - especially those related to survival decisions (Koenig, Bourron, & Royet, 2000) - which appears to be detached from higher mental functions. Support to this contention comes also from the fact that olfaction is the only sense which bypass first-relay/direct connections with the thalamus, a structure apparently involved in conscious processes (Plailly et al., 2008).

The connections between amygdala and OFC suggest that this latter region also contributes to subliminal olfactory perception (Price, 1990). Moreover, its role in multisensory integration of stimuli serving the guidance of goal-directed behaviour well fit with the implicit nature of the odour processing here described. These two areas might work in team as the amygdala may encode the significance of cues and subsequently the OFC might work as a center for multisensory appraisal, guiding

functional goal-directed behaviours rooted on information accessed through various interconnected structures, amygdala *in primis* (Schoenbaum, Chiba, & Gallagher, 1998).

This hypothesis seems to be supported by the interference effects found in the TBI patients when the visual and the olfactory stimuli did not match. Evidence from neuroimaging (Gottfried & Dolan, 2003; Österbauer, Matthews, Jenkinson, Beckmann, Hansen, & Calvert, 2005) and neurophysiological studies (Grigor, 1995; Grigor, Van Toller, Behan, & Richardson, 1999; Rolls & Baylis, 1994; Sarfarazi, Cave, Richardson, Behan, & Sedgwick, 1999; Stein & Meredith, 1990) indicate that the manipulation of the level of congruency between visual and olfactory stimuli correlates with a compatible modulation of the neural activity of OFC.

In order to fully account for the present results, the visuo-olfactory representation formed within the OFC on the basis of amygdala odour inputs needs to be translated in motor terms. In this respect direct connection between OFC and motor areas involved in arm-hand movement control have been traced (Cavada et al., 2000; Morecraft & Van Hoesen, 1993). In the light of the commonly accepted homology between cerebral regions underlying reach-to-grasp movements in monkeys and humans (for review see Castiello, 2005), it is tempting to posit that the corticocortical connections between OFC and motor areas (e.g., Bates & Goldman-Rakic, 1993) can justify the multisensory modulation of olfactory-visual information on motor behaviour in general and, specifically, on grasping actions (Rossi et al., 2010).

To sum up, TBI patients' prehensile movements may be affected by the chain of neural events beginning with implicit odorant encoding occurring at the amygdala level, continuing within OFC and, finally, reaching central motor areas. This is the hypothesized mechanisms at the basis of the preserved implicit olfactory processing in TBI patients.

However, before drawing definite conclusions on this issue, some limitations of the present study should be outlined. Most importantly, it would be of help increasing the sample size. For the sake of homogeneity, we were forced to exclude a number of potential participants. As an example, the severity of the impairment did not allow for sufficient compliance to task instructions. In this respect, think of the difficulties showed by patients presenting frontal and temporal lobe lesions in planning and executing chains of tasks such as that described in the present study. As another example, patients diagnosed with severe head trauma might become easily tired and, therefore, might not complete the experimental session and quit the evaluation. In the second instance, it would be interesting to administer these patients with odours conveying biologically relevant information. This in order to shape how TBI patients deal with social chemosignals and to evaluate whether this might be of some help in facilitating adequate social skills, that are frequently affected in these patients. Moreover, even though odours are almost fully neglected in rehabilitation, the present paradigm might be used to train crossmodal attention, an ability which allow us to adaptively navigate the environment. Finally, in the light of the strict link between neural structures regulating emotions and olfactory stimuli, odours might serve

to rehearse autobiographical events, contributing to personal orientation and an improved quality of life.

CHAPTER 7

IMPLICIT OLFACTORY PROCESSING ATTENUATES MOTOR DISTURBANCES IN IDIOPATHIC PARKINSON'S DISEASE [3]

7.1 ABSTRACT

Many reports in the literature indicate that idiopathic Parkinson's disease patients have substantial olfactory dysfunctions even before motor symptoms become evident. It has not yet been clarified, however, if some form of implicit olfactory processing is preserved in this population. An olfacto-motor priming paradigm, which detects implicit olfactory processing in neurologically healthy participants, was utilized to investigate motor control in relation to olfactory signals in a group of idiopathic Parkinson's disease patients. Two control groups were also considered: 12 vascular Parkinson's disease patients in whom normal olfactory abilities are typically reported and 12 neurologically healthy participants. All of the participants were asked to perform reach-to-grasp movements towards large or small targets following olfactory cues delivered by a computer-controlled olfactometer. The odour was either 'size' congruent with the target (e.g., strawberry or apple, respectively) or incongruent (e.g., apple or strawberry, respectively). A bend sensor glove (CyberGlove) was used to measure the hand kinematics. Facilitation

[3] *Submitted*: Parma, V., Bulgheroni, M., Scaravilli, T., Tirindelli, R., & Castiello, U. (2011). Implicit olfactory processing attenuates motor disturbances in idiopathic Parkinson's disease. *Cortex*.

effects were noted in all the groups with regard to movement time. If a congruent rather than an incongruent odour was delivered, the movement time of the reach-to-grasp was shortened and facilitation effects in maximum grip amplitude were noted in both the idiopathic Parkinson's disease and the vascular Parkinson's disease groups. The maximum grip amplitude was smaller when no odour, as compared to a congruent odour, was delivered. The present results suggest that implicit olfactory processing affects motor control in idiopathic Parkinson's disease patients favoring less severe bradykinesia and hand movement hypometria. Once confirmed, these findings could be useful when rehabilitation strategies are being hypothesized for these patients.

7.2 Introduction

PD is principally characterized by motor disturbances which are often the reason these patients seek their physicians' attention. These disturbances reflect, at least in part, a pathological loss of dopaminergic neurons in the ventral midbrain and nerve terminal degeneration in the striatum (Bernheimer, Birkmayer, Hornykiewicz, Jellinger, & Seitelberger, 1973). The greater the neuronal loss in the substantia nigra, the lower the concentration of dopamine in the striatum, and the more severe symptoms are in these patients. Typically, by the time PD is clinically diagnosed, a significant loss of dopaminergic neurons has already occurred.

Although a progressive loss of nigral neurons is considered an essential neuropathological feature, recent findings in the literature seem to suggest that PD is characterized by a variety of symptoms which go beyond motor disturbances (Braak, Del Tredici, Rüb, de Vos, Jansen Steur, & Braak, 2003; Braak et al., 2004; Chaudhuri, Healy, & Schapira, 2006; Ziemssen & Reichmann, 2007). A great deal of attention has been paid to PD-related non-motor symptoms such as sensory disorders, autonomic dysfunctions, mood and sleep disorders, cognitive deficits and hyposmia which appear to be perceptible even before motor parkinsonism becomes explicit (Braak et al., 2003, 2004; Wolters & Braak, 2006).

Olfactory dysfunction is a non-motor symptom that has long been described in patients with PD (Doty, 2003). A significant decrease in odour detection, discrimination, and identification has, in fact, frequently been reported in PD patients with respect to neurologically healthy controls (Ansari & Johnson, 1975; Double et al., 2003; Hawkes, Shephard, & Daniel, 1997; Doty et al., 1999; Korten & Meulstee, 1980; Quinn, Rossor, & Marsden, 1987).

Structures such as olfactory bulbs, olfactory tracts, and/or the anterior olfactory nuclei appear to be affected early during disease development (Braak et al., 2003, 2004; Del Tredici, Rüb, de Vos, Bohl, & Braak, 2002; Tissingh et al., 2001). Although olfactory deficits could be related to dopaminergic loss, Huisman and colleagues (Huisman, Uylings, & Hoogland, 2004) used tyrosinedroxylase immunohistochemistry to show that the number of dopaminergic cells within the olfactory bulbs of PD patients was doubled with respect to that generally found in

neurologically healthy participants. This finding led to the hypothesis that increased levels of dopamine within the olfactory glomeruli might determine an inhibitory transmission in the olfactory bulb. Possibly responsible for this condition in PD, the inhibitory process described might explain why hyposmia in these patients is not levodopa-responsive (Huisman et al., 2004).

Although it is well established that the majority of patients with idiopathic Parkinson's disease (IPD) have a defective sense of smell, a large number of investigations have utilized olfactory tests which require an explicit report of odour features (e.g., Daum, Sekinger , Kobal, & Lang, 2000; Doty et al., 1988). Such explicit report implies specific forms of odour memory involving the generation of a name or the odour identification for the participant to respond (Olsson et al., 2002). This aspect is particularly relevant in PD, given that studies addressing odour recognition memory performance seems to suggest that such function in PD patients is impaired (Corwin, Serby, Conrad, & Rotrosen, 1985; Kesslak et al., 1988; Mesholam et al., 1998; Zucco, Zaglis, & Wambsganss, 1991). At a neural level, this finding seems to be supported by studies reporting that olfactory perception may preferentially recruit the hippocampus, possibly reflecting its role in the working memory element of odour related tasks (Bohnen, Gedela, Herath, Constantine, & Moore, 2008; Bohnen et al., 2010; Kareken, Mosnik, Doty, Dzemidzic, & Hutchins, 2003).

In everyday life, nevertheless, odours are rarely encountered in isolation and generally exist in a contextual relationship with other

details. In most cases odours are learned unintentionally and unconsciously (Issanchou et al., 2002; Wilson & Stevenson, 2006). As a result, it is difficult to describe odours in terms of specific constituents, and attention is generally focused on individuals' reactions to odour-related events rather than on the identity or the names of odours per se (de Wijk & Cain, 1994; Engen, 1987). Not surprisingly, while people seem to have more difficulty naming objects via smell than via sight (Cain, Stevens, Nickou, Giles, Johnston, & Garcia-Medina, 1995), they nevertheless negotiate the world of odours quite successfully. While means of encoding odours other than language seem to be utilized, both explicit and implicit processing could be involved in forging the rather complex relationship between odours, their sources, and behaviors connected to them.

Until now, no studies have attempted to assess if any kind of implicit odour processing occurs in PD patients, but recent findings concerning the role played by olfactory stimuli in shaping motor behavior can provide some insight into the direction research should take (Castiello et al., 2006; Tubaldi, Ansuini, Demattè, et al., 2008; Tubaldi, Ansuini, Tirindelli, et al., 2008). Experiments were devised by some investigators to study reach-to-grasp movements performed in the presence or absence of an orthonasal olfactory task-irrelevant stimulus. In some of the experiments the olfactory stimulus evoked an object that was smaller or larger than the visual target utilized. The maximum distance between the index finger and the thumb (i.e., maximum grip amplitude) was found to be affected in different ways depending on the stimulus. If

the olfactory stimulus evoked an object that was smaller than the visual target, the maximum grip amplitude was smaller than the one associated to a no-odour clue, but if it evoked an object that was larger than the visual target utilized, the maximum grip amplitude was larger than that associated to a no-odour clue. Moreover, when the 'size' of the odour stimulus and the size of the visual target corresponded, facilitation effects were noted: movement time was, in fact, shorter compared to situations in which the visual target did not correspond to the olfactory stimulus or when there was no olfactory clue. Taken together, these findings seem to indicate that although an olfactory stimulus is irrelevant as far as task performance is concerned, it is nevertheless implicitly elaborated in motor terms to facilitate - or interfere with - the motor plan prepared for the visual target.

Based on the hypothesis that if some sort of implicit olfactory processing still takes place in PD patients this would be reflected in their motor behavior, we designed a reach-to-grasp experiment (e.g., Castiello, Stelmach, & Lieberman, 1993; Gordon, Ingvarsson, & Forssberg, 1997; Gordon, 1998; Müller & Stelmach, 1992; Saling, Adler, Alberts, & Stelmach, 1996; Tresilian, Stelmach, & Adler, 1997) and added an olfactory stimulus. This population has commonly been found to be slower and to reach a smaller peak amplitude than age-matched control participants but, in other respects, task performance appears to be similar in the two groups. At the same time, studies concerning the influence of olfactory stimuli on reach-to-grasp movements in neurologically healthy individuals have reported alterations in the same specific movements

parameters found in the PD patients (Castiello et al., 2006; Tubaldi, Ansuini, Dematté, et al., 2008; Tubaldi, Ansuini, Tirindelli, et al., 2008).

IPD patients were thus asked to carry out reach-to-grasp movements in the direction of visual targets of different sizes in the absence or presence of preliminary olfactory stimuli that were size congruent or incongruent with the visual targets. The performance of these patients was compared with that in vascular PD (VPD) patients with no specific olfactory deficits but demonstrating similar motor symptoms. A group of neurologically healthy participants was also assessed for comparison purposes.

Basing our premise on already published findings on olfactory stimuli and reach-to-grasp movements, we hypothesized that if implicit olfactory processing is preserved in IPD, the size information conveyed by an odour stimulus would affect the reach-to-grasp movements in different ways depending on the congruency between the motor plans elicited by the odour 'size' and the sight of the visual target. We expected to see that in incongruent situations the motor plan elicited by the visual target would interfere with that elicited by the olfactory stimulus. In congruent conditions in which both the olfactory and visual information elicit a similar motor plan, we expected to see facilitation effects reflected in the degree of bradykinesia and hand movement hypometria in both IPD and VPD patients. Finally, for the neurologically healthy group, we expected to see a similar odour facilitation/interference pattern noted in the two PD groups, but only with regard to movement time.

7.3 METHODS

7.3.1 PARTICIPANTS

Three groups of participants were recruited for the study. Those in the first group (N = 12; mean age 67.75 years, average disease duration 2.33 years, mean age at onset 65.42 years) were all diagnosed with IPD and being treated with dopaminergic drugs known to have no effect on olfaction (Doty, Stern, Pfeiffer, Gollomp, & Hurtig, 1992; Table 7.1). Patients with vascular lesions detected on MRI were excluded from the study with the exception of those with minimal evidence of small vessel disease considered normal for the patient's age and in areas other than the basal ganglia (Katzenschlager, Tischler, Kalchhauser, Panny, & Hirschl, 2009). Evaluation of the scans was made by an independent radiologist who was blinded to the study design and modality. The second group (N = 12; mean age 68.58 years) was composed of age- and gender- matched VPD patients. Demographic information, clinical data, vascular risk factors (Winikates & Jankovic, 1999) and imaging details for these patients are outlined in Table 7.2. The severity of PD symptoms in the patients studied was assessed by a board certified neurologist using two different measures: the Hoehn and Yahr (1967) severity scale and the Unified Parkinson's Disease Rating Scale (UPDRS; Fahn & Elton, 1987). All of the IPD and three of the VPD patients were tested after they had taken their medication. The fact that levodopa was producing optimal therapeutic responses was provided by the UPDRS which was administered to those patients prior to their respective experimental session. None of the

participants showed therapy-related motor complications that could interfere with the study task. A third group (N = 12; mean age 65.83 years) was made up of normal participants without neurological or skeletomotor dysfunctions. The MMSE was used to provide an index of the patients' current global cognitive state, Folstein et al., 1975). The scores of the IPD and VPD patients ranged between 29 and 30 (Tables 7.1 and 7.2) while all the neurologically healthy participants had a score of 30. Mean age was not significantly different in the groups studied nor were there significant differences in terms of disease duration in the two patient groups. Both the IPD and VPD patients scored an average of 18 out of 20 on the visual acuity test, while the neurologically healthy participants scored 20 out of 20. All the patients and the controls were non-smokers. Patients with a history of nasal or sinus surgery, severe head trauma, obstructive pulmonary disease, or allergies causing nasal congestion were excluded from the study. Olfactory function was tested using the UPSIT (Sensonics, Haddon Heights, New Jersey, USA) consisting of 40 odours, which are microencapsulated in paper strips and released when they are scratched with a pencil. Participants are asked which of four words best describes the odour. The maximum score, corresponding to normosmia, is 40. According to the literature, normal values decrease with age and are lower in men (Doty, Shaman, Appelbaum, et al., 1984). All the participants showed right-handed dominance (Edinburgh Inventory; Oldfield, 1971; Appendix D).

Table 7.1. *Demographic data and clinical features of the Idiopathic Parkinson's disease (IPD) patients studied.*

PD patients	Age (years)	Gender	Years since diagnosis	Stage of the disease	Most affected upper limb	UPDRS (upper limb)	UPSIT score	MMSE score	Dopaminergic medication	Clinical signs						
										T	R	B	A	P	O	F
1	66	M	3	II	L	2	17	28	***0 – 0 - 0***	'	-	+	+	-	-	-
2	68	F	2	II	R	8	14	30	***1 – 1 – 1****	'	-	+	+	-	-	-
3	71	F	1	I	R	6	14	30	***0 – 0 - 0***	'	+	R	-	-	-	-
4	67	M	2	II	L	5	17	30	***1 – 0 – 1****	'	+	+	+	-	-	-
5	68	M	2	I	L	3	15	30	***1 – 1 – 1****	R	+	+	+	-	-	-
6	66	M	3	II	L	10	17	29	***1 – 1 – 1****	-	+	R	+	-	-	-
7	65	F	4	II	L	4	18	30	***0 – 0 - 0***	-	-	+	-	-	-	-
8	69	M	2	I	R	8	12	30	***0 – 0 - 0***	-	-	+	-	-	-	-
9	68	F	3	I	R	5	15	29	***0 – 0 - 0***	-	-	R	L	-	-	-
10	66	F	1	II	L	9	15	30	***0.5 – 0.5 – 0.5***†	-	-	+	+	-	-	-
11	71	F	2	II	L	12	13	30	***1 – 1 - 1***	R	R	+	+	-	-	-
12	68	M	3	I	L	2	17	30	***0 – 0 – 0***	-	-	R	-	-	-	-

Medication: number of tablets morning–midday–evening (dopaminergic medication, *50 mg; †125 mg). Clinical signs: signs when medicated, according to examination at time of testing and self report: T = resting and/or postural tremor, R = rigidity, B = bradykinesia, A = akinesia, P = problems with static and dynamic upright posture, O = on–off phenomenon, F = freezing; '+' = both sides affected; '-' = neither side noticeably affected; 'L' = left side mainly affected; 'R' = right side mainly affected. MMSE = Mini-Mental State Examination. Stage of the disease was determined on the basis of the Hoehn and Yahr's scale.

The experimental sessions were individual and lasted an hour. Approved by the ethics committee of the University of Padova, this study was carried out in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all of the participants.

Table 7.2. Demographic data and clinical features of the patients with vascular parkinsonism (VPD) studied.

PD patient	Age (years)	Gender	Years since diagnosis	Most affected upper limb	UPDRS (upper limb)	UPSIT score	MMSE score	Clinical signs						
								T	R	B	A	P	O	F
1	66	F	3	L	4.4	35	30	-	-	-	-	-	-	-
2	68	F	3	L	3.3	37	30	-	-	-	-	-	-	-
3	68	M	2	L	6.2	32	29	L	-	-	-	-	-	-
4	69	F	4	L	4.8	34	30	R	-	+	-	-	-	-
5	66	M	4	R	5	36	30	L	+	+	+	-	-	-
6	70	F	3	R	8	36	29	L	-	+	-	-	-	-
7	72	F	2	L	3	35	28	R	+	+	-	-	-	-
8	68	F	2	L	6	31	30	-	-	+	-	-	-	-
9	69	M	3	L	4	37	30	-	-	L	-	-	-	-
10	71	M	2	L	8	35	30	-	-	+	+	-	-	-
11	67	M	2	L	10	34	29	R	-	+	+	-	-	-
12	69	F	1	L	3	33	29	-	-	+	-	-	-	-

Conventions as for Table 8.

Table 7.2. (continued) Other clinical features and magnetic resonance imaging findings in the patients with vascular parkinsonism (VPD) studied.

Patient	Onset	Clinical features	MRI	Vascular risk factors	L-dopa response
1	Insidious	Hemiparkinsonism following stroke, bradykinesia	DWML, PWML	Hypertension	Not tried
2	Insidious	Asymmetric parkinsonism with tremor, bradykinesia	DWML, PWML	Hypertension	Good
3	Acute	Hemiparkinsonism following stroke, bradykinesia	Bilateral GP lesion	Hypertension, diabetes	Not tried
4	Acute	Asymmetric parkinsonism with tremor, bradykinesia	Bilateral GP lesion	Hypertension, stroke	Not tried
5	Acute	Shuffling gate, bradykinesia	Lesion contralateral LN	Stroke	Not tried
6	Acute	Hemiparkinsonism following stroke, bradykinesia	Bilateral GP lesion	Hypertension, stroke	Poor
7	Insidious	Hemiparkinsonism following stroke, bradykinesia	DWML, PWML	Family history of stroke	Good
8	Acute	Hemiparkinsonism following stroke, bradykinesia	Lesion contralateral LN	Hypertension	Not tried
9	Insidious	Shuffling gate, asymmetrical parkinsonism with rest tremor, bradykinesia	DWML, PWML	Hypertension	Good
10	Acute	Hemiparkinsonism following stroke, bradykinesia	Lesion contralateral GP	Stroke	Not tried
11	Insidious	Lower body parkinsonism, bradykinesia	DWML, PWML	Family history of stroke	Good
12	Acute	Hemiparkinsonism following stroke, bradykinesia	Lesion contralateral GP	Stroke	Not tried

DWML, deep subcortical white matter (bilaterally); GP, globus pallidus; LN, lentiform nucleus; MMSE, mini-mental state examination; MRI, magnetic resonance imaging; PWML, periventricular white matter lesions (bilaterally).

7.3.2 STIMULI AND APPARATUS

The visual stimuli (i.e., targets) consisted of four plastic objects grouped on the basis of their natural sizes: large (apple, orange) and small (almond, strawberry). Imitations rather than real fruits were used in order to maintain consistent visual features and sizes throughout the experimentation period. Odours evoking strawberries, almonds, oranges, and apples were obtained by mixing 6000 µl of propylene glycol and 180 µl (3%), 60 µl (1%), 420 µl (7%), and 45 µl (0.75%) of the specific compound, respectively. A custom-built, computer-controlled

olfactometer (Department of Experimental Psychology, University of Oxford, United Kingdom) was used to deliver the odour stimulus or odourless air. Each odour generator consisted of a glass boat containing one of the four odour stimuli. A fifth glass boat containing propylene glycol was used to deliver odourless air. Passed over the odour solutions and propylene glycol at a flow rate of 8 l/min, the air mixture was delivered to a face mask attached to Teflon tubing (Figure 7.1). Data from a pilot study showed that the objects associated with the odour stimuli administered were all correctly identified by those individuals who were not anosmic. Further, the odour stimuli were judged to be equally perceivable, intense, and familiar. At the beginning of the session each individual was asked to place his/her right hand on a starting platform within which a pressure sensitive switch was embedded (i.e., starting switch). The platform was designed with slight convexities dictating a natural flexed posture of the fingers (Figure 7.1). The target object was placed on a second pressure sensitive switch (i.e., the ending switch) embedded within the working surface (Figure 7.1). To control vision, the participants were asked to wear spectacles fitted with liquid crystal lenses (Translucent Technologies Inc., Toronto, Ontario, Canada) which changed from opaque to transparent (Figure 7.1). Participants were told that pressing the starting switch, which would determine visual availability of the target (i.e., opening of the spectacles), should correspond to the onset of the reaching movement towards the target. Movement amplitude was measured at the time the ending switch was released as the object was being grasped. Movement time was calculated

as the interval between the times that the starting and ending switches were pressed.

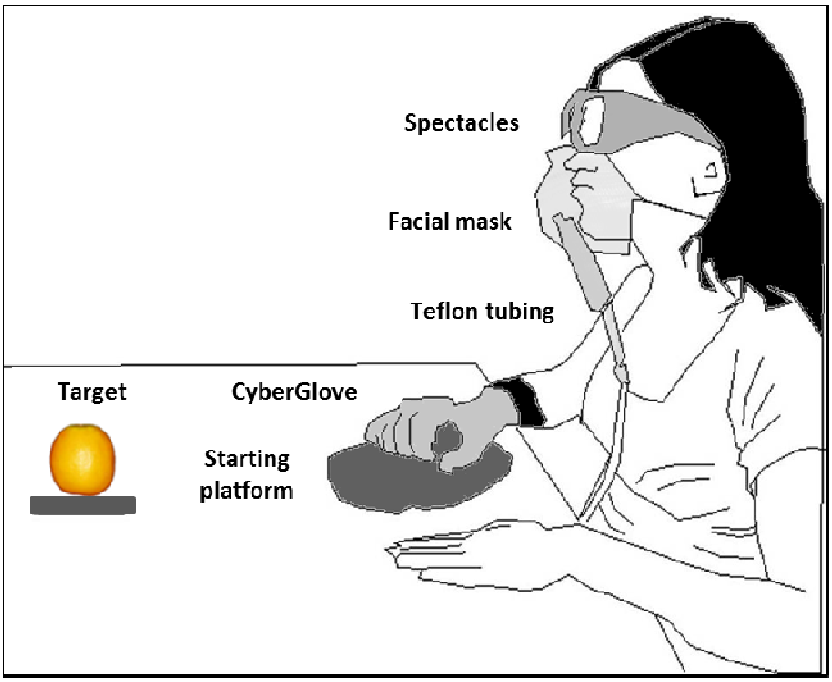


Figure 7.1. *Graphical representation of the experimental set-up. Legends indicate the relevant details.*

Hand kinematics was measured by a flex sensor glove (CyberGlove, Virtual Technologies, Palo Alto, CA, USA) worn on the participant's right hand (Figure 7.1). The sensors' linearity was 0.62% of maximum nonlinearity over the full range of hand motion. The sensors' resolution was 0.5° remaining constant over the entire range of joint motion. The output of the transducers was sampled at 12-ms intervals.

In accordance with previous reports assessing the effects of olfactory stimuli on movement performance (Castiello et al., 2006; Tubaldi, Ansuini, Dematté, et al., 2008; Tubaldi, Ansuini, Tirindelli, 2008), the dependent variables specifically relevant to test our hypothesis

were movement time and maximum grip amplitude. These variables were considered particularly appropriate to test our hypotheses because PD patients typically show slowness of movement (bradykinesia) and hand opening alterations (hypometria) when asked to perform reach-to-grasp movements (Rand & Stelmach, 2005), while other aspects of kinematic parameterization appear to be largely unaltered with respect to neurologically healthy participants (e.g., Castiello et al., 1993; Tresilian et al., 1997).

7.3.3 PROCREDURES

At the beginning of the session the participant was positioned with his/her elbow and wrist resting on a flat surface, the forearm horizontal, the arm was oriented in a natural parasagittal plane passing through the shoulder, and the right hand was placed in a pronated position with the palm toward the working surface on the starting switch. The target was aligned with the participant's body midline, located 33 cm from the hand starting position to the left of the participant's right shoulder (Fig. 1). The sequence of events for each trial was the following: (i) once correctly positioned, the participant's vision was occluded while the target was being placed on the working surface; (ii) an auditory signal was sounded (850 ms duration, 65 dB sound pressure, 800 Hz frequency) indicating that the odour was about to be released; (iii) after 3 s a similar signal was sounded to indicate the odour had been released; (iv) 500 ms later the signal was sounded again; (v) participants were instructed to reach

towards, to grasp, and to lift the target when they heard the third tone. Sufficient time interval (10 s) was scheduled between trials to permit the odour to dissipate (Hummel et al., 1996). This sequence of events was adopted because findings in the literature have indicated that the effects of olfactory stimuli on reach-to-grasp kinematics are maximized when the olfactory stimuli/cues are presented slightly before the object is visually grasped (Tubaldi, Ansuini, Tirindelli, et al., 2008). The participants were instructed to reach for the visual target at a natural speed and not to grasp it by the stem. An experimenter visually monitored all of the trials to ensure that participants complied with instructions. The experimenter noted that the participants naturally grasped the small visual targets between the thumb and the index, at times also with the help of the middle fingers, while the large visual targets were grasped using the thumb and the rest of the fingers. The task was performed under six experimental conditions: (i) 'Congruent-Large' (LL) condition: an odour associated with a large size object was presented before a reach-to-grasp movement towards a large target was initiated; (ii) 'Congruent-Small' (SS) condition: an odour associated with a small size object was presented before a reach-to-grasp movement towards a small target was initiated; (iii) 'Incongruent Small' (SL) condition: an odour associated with a small size object was presented before a reach-to-grasp movement towards a large target was initiated; (iv) 'Incongruent Large' (LS) condition: an odour associated with a large size object was presented before a reach-to-grasp movement towards a small target was initiated; (v) 'No odour-Large' (NoL) condition: odourless air was released before a

reach-to-grasp movement towards a large target was initiated; (vi) 'No odour-Small' (NoS) condition: odourless air was released before a reach-to-grasp movement towards a small target was initiated (Figure 6.1).

Each participants took part in a total of 48 trials (8 for each experimental condition) which were presented in randomized order.

7.3.4 DATA ANALYSIS

For each dependent measure, a mixed ANOVA with 'Groups' (IPD, VPD, controls) as between-subjects factor and 'Olfactory condition' (congruent, incongruent, control) and 'Target size' (large, small) as within-subjects factors was performed. The main assumptions behind this statistical model (i.e., normality and sphericity) were checked before running the ANOVA. The Kolmogorov-Smirnov test showed that the normality assumption was satisfied (α-level: $p < 0.05$). The Mauchly test showed that the sphericity assumption was not violated. Results from the ANOVA performed on the slope absolute values were assessed through *post-hoc* comparisons using t-tests. The Bonferroni correction was applied (α-level: $p < 0.05$). A *post-hoc* analysis was also performed to assess possible gender differences in selective odour identification between VPD and control participants. No significant effect was detected with reference to gender.

7.4 RESULTS

7.4.1 MOVEMENT TIME

The main 'Group' effect was significant, $F(2, 22) = 171.20$, $p < 0.0001$, $\eta_p^2 = 0.94$. Post hoc comparisons revealed that movement times were longer for both the IPD and the VPD than for the controls ($p_s < 0.0001$; 1598, 1587 and 896 ms, respectively). The movement times of the IPD and the VPD were not significantly different ($p > 0.05$; Figure 7.2). As indicated by the main 'Target size' effect, $F(1, 11) = 847.14$, $p < 0.0001$, $\eta_p^2 = 0.99$, movement times were shorter for the larger than for the smaller targets (1329 vs 1392 ms). The main 'Olfactory condition' effect was also significant, $F(2, 22) = 203.68$, $p < 0.0001$, $\eta_p^2 = 0.95$. Movement times for the congruent condition were significantly shorter than for the no odour and the incongruent conditions ($p_s < 0.0001$; 1288, 1354, and 1439 ms, respectively). A significant difference was also found in movement times when the no odour and the incongruent conditions were compared ($p < 0.0001$). These results indicate that PD patients are slower than controls and that congruent odours evoke shorter movement times, while incongruent odours determine longer ones. The no odour condition was associated with intermediate values. The similarity of the revealed effects across the three groups is highlighted in Figure 7.2.

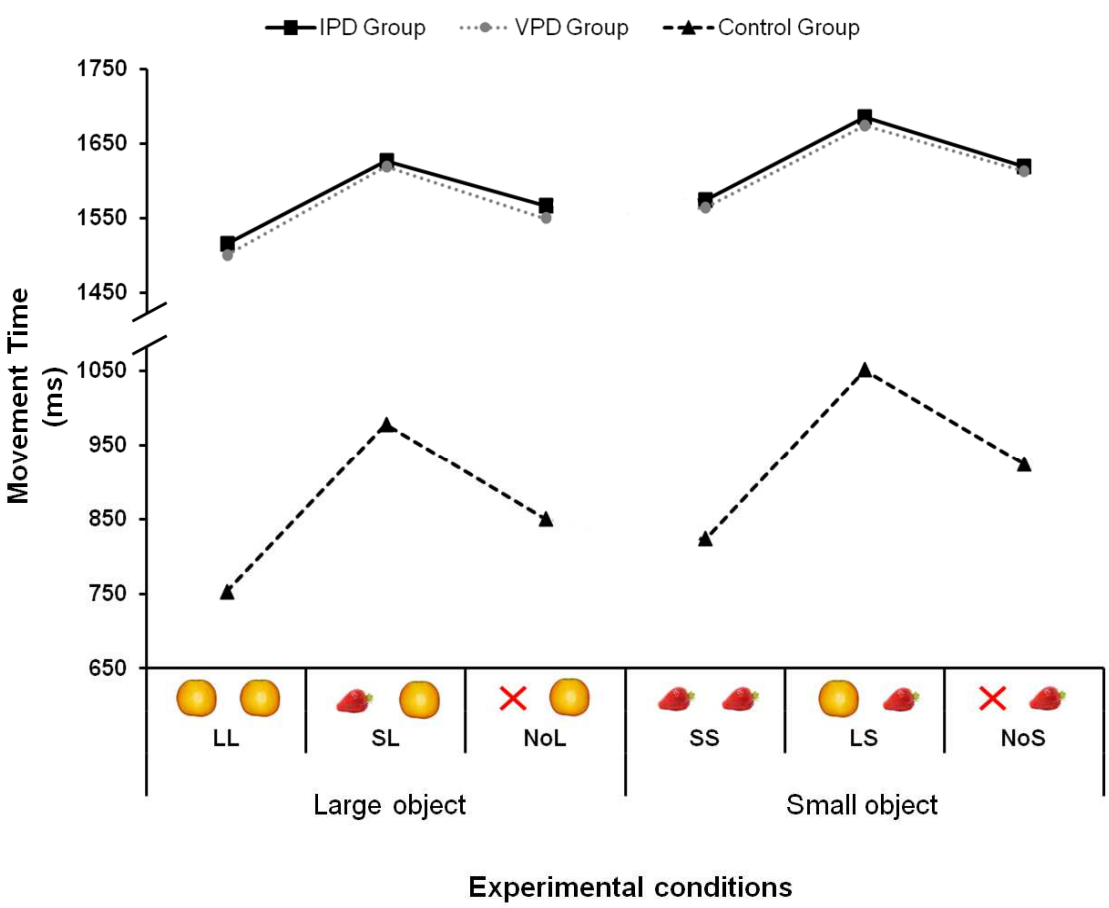


Figure 7.2. *Lines represent the duration of the reach-to-grasp movement expressed in ms for the IPD (black solid line), VPD (grey dotted line) and healthy participants (black dashed line) for the six experimental conditions tested (from left to right: congruent, incongruent, no odour condition for the large and for the small targets, respectively). LL: congruent large condition; SS: congruent small condition; SL: incongruent large condition; LS: incongruent small condition; NoL: no odour large condition; NoS: no odour small condition.*

7.4.2 MAXIMUM GRIP AMPLITUDE

The main 'Group' effect was significant, $F(2, 22) = 44.73$, $p < 0.0001$, $\eta_p^2 = 0.80$, as both the IPD and the VPD patients showed smaller grip amplitudes with respect to the controls ($p < 0.0001$; 81, 80, and 90 mm, respectively). The maximum grip amplitude did not differ in the IPD and VPD patients ($p > 0.05$). The main 'Target size' effect was also significant, $F(2, 22) = 1299.03$, $p < 0.0001$, $\eta_p^2 = 0.99$. The maximum grip amplitude was

wider for the larger than for the smaller targets (92 vs 75 mm). Analysis of the main 'Olfactory condition' effect, $F(2, 22) = 43.29$, $p < 0.0001$, $\eta_p^2 = 0.80$, indicated that the maximum grip amplitude was smaller for the no odour than for the incongruent and congruent conditions ($p_s < 0.0001$; 82, 84 and 85 mm, respectively). The three-way 'Group by Olfactory condition by 'Target size' interaction was significant, $F(4, 44) = 24.16$, $p < 0.0001$, $\eta_p^2 = 0.69$.

Large targets. Post-hoc comparisons indicate that in both PD groups the maximum grip amplitude was greater for the congruent than for the no odour condition ($p < 0.0001$; Figure 7.3). For incongruent conditions in which a 'small' odour was released before a large target was presented, the maximum grip amplitude was smaller in the PD patients compared to that for the no odour and congruent conditions ($p < 0.0001$; Figure 7.3). There were no significant differences across the congruent and the no odour conditions ($p > 0.05$; Figure 7.3), but the maximum grip amplitude was smaller for the incongruent than for the no odour and congruent conditions ($p < 0.0001$; Figure 7.3).

Small targets. Post-hoc comparisons indicate that in both PD groups the maximum grip amplitude was greater for the congruent than for the no odour condition ($p < 0.0001$; see Figure 7.3). For the incongruent condition in which a 'large' odour was delivered before a small target was presented, the maximum grip amplitude in the PD patients was wider than it was for the no odour and congruent conditions ($p < 0.0001$; Figure

7.3). There were no significant differences across the congruent and the no odour conditions in the controls ($p > 0.05$; Figure 7.3), but the maximum grip amplitude was wider for the incongruent than for the no odour and the congruent conditions ($p < 0.0001$; Figure 7.3).

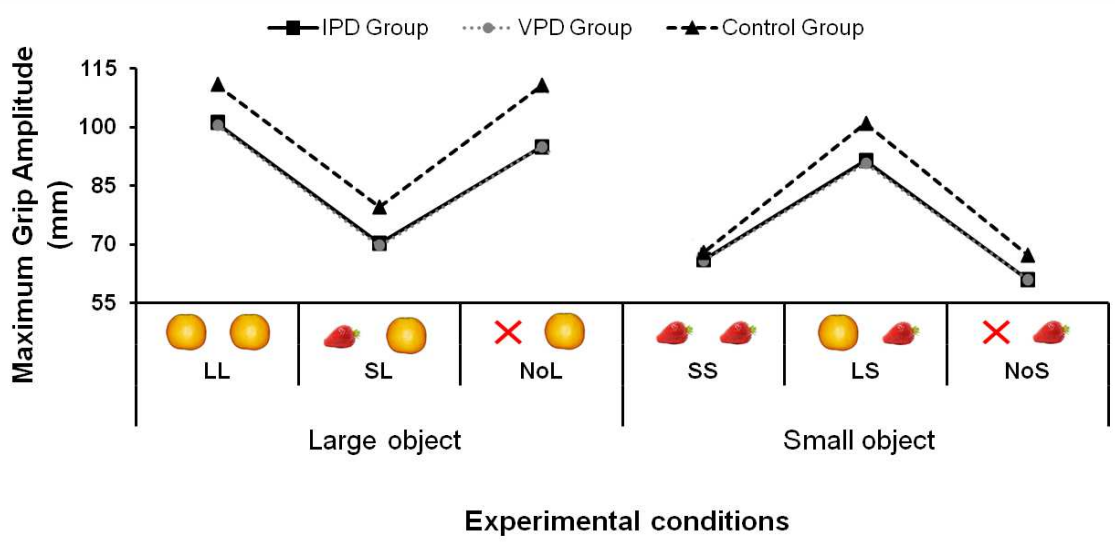


Figure 7.3. Lines represent the maximum grip amplitude expressed in mm for the IPD (black solid line), VPD (grey dotted line) and healthy participants (black dashed line) for the six experimental conditions tested (from left to right: congruent, incongruent, no odour condition for the large and for the small targets, respectively). LL: congruent large condition; SS: congruent small condition; SL: incongruent large condition; LS: incongruent small condition; NoL: no odour large condition; NoS: no odour small condition.

7.5 Discussion

The aim of this study was to assess implicit olfactory processing in IPD patients. The results indicate that although these patients generally have severe forms of olfactory loss, they do continue to process olfactory stimuli implicitly. Just as neurologically healthy and VPD groups, IPD patients were found to be facilitated in their actions when they were

exposed to an odour evoking an object that was similar in size with respect to a target. Olfactory priming, in fact, seemed to determine an improvement in bradykinesia of hand transport movement and hypometria of the grip amplitude in these patients. If, instead, the odour evoked a different sized object with respect to the visual target there were interference effects in the movement pattern in the IPD patients just as in the other two groups studied.

The results concerning the conditions in which presentation of visual targets was not preceded by olfactory information also provide insight about some aspects of olfactory processing. In fact, in order to ascertain the effects of size olfactory information on movement kinematics it is necessary to demonstrate that the size of the visual target affects movement timing and grip amplitude. And, in fact, significantly different kinematic patterns were found for the two target sizes in all the groups studied. The movement time was longer and the maximum grip amplitude was reduced for smaller with respect to larger targets in both groups of PD patients (e.g., Castiello et al., 1993; Tresilian et al., 1997) as well as in the neurologically healthy participants (Gentilucci, Castiello, Corradini, Scarpa, Umiltà, & Rizzolatti., 1991; Jakobson & Goodale, 1992; Jeannerod, 1984). With specific reference to the PD group, previous evidence demonstrating that their reach-to-grasp movements were slower (e.g., Castiello et al., 1993; Tresilian et al., 1997) and their maximum grip amplitude smaller (Rand & Stelmach, 2005) with respect to control participants was confirmed.

The results outlined here indicate that reach-to-grasp movement planning was carried out on the basis of olfactory information in all three groups studied (e.g., Castiello et al., 2006; Tubaldi, Ansuini, Dematté, et al., 2008; Tubaldi, Ansuini, Tirindelli, et al., 2008). In those cases in which the size of the visual target and that of the object elicited by the olfactory stimulus did not match, the motor plan elicited by odour did not appear to be totally superseded by that later elicited by the visual target. In other words, some aspects of the motor plan implicitly elicited by an incongruent olfactory stimulus persist in the prehensile movement made to grasp the visual target. It is important to remember that in these situations the movement elicited by the olfactory stimuli is different from the one visually needed. Parallel preparations appear to be made for both types of movements: one for the visual target and one for the olfactory stimulus, and this might explain the differences found in action kinematics. Conversely, when an odour elicits a motor plan which is congruent with the plan made subsequently for the visual target, facilitation effects were noted. The hand movement plan triggered by the olfactory stimulus seems to pave the way for the plan made for the visual target. Taken together, these results are particularly important with regard to the IPD group as they demonstrate that although these patients are unable to explicitly process olfactory information, some sort of implicit olfactory processing does take place. Not only, when primed by a congruent olfactory stimulus, IPD patients are faster and better able to increase hand amplitude thus diminishing the tendency to produce movements that are slower (bradykinesia) and smaller (hypometria).

These results clearly confirm that there is some kind of olfactomotor activity in PD patients despite the fact that the hypothesis has been made that their olfactory impairment depends at least in part on less vigorous sniffing (Sobel et al., 2001). The results presented here suggest that even though motor problems can inhibit sniffing behavior, they do not preclude odour elaboration and an appropriate behavioral reaction to olfactory cues from the external world.

These findings also evince a dissociation between explicit and implicit olfactory processing in these patients. Olfactory deficits in PD have been described as far as odour identification, odour discrimination, odour threshold detection, and odour recognition memory are concerned (Haehner et al., 2009; Mesholam et al., 1998), even though there is considerable inconsistency in the reliability of olfactory testing (Doty, Smith, McKeown, & Raj, 1994). It is possible that some tests assessing olfactory function are unable to provide reliable results because the operational processes involved depend in part on the integrity of brain structures involved in cognition or memory, such as the hippocampus (Larsson et al., 2004; Wang, Eslinger, Smith, & Yang, 2005). Odour impairment in early stages of PD has been found to correlate with hippocampal dopaminergic denervation (Bohnen et al., 2008). It is possible then that the implicit olfactory processing observed in IPD patients may not require conscious recollection of olfactory stimuli or the integrity of structures involved in memory functions, but that it requires the integrity of amygdala, an area which is physically closer to the

olfactory sensory modality and may not be compromised during early stages of the disease (Bohnen et al., 2008; Braak et al., 2003, 2004).

One of two large limbic system structures, the amygdala forms a more primitive, emotional part of the brain. Olfaction is, in fact, considered a primitive survival system serving to quickly categorize experiences and it appears to be unconnected to higher mental functions. It is the only sense whose primary areas are not directly connected to the thalamus, the structure apparently involved in conscious processes (Plailly et al., 2008). This, of course, implies that there are other means of encoding odours not requiring access to higher cognitive functions for recognition. Implicit processing may have been preserved in view of life-and-death decisions needing to be made on the basis of olfactory information (Koenig et al., 2000).

There are, moreover, interconnections between the amygdala and the OFC which might be critical for the multisensory integration of stimuli utilized to guide behavior. The amygdala encodes the significance of cues while the OFC serves as a center for appraisal, guiding adaptive goal-directed behavior based on information accessed through its connections with the amygdala as well as with other structures (Schoenbaum et al., 1998). It is possible that the effects found in the present study are mediated by an implicit olfactory encoding occurring at the level of the amygdala which is conveyed to the OFC where visual-olfactory representations are formed. This hypothesis seems to be supported by the facilitation effects found in the IPD patients when the visual and the olfactory stimuli were congruent. Neuroimaging findings

(Gottfried & Dolan, 2003; Österbauer et al., 2005) and neurophysiological studies (Grigor, 1995; Grigor, Van Toller et al., 1999; Rolls & Baylis, 1994; Sarfarazi et al., 1999; Stein & Meredith, 1990) indicate that facilitation effects, associated with enhanced neural activity within the OFC, are obtained by manipulating the degree of correspondence between olfactory and visual stimuli.

Confirmation of a direct connection between OFC and motor areas involved in arm-hand movement control (Cavada et al., 2000; Morecraft & Van Hoesen, 1993) is of particular importance for our study in view of the well-known homology between cerebral regions underlying reach-to-grasp movements in monkeys and humans (for review see Castiello, 2005). It can be hypothesized that the corticocortical connections between OFC and motor areas affecting motor output (e.g., Bates & Goldman-Rakic, 1993) can account for the influence multisensory integration of olfactory-visual information has on motor behavior and more specifically on prehensile actions (Rossi et al., 2010). In this perspective, IPD patients' prehensile movements may be affected by the chain of neural events beginning with implicit odorant encoding occurring at the amygdala level.

The results presented here seem to indicate that implicit olfactory processing is preserved in IPD patients. The fact that there appears to be a dissociation between explicit and implicit olfactory processing may have important clinical implications permitting clinicians to distinguish IPD from pathologies sharing similar motor and explicit olfactory symptoms. Even more importantly, the finding that implicit odour processing in IPD patients is preserved and associated to bradykinesia

and hypometric amplitude of the grip, the classical deficits in the reach-to-grasp movements in these patients, has important implications in terms of rehabilitation. The residual ability to perceive olfactory stimuli and to respond subconsciously to them could hypothetically be utilized to design rehabilitation strategies to improve upper limb motor control. Although the idea of using olfactory cues in clinically relevant contexts is not a new one, they have been used until now only to add emotional color to perceptions (e.g., Bordnick et al., 2008; Gerardi, Rothbaum, Ressler, Heekin, & Rizzo, 2008; Kawai & Noro, 1996; Ryan, Kreiner, Chapman, & Stark-Wroblewski, 2009). The findings presented here imply that olfaction could serve as a conditioned stimulus for some voluntary, goal-directed actions. In accordance with classical conditioning paradigms, the association between an object's odour (e.g. a peach) and the action needed to grasp it (e.g. a whole-hand grip) can be easily established. Patients can hypothetically be trained, following congruent olfactory stimulation, to speed up reaching movements and to shorten or lengthen their grip amplitude. Continuous, constant practice might help make the movement automatic and facilitate the patient's performance in an ecological environment even in the absence of olfactory prompts. Sensory training of this kind might help patients become more autonomous during some periods of the day (for example, meal times), increasing their self-perceived quality of life and, at the same time, relieve some of the duties of caregivers. Future studies would seem warranted in view of this prospect.

CHAPTER 8

SMELLING OWN MOTHER'S BODY ODOUR OPENS UP TO AUTOMATIC IMITATION IN CHILDREN WITH HIGH FUNCTIONING AUTISM [4]

8.1 ABSTRACT

Recent theories on the causes underlying autism postulate a relationship between olfactory performance and a deficiency of the mirror neuron system - which is thought to be responsible for the lack of imitative abilities in autism. With this in mind, the aim of the present study is to investigate whether body odours can modulate automatic imitation in children diagnosed with high functioning autism (HFA) as measured by a visuomotor priming paradigm. We recruited a group of 20 HFA and 20 typically developing (TD) children. Body odours from the children's mother axillæ were collected. We asked the children to observe a model (either their mother or the mother of another participant) executing a reach-to-grasp action towards an object and to perform the observed action in the absence of specific instructions to imitate. The object could be impregnated of the children' mother's odour, the odour of the mother of another participant or no odour. The model-child-object interactions

[4] *Submitted:* Parma, V., Bulgheroni, M., Tirindelli, R., Mari, M., & Castiello, U. (2011). Smelling own mother's body odour opens up to automatic imitation in children with high functioning autism. *Science.*

were videotaped and arm kinematics extracted post-hoc via a digitalization technique. Familiar body odours modulate visuomotor priming effects, indicating that in all cases a previously observed action facilitates in terms of movement speed the execution of a similar action in TD participants. Noticeably, in HFA such facilitation effects were evident only when they acted upon the object impregnated by their own mother's odour. We suggest that the familiar odour conveys some social significance to the object, indicating that olfaction may have the potency to help HFA in forging social interactions.

8.2 Introduction

It is now established that olfactory cues are essential to the formation of social behaviors - such as kin recognition (e.g., Pause, 2011). Behavioral investigations revealed that olfactory cues are fundamental for grounding maternal-offspring interactions in certain animal species (e.g., Van-Laillet & Norwak, 2008) as well as in humans (Schaal, Montagner, Hertling, Bolzoni, Moyse, & Quichon, 1980). In this respect, evidence exists that human newborns and infants are able to discriminate their own mother's odour. They show more preference and they are more easily comforted when exposed to it than when exposed to the odour of an unfamiliar mother (Doucet, Soussignan, Sagot, & Schaal, 2007; Ferdenzi, Soussignan, Sagot, & Schaal, 2008; Macfarlane, 1975; Montagner, 1974; Schaal et al., 1980).

It has also been reported that humans are far more sensitive to odours produced by body fluids than other animals (Laska, Wieser, & Salazar, 2006) and that, in the human brain, body odours deserve specialized neural processing (Lundström et al., 2008, 2009; Pause, 2011). Specifically, they recruit cortical and subcortical areas outside the olfactory circuits activated by common odours (i.e., odours produced by inanimate sources; Lundström et al., 2008). Furthermore, some of the brain regions involved in the processing of common and body odour are also involved in the regulation of social behaviors (e.g., Rolls, 2000). In this respect, lesion studies indicate that damaged medial temporal lobe structures brings to a lack in olfactory identification ability which reflects in social indifference and stereotyped behaviors (Bachevalier, 1994). Similarly, an impairment at the level of the orbitofrontal cortex determines a significant decrement in olfactory identification ability (Savage, Combs, Pinkstone, Advokat, & Gouvier, 2002) as well as decreased empathy, inappropriate social interaction and increased obsessive behavior (Eslinger & Damasio, 1985). As another piece of evidence, both the anterior and the posterior cingulate cortex together with the angular gyrus are activated under the exposure of body odours (Lundström et al., 2009). Of relevance, these latter regions are also involved in tasks such as social evaluation (Allman, Hakeem, Erwin, Nimchinsky, & Hof, 2001), person recognition (Maddock, Garret, & Buonocore, 2001) and social perception (Allison, Puce, & McCarty, 2000), respectively.

In light of the above evidence, it is not surprising that some pathological conditions present both olfactory and social impairments (Malaspina & Coleman, 2003). For one, when considering schizophrenia spectrum disorders, smell identification deficits positively correlate to a low social drive (Malaspina & Coleman, 2003), which is operationally described as a lack in the ability to conceive, initiate and maintain/complete goal directed activities (Malaspina & Coleman, 2003). For another, and specifically relevant for the present study, ASD present social deficits such as impaired communication, restricted and repetitive behaviors in association with abnormal smell reactions (American Psychiatric Association, 2000).

It is this latter aspect of ASD which is at the core of the present investigation. Anecdotal reports (Grandin, 1992), questionnaire (Kern et al. 2006; Lane, Young, Baker, & Angley, 2010; Lane, Dennis, & Geraghty, 2011; Leekam, Nieto, Libby, Wing, & Gould, 2007; Tomchek & Dunn 2007; Nieminen-von Wendt et al., 2005; Williams, 2005) and behavioural (Bennetto et al., 2007; Brewer, Brereton, & Tonge, 2008; Dudova, Vodicka, Havlovicova, Sedlacek, Urbanek, & Hrdlicka, 2011; Hrdlicka, Vodicka, Havlovicova, Urbanek, Blatny, & Dudova, 2011; May, Brewer, Rinehart, Enticott, Brerenton, & Tonge, 2010; Tavassoli & Baron-Cohen, 2011; Suzuki, Critchley, Rowe, Howlin, & Murphy, 2003) investigations indicate that the vast majority of people diagnosed with ASD present smell abnormal reactions. They manifest either in the direction of under- or over-estimation of the olfactory stimuli as compared to age- and gender-matched healthy controls.

When considering the possible link between social behaviour and olfactory functions, there are studies indicating that ASD people can use odours to define environmental boundaries and identify people by repetitively sniffing family members' body or clothes (Bogdashina, 2003). In the light of the ASD restricted interpersonal experience, this might be viewed as an attempt of shaping social interactions. In addition, the odour of a family member might be considered as a relevant stimulus possibly facilitating social behavior.

But how can this hypothesis be tested? A possibility is to investigate how a milestone of social abilities is modulated by the exposure to a body odour. One of the abilities necessary for the development of adequate social skills is imitation (Hurley & Chater, 2005). To date, it has been proposed that imitation might be the primary deficit underlying the abnormal socio-communicative behaviors shown by ASD patients (Meltzoff & Gopnik, 1994; Rogers, 1999; Rogers & Pennington, 1991; Smith & Bryson, 1994). In this respect, one of the experimental paradigms used to ascertain imitation in ASD, in a controlled but sufficiently ecological environment, is the visuomotor priming paradigm (e.g., Craighero et al., 1996). This paradigm reveals a motor facilitation effect induced by the pure observation of a movement on the execution of a similar action, without the explicit instruction to imitate (i.e., automatically). And, it is thought to provide a behavioral correlate of the mirror neuron system (Rizzolatti & Craighero, 2004; Hayes, 2011), which is a system responding to both the observation and the execution of actions (Gallese, Fadiga, Fogassi, & Rizzolatti, 1996) and

which can also be alerted by olfactory cues (Rossi et al., 2008; Tubaldi, Turella, Pierno, Grodd, Tirindelli, & Castiello, 2010).

Although the administration of such paradigm to ASD children indicates that they are impermeable to the observation of other people's actions (Pierno, Mari, Glover, Georgiou, & Castiello, 2006; Pierno, Mari, Lusher, & Castiello, 2008; Becchio, Pierno, Mari, Lusher, & Castiello, 2007), when the degree of familiarity of the considered stimuli is taken into account visuomotor priming effects do emerge (e.g., Oberman, Ramachandran, & Pineda, 2008). As to appropriately understand actions, individuals with ASD appear to rely on familiarity, not just with the action, but also with the model (Le Bel, Pineda, & Sharma, 2009; Oberman et al., 2008). As an example, Oberman and coworkers (2008) revealed that during action observation the μ rhythm – a physiological marker of the mirror neuron system activity (Marshall & Meltzoff, 2010) - was suppressed in TD participants, but far less for the ASD group. However, the degree of familiarity with the model performing the action modulated this effect in both groups. In other words, μ rhythm suppression was more pronounced when the actor was familiar. The proposal is that the mirror neuron system responds to observed actions in individuals with ASD only when they can identify in some personal way with the stimuli. Therefore, it is the social relevance of the stimuli presented to ASD children, which might acquire central importance as to appropriately delineate their intact and impaired abilities across tasks (Frith & Happé, 1994; Happé & Frith, 1996a, 1996b).

In keeping with the idea that familiarity in general, and familiar odours in particular, might help ASD patients in establishing social interactions here we undertake the important challenge to uncover behavioral evidence for the link between olfaction, imitation and social interactions in ASD. Support to this idea comes from theoretical and empirical evidence. First, it has been proposed that the link between olfactory behavior and impaired mirror neuron system activity in ASD might set the basis for a sufficiently complex and apparently exhaustive framework to explain most of the symptoms present in ASD (Brang & Ramachandran, 2010). Second, consistent evidence has demonstrated that the mirror neuron system responds to action-related information conveyed via olfaction (Rossi et al., 2008; Tubaldi, Turella, et al., 2010).

The aim of the present study was to investigate whether body odours (e.g., the body odour collected from the participants' mothers) have the ability to modulate automatic imitation in ASD children diagnosed with HFA and age- and gender- matched TD children. To this end, we capitalized on a modified version of the visuomotor priming paradigm, including, for the first time, an olfactory component. We collected body odours from the mothers' axillæ of 20 HFA and 20 TD children. We asked the children to observe a model (either their mother or the mother of another participant) executing a reach-to-grasp action towards an object which could be impregnated of the children mother's odour, the odour of the mother of another participant or no odour. Then they were requested to perform the observed action in the absence of

specific instructions to imitate. The model-child-object interactions were videotaped and subsequently analyzed via digitalization techniques.

If the hypothesis of an olfactory driven social behavior (i.e., automatic imitation) in ASD is correct, then we should be able to reveal visuomotor priming effects in HFA when they will be asked to act upon a stimulus impregnated by a familiar body odour, that is the participant own mother's odour. We also expect that these facilitation effects should emerge independently of whether their own mother acted as a model. Facilitation here is intended as a reduction in the time to initiate and perform the action following the observation of a model performing a similar movement, compared to when such prompting is not present.

8.3 METHODS

8.3.1 PARTICIPANTS

The study included 20 children diagnosed with HFA, 20 TD children and their mothers. Children were matched with respect to age, gender, full scale IQ (Wechsler Intelligence Scale for Children, WISC-R; Wechsler, 1991), socioeconomic status (Hollingshead, 1975) and handedness (Oldfield, 1971; Table 8.1). Participants were recruited from the community or from a database of families who had taken part in previous studies.

The diagnosis of HFA was based on the DSM-IV-TR criteria (American Psychiatric Association, 2000) and was obtained by means of the Autism Diagnostic Observation Schedule (ADOS; Lord, Rutter,

DiLavore, & Risi, 2002) with the child and the Autism Diagnostic Interview-Revised with the caregiver (ADI-R; Le Couteur, Lord, & Rutter, 2003). Only the participants who met diagnostic criteria on these standardized measures as well as clinician opinion of HFA, were included in the experimental sample. Participants with HFA were not diagnosed with any neurological, genetic syndrome resulting in the deficits ascribable to HFA or to smell disturbance (Appendix A). The administration of UPSIT (Doty, Shaman, & Dann, 1984) revealed that, on average, HFA participants present a decreased sense of smell [microsmia; UPSIT mean(sd) scores: 23,55(5,75)]. TD participants had no history of autism as witnessed by the scores obtained from the administration of the ADOS and ADI-R and UPSIT showed a normal sense of smell [mean(sd) scores: 33,67(4,31)]. Furthermore, no concerns about ASD were acknowledged within participants' first or second degree relatives. All children were right-handed, reported normal or corrected-to-normal vision, no-hearing impairments, and were naive as to the purpose of the experiment. None was on medication. All the participants, both children and their mothers, did not show any motor impairment to the upper limbs which might have interfered with the execution of a reach-to-grasp movement. Imitation abilities were assessed by an expert clinical psychologist by means of structured observation.

Table 8.1. *Descriptive characteristics for the TD and HFA children groups. Means and standard deviations (in parentheses) are shown along with corresponding F or χ^2 values.*

	TD M (SD)	HFA M (SD)	F or χ2	P
N	20	20	-	-
Age	13.4 (1.76)	13.2 (1.82)	.05	.58
Full Scale IQ	109 (8.52)	103.5 (10.38)	1.13	.22
Socioeconomic Status	52.18 (6.55)	51.23 (6.18)	.22	.35
Handedness (R:L)	20:0	20:0	.26	.31
Gender (M:F)	10:10	10:10	.22	.37
CARS	-	36.7 (3.78)	-	-

HFA: high functioning autism; TD: typically developing children; CARS: Childhood Autism Rating Scale.

8.3.2 STIMULI AND APPARATUS

The olfactory stimuli were body odours resulting from axillary secretions, which are considered one of the main ingredients in the determination of human body odours (Natsch, Derrer, Flachsmann, & Schmid, 2006). The body odours were obtained from both HFA and TD participants' mothers. A body odour was classified as 'familiar' to the participant when it was collected from his/her own mother and 'unfamiliar' when it was collected from the mother of another child participating in the study. Before the experimental sessions took place, the mothers of both the HFA and TD children were provided with perfume free body and laundry detergents and were instructed to bath themselves and wash their clothes with these products for the whole period of the experimentation. A day before the schedule of the experimental session, the mothers were asked to wear cotton pads under their axillæ as to make them be permeated of their body odour while shielding them from external odour sources (Stern & McClintock, 1998). Great care was paid in avoiding the collection of body odour in particular emotional conditions (e.g., fear, moderate to high

anxiety, …) as to avoid the effect of a predominant emotional stimulation (Chen & Haviland-jones, 2000; Mujica-Parodi et al., 2009; Prehn-Kristensen et al., 2009). Donors were instructed not to be involved in activities which generate moderate to high anxiety (e.g., exams, trials, sport sessions,…) and were debriefed at the end of the 'collection time'. At that time, the mothers were trained to remove the pad put it in a glass jar and then wrap, hot seal and freeze it to prevent the odour to be dissolved. The day of the experiment, each pad was defrosted and cut into four sections for distribution to different recipients, treated with 4 drops of 70% isopropyl alcohol and then re-frozen immediately at -80°C in a glass vial (Stern & McClintock, 1998). The day of the experimental session, the models were asked to bring freshly washed clothes within a plastic bag and to wear them in the experimental room, just before the beginning of the session. This minimized the effect played by other familiar odours coming from the familiar environment of the child or of the model.

As to preserve ecological validity, each model-child-object interaction was videotaped and kinematics were extracted post-hoc by means of digitalization procedures (Zoia et al., 2007). Two dependent measures were considered as to the specifically test the effects that observing another person's action might have on the performance of the same action, namely initiation time and movement time. Initiation time allows to understand whether visuomotor priming effects were already present at the time the action was planned (Edwards, Humphreys, & Castiello, 2003; Pierno et al., 2008). It was calculated as the time elapsing from the presentation of the 'go' signal and the start of the action, defined

as the wrist reaching towards the object for two consecutive frames (28 ms). Movement time is the dependent measure which is particularly sensitive to visuomotor priming effect concerning the movement execution phase (Edwards et al., 2003; Pierno et al., 2008). It was calculated as the time between the start of the action and the time at which the index finger and the thumb closed on the object and remained stationary for at least two frames (28 ms).

8.3.3 *Procedures*

The participant and the model - either the participant's mother or another participant's mother - sat at a table in front of each other. The object (e.g., a glass) was aligned with the participant and the model's body midline and located at a 20-cm-distance from both the participant and the model's hand starting position (Figure 8.1). The right hand of each participant rested on a starting pad with the index finger and the thumb gently opposed. First, the model and the participant were asked to smell the object on the table. The pads impregnated with either the participant mother's odour, another participant mother's odour or no odour were fastened by means of a tubular net bandage around the object used in the experimentation as to allow for the dispersion of the odour.

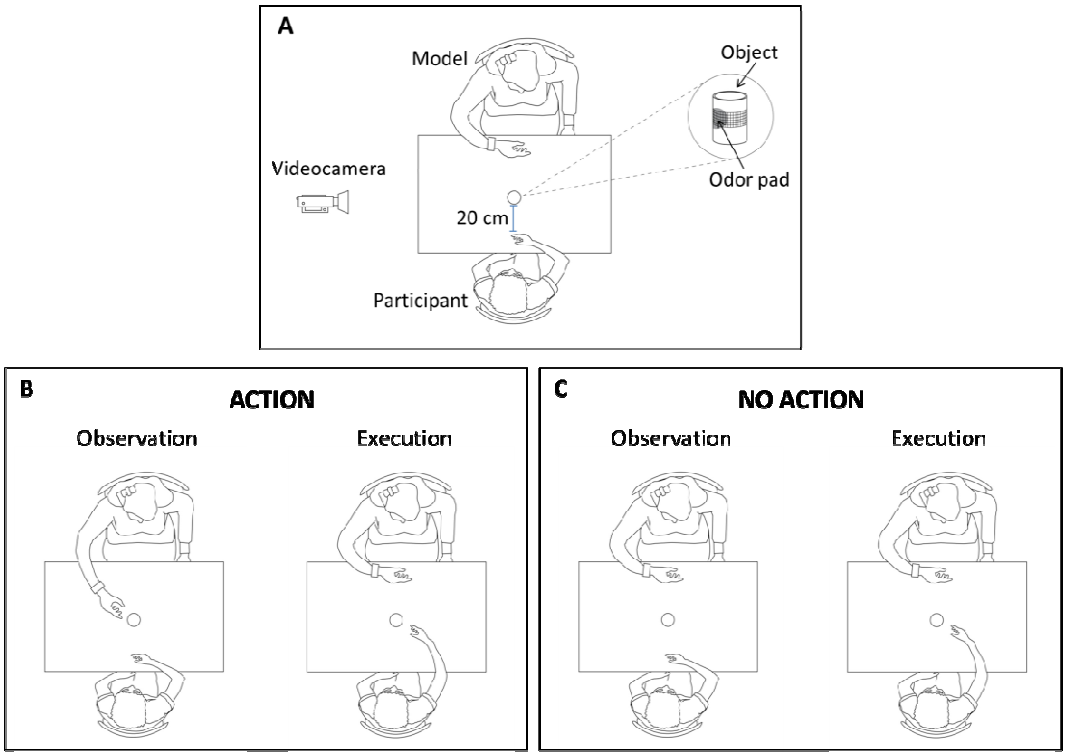


Figure 8.1. *Graphical representation of the experimental set-up. Panel 'A' graphically represent the pad impregnated with the odour was fastened via a tubular net to the to-be-grasped object. Panel 'B' shows the child performs the same reach to grasp movement after having observed a model executing a reach-to-grasp action towards the object. Panel 'C' shows the child reaches and grasps the object in the absence of the observation of any preceding action performed by the model.*

Following the presentation of a 'go' signal (e.g., 850 ms duration, 65 dB sound pressure, and 800 Hz frequency), the model could either naturally reach and grasp the object, lift it and put it back to the original position or remain still in the starting position, without performing any action. Then, the same auditory signal was presented as to indicate the child - who was not explicitly instructed to imitate the observed action - to reach and grasp the object.

Participants performed a total of 120 trials (10 for each experimental condition, Table 8.2) which were presented in randomized order within four blocks. The experimental session lasted from a

minimum of 60 to a maximum of 90 min depending on participants compliance to the task and the setting.

Table 8.2. *Factor combination for each of the 12 experimental conditions administered to both the HFA and TD groups.*

Model	Stimulus	Observed behavior
M	O	A
M	O	nA
M	o	A
M	o	nA
M	nO	A
M	nO	nA
m	O	A
m	O	nA
m	o	A
m	o	nA
m	nO	A
m	nO	nA

M = participant's mother; m = another participant's mother; O = participant mother's odour; o = another participant mother's odour; nO = no odour; A = action performed by the model; nA = no action performed by the model.

The experimental procedures were in accordance with the Declaration of Helsinki and were approved by the Institutional Review Board at the University of Padova. Prior to testing, written informed consent was obtained from the mothers and from each participant. Mothers and participants were informed that their withdrawal from the study may occur whether and whenever they want prior or during the experimental session, without giving any further reason.

8.3.4 Data Analysis

Prior to inferential statistics, explorative data analysis was performed. The final experimental design considered the between-subjects factor

'Group' (HFA, TD) and three within-subjects factors: 'Model' (familiar, unfamiliar), 'Stimulus' (own mother's body odour, another mother body odour), and 'Observed action' (action, no action).

Factor differences were evaluated by means of a mixed ANOVA. Mauchly's test have been applied as to assess sphericity, which was not confirmed ($p < 0.05$). Therefore, the violation of sphericity was adjusted by using the Greenhouse-Geisser correction. Effect sizes were calculated and reported as partial eta squared (η_p^2).

8.4 Results

For both dependent measures the mixed ANOVA revealed a four-way interaction 'Model by Stimulus by Observed action by Group' which reached out the significance level (Initiation time: $F(2, 76) = 29.41$, $p < 0.0001$, $\eta_p^2 = 0.44$; Movement time: $F(2, 76) = 4.87$, $p < 0.05$, $\eta_p^2 = 0.11$). Results from significant post-hoc contrasts are reported below.

8.4.1 Initiation time

As shown in Figure 8.2 for the TD participants the effects of observing an action prior execution determined a speeded up of the time spent to initiate the movement with respect to when no action was observed. The comparison between the action and the no action conditions was significant independently from the type of model and the type of smelled odour ($p_s < 0.01$). For the HFA participants, however, such significant decrease in initiation time for the action versus the no action condition

was evident only when the familiar odour was smelled and the model was unfamiliar. This can be observed by inspecting the top right panel in Figure 8.2. Furthermore, for this group, exposure to the familiar odour - rather than to the unfamiliar or no odour - brought to a decrease in the absolute time spent to initiate the movement when interacting with the familiar model. Such reduction applied to both the action and the no action conditions.

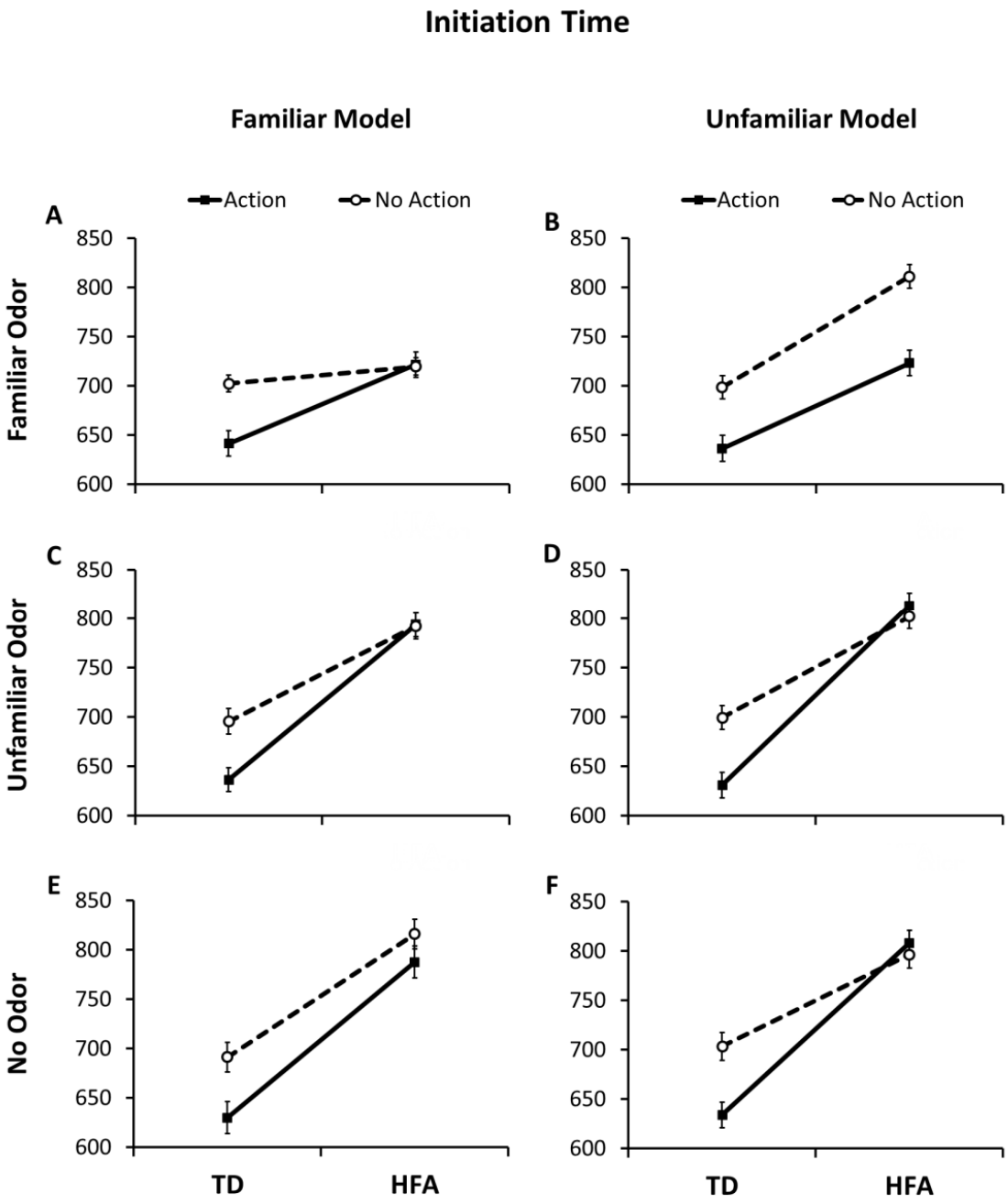


Figure 8.2. *Top panels show the means of initiation time of the action performed interacting with either the familiar (A) or unfamiliar (B) model under the exposure of the familiar odour for both the TD and the HFA participants. Central panels represent the mean initiation times for both TD and HFA groups when exposed to the unfamiliar odour and interacting with a familiar (C) or unfamiliar (D) model. Bottom panels report the means of initiation time of the action performed interacting with a familiar (E) or unfamiliar (F) model when no odour was presented for the TD and the HFA participants. ms: milliseconds. Bars represent standard error of means.*

8.4.2 *Movement time*

As shown in Figure 8.3, for the TD participants the effect of observing an action prior execution determined a speeded up of the time spent to perform the action. The comparison between the action and the no action conditions was significant independently from the model and the smelled odour ($p_s < 0.01$). For the HFA participants, however, such significant decrease in movement time for the action versus the no action condition was evident only when the familiar odour was smelled. This occurred independently from the model performing the observed action. This effect can be noticed by inspecting the top panels for the left and the right columns in Figure 8.3.

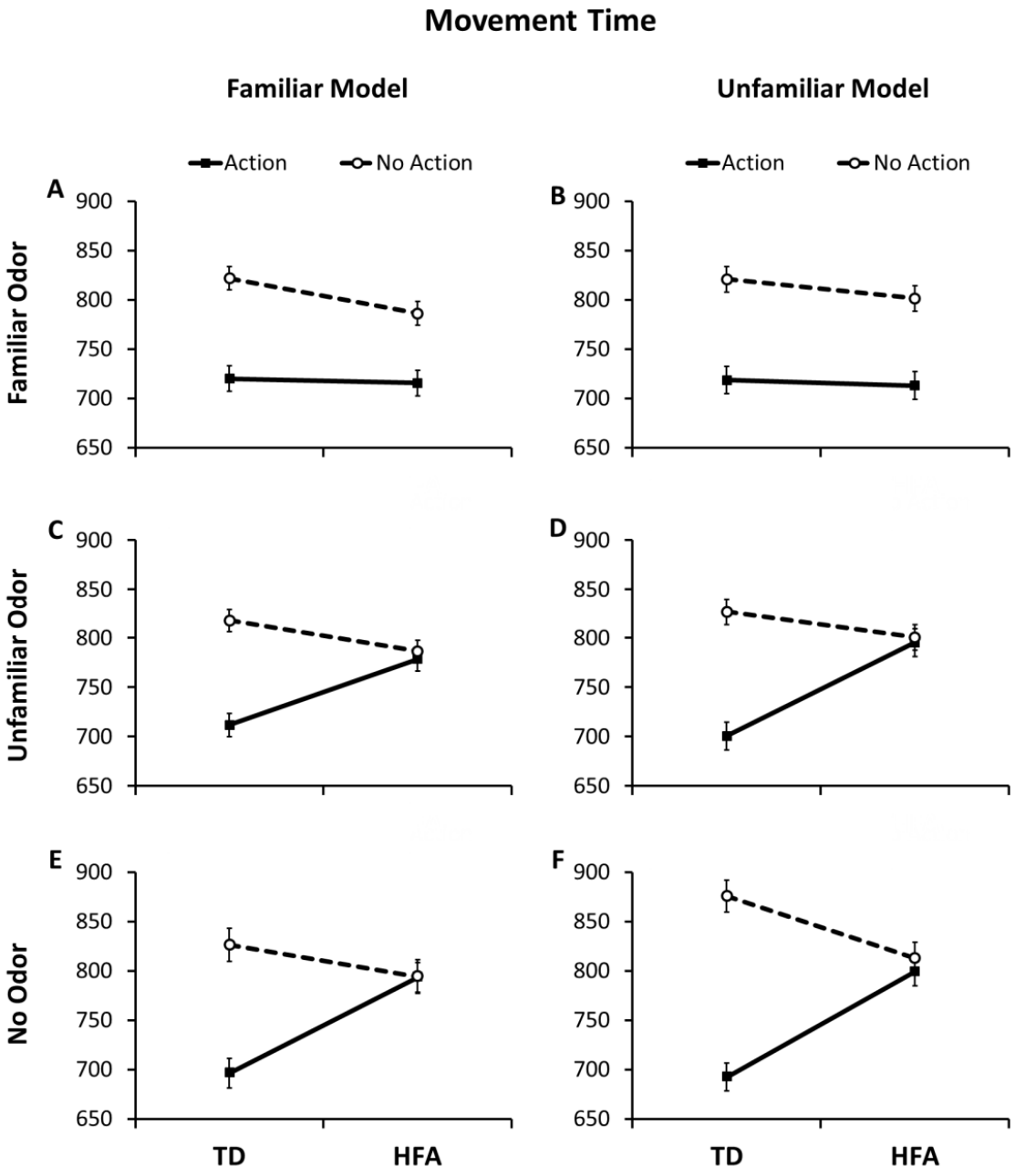


Figure 8.3. *Top panels represent the mean movement times for both TD and HFA groups when exposed to the familiar odour and interacting with a familiar (A) or unfamiliar (B) model. Central panels show the means of movement time of the action performed interacting with either the familiar (C) or unfamiliar (D) model under the exposure of the familiar odour for both the TD and the HFA participants. Bottom panels report the means of movement time of the action performed interacting with a familiar (E) or unfamiliar (F) model when no odour was presented for both the TD and the HFA groups. ms: milliseconds. Bars represent standard error of means.*

8.5 Discussion

The aim of the present study was to evaluate whether body odours have the ability to elicit automatic imitation in HFA children as measured via a crossmodal visuomotor priming paradigm.

The results for the 'no odour' condition are consistent with what has been previously demonstrated in 'classic' visuomotor priming experiments in TD and HFA children. Children for the TD group were facilitated following the observation of an action performed by a model, either familiar or unfamiliar (Pierno et al., 2006, 2008; Becchio et al., 2007). Conversely, HFA participants were impermeable to visuomotor priming effects irrespectively from the model they were interacting with.

Our crossmodal version of the paradigm did not bring to any clause in the pattern of results found for TD children. That is, visuomotor priming effects were present but not modulated depending on the body odour (familiar or unfamiliar) participants were exposed to. Exposure to a familiar body odour, however, changed dramatically the performance of HFA children. We found that exposure to a familiar body odour (but not to an unfamiliar body odour) determined a reduction in initiation and movement times, thus opening to the appearance of automatic imitation abilities.

The present results, disclosing that a familiar body odour can trigger automatic imitation in HFA children, can be considered innovative for several reasons. First, this study considers the effects of human body odours in ASD, by revealing a preferential processing for this kind of

stimuli as compared to common odours. Second, it is the first study suggesting that olfactory-induced 'emotion' in HFA children is possible. Third, the present study indicates that the beneficial effect of familiar stimuli can be triggered via crossmodal cues, even though difficulties in multisensory integration has been extensively reported for HFA individuals (Iarocci & McDonald, 2006; Benaroya, 1977, 1979; Chan, Fung, & Tong, 2005).

One might be surprised by the present outcomes given that HFA children have been attributed with scarce olfactory abilities (Suzuki et al., 2003; Bennetto et al., 2007; Brewer et al., 2008; May et al., 2010; Dudova et al., 2011; Hrdlicka et al., 2011; Tavassoli & Baron-Cohen, 2011). However, it should be noted that the studies grounding the idea of scant olfactory abilities in ASD are based on standardized tests which require explicit recognition of the stimulus (Doty, Shaman, Kimmelman, et al., 1984) and utilize common odorants, but do not include any body odours (Suzuki et al., 2003; Bennetto et al., 2007). To date, in none of the previous studies ASD participants have been tested via implicit methodologies - not requiring verbal abilities which are known to be lacking in this population (American Psychiatric Association, 2000) and with odours derived from human body fluids (Suzuki et al., 2003; Bennetto et al., 2007; Brewer et al., 2008; May et al., 2010; Dudova et al., 2011; Hrdlicka et al., 2011; Tavassoli & Baron-Cohen, 2011).

Although the poor olfactory recognition performance of HFA participants might depend on the assessing methodology, a point worth noting is that common and body odours as rather different olfactory

stimuli (Lundström et al., 2008, 2009; Pause, 2011). The strongest evidence supporting this view is the fact that common and body odours are elaborated via different neural pathways (Lundström et al., 2008, 2009; Pause, 2011). Specifically, body odours do not activate primary (entorhinal) and secondary (orbitrofrontal) olfactory cortices (Zatorre, Jones-Gotman, Evans, & Meyer, 1992), but they recruit cortical and subcortical areas outside the circuit known to be active during common and conscious olfactory processing (Lundström et al., 2008, 2009). In this respect, it has been argued that body odours might receive such a differential treatment in neural terms because of the heightened attention and emotional valence they elicit (Chen & Haviland-Jones, 1999). Evolutionarily speaking, body odours can be considered stimuli conveying highly relevant information (e.g., presence of in-group members in the vicinity), which deserve a quick and reliable processing as to preserve survival. Evidence is accumulating that the chemical complexity of the social environment was one of the main forces motivating brain development (Rowe, Macrini, & Luo, 2011) and that some social emotions - such as moral disgust - might evolutionary originate from the processing of chemical signals (Chapman, Kim, Susskind, & Anderson, 2009). As witnessed by the automatic nature of the imitation abilities triggered by a familiar body odour in the HFA group tested here, body odour processing appears to be most subliminal in nature and it does not require a conspicuous involvement of explicit learning (Lundström et al., 2009).

Evaluating the results of the present study in the light of the aforementioned evidence, we suggest that some form of social

communication mediated via chemosensory signals might be preserved and adaptively function in HFA participants. A conjecture on the regulation of this kind of nonconscious chemo-social communication considers that body odours become socially relevant in virtue of an automatic self-referent phenotype matching (Lundström et al., 2008, 2009; Pause, Krauel, Sojka, & Ferst, 1999). In other words, as for other animal species (Mateo & Johnston, 2000), the HFA participants might use their own body odour as a template to evaluate the familiarity of individuals in the vicinity (Lundström et al., 2009) and therefore congruently react to that.

The issue of familiarity is particularly debated in reference to ASD (Meirsschaut, Roeyers, & Warreyn, 2011). Of relevance for the present investigation is the demonstration that individuals with autism, as to appropriately understand actions, do rely on familiarity, not just with the action, but also with the model (Le Bel et al., 2009; Oberman et al., 2008). During action observation, the degree of familiarity with the model performing the action had the ability to elicit some form of mirror neuron system activity which was not evident when the actor was unfamiliar. In other words, the mirror neuron system responds to observed actions in individuals with ASD only when they can identify in some personal way with the stimuli (Oberman et al., 2008).

Here we crucially extend this notion by demonstrating that familiar body odours might alert the neural systems concerned with a kind of 'mirror' activity. Therefore we are tempted to speculate that the automatic imitation task reported here might allow to access the mirror

neuron system via olfactory processing. Indeed previous evidence have demonstrated that common odours activate the mirror neuron system (Rossi et al., 2008; Tubaldi, Turella, et al., 2010) and that body odours activate areas, such as the inferior frontal gyrus and the superior temporal sulcus (STS), which are recognized to be involved in the mirror neuron system (Lundström et al., 2008, 2009; Pause, 2011). Thus, one might agree that the results of the present study can suggest that maternal body odour processing can constitute a way to overcome mirror neuron system activation impairment in HFA children (e.g., Oberman & Ramachandran, 2007; Williams, Whiten, Suddendorf, & Perret, 2001).

Given the evolutionary relevance of body odours, especially proper mother's body odour within the childhood temporal window (Ferdenzi, Schaal, & Roberts, 2010), it might be advanced that this is a means to convey a sufficient emotional contagion which facilitates automatic imitation. In addition, the repeated exposure to the maternal body odour during development makes it a very familiar stimulus, which has the ability to open to an adaptive functioning of the mirror neuron system.

Taken together the evidence emerging from the present study could be useful when rehabilitation strategies are being hypothesized for autistic patients. Nevertheless, further research is needed to clarify a number of questions that has been left opened. First, before definite conclusions on the neural substrates underlying the effects of familiar body odour on imitation in HFA can be drawn, direct investigation using neuroimaging techniques is needed. A crossmodal approach would also facilitate the simultaneous consideration of different aspects, providing a

more exhaustive framework within which analyze the current knowledge on sensory processing, imitation and mirror neuron system functioning in ASD. Second, it will be of interest to compare the effects of different kin body odours (e.g., father, siblings,..) on the visuomotor priming effect. This would allow to reveal whether the facilitation of automatic imitation under the exposure of familiar body odours in HFA emerged due to a general familiarity effect or to a specific influence of the 'source' of odour. Whether the former hypothesis is correct, then we would expect that the exposure to either mother, father or siblings' odours equally prompt the appearance of the visuomotor priming effects in HFA children. On the other hand, if the visuomotor priming facilitation is accounted for a specific chemosensory communication, we foresee differences in the appearance of the visuomotor priming effects when exposed to the different familiar odours. It might be the case, for example, that HFA children would be selectively facilitated in automatic imitation by social odours to which they have been exposed to early in the development, such as their own mother's odour. Finally, it would be of interest to evaluate whether the effect of familiar body odours is stable during development. It might be plausible that the preference for maternal odour serving attachment purposes throughout childhood may shift in sexually-mature adults towards a dislike congruent with inbreeding avoidance (Ferdenzi et al., 2010), and a consequent inhibitory effect. Whether this may outcome in a change of the familiar body odour effect in the visuomotor priming requires further testing.

CHAPTER 9

GENERAL CONCLUSIONS

During our daily activities most of the times we are not aware of the odours we are exposed to. Nevertheless, we negotiate the world of odours in an effortless way, even though olfactory elaboration has been described as a multi-component and rather complex task. Such complexity is dictated by several factors, namely the characteristics of the olfactory system, the properties of the odorant, the biological relevance of the odour, the environment within which the odour is released and the degree of intentionality which characterizes olfactory elaboration. All these aspects contribute to a successful odour perception, which is an essential ability as to maximize survival chances.

The experimental work included in the present thesis aimed at extending the knowledge on the functioning of human olfactory perception with particular reference to some of the abovementioned factors. I addressed this issue capitalizing on selective olfactory deficits exhibited by patients suffering from different neurological pathologies, such as MS, TBI, PD, and HFA. The patients, as weel as the age- and gender-matched controls, were administered with explicit (requiring conscious elaboration) and implicit (requiring subliminal elaboration) olfactory tests to explore their olfactory (residual) abilities. The olfactory

stimuli utilized varied in their degree of biological relevance, going from common odours to body odours functioning as social chemosignals.

An overview of the experiments carried out, their implications for the understanding of the mechanisms underlying human olfactory perception and some final considerations are outlined in the following sections.

9.1 Dissociation of explicit and implicit olfactory processing

The use of explicit standardized olfactory tests is a common fact in clinical practice. They provide a quick and reliable tool as to screen for the possibility of olfactory abnormalities. Nevertheless, as illustrated by the results reported in Chapter 5, they can also lead to controversial and mixed outcomes.

The results described in the subsequent chapters of the present thesis (Chapter 6, 7 and 8) point out the presence of a dissociation between the performances to explicit and implicit olfactory testing in different groups of neurological patients, whose characterizing symptoms are not overlapping. This is in line with previous evidence of the well-known case of patient H.M., who failed in the conscious recollection - but succeeded in implicit recognition - of items previously presented (Roediger, 1990). In other words, it is plausible that the performance to an explicit test is impaired whereas the performance to an implicit test is preserved and falls within the range of normal scores.

The fact that some sort of olfactory processing is preserved in patients who are believed incapable of smelling any odour explain the reason why, although unable to consciously elaborate odours, these patients can react, in some environmental situations, as neurologically intact participants.

Even though the present thesis does not provide direct information about the brain mechanisms underlying olfactory perception (exeption made for Chapter 5), the findings reported in Chapter 6, 7, and 8 might allow to speculatively suggest that different neural systems are involved in explicit vs. implicit olfactory processing.

Explicit odour processing might recruit, besides olfactory cortices, brain areas involved in high cognitive functions, such as memory (e.g., hippocampus) and conscious perception of stimuli (e.g., thalamus). Given that those functions have necessarily to be intact as to successfully respond to the standardized olfactory tests commonly used to assess olfactory abilities, then it is not surprising that patients disgnosed with different neurological pathologies do fail to such tests. For instance, this might be the case of the anosmic TBI and PD patients as well as the hyposmic ASD children described here (Chapters 6, 7, and 8). These patients, who were able to appropriately react to the exposure of odours, might have processed odours implicitly, via different cerebral regions than those involved in explicit odour processing. The amygdala represents the best candidate upon which implicit odour processing circuit might be rooted. In the first instance, it is highly interconnected with olfactory cortices, especially beacause of its location (Price, 1990). In the second

instance, it mediates automatic reactions, postponing a conscious (thalamus-mediated) evaluation of the stimuli (LeDoux, 2000).

The presence of two brain networks elaborating olfactory information might be plausible from an evolutionary perspective. The fact that odours (both common and biologically relevant) are redundantly represented at a neural level might indicate that different neural mechanisms have evolved in order to guarantee a certain degree of elaboration of olfactory stimuli, which might be crucial for survival purposes (Koenig et al., 2000).

To fully account for this issue, future neuroimaging research on human participants is needed as to empirically validate the presence of explicit and implicit olfactory networks. Furthermore, behavioural studies should focus on the direct comparison of explicit and implicit olfactory measures (e.g., normalization procedures) as to ascertain the degree of overlapping that these two processes do share.

9.2 Common vs. biologically relevant odours

Explicit olfactory tests are constituted by common odorants, leaving aside the possibility of investigating the effect of biologically relevant olfactory stimuli, which are known to be preferentially and differently elaborated by the human brain. The results reported in Chapter 8 underline the need for a careful assessment of the social role of odours. A point worth noting is that, even in those populations presenting impaired social skills,

biologically relevant odours are able to prompt a social contact, in the form of chemosignal communication.

Further research elaborating on the level of biological relevance of odours on explicit and implicit olfactory prcessing would be of help in predicting the modulation of dours on manifest (social) behaviour.

9.3 Clinical relevance

Taken together, the results reported in Chapters 6, 7 and 8 provide evidence which can be taken into account from developers of new rehabilitation strategies. As a general point, olfactory cues are only rarely included within rehabilitation trainings. Nevertheless, on the basis of the effects the experiments of the present thesis are able to trigger, they are worth of being included in such treatments. As specific concerns, each pathology might benefit from different aspects of olfactory cues. As an example, repetitive exposure to common odours might be used as to train traumatic brain injured patients' olfactory sensitivity. As anoter example, idiopathic Parkinson's disease might profit from the use of olfactory cues congruent to objects as conditioned stimuli in order to favour upper limb motor control. Finally, HFA patients would take advantage from the exposure to biologically relevant odours in the light of their social properties.

9.4 Epilogue

It was more the premonition of a scent
than the scent itself-and at the same time
it was definitely a premonition of something
he had never smelled before.

Patick Süskind
Perfume

The core message stemming from the present thesis is that we all have a sense of smell, which we use and trust more than we expect, whether we deliberately realize it or not. Although the majority of the literature on human olfaction has focused on explicit/verbally mediated olfactory abilities, I have attempted to adopt a novel approach which have so far received little attention. Specifically, I focused on implicit olfactory testing methods as to obtained an unbiased behavioural measure of how humans, with either preserved or impaired sense of smell, do elaborate olfactory stimuli. This new methodology lead me to uncover that some sort of implicit olfactory processing is preserved in patients who are diagnosed with smell loss by means of explicit tools. This was confirmed for odours with different properties, such as biological relevance. The preservation of a form of implicit odour processing is then in line with a successful navigation of the environment, which may require life-and-death decisions needing to be made in the absence of other sensory cues, merely on the basis of olfactory information (Koenig et al., 2000).

Bibliography

Adams, J.H., Doyle, D., Ford, I., Gennarelli, T.A., Graham, D.I., & McLellan, D.R. (1989). Diffuse axonal injury in head injury: definition, diagnosis and grading. *Histopathology*, *15*, 49-59.

Akerlund, A., Bende, M., & Murphy, C. (1995). Olfactory threshold and nasal mucosal changes in experimentally induced common cold. *Acta Oto-Laryngologica*, *115*, 88–92.

Allison, T., Puce, A., & McCarty, G. (2000). Social perception from visual cues: Role of the STS region. *Trends in Cognitive Science*, *4*, 267–278.

Allman, J.M., Hakeem, A., Erwin, J.M., Nimchinsky, E., & Hof, P. (2001). Anterior cingulate cortex: the evolution of an interface between emotion and cognition. *Annals of the New York Academy of Sciences*, *935*, 107–117.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual Of Mental Disorders (DSM-IV-TR)*, 4th ed., Text revision. Washington, DC: American Psychiatric Association.

Amoore, J.E., & Ollman, B.G. (1983). Practical test kits for quantitatively evaluating the sense of smell. *Rhinology*, *21*, 49–54.

Andersen, R. A., Snyder, L. H., Bradley, D. C., & Xing, J. (1997). Multimodal representation of space in the posterior parietal cortex

and its use in planning movements. *Annual Review of Neuroscience*, *20*, 303– 330.

Ansari, K.A. (1976). Olfaction in multiple sclerosis. With a note on the discrepancy between optic and olfactory involvement. *European Neurology*, *14*, 138–145.

Ansari, K.A., & Johnson, A. (1975). Olfactory function in patients with Parkinson's disease. *Journal of Chronic Diseases*, *28*, 493–497.

Arshamian, A., Willander, J., & Larsson, M. (2011). Olfactory awareness is positively associated to odor memory. *Journal of Cognitive Psychology*, *23*, 220-226.

Atanasova, B., Graux, J., El-Hage, W., Hommet, C., Camus, V., & Belzung, C. (2008). Olfaction: a potential cognitive marker of psychiatric disorders. *Neuroscience Biobehavior Review*, *32*, 1315-1325.

Atighechi, S., Salari, H., Baradarantar, M.H., Jafari, R., Karimi, G., & Mirjali, M. (2009). A comparative study of brain perfusion single-photon emission computed tomography and magnetic resonance imaging in patients with post-traumatic anosmia. *American Journal of Rhinology & Allergy*, *23*, 409–412.

Bachevalier, J. (1994). Medial temporal lobe structures and autism: a review of clinical and experimental findings. *Neuropsychologia*, *32*, 627-648.

Baddeley, A. (1992). Working Memory. *Science*, *255*, 556–559.

Bargh, J.A., Chen, M., & Burrows, L. (1996). Automaticity of social behavior: direct effects of trait construct and stereotype activation on action. *Journal of Personality and Social Psychology*, *71*, 230–244.

Bargh, J.A., Gollwitzer, P.M., Lee-Chai, A., Barndollar, K., & Trotschel, R. (2001). The automated will: non-conscious activation and pursuit of behavioral goals. *Journal of Personality and Social Psychology*, *81*, 1014–1027.

Bargmann, W., & Schadé, J.P. (1963). *The Rhinencephalon and Related Structures*. Amsterdam; Elsevier.

Barkhof, F. (2002). The clinico-radiological paradox in multiple sclerosis revisited. *Current Opinion in Neurology*, *15*, 239–245.

Bates, J.F., & Goldman-Rakic, P.S. (1993). Prefrontal connections of medial motor areas in the rhesus monkey. *Journal of Comparative Neurology*, *336*, 211-228.

Becchio, C., Pierno, A., Mari, M., Lusher, D., & Castiello, U. (2007). Motor contagion from gaze: The case of autism. *Brain*, *130*, 2401-2411.

Beck, A.T., & Steer, R.A. (Ed.). (1990). *Manual for the Beck Anxiety Inventory*. San Antonio, TX: Psychological Corporation.

Beck, A.T., Steer, R.A., & Brown, G.K. (Ed.). (1996). *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation.

Beites, C.L., Kawauchi, S., Crocker, C.E. & Calof, A.L. (2005). Identification and molecular regulation of neural stem cells in the olfactory epithelium. *Experimental Cell Research*, *306*, 309–316.

Benaroya, S. (1977). Sign language and multisensory input training of children with communication and related developmental disorders. *Journal of Autism and Childhood Schizophrenia*, *7*, 23–31.

Benaroya, S. (1979). Sign language and multisensory input training of children with communication and related developmental disorders: Phase II. *Journal of Autism and Developmental Disorders*, *9*, 219–220.

Bende, M., & Nordin, S. (1997). Perceptual learning in olfaction: Professional wine tasters versus controls. *Physiology & Behavior*, *62*, 1065-1070.

Bennetto, L., Kuschner, E.S., & Hyman, S.L. (2007). Olfaction and taste processing in autism. *Biological Psychiatry*, *62*, 1015-1021.

Bensafi, M., & Rouby, C. (2007). Individual differences in odor imaging ability reflect differences in olfactory and emotional perception. *Chemical Senses*, *32*, 237-244.

Bensafi, M., Porter, J., Pouliot, S., Mainland, J., Johnson, B., Zelano, C., Young, N., …, Sobel, N. (2003). Olfactomotor activity during

imagery mimics that during perception. *Nature Neuroscience*, 6, 1142–1144.

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndrome of parkinsonism and Huntington: clinical, morphological and neurochemical correlations. *Journal of Neurological Sciences*, *20*, 415-455.

Bogdashina, O. (2003). *Sensory Perceptual Issues in Autism and Asperger Syndrome: Different Sensory Experiences - Different Perceptual Worlds*. London: Jessica Kingsley Publishers.

Bohnen, N.I., Gedela, S., Herath, P., Constantine, G.M., & Moore, R.Y. (2008). Selective hyposmia in Parkinson disease: association with hippocampal dopamine activity. *Neuroscience Letters*, *447*, 12–16.

Bohnen, N.I., Müller, M.L.T.M., Kotagal, V., Koeppe, R.A., Kilbourn, M.A., Albin, R.L., & Frey, K.A. (2010). Olfactory dysfunction, central cholinergic integrity and cognitive impairment in Parkinson's disease. *Brain*, *133*, 1747–1754.

Bonanni, E., Borghetti, D., Fabbrini, M., Maestri, M., Cignoni, F., Sartucci, F., & Murri, L. (2006). Quantitative EEG analysis in post-traumatic anosmia. *Brain Research Bulletin*, *71*, 69–75.

Bordnick, P.S., Traylor, A., Copp, H.L., Graap, K.M., Carter, B., Ferrer, M., & Walton, A.P. (2008). Assessing reactivity to virtual reality alcohol based cues. *Addictive Behaviors*, *33*, 743–756.

Bozza, T., Feinstein, P., Zheng, C. & Mombaerts, P. (2002). Odorant receptor expression defines functional units in the mouse olfactory system. *Journal of Neuroscience*, *22*, 3033–3043.

Braak, H., Del Tredici, K., Rüb, U., de Vos, R.A.I., Jansen Steur, E.N.H., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*, 197–211.

Braak, H., Ghebremedhin, E., Rüb, U., Bratzke, H., & Del Tredici, K. (2004). Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Research*, *318*,:121-134.

Brämerson, A., Johansson, L., Ek, L., Nordin, S., & Bende, M. (2004). Prevalence of olfactory dysfunction: the Skövde population-based study. *Laryngoscope*, *114*, 733–737.

Brang, D., & Ramachandran, V.S. (2010). Olfactory bulb dysgenesis, mirror neuron system dysfunction, and autonomic dysregulation as the neural basis for autism. *Medical Hypothesis*, *74*, 919-921.

Brewer, W., Brereton, A. V., & Tonge, B. J. (2008). Dissociation of age and ability on a visual analogue of the University of Pennsylvania Smell Identification Test in children with autism. *Research in Autism Spectrum Disorders*, *158*, 107–115.

Briand, L., Eloit, C., Nespoulous, C., Bézirard, V., Huet, J.C., Henry, C., …, Pernollet, J.C. (2002). Evidence of an odorant-binding protein in the human olfactory mucus: location, structural characterization, and odorant-binding properties. *Biochemistry*, *41*, 7241–7252.

Briner, H.R., Simmen, D., & Jones, N. (2003). Impaired sense of smell in patients with nasal surgery. *Clinical Otolaryngology*, *28*, 417-419.

Brooks, D. N., & Baddeley, A. (1976). What can amnesic patients learn. *Neuropsychologia*, *14*, 111–122.

Bruns, J., & Hauser, W.A. (2003). The epidemiology of traumatic brain injury: A review. *Epilepsia*, *44*, 2-10.

Buck, L.B. (2000). Smell and tase: The chemical senses. In E.R. Kandel, J.H. Schwartz, & T.M. Jessel (Eds.), *Principles of Neural Science, IV edition* (p. 632). New York, NY: McGraw-Hill.

Buck, L.B. (2004). Olfactory receptors and odor coding in mammals. *Nutrition Reviews*, *62*, S184–S188.

Buck, L.B., & Axel, R. (1991). A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell*, *65*, 175–187.

Cain, W.S., Gent, J.F., Goodspeed, R.B., & Leonard, G. (1988). Evaluation of Olfactory Dysfunction in the Connecticut Chemosensory Clinical Research Center. *Laryngoscope*, *98*, 83–88.

Cain, W.S., Stevens, J.C., Nickou, C.M., Giles, A., Johnston, I., & Garcia-Medina, M.R. (1995). Life-span development of odor identification, learning, and olfactory sensitivity. *Perception*, *24*, 1457-1472.

Callahan, C.D., & Hinkebein, J. (1999). Neuropsychological significance of anosmia following traumatic brain injury. *Journal of Head Trauma Rehabilitation*, *14*, 581–587.

Callahan, C.D., & Hinkebein, J. (2002). Assessment of anosmia after traumatic brain injury: Performance characteristics of the University of Pennsylvania Smell Identification Test. *Journal of Head Trauma Rehabilitation*, *17*, 251–256.

Carmichael, S.T., Clugnet, M.C., & Price, J.L. (1994). Central olfactory connections in the macaque monkey. *Journal of Comparative Neurology*,*346*, 403–434.

Carrasco, M., & Ridout, J.B. (1993). Olfactory perception and olfactory imagery: A multidimensional analysis. *Journal of Experimental Psychology: Human Perception and Performance*, *19*, 287–301.

Castiello, U. (2005). The neuroscience of grasping. *Nature Review Neuroscience*, *6*, 726-736.

Castiello, U., Stelmach, G.E., & Lieberman, A.N. (1993). Temporal dissociation of the prehension pattern in Parkinson's disease. *Neuropsychologia*, *31*, 395–402.

Castiello, U., Zucco, G.M., Parma, V., Ansuini, C., & Tirindelli, R. (2006). Cross-modal interactions between olfaction and vision when grasping. *Chemical Senses*, *31*, 665-671.

Cavada, C., Company, T., Tejedor, J., Cruz-Rizzolo, R.J., & Reinoso-Suarez, F. (2000). The anatomical connections of the macaque monkey orbitofrontal cortex. A review. *Cerebral Cortex*, *10*, 220–242.

Cermak, L.S., Talbot, N., Chandler, K., & Wolbarst, L.R. (1985). The perceptual priming phenomenon in amnesia. *Neuropsychologia*, *23*, 615–622.

Chan, S., Fung, M. Y., & Tong, C. W. (2005). The clinical effectiveness of a multisensory therapy on clients with developmental disability. *Research in Developmental Disabilities*, *26*, 131–142.

Chapman, H.A., Kim, D.A., Susskind, J.M., & Anderson, A.K. (2009). In bad taste: evidence for the oral origins of moral disgust. *Science*, *323*, 1222–1226.

Chaudhuri, K.R., Healy, D.G., & Schapira, A.H. (2006). Non-motor symptoms of Parkinson's disease: diagnosis and management. *Lancet Neurology*, *5*, 235–245.

Chen, D., & Haviland-Jones, J. (2000). Human olfactory communication of emotion. *Perceptual and Motor Skills*, *91*, 771–781.

Cleland, T.A. (2008). The construction of olfactory representations . In C. Hölscher & M. Munk (Eds.), *Information processing in neuronal populations* (pp. 247-280). Cambridge: Cambridge University Press.

Cohen, N.J., & Squire, L.R. (1980). Preserved learning and retention of pattern-analyzing skill in amnesia: Dissociation of knowing how and knowing that. *Science*, *210*, 207– 210.

Colebatch, J.G., & Gandevia, S.C. (1989). The distribution of muscular weakness in upper motor neuron lesions affecting the arm. *Brain*, *112*, 749-763.

Collet, S., Grulois, V., Bertrand, B., & Rombaux, P. (2009). Post-traumatic olfactory dysfunction: a cohort study and update. *B-ENT*, *5*, 97-107.

Compston, A. (1994). The epidemiology of multiple sclerosis: principles, achievements, and recommendations. *Annals of Neurology*, *36*, S211–217.

Confaveaux, C., Vukusic, S., Moreau, T., & Adeleine, P. (2000). Relapses and progression of disability in multiple sclerosis. *New England Journal of Medicine*, *343*, 1430–1438.

Connelly, T., Farmer, J.M., Lynch, D.R., & Doty, R.L. (2003). Olfactory dysfunction in degenerative ataxias. *Journal of Neurology, Neurosurgery, and Psychiatry*, *74*, 1435–1437.

Constantinescu, C.S., Raps, E.C., Cohen, J.A., West, S.E., & Doty, R.L. (1994). Olfactory disturbances as the initial or most prominent symptom of multiple sclerosis. . *Journal of Neurology, Neurosurgery, and Psychiatry*, *57*, 1011–1012.

Corcoran, C., Whitaker, A., Coleman, E., Fried, J., Feldman, J., Goudsmit, N., & Malaspina, D. (2005). Olfactory deficits, cognition and negative symptoms in early onset psychosis. *Schizophrenia Research*, *80*, 283–293.

Corwin, J., Serby, M., Conrad, P., & Rotrosen, J. (1985). Olfactory recognition deficit in Alzheimer's and parkinsonian dementias. *IRCS Medical Science: Psychology & Psychiatry*, *13*, 260.

Corydon Hammond, D. (2007). Can LENS neurofeedback treat anosmia resulting from a head injury? *Journal of Neurotherapy*, *11*, 57-62.

Costanzo, R.M., & Becker, D.P. (1986). Smell and taste disorders in head injury and neurosurgery patients. In H.L. Meiselman & R.S. Rivlin (Ed.), *Clinical Measurements of Taste and Smell* (pp. 565–578). New York, NY: MacMillian Publishing Company.

Costanzo, R.M., & Zasler, N.D. (1991). Head trauma. In T.V. Getchell, R.L. Doty, L.M. Bartoshuk, J.B. Jr Snow (Eds.), *Smell and Taste in Health and Disease* (pp. 711-730). New York, NY: Raven Press

Craighero, L., Fadiga, L., Umiltà, C., & Rizzolatti, G. (1996). Evidence of visuomotor priming effect. *Neuroreport*, *7*, 347–349.

Critchley, H.D. & Rolls, E.T. (1996a). Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. *Journal of Neurophysiology*, *75*, 1673–1686 .

Critchley, H.D. & Rolls, E.T. (1996b). Olfactory neuronal responses in the primate orbitofrontal cortex: analysis in an olfactory discrimination task. *Journal of Neurophysiology*, *75*, 1659–1672.

Cunningham, J. M., Chan, F., Jones, J., Kramnetz, B., Stoll, J., & Calabresa, J. (1999). Brain injury rehabilitation: A primer for case managers. In F. Chan & M. J. Leahy (Eds), *Health Care and Disability Case Management* (pp. 475-526). Lake Zurich, IL: Vocational Consultants Press.

Curtis, V., & Biran, A. (2001). Dirt, disgust and disease: Is hygiene in our genes? *Perspectives on Biological Medicine*, *44*, 17–31.

Curtis, V., Aunger, R., & Rabie, T. (2004). Evidence that disgust evolved to protect from risk of disease. *Proceedings of the Royal Society B*, *271*, S131–S133.

Damm, M., Temmel, A., Welge-Lüssen, A., Eckel, H.E., Kreft, M.P., Klussmann, J.P., ..., Hummel., T. (2004). Epidemiologie und Therapie von Riechstörungen in Deutschland, Österreich und der Schweiz. *HNO*, 52, 112–120.

Daum, R.F., Sekinger, B., Kobal, G., & Lang, C.J. (2000). Olfactory testing with 'Sniffin' Sticks' for clinical diagnosis of Parkinson's disease. *Nervenarzt*, *71*, 643–650.

de Kruijk, J.R., Leffers, P., Menheere, P.P., Meerhoff, S., Rutten, J., & Twijnstra, A. (2003). Olfactory function after mild traumatic brain injury. *Brain Injury*, *17*, 73–78.

de Wijk, R.A., & Cain, W.S. (1994). Odor quality: discrimination versus free and cued identification. *Perception and Psychophysics*, *56*, 12-18.

Deems, D.A., Doty, R.L., Settle, R.G., Moore-Gillon, V., Shaman, P., Mester, A.F., …, Snow, J.B.J. (1991). Smell and taste disorders: a study of 750 patients from the Uni versity of Pennsylvania Smell and Taste Center. *Archives of Otolaryngology - Head and Neck Surgery*, *117*, 519–528

Degel, J. & Köster, E.P. (1999). Odors: implicit memory and performance effects. *Chemical Senses*, *24*, 317–325.

Degel, J., Piper, D., & Köster, E.P. (2001). Implicit learning and implicit memory for odors: the influence of odor identification and retention time. *Chemical Senses*, *26*, 267–280.

Del Tredici, K., Rüb, U., de Vos, R.A.I., Bohl, J.R.E., & Braak, H. (2002).. Where does Parkinson disease pathology begin in the brain? *Journal of Neuropathology & Experimental Neurology*, *61*, 413–426.

Delank, K.W., & Fechner, G. (1996). Pathophysiology of post-traumatic anosmia. *Laryngorhinootologie*, *75*, 154–159.

Delank, K.W., & Stoll, W. (1998). Olfactory function after functional endoscopic sinus surgery for chronic sinusitis. *Rhinology*, *36*,15–19.

Demattè, M.L., Endrizzi, I., Biasioli, F., Corollaro, M.L., Zampini, M. & Gasperi, F. (2011). Individual variability in the awareness of odors: Demographic parameters and odor identification ability. *Chemosensory Perception*. doi: 10.1007/s12078-011-9103-7

Dijksterhuis, A., & Aarts, H. (2010). Goals, attention, and (un)consciousness. *Annual Reviews of Psychology*, *61*, 467–490.

Dijksterhuis, A., & Nordgren, L.F.. (2006). A theory of unconscious thought. *Perspectives on Psychological Science*, *1*, 95–109

Doty, R.L. (1991). Olfactory dysfunction in neurodegenerative disorders. In T.V. Getchell, R.L. Doty, L.M. Bartoshuk & J.B. Snow (Eds.), *Smell and taste in health and disease* (pp.735- 751). New York, NY: Raven.

Doty, R.L. (2001). Olfaction. *Annual Review of Psychology*, *52*,423–452.

Doty, R.L. (2003). Odor perception in neurodegenerative disease and schizophrenia. In R.L. Doty (Ed.), *Handbook of olfaction and gustation. 2nd ed* (pp.479- 502). New York, NY: Marcel Dekker.

Doty, R.L. (2006) Assessment of Olfaction. In W.J. Brewer, D. Castle & C. Pantelis (Eds.), *Olfaction and the Brain* (pp. 235-258). Cambridge, MA: Cambridge University Press.

Doty, R.L. (2007). Olfaction in Parkinson's disease. *Parkinsonism and Related Disorders*, *3*, 225–228.

Doty, R.L. (2009). The olfactory system and its disorders. *Seminars in Neurology*, 29, 74–81.

Doty, R.L., & Frye, R. (1989). Influence of nasal obstruction on smell function. *Otolaryngology Clinics of North America*, *22*, 397–411.

Doty, R.L., & Mishra, A. (2001). Influences of nasal obstruction, rhinitis, and rhinosinusitis on the ability to smell. *Laryngoscope*, *111*, 409–423.

Doty, R.L., Bartoshuk, L.M., & Snow, J.B. (1991). Causes of olfactory and gustatory disorders. In T.V. Getchell, R.L. Doty, L.M. Bartoshuk, & J.B. Snow (Eds.), *Smell and taste in health and disease* (pp. 449-462). New York, NY: Raven.

Doty, R.L., Deems, D.A., & Stellar, S. (1988). Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology*, *38*, 1237–1244.

Doty, R.L., Li, C., Mannon, L.J., & Yousem, D.M. (1997). Olfactory dysfunction in MS. *New England Journal of Medicine*, *336*, 1918–1919.

Doty, R.L., Li, C., Mannon, L.J., & Yousem, D.M. (1998). Olfactory dysfunction in MS. Relation to plaque load in inferior frontal and temporal lobes. *Annals of the New York Academy of Sciences*, *855*, 781–786.

Doty, R.L., Li, C., Mannon, L.J., & Yousem, D.M. (1999). Olfactory dysfunction in multiple sclerosis: Relation to longitudinal changes in plaque numbers in central olfactory structures. *Neurology*, *53*, 880–882.

Doty, R.L., McKeown, D.A., Lee, W.W., & Shaman, P. (1995). A study of the test-retest reliability of ten olfactory tests. *Chemical Senses*, *20*, 645–656.

Doty, R.L., Shaman, P., & Dann, M. (1984). Development of the University of Pennsylvania Smell Identification Test: A standardized microencapsulated test of olfactory function [Monograph]. *Physiology & Behavior*, *32*, 489-502.

Doty, R.L., Shaman, P., Applebaum, S.L., Gibersen, R., Siksorski, L., & Rosenberg, R. (1984). Smell identification ability: changes with age. *Science*, *226*, 1441–1443.

Doty, R.L., Shaman, P., Kimmelman, C.P., & Dann, M.S. (1984). University of Pennsylvania Smell Identification Test: A rapid quantitative olfactory function test for the clinic. *Laryngoscope*, *94*, 176–178.

Doty, R.L., Smith, R., McKeown, D.A., & Raj, J. (1994). Tests of human olfactory function: principal components analysis suggests that most measure a common source of variance. *Perception & Psychophysics*, *56*, 701–707.

Doty, R.L., Stern, M.B., Pfeiffer, C., Gollomp, S.M., & Hurtig, H.I. (1992). Bilateral olfactory dysfunction in early stage treated and untreated idiopathic Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *55*, 138–42.

Doty, R.L., Yousem, D.M., Pham, L.T., Kreshak, A.A., Geckle, R., & Lee, W.W. (1997). Olfactory dysfunction in patients with head trauma. *Archives of Neurology*, *54*, 1131–1140.

Double, K.L., Rowe, D.B., Hayes, M., Chan, D.K.Y., Blackie, J., Corbett, A., …, Halliday GM. (2003). Identifying the pattern of olfactory deficits in Parkinson's disease using the brief smell identification test. *Archvies of Neurology*, *60*, 545-549.

Doucet, S., Soussignan, R., Sagot, P., & Schaal, B. (2007). The "smellscape" of mother's breast: effects of odor masking and selective unmasking on neonatal arousal, oral, and visual responses. *Developmental Psychobiology*, *49*, 129–138.

Downey, L.L., Jacobs, J.B., & Lebowitz, R.A. (1996). Anosmia and chronic sinus disease. *Archives of Otolaryngology - Head and Neck Surgery*, *115*, 24–28.

Dudova, I., Vodicka, J., Havlovicova, M., Sedlacek, Z., Urbanek, T., & Hrdlicka, M. (2011). Odor detection threshold, but not odor identification, is impaired in children with autism. *European Child and Adolescent Psychiatry*, *20*, 333–340.

Edwards, M.G., Humphreys, G.W., & Castiello, U. (2003). Motor facilitation following action observation: A behavioural study in prehensile action. *Brain and Cognition*, *53*, 495–502.

Eisthen, H.L. (2002). Why are olfactory systems of different animals so similar? *Brain Behavior Evolution*, *59*, 273–293.

Elsaesser, R. & Paysan, J. (2005). Morituri te salutant? Olfactory signal transduction and the role of phosphoinositides. *Journal of Neurocytology*, *34*, 97–116

Engen, T. (1987). Remembering odors and their names. *American Scientist*, *75*, 497-503.

Eslinger, P.J., & Damasio, A.R. (1985). Severe disturbance of higher cognition after bilateral frontal lobe ablation: Patient E.V.R. *Neurology*, *35*, 1731-1741

Fahn, S., & Elton, R.L. (1987). UPDRS program members. Unified Parkinson's disease rating scale. In S. Fahn, C.D. Marsden, M. Goldstein, D.B. Calne (Eds.), *Recent Developments in Parkinson's*

Disease, Vol 2 (pp. 152- 162). Florham Park, NJ: Macmillan Healthcare Information.

Faulcon, P., Portier, F., Biacabe, B., & Bonfils, P. (1999). Anosmie secondaire à une rhinite aiguë: sémiologie et évolution à propos d'une série de 118 patients. *Annales d'Otolaryngologie et de Chirurgie Cervico-faciale*, *116*, 351–357.

Fedoroff, I.C., Stoner, S.A., Andersen, A.E., Doty, R.L., & Rolls, B.J. (1995). Olfactory dysfunction in anorexia and bulimia nervosa. International *Journal of Eating Disorders*, *18*, 71–77.

Ferdenzi, C., Coureaud, G., Camos, V., & Schaal, B. (2008). Human awareness and uses of odor cues in everyday life: Results from a questionnaire study in children. *International Journal of Behavioral Development*, *32*, 422-431.

Ferdenzi, C., Schaal, B., & Roberts, S.C. (2010). Family scents: developmental changes in the perception of kin body odor? *Journal of Chemical Ecology*, *36*, 847–854.

Ferreyra-Moyano, H., & Barragan, E. (1989). The olfactory system and Alzheimer's disease. *International Journal of Neuroscience*, *49*, 157–197.

Ferry, B., Ferreira, G., Traissard, N., & Majchrzak, M. (2006). Selective involvement of the lateral entorhinal cortex in the control of the

olfactory memory trace during conditioned odor aversion in the rat. *Behavioral Neuroscience*, *120*, 1180–1186.

Firestein, S. (2001). How the olfactory system makes sense of scents? *Nature*, *413*, 211–218.

Fitzsimon, J.S., Waring, S.C., Kokmen, E., McLaren, J.W., & Brubaker, R.F. (1997). Response of the pupil to tropicamide is not a reliable test for Alzheimer disease. *Archives of Neurology*, *54*, 155–159.

Fogassi, L., & Gallese, V. (2004). Action as binding key to multisensory integration. In G. Calvert, C. Spence, & B. E. Stein (Eds.), *The Handbook of Multisensory Processes* (pp. 425-441). Cambridge, MA: MIT Press.

Folstein, M.F., Folstein, S.E., & McHugh, P.R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, *12*, 189-198.

Fortin, A., Lefebvre, M.B., & Ptito, M. (2010). Traumatic brain injury and olfactory deficits: The tale of two smell tests. *Brain Injury*, *24*, 27–33.

Franselli, J., Landis, B.N., Heilmann, S., Hauswald, B., Huttenbrink, K.B., Lacroix, J.S., …, Hummel, T. (2004). Clinical presentation of qualitative olfactory dysfunction. *European Archives of Otohinolaryngology*, *261*, 411–415.

Freud, S. (1978). *Minutes de la société psychanalytique de Vienne (17-11-1909)*. Paris: Gallimard.

Frith, U., & Happé´, F. (1994). Autism: Beyond "theory of mind". *Cognition*, *50*, 115–132.

Fujii, M., Fukazawa, K., Takayasu, S., & Sakagami, M. (2002). Olfactory dysfunction in patients with head trauma. *Auris Nasus Larynx*, *29*, 35–40.

Fujiwara, E., Schwartz, M.L., Gao, F., Black, S.E., & Levine, B. (2008). Ventral frontal cortex functions and quantified MRI in traumatic brain injury. *Neuropsychologia*, *46*, 461–474.

Gallese, V., Fadiga, L., Fogassi, L. & Rizzolatti, G. (1996). Action recognition in the premotor cortex. *Brain*, *119*, 593–609.

Gansler, D.A., Fucetola, R., Krengel, M., Stetson, S., Zimering, R., & Makary, C. (1998). Are there cognitive subtypes in adult attention deficit/hyperactivity disorder? *Journal of Nervous & Mental Disease*, *186*, 776–781.

Geisler, M.W., Schlotfeldt, C.R., Middleton, C.M., Dulay, M.F., & Murphy, C., (1999). Traumatic brain injury assessed with olfactory event-related brain potentials. *Journal of Clinical Neurophysiology*, *16*, 77-86.

Gentilucci, M., Castiello, U., Corradini, M.L., Scarpa, M., Umiltà, C., & Rizzolatti, G. (1991). Influence of different types of grasping on the transport component of prehension movements. *Neuropsychologia*, *29*, 361-378.

Gentilucci, M., Daprati, E. & Gangitano, M. (1998). Haptic information differentially interferes with visual analysis in reaching-grasping control and in perceptual processes. *Neuroreport*, *9*, 887–891.

Gerardi, M., Rothbaum, B.O., Ressler, K., Heekin, M., & Rizzo, A. (2008). Virtual reality exposure therapy using a virtual Iraq: Case report. *Journal of Trauma Stress*, *21*, 209-213.

Gibson, E.J. (1969). *Principles of Perceptual Learning and Development*. New York: Appleton-Century-Crofts.

Gilbert, A.N., & Wysocki, C.J. (1987, October). The smell survey results. *National Geographic Magazine*, p. 514.

Gilbert, A.N., Crouch, M., & Kemp, S.E. (1998). Olfactory and visual mental imagery. *Journal of Mental Imagery*, *22*, 137- 146.

Goldstein, E.B. (2011). Perception. In E.B. Goldstein (Ed.), *Cognitive Psychology: Connecting Mind, Research, and Everyday Experience*. (pp. 47-79). Belmont, CA: Wadsworth.

Goldstone, R.L. (1998). Perceptual learning. *Annual Review of Psychology*, *49*, 585–612.

Gordon, A.M. (1998). Task-dependent deficits during object release in Parkinson's disease. *Experimental Neurology*, *153*, 287-298.

Gordon, A.M., Ingvarsson, P.E., & Forssberg, H. (1997). Anticipatory control of manipulative forces in Parkinson's disease. *Experimental Neurology*, *145*, 477-488.

Gottfried, J. A. (2010). Central mecha-nisms of odour object perception. *Nature Reviews Neuroscience*, *11*, 628–641.,

Gottfried, J.A., & Dolan, R.J. (2003). The nose smells what the eye sees: crossmodal visual facilitation of human olfactory perception. *Neuron*, *39*, 375-386.

Gouvier, W.D., Blanton, P.D., LaPorte, K.L., & Nepomuceno, C. (1987). Reliability and validity of the Disability Rating Scale and the Levels of Cognitive Functioning Scale in monitoring recovery from severe head injury. *Archives of Physical Medical Rehabilitation*, *68*, 947.

Graf, P., & Schacter, D.L. (1985). Implicit and explicit memory for new associations in normal and amnesic subjects. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *11*, 501–518.

Graf, P., Shimamura, A. P., & Squire, L. R. (1985). Priming across modalities and priming across category levels: Extending the domain of preserved function in amnesia. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *11*, 386–396.

Graf, P., Squire, L. R., & Mandler, G. (1984). The information that amnesic patients do not forget. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 10*, 164– 178.

Grandin, T. (1992). An inside view of autism. In E. Schopler & G. B. Mesibov (Eds.). *High functioning Individuals with Autism* (pp. 105–126). New York, NY: Plenum Press.

Gray, H. (1918). Anatomy of the human body. Philadelphia: Lea & Febiger. Retrieved from: http://www.bartleby.com/107/.

Graziadei, P.P., & Monti Graziadei, G.A. (1985). Neurogenesis and plasticity of the olfactory sensory neurons. *Annals of the New York Academy of Sciences, 457*, 127–142.

Graziadei, P.P., Levine, R.R. & Graziadei, G.A. (1978). Regeneration of olfactory axons and synapse formation in the forebrain after bulbectomy in neonatal mice. *Proceedings of the National Academy of Sciences USA, 75*: 5230–5234.

Green, P., & Iverson, G.L. (2001). Effects of injury severity and cognitive exaggeration on olfactory deficits in head injury compensation claims. *NeuroRehabiliation, 16*, 237–243.

Green, P., Rohling, M.L., Iverson, G.L., & Gervais, R.O. (2003). Relationships between olfactory discrimination and head injury severity. *Brain Injury, 17*, 479–496.

Grevers, G. (2006). Diagnostic evaluation of the nose and paranasal sinuses. In R. Probst, G. Grevers & H. Iro (Eds.), *Basic otolaryngology. A step-by-step learning guide* (pp. 15-26). Stuttgart: Thieme.

Grigor, J. (1995). Do the eyes see what the nose knows? An investigation of the effects of olfactory priming on visual event related potentials. *Chemical Senses*, *20*, 163.

Grigor, J., Van Toller, S., Behan, J., & Richardson, A. (1999). The effect of odour priming on long latency visual evoked potentials of matching and mismatching objects. *Chemical Senses*, *24*, 137-144.

Gross-Isseroff, R., Luca-Haimovici, K., Sasson, Y., Kindler, S., Kotler, M., & Zohar, J. (1994). Olfactory sensitivity in major depressive disorder and obsessive compulsive disorder. *Biological Psychiatry 35*, 798–802.

Haehner, A., Boesveldt, S., Berendse, H.W., Mackay-Sim, A., Fleischmann, J., Silburn, P.A., …, Hummel, T. (2009). Prevalence of smell loss in Parkinson's disease - a multicenter study. *Parkinsonism and Related Disorders*, *15*, 490–494.

Haist, F., Musen, G., & Squire, L. R. (1991). Intact priming of words and nonwords in amnesia. *Psychobiology*, *19*, 275–285.

Happé, F.G.E., & Frith, U. (1996a). The neuropsychology of autism, *Brain*, *119*, 1377–1400.

Happé, F.G.E., & Frith, U. (1996b). Theory of mind and social impairment in children with conduct disorder. *British Journal of Developmental Psychology*, *14*, 385–398.

Havlicek, J., & Roberts, S.C. (2009). MHC-correlated mate choice inhumans: a review. *Psychoneuroendocrinology*, *34*, 497–512.

Hawkes, C.H., Shephard, B.C., & Daniel, S.E. (1997). Olfactory dysfunction in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry*, *62*, 436–446.

Hawkes, C.H., Shephard, B.C., & Daniel, S.E. (1999). Is Parkinson's disease a primary olfactory disorder? *Quarterly Journal of Medicine*, *92*, 473–480.

Hawkes, C.H., Shephard, B.C., & Kobal, G. (1997). Assessment of olfaction in multiple sclerosis: Evidence of dysfunction by olfactory evoked response and identification tests *Journal of Neurology, Neurosurgery, and Psychiatry*, *63*, 145–151.

Haxel, B.R., Grant, L., & Mackay-Sim, A. (2008). Olfactory dysfunction after head injury. *Journal of Head Trauma Rehabilitation*, *23*, 407–413.

Hayes, C. (2011). Automatic imitation. *Psychological Bullettin*, *137*, 463-483.

Heaton, R.K., Chelune, G.J., Talley, J.L., Kay, G.G., & Curtiss, G. (1993). *Wisconsin Card Sorting Test manual: Revised and expanded*. Odessa. FL: Psychological Assessment Resources.

Henkin, R.I. (1967). Abnormalities of taste and olfaction in patients with chromatin negative gonadal dysgenesis. *Journal of Clinical Endocrinology and Metabolism*, *27*, 1436–1440.

Henkin, R.I. (1968). Impairment of olfaction and of the tastes of sour and bitter in pseudohypoparathyroidism. *Journal of Clinical Endocrinology and Metabolism*, *28*, 624–628.

Henkin, R.I. (1975). The role of adrenal corticosteroids in sensory processes. In H. Blaschko, A.D. Smith & G. Sayers (Eds.), *Handbook of Physiology* (pp. 209–230). Washington, DC: American Physiological Society.

Henkin, R.I., & Bartter, F.C. (1966). Studies on olfactory thresholds in normal man and in patients with adrenal cortical insufficiency: the role of adrenal cortical steroids and of serum sodium concentration. *Journal of Clinical Investigation*, *45*, 1631–1639.

Herz, R.S. (1998). Are odors the best cues to memory? A cross-modal comparison of associative memory stimuli. *Annals of New York Academy of Science*, *855*, 670–674.

Herz, R.S. (2002). Influences of odors on mood and affective cognition. In C. Rouby, B. Schaal, D. Dubois, R. Gervais, & A. Holley (Eds.), *Olfaction, Taste, and Cognition* (pp. 160–177). Cambridge: Cambridge University Press.

Herz, R.S., Eliassen, J., Beland, S., & Souza, T. (2004). Neuroimaging evidence for the emotional potency of odor-evoked memory. *Neuropsychologia*, *42*, 371–378.

Herz, R.S., & Engen, T. (1996). Odor memory: review and analysis. *Psychonomic Bulletin Review*, *3*, 300–313.

Herz, R.S., & Inzlicht, M. (2002). Sex differences in response to physical and social factors involved in human mate selection: The importance of smell for women. *Evolution and Human Behavior*, *23*, 359–364.

Hildebrand, J.G. & Shepherd, G.M. (1997). Mechanisms of olfactory discrimination: converging evidence for common principles across phyla. *Annual Review of Neuroscience*, *20*, 595–631.

Ho, W.K., Kwong, D.L., Wei, W.I., & Sham, J.S. (2002). Change in olfaction after radiotherapy for nasopharyngeal cancer - A prospective study. *American Journal of Otolaryngology*, *23*, 209–214.

Hoehn, M.M., & Yahr, M.D. (1967). Parkinsonism: onset, progression and mortality. *Neurology*, *17*, 427– 442.

Hollingshead, A.B. (1975). *Four factor index social status.* Unpublished working paper. New Haven, CT: Yale University, Department of Sociology. Available at: http://www.yale.edu/sociology/faculty/

Hoseman, W., Goertzen, W., Wohlleben, R., Wolf, S., & Wigand, M. E. (2000). Olfaction after endoscopic endonasal ethmoidectomy. *American Journal of Rhinology*, *7*, 11–15.

Howard, J.D., Plailly, J., Grueschow, M., Haynes, J.D., & Gottfried, J.A. (2009). Odor quality coding and categorization in human posterior piriform cortex. *Nature Neuroscience*, *12*, 932–938.

Hrdlicka, M., Vodicka, J., Havlovicova, M., Urbanek, T., Blatny, M., & Dudova, I. (2011). Brief Report: Significant Differences in Perceived Odor Pleasantness Found in Children with ASD. *Journal of Autism and Developmental Disorders*, *41*, 524–527.

Huard, J.M., Youngentob, S.L., Goldstein, B.J., Luskin, M.B. & Schwob, J.E. (1998) Adult olfactory epithelium contains multipotent progenitors that give rise to neurons and non-neural cells. *Journal of Comparative Neurology*, *400*, 469–486.

Hudry, J., Saoud, M., Amato, T., Daléry, J., & Royet, J.P. (2002). Ratings of different olfactory judgements in schizophrenia. *Chemical Senses*, *27*, 407–416.

Huisman, E., Uylings, H.B., & Hoogland, P.V. (2004). A 100% increase of dopaminergic cells in the olfactory bulb may explain hyposmia in Parkinson's disease. *Movement Disorders*, *19*, 687– 692.

Hummel, T. (2000). Perspectives in olfactory loss following viral infections of the upper respiratory tract. *Archives of Otolaryngology - Head and Neck Surgery*, *126*, 802–803.

Hummel, T., & Kobal, G. (2001). Olfactory event-related potentials. In S.A. Simon & M.A.L. Nicolelis (Eds.), *Methods and Frontiers in Chemosensory Research* (pp 429–464). Boca Raton, FL: CRC Press.

Hummel, T., & Nordin, S. (2005). Olfactory disorders and their consequences for quality of life. *Acta Otolaryngologica*, *125*, 116-121.

Hummel, T., & Welge-Lüssen, A. (2006). Assessment of olfactory function. In T. Hummel, & A. Welge-Lüssen (Eds.), *Taste and Smell. An Update* (pp. 85-99). Basel: Karger.

Hummel, T., Knecht, M., & Kobal, G. (1996). Peripherally obtained electrophysiological responses to olfactory stimulation in man: Electro-olfactograms exhibit a smaller degree of 501 desensitization compared with subjective intensity estimates. *Brain Research*, *117*, 160-164.

Hummel, T., Kobal, G., Gudziol, H., & Mackay-Sim. (2007). A. Normative data for the "Sniffin' Sticks" including tests of odour identification, odour discrimination, and olfactory thresholds: An upgrade based

on a group of more than 3,000 subjects. *European Archives of Otorhinolaryngoly*, *264*, 237–243.

Hummel, T., Konnerth, C.G., Rosenheim, K., & Kobal, G. (2001). Screening of olfactory function with a four-minute odor identification test: reliability, norms given and investigations in patients with olfactory loss. *Annals of Otology, Rhinology and Laryngology*, *110*, 976–981.

Hummel, T., Rothbauer, C., Barz, S., Grosser, K., Pauli, E., & Kobal, G. (1998). Olfactory function in acute rhinitis. *Annals of the New York Academy of Sciences*, *855*, 616–624.

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

Hurley, S., & Chater, N. (2005). *Perspective on imitation: From neuroscience to social science*. Cambridge, MA: MIT Press.

Iarocci, G., & McDonald, J. (2006). Sensory integration and the perceptual experience of persons with autism. *Journal of Autism and Developmental Disorders*, *36*, 77–90.

Isaacson, J.S. (2010).Odor representations in mammalian cortical circuits. *Current Opinion in Neurobiology*. doi:10.1016/j.conb.2010.02.004.

Issanchou, S., Valentin, D., Sulmont, C., Degel, J., & Koster, E.P.(2002). Testing odor memory: Incidental versus intentional learning, implicit versus explicit memory. In C. Rouby, B. Schaal, D.Dubois, R. Gervais, & A. Holley (Eds.), *Olfaction, Taste, and Cognition* (pp. 211–230). New York, NY: Cambridge University Press.

Jafek, B.W., Moran, D.T., Eller, P.M., Rowley, J.C., & Jafek, T.B. (1987). Steroid-dependent anosmia. *Archives of Otolaryngology - Head and Neck Surgery*, *113*, 547–549.

Jakobson, L.S., & Goodale, M.A. (1991). Factors affecting higher-order movement planning: A kinematic analysis of human prehension. *Experimental Brain Research*, *86*, 199-208.

Jeannerod, M. (1981). Intersegmental coordination during reaching at natural visual objects. In J. Long & A. Baddeley (Eds.), *Attention and Performance IX* (pp. 153–168). Hillsdale, NJ: Erlbaum.

Jeannerod, M. (1984). The timing of natural prehension movements. *Journal of Motor Behavior*, *16*, 235-254.

Jennet, B., & Teasdale, G. (1981). *Management of Head Injuries*. Philadelphia, PA: FA Davies.

Jones-Gotman, M., & Zatorre, R.J. (1988). Olfactory identification deficits in patients with focal cerebral excision. *Neuropsychologia*, *26*, 387–400.

Kareken, D.A., Mosnik, D.M., Doty, R.L., Dzemidzic, M., & Hutchins, G.D. (2003). Functional anatomy of human odor sensation, discrimination, and identification in health and aging. *Neuropsychology*, *17*, 482–495.

Katzenschlager, R., Tischler, R., Kalchhauser, G., Panny, M., & Hirschl, M. (2009). Angio-Seal use in patients with peripheral arterial disease (ASPIRE). *Angiology*, *60*, 536–538.

Kawai, T., & Noro, K. (1996). Psychological effect of stereoscopic 3-D images with fragrances. *Ergonomics*, *39*, 1364–1369.

Kay, L.M., Crk, T., & Thorngate, J. (2005). A redefinition of odor mixture quality. *Behavioral Neuroscience*, *119*, 726–733.

Keller, A., & Vosshall, L.B. (2004). Human olfactory psychophysics. *Current Biology*, *14*, R875–R878.

Kern, J.K., Trivedi, M.H., Garver, C.R., Grannemann, B.D., Andrews, A.A., Savla, J.S., …, Schroeder, J.L. (2006). The pattern of sensory processing abnormalities in autism. *Autism*, *10*, 480–494.

Kesslak, J.P., Cotman, C.W., Chui, H.C., Van Den Noort, S., Fang, H., Pfeffer, R., & Lynch, G. (1988). Olfactory tests as possible probes for detecting and monitoring Alzheimer's disease. *Neurobiology of Aging*, *9*, 399-403.

Kimmelman, C.P. (1994). The risk to olfaction from nasal surgery. *Laryngoscope*, *104*, 981–988.

Knudsen, E.I., & Brainard, M.S. (1995). Creating a unified representation of visual and auditory space in the brain. *Annual Review of Neuroscience*, *18*, 19–43.

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

Kobal, G., Klimek, L., Wolfensberger, M., Gudziol, H., Temmel, A., Owen, C.M., …, Hummel, T. (2000). Multicenter investigation of 1,036 subjects using a standardized method for the assessment of olfactory function combining tests of odour identification, odour discrimination, and olfactory thresholds. *European Archives of Otorhinolaryngoly*, *257*, 205–211.

Koch, K., Pauly, K., Kellermann, T., Seiferth, N.Y., Reske, M., Backes, V., …, Habel, U. (2007) Gender differences in the cognitive control of emotion: An fMRI study. *Neuropsychologia*, *45*, 2744–2754.

Koenig, O., Bourron, G., & Royet, J.P. (2000). Evidence of separate perceptive and semantic memories for odors: A priming experiment. *Chemical Senses*, *25*, 703-708.

Koffka, K. (1935). *Principles of Gestalt Psychology*. New York: Harcourt Brace Jovanovich

Kopala, L.C., & Good, K.P. (1996). Olfactory identification ability in patients with panic disorder. *Journal of Psychiatry & Neuroscience*, *21*, 340–342.

Kopala, L.C., Good, K., Goldner, E.M., & Birmingham, C.L. (1995). Olfactory identification ability in anorexia nervosa. *Journal of Psychiatry & Neuroscience*, *20*, 283–286.

Korten, J.J., & Meulstee, J. (1980). Olfactory disturbances in parkinsonism. *Clinical Neurology and Neurosurgery*, *82*, 113-118.

Kostic, J., Stefanova, E., Pekmezovic, T., & Drulovic, J. (2009). Correlation between olfactory dysfunction and linical parameters in patients with multiple sclerosis. *Vojnosanitetski pregled*, *66*, 203–206.

Kubovy, M. & Van Valkenburg, D. (2001). Auditory and visual objects. *Cognition*, *80*, 97–126.

Kurtzke, J.F. (1983). Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). *Neurology*, *33*, 1444–1452.

Laing, D.G., & Francis, G.W. The capacity of humans to identify odors in mixtures. *Physiology & Behavior*, *46*, 809–814.

Lancet, D. (1994). Olfaction. Exclusive receptors. *Nature*, *372*, 321–322.

Landis, B.N., Konnerth, C.G., & Hummel, T. (2004). A study on the frequency of olfactory dysfunction. *Laryngoscope*, *114*, 1764–1769.

Landis, B.N., Scheibe, M., Weber, C., Berger, R., Brämerson, A., Bende, M., Nordin, S., & Hummel., T. (2010). Chemosensory interaction: acquired olfactory impairment is associated with decreased taste function. *Journal of Neurology*, *253*, 1303-1308.

Lane, A.E., Dennis, S.J., & Geraghty, M.E. (2011). Brief report: Further evidence of sensory subtypes in autism. *Journal of Autism and Developmental Disorders*, *41*, 826–831.

Lane, A.E., Young, R.L., Baker, A.E. Z., & Angley, M. (2010). Sensory processing subtypes in autism: Association with adaptive behavior. *Journal of Autism and Developmental Disorders*, *40*, 112–122.

Larsson, M. (2002). Odor memory: a memory system approach. In: C. Rouby, B. Schaal, D. Dubois, R. Gervais, & A. Holley (Eds.), *Olfaction, Taste, and Cognition* (pp. 231–245). Cambridge: Cambridge University Press.

Larsson, M., Nilsson, L.G., Olofsson, J.K., & Nordin, S. (2004). Demographic and cognitive predictors of cued odor identification: evidence from a population based study. *Chemical Senses*, *29*, 547–554.

Laska, M., Wieser, A., & Salazar, L.T. (2006). Sex-specific differences in olfactory sensitivity for putative human pheromones in nonhuman primates. *Journal of Comparative Psychology*, *120*, 106-112.

Le Bel, R., Pineda, J., & Sharma, A. (2009). Motor-auditory-visual integration: the role of the human mirror neuron system in

communication and communication disorders. *Journal of Communicative Disorders*, *42*, 299–304.

Le Couteur, A., Lord, C., & Rutter, M. (2003). *Autism Diagnostic Interview–Revised (ADI-R)*. Los Angeles: Western Psychological Services.

Le Guérer, A. (2002). Olfaction and cognition: A philosophical and psychoanalytic view. In C. Rouby, B. Schaal, D. Dubois, R. Gervais, & A. Holley (Eds.), *Olfaction, Taste, and Cognition* (pp. 3–15). Cambridge: Cambridge University Press.

LeDoux, J.E. (2000). The amygdala and emotion: a view through fear. In J.P. Aggleton (Ed.), *The Amygdala* (pp. 289– 310). New York, NY: Oxford University Press.

Leekam, S.R., Nieto, C., Libby, S.J., Wing, L., & Gould, J. (2007). Describing the sensory abnormalities of children and adults with autism. *Journal of Autism and Developmental Disorders*, *37*, 894–910.

Leonard, G., Cain, W. S., & Clavet, G. (1988). Surgical correction of olfactory disorders. *Chemical Sense*, *13*, 708.

Levy, D.A., Manns, J.R., Hopkins, R.O., Gold, J.J., Broadbent, N.J.,& Squire, L.R. (2003). Impaired visual and odor recognition memory span in patients with hippocampal lesions. *Learning & Memory*, *10*, 531–536.

Libet, B., Gleason, C.A., Wright, E.W., & Pearl, D.K. (1983). Time of conscious intention to act in relation to onset of cerebral activity (readiness-potential): the unconscious initiation of a freely voluntary act. *Brain*, *106*, 623–642.

Livermore, A. & Laing, D. G. (1996). Influence of training and experience on the perception of multicomponent odor mixtures. *Journal of Experimental Psychology: Human Perception and Performance*, *22*, 267–277.

Lombion-Pouthier, S., Vandel, P., Nezelof, S., Haffen, E., & Millot, J.L. (2006). Odor perception in patients with mood disorders. *Journal of Affective Disorders*, *90*, 187–191.

London, B., Nabet, B., Fisher, A.R., White, B., Sammel, M.D., & Doty, R.L. (2008). Predictors of prognosis in patients with olfactory disturbance. *Annals of Neurology*, *63*, 159–166.

Lord, C., Rutter, M., DiLavore, P.C., & Risi, S. (2002). *Autism diagnostic observation schedule: ADOS*. Los Angeles: Western Psychological Services

Lumsden, C.E. (1970). The neuropathology of multiple sclerosis. In P.J. Vinken, & G.W. Bruyn (Eds.), *Handbook of Clinical Neurology* (pp 217–309). Amsterdam: Elsevier.

Lundström, J.N., Boyle, J.A., Zatorre, R.J., & Jones-Gotman, M. (2008). Functional neuronal processing of body odors differ from that of similar common odors. *Cerebral Cortex*, *18*, 1466–1474.

Lundström, J.N., Boyle, J.A., Zatorre, R.J., & Jones-Gotman, M. (2009). The neuronal substrates of human olfactory based kin recognition. *Human Brain Mapping*, *30*, 2571–2580.

Lynch, P. J. Medical illustrator. Retrieved from: http://commons.wikimedia.org/wiki/File:Head_olfactory_nerve.jpg

Lynch, R. T. (1986). Traumatic brain injury: Implications for rehabilitation counseling. In T. F. Riggar, D. R. Maki, & A. W. Wolf (Eds.), *Applied Rehabilitation Counseling* (pp. 262-270). New York, NY: Springer.

Macfarlane, A. (1975). Olfaction in the development of social preferences in the human neonate. In *CIBA Foundation Symposium 33: Parent-infant interaction*. New York, NY: Elsevier

Mackay-Sim, A. & Kittel, P.W. (1991). On the life span of olfactory receptor neurons. European Journal of Neuroscience, 3, 209–215.

Maddock, R.J., Garrett, A.S., & Buonocore, M.H. (2001). Remembering familiar people: the posterior cingulate cortex and autobiographical memory retrieval. *Neuroscience*, *104*, 667–676.

Malaspina, D., & Coleman, E. (2003). Olfaction and social drive in schizophrenia. *Archives of General Psychiatry*, *60*, 578–584.

Males, J.L., & Schneider, R.A. (1972). Hypergonadotrophic hypogonadism with anosmia. *Acta Endocrinologica. 71*, 7–12.

Malnic, B., Godfrey, P.A., & Buck, L.B. (2004). The human olfactory receptor gene family. *Proceedings of the National Academy of Sciences USA*, *101*, 2584–2589.

Malnic, B., Hirono, J., Sato, T., & Buck, L.B. (1999). Combinatorial receptor codes for odors. *Cell*, *96*, 713–723.

Mann, N.M. (2002). Management of smell and taste problems. *Clevlande Clinic Journal of Medicine*, *69*, 329–336.

Mann, N.M., & Vento, J.A. (2006). A study comparing SPECT and MRI in patients with anosmia after traumatic brain injury. *Clinical Nuclear Medicine*, *31*, 458–462.

Marshall, P.J., & Meltzoff, A.N. (2011). Neural mirroring systems: Exploring the EEG μ rhythm in human infancy. *Developmental Cognitive Neuroscience*, *1*, 110-123.

Mateo, J.M., & Johnston, R.E. (2000). Kin recognition and the 'armpit effect': evidence of self-referent phenotype matching. *Proceedings of the Royal Society London: Series B: Biological Sciences*, *267*, 695-700.

Maturana, H.R., & Varela, F.J. (1987). *The Tree of Knowledge: The Biological Roots of Human Understanding.* Boston, MA: Shambhala.

May, T., Brewer, W.J., Rinehart, N., Enticott, P.G., Brerenton, A.V., & Tonge, B.J. (2011). Differential olfactory identification in children with autism and Asperger's disorder: A comparative and longitudinal study. *Journal of Autism and Developmental Disorders, 41*, 837–847.

McCormack, L.J., & Harris, H.E. (1955). Neurogenic tumors of the nasal fossa. *Journal of American Medical Association, 157*, 318–321.

McDonald, I., Compston, A., Edan, G., Goodkin, D., Hartung, H.P., Lublin, F.D., …, Wolinsky, J.S. (2001). Recommended diagnostic criteria for multiple sclerosis: Guidelines from the internatioal panel on the diagnosis of multiple sclerosis. *Annals of Neurology, 50*, 121–127.

McGurk, H., & MacDonald, J. (1976). Hearing lips and seeing voices. *Nature, 264*, 746–748.

Meirsschaut, M., Roeyers, H., & Warreyn, P. (2011). The social interactive behaviour of young children with autism spectrum disorder and their mothers. *Autism, 15*, 43-64.

Melcher, J.M., & Schooler, J.W. (1996). The mis-remembrance of wines past: Verbal and perceptual expertise differentially mediate verbal overshadowing of taste memory. *Journal of Memory and Language, 35*, 231-245.

Meltzoff, A.N., & Gopnik, A. (1994). The role of imitation in understanding persons and developing a theory of mind. In: S. Baron-Cohen, H. Tager-Flusberg, & D. Cohen (Eds.), *Understanding Other Minds; Perspectives from Autism* (pp. 335--366). Oxford: Oxford University Press.

Menco, B.P., & Morrison, E.E. (2003). Morphology of the mammalian olfactory epithelium: Form, fine structure, function and pathology. In R.L. Doty (Eds.) *Handbook of Olfaction and Gustation* (pp. 17-49). New York, NY: Marcel Dekker.

Mesholam, R.I., Moberg, P.J., Mahr, R.N., & Doty, R.L. (1998). Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of Neurology*, *55*, 84–90.

Miller, D.H., Grossman, R.I., Reingold, S.C., & McFarland, H.F. (1998). The role of magnetic resonance techniques in understanding and managing multiple sclerosis. *Brain*, *121*, 3–24.

Miwa, T., Furukawa, M., Tsukatani, T., Costanzo, R.M., DiNardo, L.J., & Reiter, E.R. (2001). Impact of olfactory impairment on quality of life and disability. *Archives of Otolaryngology- Head and Neck Surgery*, *127*, 497–503.

Moberg, P.J., & Doty, R.L. (1997). Olfactory function in Huntington's disease patients and at-risk offspring. *International Journal of Neuroscience*, *89*, 133–139.

Moberg, P.J., Arnold, S.E., Doty, R.L., Kohler, C., Kanes, S., Seigel, S.,…, Turetsky, B.I. (2003). Impairment of odor hedonics in men with schizophrenia. American Journal of Psychiatry, 160, 1784–1789.

Mombaerts P. (2004) Odorant receptor gene choice in olfactory sensory neurons: the one receptor-one neuron hypothesis revisited. *Current Opinion in Neurobiology*, *14*, 31–36

Mombaerts, P. (2006). Axonal wiring in the mouse olfactory system. *Annual Review of Cell Developmental Biology*, *22*, 713–737.

Mombaerts, P., Wang, F., Dulac, C., Chao, S.K., Nemes, A., Mendelsohn, M., …, Axel, R. (1996). Visualizing an olfactory sensory map. *Cell*, *87*, 675-686.

Montagner, H. (1974). Communication non verbale et discrimination olfactive chez le jeune enfant: approche éthologique. In E. Morin & M. Piatell-Palmarini (Eds.), *L'Unité de l'Homme* (pp. 246– 270). Paris: Seuil.

Montani, G., Tonelli, S., Elsaesser, R., Paysan, J., & Tirindelli, R. (2006). Neuropeptide Y in the olfactory microvillar cells. *European Journal of Neuroscience*, *24*, 20–24.

Moran, D.T., Rowley, J.C., Jafek, B.W. & Lovell, M.A. (1982). The fine structure of the olfactory mucosa in man. *Journal of Neurocytology*, *11*, 721–746.

Morecraft, R.J., & Van Hoesen, G.W. (1993). Frontal Granular cortex input to the cingulate (M3), supplementary (M2) and primary (M1) motor cortices in the rhesus monkey. *Journal of Comparative Neurology*, *337*, 669-689.

Mori, K., Takahashi, Y.K., Igarashi, K.M., & Yamaguchi, M. (2006). Maps of odorant molecular features in the Mammalian olfactory bulb. *Physiological Reviews*, *86*, 409–433.

Mueller, C., & Hummel, T. (2009). Recovery of olfactory function after nine years of post-traumatic anosmia: a case report. *Journal of Medical Case Reports*, *3*, 9283.

Mujica-Parodi, L.R., Strey, H.H., Frederick, B., Savoy, R., Cox, D., Botanov, Y., …, Weber, J. (2009). Chemosensory cues to conspecific emotional stress activate amygdala in humans. *PLoS One*, *4*, e6415.

Müller, A., Müngersdorf, M., Reichmann, H., Strehle, G., & Hummel, T. (2002). Olfactory function in Parkinsonian syndromes. *Journal of Clinical Neuroscience*, *9*, 521-524.

Müller, F., & Stelmach, G.E. (1992). Prehension movements in Parkinson's disease. In: G.E. Stelmach & J. Requin (Eds.), *Tutorials in Motor Behavior II* (pp. 307-319). Amsterdam: North-Holland.

Murphy, C. (1999). Loss of olfactory function in dementing disease. *Physiology & Behavior*, 66, 177–182

Murphy, C., Davidson, T. M., Jellison, W., Austin, S., Mathews, W. C., Ellison, D. W., & Schlotfeldt, C. (2000). Sinonasal disease and olfactory impairment in HIV disease: endoscopic sinus surgery and outcome measures. *Laryngoscope*, *110*, 1707–1710.

Murphy, C., Doty, R.L., & Duncan, H.J. (2005). Clinical disorders of olfaction. In R.L. Doty (Eds.) *Handbook of Olfaction and Gustation* (pp. 461-478). New York, NY: Marcel Dekker.

Murphy, C., Schubert, C.R., Cruickshanks, K.J., Klein, B.E., Klein, R., & Nondahl, D.M. (2002). Prevalence of olfactory impairment in older adults. *Journal of the American Medical Association*, *288*, 2307–2312.

National Institutes of Health. (1999). *Report of the NIH Consensus Development Conference on the Rehabilitation of Persons with Traumatic Brain Injury*. Bethesda, MD: U.S. Department of Health and Human Services.

Natsch, A., Derrer, S., Flachsmann, F., & Schmid, J. (2006). A broad diversity of volatile carboxylic acids, released by a bacterial aminoacylase from axilla secretions, as candidate molecules for the

determination of human body odor type. *Chemistry & Biodiversity*, *3*, 1-20.

Newell, F.N. (2004). Cross-modal object recognition. In G. Calvert, C. Spence, & B.E. Stein (Eds.), *The Handbook of Multisensory Processes* (pp. 123-139). Cambridge, MA: MIT Press.

Nieminen-von Wendt, T., Paavonen, J.E., Ylisaukko-Oja, T., Sarenius, S., Källman, T., Järvelä, I., & von Wendt, L. (2005). Subjective face recognition difficulties, aberrant sensibility, sleeping disturbances and aberrant eating habits in families with Asperger syndrome. *BMC Psychiatry*, *5*, 20.

Nordin, S., & Brämerson, A. (2008). Complaints of olfactory disorders: epidemiology, assessment and clinical implications. *Current Opinion in Allergy and Clinical Immunology*, *8*, 10-15.

Nordin, S., Monsch, A.U., & Murphy, C. (1995). Unawareness of smell loss in normal aging and Alzheimer's disease: discrepancy between self-reported and diagnosed smell sensitivity. *Journal of Gerontology*, *50*, P187-P192.

Nordin, S., Murphy, C., Davidson, T.M., Quinonez, C., Jalowayski, A.A., & Ellison, D.W. (1996). Prevalence and assessment of qualitative olfactory dysfunction in different age groups. *Laryngoscope*, *106*, 739–744.

Nyberg, L., & Tulving, E. (1996). Classifying Human Long-Term Memory: Evidence from Converging Dissociations. *European Journal of Cognitive Psychology*, *8*, 163–183.

Nyul, L.G., & Udupa, J.K. (1999). On standardizing the MR image intensity scale. *Magnetic Resonance in Medicine*, *42*, 1072-1081.

O'Regan, J.K., & Noe, A. (2001). A sensorimotor account of vision and visual consciousness. *Behavioral and Brain Sciences*, *24*, 939–973.

Oberman, L.M., & Ramachandran, V.S. (2007). The simulating social mind: The role of the mirror neuron system and simulation in the social and communicative deficits of autism spectrum disorders. *Psychological Bulletin*, *133*, 310–327.

Oberman, L.M., Ramachandran, V.S., & Pineda, J.A. (2008). Modulation of mu suppression in children with autism spectrum disorders in response to familiar or unfamiliar stimuli: the mirror neuron hypothesis. *Neuropsychologia*, *46*, 1558-1565.

Oka, Y., Katada, S., Omura, M., Suwa, M, Yoshihara, Y., & Touhara, Z. (2006). Odorant receptor map in the mouse olfactory bulb: in vivo sensitivity and specificity of receptor-defined glomeruli. *Neuron*, *52*, 857-869.

Oldfield, R.C. (1971). The assessment and analysis of handedness: the Edinburgh inventory. *Neuropsychologia*, *9*, 97–113.

Olsson, M.J., & Friden, M. (2001). Evidence of odor priming: edibility judgements are primed differently between the hemispheres. *Chemical Senses*, *26*, 117-123.

Olsson, M.J., Jonsson, F.U., & Faxbrink, M. (2002). Repetition priming in odor memory. In: Rouby C, Schaal B, Dubois D, Gervais R, & Holey A (Eds.), *Olfaction, Taste, and Cognition* (pp.246-260). London: University Press.

Olsson, P., Berglind, N., Bellander, T., & Stjärne, P. (2003). Prevalence of self-reported allergic and non-allergic rhinitis symptoms in Stockholm: relation to age, gender, olfactory sense, and smoking. *Acta Otolaryngologica*, *123*, 75–80.

Österbauer, R.A., Matthews, P.M., Jenkinson, M., Beckmann, C.F., Hansen, P.C., & Calvert, G. (2005). Color of scents: chromatic stimuli modulate odor responses in the human brain. *Journal of Neurophysiology*, *93*, 3434-3441.

Panel on Communicative Disorders to the National Advisory Neurological and Communicative Disorders and Stroke Council (1979). (NIH Publication No 79–1914). Washington, DC: Public Health Service.

Parma, V., Ghirardello, D., Tirindelli, R., & Castiello, U. (2010). Grasping a fruit. Hands do what flavour says. *Appetite*, *56*, 249-254.

Parma, V., Roverato, R., Ghirardello, D., Bulgheroni, M., Tirindelli, R., & Castiello, U. (2011). When flavor guides motor control: an effector independence study. *Experimental Brain Research*, *212*, 339-346.

Patchay, S., Castiello, U., & Haggard, P. (2003). A crossmodal interference effect in grasping objects. *Psychological Bulletin Review*, *10*, 924–931.

Patchay, S., Haggard, P., & Castiello, U. (2005). Cross-modal links in action: evidence for an object-centred reference frame for control of grasping. *Experimental Brain Research*, *23*, 1–11.

Pause, B. (2011). Processing of body odor signals by the human brain. *Chemosensory Perception*, doi: 10.1007/s12078-011-9108-2.

Pause, B.M., Krauel, K., Sojka, B., & Ferstl, R. (1999). Body odor evoked potentials: a new method to study the chemosensory perception of self and non-self in humans. *Genetica*, *104*, 285-294.

Pause, B.M., Miranda, A., Göder, R., Aldenhoff, J.B., & Ferstl, R. (2001). Reduced olfactory performance in patients with major depression. *Journal of Psychiatric Research*, *35*, 271–277.

Pierno, A.C., Mari, M., Glover, S., Georgiou, I., & Castiello, U. (2006). Failure to read motor intentions from gaze in children with autism. *Neuropsychologia*, *44*, 1483-1488.

Pierno, A.C., Mari, M., Lusher, D., & Castiello, U. (2008). Robotic movement elicits visuomotor priming in children with autism. *Neuropsychologia*, *46*, 448-454.

Pinching, A.J. (1977). Clinical testing of olfaction reassessed. *Brain*, *100*, 377–388.

Pitts, L.H., & McIntosh, T.H. (1990). Dynamic changes after brain trauma. In P.J. Vinken, G.W. Bruyn, & H.L. Klawans, (Eds.), *Handbook of Clinical Neurology* (pp. 65-100). Amsterdam: Elsevier.

Plailly, J., Howard, J.D., Gitelman, D.R., & Gottfried, J.A. (2008). Attention to odor modulates thalamocortical connectivity in the human brain. Journal of Neuroscience, 28, 5257–5267.

Porter, J., Craven, B., Khan, R.M., Chang, S.J., Kang, I., Judkewitz, B., …, Sobel, N. (2006). Mechanisms of scent-tracking in humans. Nature *Neuroscience*, *10*, 27–29.

Porter, R.H. (1999). Olfaction and human kin recognition. *Genetica*, *104*, 259–263.

Porter, R.H., & Schaal, B. (2003). Olfaction and the development of social behavior in neonatal mammals. In R.L. Doty (Ed.), *Handbook of Olfaction and Gustation, 2nd edition* (pp. 309-327). New York, NY: Marcel Dekker.

Posner, M. I. (1994) Attention: The mechanisms of consciousness. *Proceedings of the National Academy of Sciences USA*, *91*, 7398–7403.

Postolache, T.T., Doty, R.L., Wehr, T.A., Jimma, L.A., Han, L., Turner, E.H., …, Rosenthal, N.E. (1999). Monorhinal odor identification and depression scores in patients with seasonal affective disorder. *Journal of Affective Disorders*, *56*, 27–35.

Postolache, T.T., Wehr, T.A., Doty, R.L., Sher, L., Turner, E.H., Bartko, J.J., & Rosenthal, N.E. (2002). Patients with seasonal affective disorder have lower odor detection thresholds than control subjects. *Archives of General Psychiatry*, *59*, 1119–1122.

Prehn-Kristensen, A., Wiesner, C., Bergmann, T. O., Wolff, S., Jansen, O., Mehdorn, H. M., Ferstl, R., & Pause, B. M. (2009). Induction of empathy by the smell of anxiety. *PLoS One*, *4*, e5987.

Prescott, J., Johnstone, V., & Francis, J. (2004). Odor-taste interactions: Effects of attentional strategies during exposure. *Chemical Senses*, *29*, 331-340.

Price, J.L. (1985). Beyond the primary olfactory cortex: olfactory-related areas in the neocortex, thalamus and hypothalamus. *Chemical Senses*, *10*, 239–258.

Price, J.L. (1990). Olfactory system. In G. Paxinos (Ed.), *The Human Nervous System* (pp. 979-1001). San Diego, CA: Academic Press.

Proust, M. (1996). *In Search of lost time, 6 vols. (*C.K.S. Morcrieff & T. Kilmartin, Trans.), revised by D.J. Enright. New York, NY and London: Vintage.

Purves, D. (2004). The chemical senses, In D. Purves, G.J. Augustine, D. Fitzpatrick, W.C. Hall, A.-S. LaMantia, J.O. McNamara, & S.M. Williams (Eds.), *Neuroscience, III edition* (p. 338). Sunderland, MA: Sinauer Associates.

Quinn, N.P., Rossor, M.N., & Marsden, C.D. (1987). Olfactory threshold in Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 50*, 88–89.

Quint, C., Temmel, A.F., Schickinger, B., Pabinger, S., Ramberger, P., & Hummel, T. (2001). Patterns of non-conductive olfactory disorders in eastern Austria: a study of 120 patients from the Department of Otorhinolaryngology at the University of Vienna. *Wiener Klinische Wochenschrift, 113*, 52–57.

Rajaram, S., & Roediger, H.L. III. (1993). Direct comparison of four implicit memory tests. *Journal of Experimental Psychology: Learning, Memory, and Cognition, 19*, 765–776.

Rand, M.K., & Stelmach, G.E. (2005). Effect of orienting the finger opposition space on the control of reach-to-grasp movements. *Journal of Motor Behaviour, 37*, 65–78.

Raven, J. C. (1938). *Progressive matrices*. London: H.K. Lewis.

Reden, J., Mueller, A., Mueller, C., Konstantinidis, I., Frasnelli, J., Landis, B., …, Hummel, T. (2006). Recovery of olfactory function following closed head injury or infections of the upper respiratory tract. *Archives of Otolaryngology - Head and Neck Surgery*, *132*, 265–269.

Reitan, R.M. (1955). The relation of the trail making test as an indicator of organic brain damage. *Perceptual and Motor Skills*, *8*, 271–276.

Rizzolatti, G.,& Craighero, L. (2004). The mirror-neuron system. *Annual Review of Neuroscience*, *27*, 169–192.

Roberts., R.J., Sheehan, W., Thurber, S., & Roberts, M.A. (2010). Functional Neuro-Imaging and Post-Traumatic Olfactory Impairment. *Indian Journal of Psychological Medicine*, *32*, 93–98.

Roediger, H.L. III. (1990). Implicit memory: Retention without remembering. *American Psychologist*, *45*, 1043–1056.

Roediger, H.L., & McDermott, K.B. (1993). Implicit memory in normal human subjects. In F. Boiler & J. Grafman (Eds.), *Handbook of Neuropsychology. Vol. 8*. Amsterdam: Elsevier.

Roediger, H.L., Buckner, R.L., & McDermott, K.B. (1999). Components of Processing. In J.K. Foster & M. Jelicic (Eds.), *Memory: Systems, Process, or Function*? (pp. 31-65). New York, NY: Oxford University Press.

Roessner, V., Bleich, S., Banaschewski, T., & Rothenberger, A. (2005). Olfactory deficits in anorexia nervosa. *European Archives of Psychiatry and Clinical Neuroscience*, *255*, 6–9.

Rogers, S. (1999). An examination of the imitation deficit in autism. In J. Nadel, & G. Butterworth (Eds.), *Imitation in infancy*. (pp. 254-279). Cambridge: Cambridge University Press.

Rogers, S., & Pennington, B. (1991). A theoretical approach to the deficits in infantile autism. *Developmental Psychology*, *3*, 137– 162.

Rolls, E.T. & Baylis, L.L. (1994). Gustatory, olfactory, and visual convergence within the primate orbitofrontal cortex. *Journal of Neuroscience*, *14*, 5437–5452.

Rolls, E.T. (2000). The orbitofrontal cortex and reward. *Cerebral Cortex*, *10*, 284-294.

Rolls, E.T., Yaxley, S., & Ienkiewcz, J. (1990). Gustatory responses of single neurons in the orbitofrontal cortex of the macaque monkey. *Journal of Neurophysiology*, *64*, 1055- 1066.

Rossi, S., De Capua, A., Pasqualetti, P., Ulivelli, M., Fadiga, L., Falzarano, V., …, Rossini, P.M. (2008). Distinct olfactory cross-modal effects on the human motor system. *PLoS One*, *3*, e1702.

Roudnitska, E. (1983). The Investigator and the Perfumer. *Perfumer and Flavorist*, *8*, 8–18.

Rowe, T.B., Macrini, T.E., & Luo, Z.X. (2011). Fossil evidence on origin of the mammalian brain. *Science*, *332*, 955–957.

Rozin, P., Haidt, J., & McCauley, C. (2000). Disgust. In M. Lewis & J.M. Haviland-Jones (Eds.), *Handbook of Emotions, 2nd edition* (pp. 637-653). New York, NY: Guilford Press.

Russell, M.J. (1976). Human olfactory communication. *Nature*, *260*, 520–522.

Russell, M.J., Cummings, B.J., Profitt, B.F., Wysocki, C.J., Gilbert, A.N., & Cotman, C.W. (1993). Life span changes in the verbal categorization of odors. *Journal of Gerontology*, *48*, 49–53.

Ryan, J.J., Kreiner, D.S., Chapman, M.D., & Stark-Wroblewski, K. (2010). Virtual reality cues for binge drinking in college students. *Cyberpsychology, Behavior, and Social Networking*, *13*, 159-162.

Saling, M., Adler, C.H., Alberts, J., & Stelmach, G.E. (1996). Kinematic properties of prehensile movements in Parkinson's disease patients [abstract]. *Neurology*, *46*, A141.

Samkoff, L.M. (1996). A. Quantitative Study of Olfaction in Multiple Sclerosis. *Neurorehabilitation and Neural Repair*, *10*, 97–99.

Sandford, A.A., Davidson, T.M., Herrera, N., Gilbert, P., Magit, A.E., Haug, K., …, Murphy, C. (2006). Olfactory dysfunction: a sequela of

pediatric blunt head trauma. *International Journal of Pediatric Otorhinolaryngology*, *70*, 1015–1025.

Sarfarazi, M., Cave, B., Richardson, A., Behan, J., & Sedgwick, E.M. (1999). Visual event related potentials modulated by contextually relevant and irrelevant olfactory primes. *Chemical Senses*, *24*, 145-154.

Savage, R., Combs, D.R., Pinkstone, J.B., Advokat, C., & Gouvier, W.M.D. (2002). The role of temporal lobe and orbitofrontal cortices in olfactory memory function. *Archives of Clinical Neuropsychology*, *17*, 305–318.

Savic, I. (2008) Brain imaging studies of the functional organization of human olfaction. *Neuroscientist*, *8*, 204–211.

Schaal, B., & Porter, R.H. (1991). "Microsmatic humans" revisited: The generation and perception of chemical signals. In P. Slater, J.S. Rosenblatt, C. Beer, & M. Milinsky (Eds.), *Advances in the study of behavior*, *20* (pp. 135–199). New York, NY: Academic Press.

Schaal, B., Montagner, H., Hertling, E., Bolzoni, D., Moyse, A., & Quichon, R. (1980). Olfactory stimulations in mother-child relations. *Reproduction, Nutrition, Development*, *20*, 843–858.

Schacter, D.L. (1987). Implicit Memory: History and Current Status. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *13*, 501–518.

Schacter, D.L., & Tulving, E. (1994). What Are the Memory Systems of 1994? In D.L. Scachter & E. Tulving (Eds.), *Memory Systems* (pp. 1–38). Cambridge, MA: MIT Press.

Schoenbaum, G. & Eichenbaum, H. (1995). Information coding in the rodent prefrontal cortex. I. Single-neuron activity in orbitofrontal cortex compared with that in pyriform cortex. *Journal of Neurophysiology*, *74*, 733–750.

Schoenbaum, G., Chiba, A.A., & Gallagher, M. (1998). Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. *Nature Neuroscience*, *1*, 155–159.

Schott, B.H., Henson, R.N., Richardson-Klavehn, A., Becker, C., Thoma, V., …, Duzel, E. (2005). Redefining implicit and explicit memory: the functional neuroanatomy of priming, remembering, and control of retrieval. *Proceedings of the National Academy of Sciences USA*, *102*, 1257-1262.

Schwob, J.E. (2002). Neural regeneration and the peripheral olfactory system. *The Anatomical Record*, *269*, 33–49.

Seiden, A.M., & Smith, D.V. (1998). Endroscopic intranasal surgery as an approach to restoring olfactory function. *Chemical Senses*, *13*, 736.

Sela, L., & Sobel, N. (2010). Human olfaction: A constant state of change-blindness. *Experimental Brain Research*, *205*, 13-29.

Serby, M. (1987). Olfactory deficits in Alzheimer's disease. *Journal of Neural Transmission*, *24*, 69–77.

Shepherd, G.M. (2004). The human sense of smell: Are we better than we think? *PLoS Biology*, *2*, 572–575.

Shimamura, A.P., & Squire, L.R. (1984). Paired-associate learning and priming effects in amnesia: A neuropsychological study. *Journal of Experimental Psychology: General*, 113, 556–570.

Shu, C.H., Hummel, T., Lee, P.L., Chiu, C.H., Lin, S.H., & Yuan, B.C. (2009). The proportion of self-rated olfactory dysfunction does not change across the life span. *American Journal of Rhinology & Allergy*, *23*, 413-416.

Sigurdardottir, S., Jerstad, T., Andelic, N., Roe, C., & Schanke, A.K. (2010). Olfactory dysfunction, gambling task performance and intracranial lesions after traumatic brain injury. *Neuropsychology*, *24*, 504–513.

Singer, W. (1998). Consciousness and the structure of neuronal representations. *Philosophical Transactions of the Royal Society of London: Series B: Biological Sciences*, *353*, 1829–1840.

Smeets, M.A.M., Hendrik, N.J., Schifferstein, S.R., Boelema, S., & Lensvelt-Mulders, G. (2008). The Odor Awareness Scale: A new

scale for measuring positive and negative odor awareness. *Chemical Senses*, *33*, 725-734.

Smith, I., & Bryson, S. (1994). Imitation and action in autism: A critical review. *Psychological Bulletin*, *116*, 259–273.

Smith, M.E., & Oscar-Berman, M. (1990). Repetition priming of words and pseudowords in divided attention. *Journal of Experimental Psychology: Learning, Memory, and Cognition*, *16*, 1033–1042.

Sobel, N., Thomason, M.E., Stappen, I., Tanner, C.M., Tetrud, J.W., Bower, J.M., …, Gabrieli, J.D. (2001). An impairment in sniffing contributes to the olfactory impairment in Parkinson's disease. *Proceedings of the National Academy of Sciences USA*, *98*, 4154–4159.

Solomon, G. (1990). Psychology of novice and expert wine talk. *American Journal of Psychology*, *105*, 495-517.

Sørensen, T.L., Frederiksen, J.L., Brønnum-Hansen, H., & Petersen, H.C. (1999). Optic neuritis as onset manifestation of multiple sclerosis: a nationwide, longterm survey. *Neurology*, *53*, 473.

Soucy, E.R., Albeanu, D.F., Fantana, A.L., Murthy, V.N., & Meister, M. (2009). Precision and diversity in an odor map on the olfactory bulb. *Nature Neuroscience*, *12*, 210–220.

Spence, C., Kettenmann, B., Kobal, G., & McGlone, F.P. (2001). Shared attentional resources for processing visual and chemosensory information. *Quarterly Journal of Experimental Psychology*, *54*, 775–783.

Spence, C., McGlone, F., Kettenmann, B., & Kobal, G. (2001). Attention to olfaction: A psychophysical investigation. *Experimental Brain Research*, *138*, 432–437.

Spinnler, H., & Tognoni, G. (Eds.). (1987). Standardizzazione e taratura italiana di test neuropsicologici [Supplement 8]. *The Italian Journal of Neurological Sciences*, *8*, 8-120.

Stein, B.E., & Meredith, M.A. (1990). Multisensory integration-neural and behavioral solutions for dealing with stimuli from different sensory modalities. *Annals of the New York Academy of Sciences*, *608*, 51-70.

Stenner, M., Vent, J., Huttenbrink, K.B., Hummel, T., & Damm, M. (2008). Topical therapy in anosmia: relevance of steroids-responsiveness. *Laryngoscope*, *118*, 1681–1686.

Stern, K., & McClintock, M.K. (1998). Regulation of ovulation by human pheromones. *Nature*, *392*, 177-178

Stettler, D.D., & Axel, R. (2009). Representations of odor in the piriform cortex. *Neuron*, *63*, 854–864.

Stevens, J.C., Cain, W.S., & Burke, R.J. (1988). Variability of olfactory thresholds. *Chemical Senses*, *13*, 643–653.

Stevenson, R.J. (2009). Phenomenal and access consciousness in olfaction. *Consciousness and Cognition*, *18*, 1004-1017.

Stevenson, R.J., & Case, T.I. (2005). Olfactory dreams: Phenomenology, relationship to volitional imagery and odor identification. *Imagination, Cognition, and Personality*, *24*, 69-90.

Stevenson, R.J., & Repacholi, B.M. (2003). Age related changes in children's hedonic response to male body odor. *Developmental Psychology*, *39*, 670–679.

Stevenson, R.J., & Repacholi, B.M. (2005). Does the source of an interpersonal odour affect disgust? A disease risk model and its alternatives. *European Journal of Social Psychology*, *35*, 375–401.

Sumner, D. (1964) Post-traumatic anosmia. *Brain*, *87*, 107–120.

Suzuki, Y., Critchley, H.D., Rowe, A., Howlin, P., & Murphy, D.G.M. (2003). Impaired olfactory identification in Asperger's syndrome. *Journal of Neuropsychiatry and Clinical Neurosciences*, *15*, 105–107.

Swann, I.J., Bauza-Rodriguez, B., Currans, R., Riley, J., & Shukla, V. (2006). The significance of post-traumatic amnesia as a risk factor in the development of olfactory dysfunction following head injury. *Emergency Medicine Journal*, *23*, 618–621.

Talairach, J., & Tourneaux, P. (1998). *Co-planar stereotaxic atlas of the human brain*. Stuttgart: Thieme.

Tavassoli, T., & Baron-Cohen, S. (2011). Olfactory detection thresholds and adaptation in adults with autism spectrum condition. *Journal of Autism and Developmental Disorders*, doi: 10.1007/s10803-011-1321-y

Taylor, K.I., Moss, H.E., Stamatakis, E.A., & Tyler, L.K. (2006). Binding crossmodal object features in perirhinal cortex. *Proceedings of the National Academy of Sciences USA*, *103*, 8239-8244.

Teasdale, G., & Jennett, B. (1974). Assessment of coma and impaired consciousness: a practical scale. *Lancet*, *2*, 81-83.

Teasdale, G., & Mendelow, D. (1984). Pathophysiology of head injuries. In N. Brooks (Ed.), *Closed Head Injury: Psychological, Social and Family Consequences* (pp. 4-36). Oxford: Oxford University Press.

Temmel, A.F., Quint, C., Schickinger-Fischer, B., Klimek, L., Stoller, E., Hummel, T. (2002). Characteristics of olfactory disorders in relation to major causes of olfactory loss. *Archives of Otolaryngology - Head and Neck Surgery*, *128*, 635–641.

Thompson, E., & Varela, F. J. (2001). Radical embodiment: neural dynamics and consciousness. *Trends in Cognitive Sciences*, *5*, 418–425.

Tirindelli, R., Dibattista, M., Pifferi, S., & Menini, A. (2009). From pheromones to behavior. *Physiological Reviews*, *89*, 921–956.

Tissingh, G., Berendse, H.W., Bergmanns, P., De Ward, R., Drukarch, B., Stoof, J.C., & Wolters, M.D. (2001). Loss of olfaction in de novo and

treated Parkinson's disease: possible implication for early diagnosis. *Movement Disorders*, *16*, 41–46.

Tomchek, S.D., & Dunn, W. (2007). Sensory processing in children with and without autism: A comparative study using the short sensory profile. *American Journal of Occupational Therapy*, *61*, 190–200.

Tresilian, J.R., Stelmach, G.E., & Adler, C.H. (1997). Stability of reach-to-grasp movement patterns in Parkinson's disease. *Brain*, *120*, 2093-2111.

Tubaldi, F., Ansuini, C., Demattè, M.L., Tirindelli, R., & Castiello, U. (2008). Effects of olfactory stimuli on arm reaching duration. *Chemical Senses*, *33*, 433–440.

Tubaldi, F., Ansuini, C., Tirindelli, R, & Castiello, C. (2008). The grasping side of odors. *PLoS One*, *3*, 1795-1808.

Tubaldi, F., Turella, L., Pierno, A.C., Grodd, W., Tirindelli, R., & Castiello, U. (2010). Smelling odors, understanding actions. *Social Neuroscience*, 6, 31-47

Tulving, E. & Schacter, D.L. (1990). Priming and human memory systems. *Science*, *247*, 301–306.

Tulving, E. (1993). Human memory. In P. Andersen, O. Hvalby, O. Paulsen, & B. Hökfelt (Eds.) *Memory Concepts. Basic and Clinical Aspects* (pp. 27–45). New York, NY: Elsevier.

Tulving, E. (1995). How many memory systems are there? *American Psychologist*, *40*, 385–398.

Udupa, J.K., Nyul, L.G., Ge, Y., & Wei, L. (2001). Multiprotocol MR image segmentation in multiple sclerosis: experience with over 1,000 studies. *Academic Radiology*, *8*, 1116–1126.

Val-Laillet, D., & Nowak, R. (2008). Early discrimination of the mother by rabbit pups. *Applied Animal Behavioural Science*, *111*, 173–182.

Van Toller, S (1999). Assessing the impact of anosmia: review of a questionnaire's findings. *Chemical Senses*, *24*, 705–712.

Verfaellie, M., Cermak, L. S., Letourneau, L., & Zuffante, P. (1991). Repetition effect in lexical decision task: The role of episodic memory in the performance of alcoholic Korsakoff patients. *Neuropsychologia*, *29*, 641–657.

Vowles, R.H., Bleach, N.R., & Rowe-Jones, J.M. (1997). Congenital anosmia. *International Journal of Pediatric Otorhinolaryngology*, *41*, 207-214.

Wang, F., Nemes, A., Mendelsohn, M., & Axel, R. (1998). Odorant receptors govern the formation of a precise topographic map. *Cell*, *93*, 47-60.

Wang, J., Eslinger, P.J., Smith, M.B., & Yang, Q.X. (2005). Functional magnetic resonance imaging study of human olfaction and normal

aging. *The Journal of Gerontology Series A: Biological Sciences and Medical Sciences*, *60*, 510–14.

Wang, L., Walker, V., Sardi, H., Fraser, C., & Jacob, T. (2002). The correlation between physiological and psychological responses to odor stimulation in human subjects. *Clinical Neurophysiology*, *113*, 542-551.

Warrington, E.K., & Weiskrantz, L. (1968). New method of testing long-term retention with special reference to amnesic patients. *Nature*, *217*, 927–974.

Warrington, E.K., & Weiskrantz, L. (1970). The amnesic syndrome: Consolidation or retrieval? *Nature*, *228*, 628–630.

Wechsler, D. (1991). WISC-III: *Wechsler intelligence scale for children III edition: Manual*. San Antonio, TX: Psychological Corporation.

Welge-Lüssen, A., & Wolfensberger, M. (2006). Olfactory disorders following upper respiratory tract infections. In T. Hummel, & A. Welge-Lüssen (Eds.), *Taste and Smell. An Update* (pp. 125-132). Basel: Karger.

Welge-Lüssen, A., Temmel, A., Quint, C., Moll, B., Wolf, S., & Hummel, T. (2001). Olfactory function in patients with olfactory groove meningioma. *Journal of Neurology, Neurosurgery, and Psychiatry*, *70*, 218–221.

Wender, M., & Szmeja, Z. (1971). Examination of the hearing, the vestibular system functions, taste and olfactory systems in patients with disseminated sclerosis. *Neurologia i Neurochirurgia Polska [Polish Journal of Neurology and Neurosurgery]*, 5, 179–184.

Williams, D. (2005). *Autism: An Inside-out Approach: An Innovative Look at the Mechanics of Autism and its Developmental Cousins.* London and Philadelphia: Jessica Kingsley Publishers.

Williams, J.H., Whiten, A., Suddendorf, T., & Perrett, D.I. (2001). Imitation, mirror neurons and autism. *Neuroscience & Biobehavioral Reviews*, 25, 287–295.

Wilson, D.A., & Stevenson, R.J. (2006). *Learning to Smell: Olfactory Perception from Neurobiology to Behavior.* Baltimore, MD: Johns Hopkins University Press.

Winikates, J., & Jankovic, J. (1999). Clinical correlates of vascular parkinsonism. *Archives of Neurology*, 56, 98–102.

Winston, J.S., Gottfried, J.A., Kilner, J.M., & Dolan, R.J. (2005). Integrated neural representations of odor intensity and affective valence in human amygdala. *Journal of Neuroscience*, 25, 8903-8907.

Wolters ,E., & Braak, H. (2006). Parkinson's disease: premotor clinico-pathological correlations. *Journal of Neural Transmission*, 70, 309-319.

Wrzesniewski, A., McCauley, C., & Rozin, P. (1999). Odor and affect: Individual differences in the impact of odor on liking for places, things and people. *Chemical Senses*, *24*, 713-721.

Yarita, H., Iino, M., Tanabe, T., Kogure, S., & Takagi, S.F. (1980). A transthalamic olfactory pathway to orbitofrontal cortex in the monkey. *Journal of Neurophysiology*, *43*, 69–85.

Yeshurun, Y. & Sobel, N. (2010). An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. *Annual Review of Psychology*, *61*, 219–241.

Yousem, D.M., Geckle, R.J., Bilker, W., McKeown, D.A., & Doty, R.L. (1996). MR evaluation of patients with congenital hyposmia or anosmia. *American Journal of Roentgenology*, *166*, 439–443.

Yousem, D.M., Geckle, R.J., Bilker, W.B., McKeown, D.A., & Doty, R.L. (1996). Posttraumatic olfactory dysfunction: MR and clinical evaluation. *American Journal of Neuroradiology*, *17*, 1171–1179.

Zald, D.H., & Pardo, J.V. (2000). Functional neuroimaging of the olfactory system in humans. *International Journal of Psychophysiology*, *36*, 165–181.

Zatorre, R.J., & Jones-Gotman, M. (1991). Human olfactory discrimination after unilateral frontal or temporal lobectomy. *Brain*, *114*, 71–84.

Zatorre, R.J., Jones-Gotman, M., Evans, A.C., & Meyer, E. (1992). Functional localization and lateralization of human olfactory cortex. *Nature*, *360*, 339–340.

Zhou W, & Coggins R. (2002). Computational models of the amygdala and the orbitofrontal cortex: a hierarchical reinforcement learning system for robotic control. In R.I. McKay, & J. Slaney, (Ed.), *Lecture Notes of Artificial Intelligence* (pp. 419–430).

Ziemssen, T., & Reichmann, H. (2007). Non-motor dysfunction in Parkinson's disease. *Parkinsonism and Related Disorders*, *13*, 323–332.

Zivadinov, R., Zorzon, M., Monti Bragadin, L., Pagliarto, G., & Cazzato, G. (1999). Olfactory loss in multiple sclerosis. *Journal of Neurological Science*, *168*, 127–130.

Zoia, S., Blason, L., D'Ottavio, G., Bulgheroni, M., Pezzetta, E., Scabar, A., & Castiello, U. (2007). Evidence of early development of action planning in the human foetus: a kinematic study. *Experimental Brain Research*, *176*, 217-226.

Zorzon, M., Ukmar, M., Bragadin, L.M., Zanier, F., Antonello, R.M., Cazzato, G. Zivadinov, R. (2000). Olfactory dysfunction and extent of white matter abnormalities in multiple sclerosis: A clinical and MR study. *Multiple Sclerosis*, *6*, 386–390.

Zucco, G.M. (2003). Anomalies in cognition: olfactory memory. *European Psychologist*, *3*, 77–86.

Zucco, G.M., Amodio, P., & Gatta, A. (2006). Olfactory deficits in patients affected by minimal hepatic encephalopathy: A pilot study. *Chemical Senses*, *31*, 273–278.

Zucco, G.M., Zaglis, D., & Wambsganss, C.S. (1991). Olfactory deficits in elderly subjects and Parkinson patients. *Perceptual and Motor Skills*, *73*, 895-898.

Zusho, H. (1982). Post traumatic anosmia. *Archives of Otolaryngology*, *108*, 90–92.

Appendices

APPENDIX A.

Questionnaire for the evaluation of the olfactory function (adapted from Zucco, Amodio, &Gatta, 2006).

1. How do you think your ability to smell is?

a) Poor
b) Sufficient
c) Good

2. Which of these changes have you noticed in your eating?

a) Alteration in taste perception
b) Difficulties in perceiving tastes
c) Lack of pleasure when eating
d) Decreased appetite
e) None

3. Have you ever experienced allergic reactions when exposed to strong-smelling substances?

a) Yes
b) No

4. Because of your job or for other reasons are you exposed to irritating substances like, powders, acids, gases, smokes?

a) Yes
b) No

5. At present are you suffering from allergic or infective rhinitis?

a) Yes
b) No

APPENDIX A. *(continued)*

6. At present are you suffering from infections to the upper respiratory tract (e.g. pharyngitis, laryngitis, tonsillitis)?

a) Yes
b) No

7. Have you suffered from head cold in the last three days?

a) Yes
b) No

8. During last month, have you daily taken medication for cancer, for rheumatism or heart disease (ACE inhibitors) by mouth?

a) Yes
b) No

If you answer 'Yes', which of these?

For heart disease:

Acepress
Acequin
Accuprin
Alapril
Bifril
Capoten
Cibacen
Converten
Delaket
Eliten
Enapren
Femipres
Fosipres
Goptem
Inibace
Naprilene
Primoxil
Prinivil
Procaptan
Renormax
Setrilan
Tensanil
Tensogard
Triatec
Unipril
Zestril
Zopranol

For rehumatisms:

Akudol
Aleve
Algofen
Algolider
Antalfort
Antalgil
Antalisin
Aulin
Buscofen
Cibalgina
Diclofenac
Dicloreum
Eufans
Feldene
Ketoprofene
Momendol
Moment
Naprosyn
Naproxene
Nimesulinde
Nurofen
Oki
Toradol
Voltadvance
Voltaren

For cancer:

Alkeran
Bleomicina
Campto
Endoxan
Eulexin
Gemzar
Hycamtin
Holoxan
Kidrolase
Leukeran
Navelbine
Paraplatin
Taxol
Taxotere
Velbe
Vepesid
Zoladex

APPENDIX A. *(continued)*

9. Have you ever undergone radiotherapy or chemotherapy?

a) Yes
b) No

10. Have you ever had head or nose surgery (e.g. because of sinusitis)?

a) Yes
b) No

11. Have you ever experienced a nose trauma (e.g. a bash hit against a surface)?

a) Yes
b) No

If you answered 'Yes': how do you judge your olfactory sensibility before and after the accident?

Before	*After*
a) Poor	a1) Poor
b) Sufficient	b1) Sufficient
c) Good	c1) Good

12. Have you been diagnosed with a deviated septum?

a) Yes
b) No

13. Are you taking or have you ever taken significant quantities of drugs such as cocaine or morphine nasally?

a) Yes
b) No

APPENDIX A. *(continued)*

14. Have you ever been diagnosed with one of the following diseases? If 'Yes', please underline the name of the disease.

Multiple Sclerosis
Diabete mellitus
Gastro esophageal Reflux
Facial Paralysis
Renal Failure
Cirrhosis
Alcoholism
Celiac Disease
Alzheimer's disease
Huntington's disease
Meningitis
Parkinson's disease
Syphilis
Depression
Schizophrenia
Down syndrome
Anorexia Nervosa
Attention Deficit Disorder

15. Do you smoke?

a) Yes
b) No

If you answer 'Yes', how many cigarettes do you smoke per day?

……

16. How long do you smoke?

……

17. You consider yourself an ex-smoker?

a) Yes
b) No

If you answer 'Yes', how many cigarettes did you smoke per day?

……

18. When did you started to smoke?

……

19. When did you stop smoking?

……

FOR WOMEN ONLY

20. Have you been diagnosed with an estrogenic deficiency?

a) Yes
b) No

If you answered 'Yes', are you following an estrogenic therapy?

a) Yes
b) No

APPENDIX B.

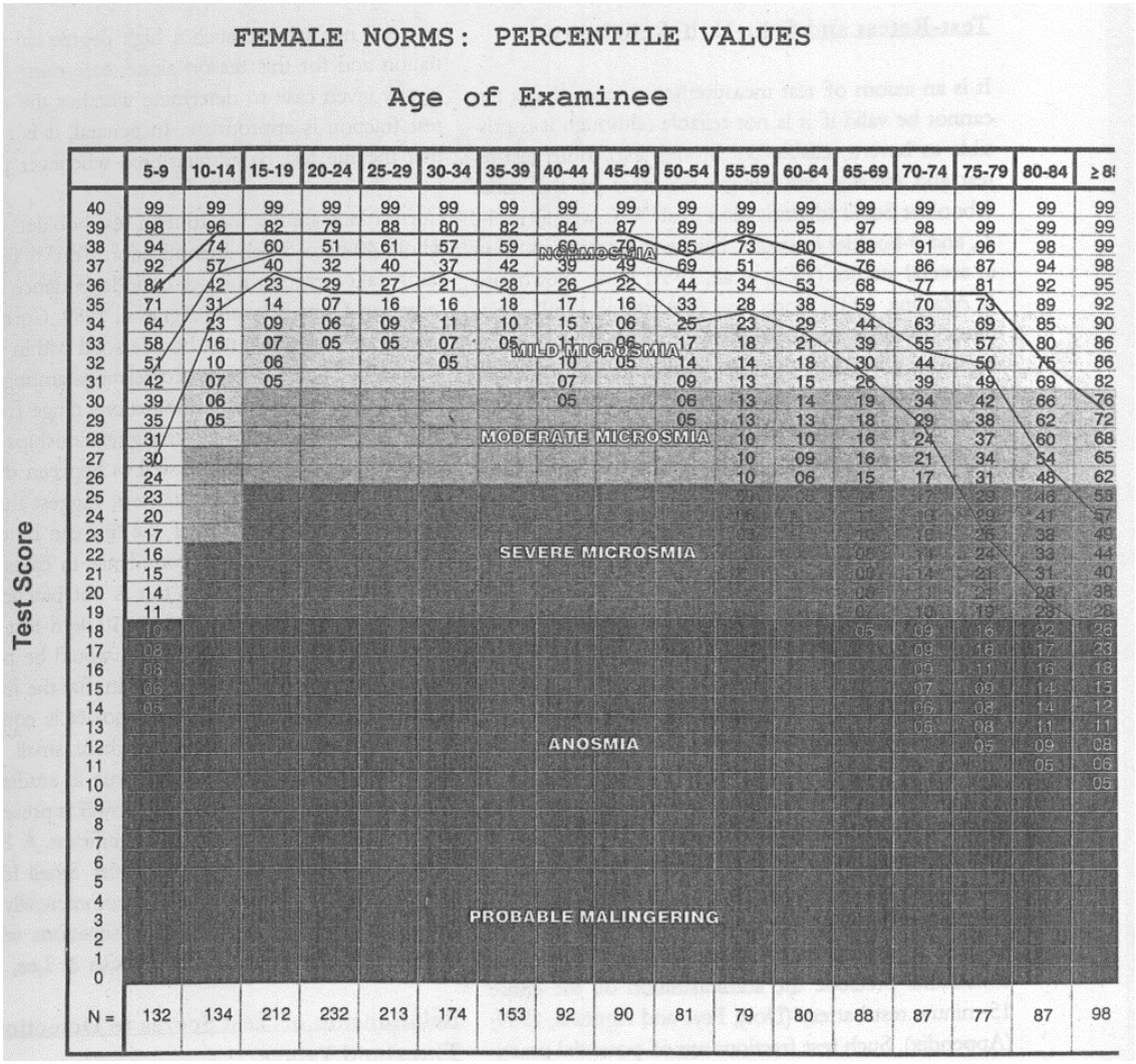
Sniffin' Sticks Extended Test normative data. Norms are listed separately for gender and age groups (from Hummel, Kobal, Gudziol, &Mackay-Sim, 2007).

		Female subjects				Male subjects			
		THR	DIS	ID	TDI	THR	DIS	ID	TDI
Age group A 5–15 years									
N		25	25	59	25	17	17	51	17
Mean		6.59	12.32	11.75	30.67	7.22	11.71	12.41	30.87
SD		2.23	1.70	1.77	3.60	2.59	1.57	1.77	4.79
Minimum		2.75	10	6	24.50	4.00	9	8	23.00
Maximum		13.50	16	15	36.50	12.00	14	16	40.00
Percentiles	5	3.13	10	8	24.58	4.00	9	8	23.00
	10	4.30	10	9	24.90	4.00	9.8	10	23.80
	25	5.00	11	11	27.75	5.00	10	12	27.88
	50	6.00	12	12	31.00	6.75	12	13	31.50
	75	8.00	14	13	33.88	9.00	13	14	32.88
	90	9.35	15	14	35.60	12.00	14	14	40.00
	95	12.30	15.7	14	36.28	12.00	14	15	40.00
Age group B 16–35 years									
N		760	741	827	704	579	587	672	552
Mean		9.39	12.91	13.68	36.06	9.24	12.61	13.48	35.31
SD		2.56	1.92	1.62	4.17	2.99	1.95	1.73	4.73
Minimum		1.75	5	8	23.00	1.00	5	6	18.00
Maximum		16.00	16	16	46.75	16.00	16	16	47.00
Percentiles	5	5.51	9	11	29.50	4.75	9	10	27.91
	10	6.50	10	11	30.50	6.00	10	11	29.50
	25	7.50	12	13	33.50	7.00	11	12	32.00
	50	9.00	13	14	36.00	8.75	13	14	35.00
	75	11.25	14	15	39.00	11.50	14	15	38.60
	90	12.50	15	16	41.50	13.75	15	16	41.50
	95	14.00	16	16	43.00	14.80	15	16	43.00
Age group C 36–55 years									
N		295	291	586	288	208	207	491	207
Mean		9.08	12.46	13.49	35.16	8.43	11.94	13.10	33.20
SD		3.09	1.96	1.56	4.52	3.47	2.24	1.88	6.05
Minimum		1.00	6	4	22.50	1.00	5	4	15.00
Maximum		16.00	16	16	45.75	16.00	16	16	44.25
Percentiles	5	4.25	9	11	26.86	2.75	7	10	20.60
	10	5.50	10	12	28.75	3.75	9	11	24.95
	25	6.75	11	13	32.50	6.25	10	12	29.50
	50	8.75	13	14	35.50	8.50	12	13	34.50
	75	11.00	14	15	38.00	10.50	14	14	37.50
	90	13.60	15	15	40.50	13.02	15	15	39.55
	95	15.30	15	16	42.89	14.91	15	16	42.48
Age group D >55 years									
N		147	143	251	143	142	139	238	139
Mean		7.44	10.66	12.06	29.83	7.15	10.69	12.20	29.81
SD		3.51	2.50	2.31	6.77	3.59	2.77	2.57	7.17
Minimum		1.00	4	4	11.00	1.00	4	3	9.00
Maximum		16.00	16	16	43.00	16.00	16	16	44.00
Percentiles	5	1.55	5.2	7	17.25	1.04	5	6.95	14.50
	10	2.75	7.4	9	19.05	2.25	7	9	19.75
	25	5.50	9	11	25.75	4.44	9	11	26.25
	50	7.25	11	12	30.50	7.50	11	13	31.00
	75	9.00	13	14	34.25	9.25	13	14	34.50
	90	12.60	13.6	14.8	37.65	11.68	14	15	37.75
	95	14.70	14	15	40.20	14.35	15	16	40.50

THR = Threshold; DIS = Discrimination; ID = Identification; TDI = composite score as the sum of results for threshold, discrimination and identification.

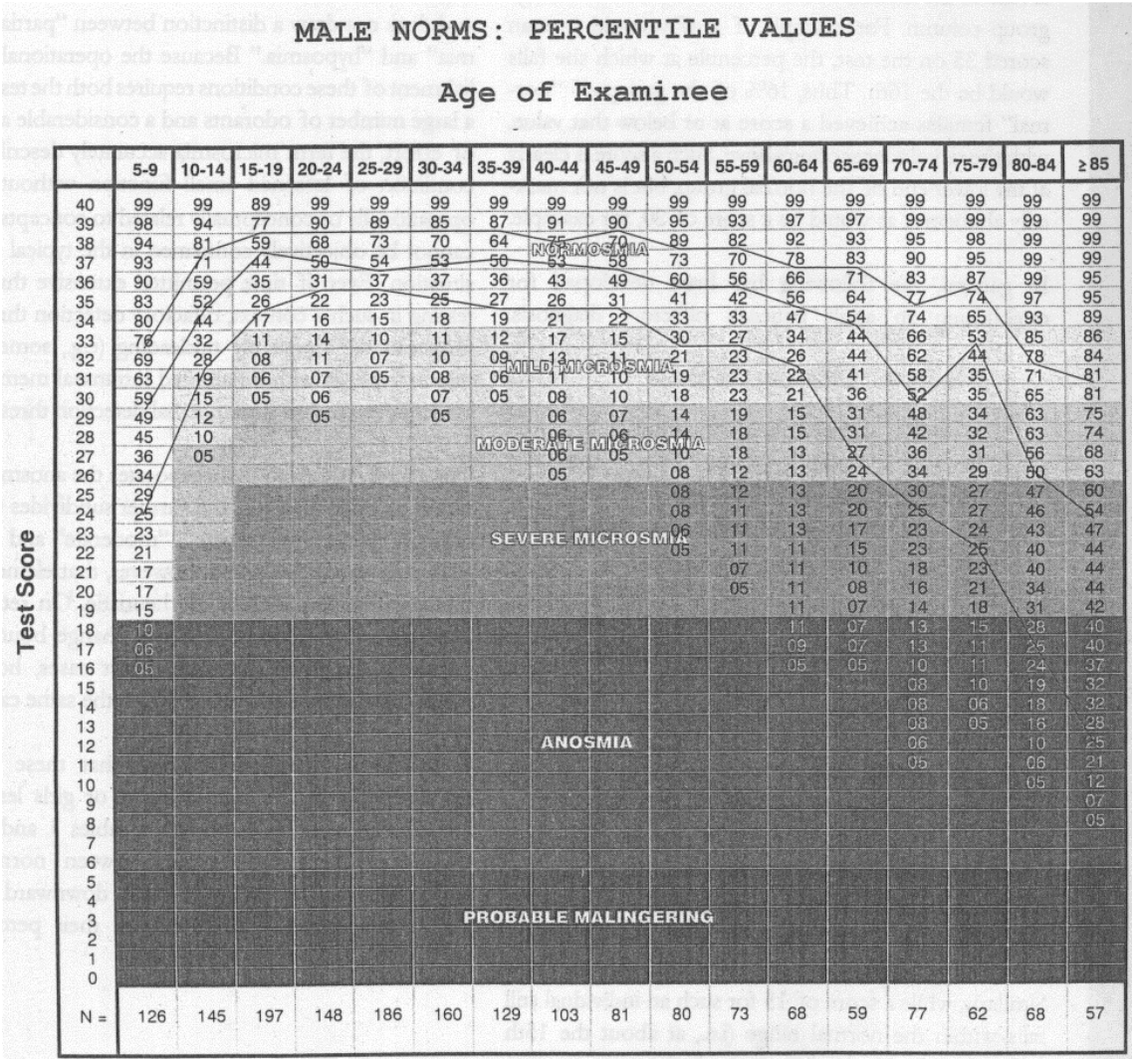
APPENDIX C.

University of Pennsylvania Smell Identification Test normative data. Norms are listed separately for gender and age groups (from Doty, Shaman, & Dann, 1984).



	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45-49	50-54	55-59	60-64	65-69	70-74	75-79	80-84	≥ 85
40	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99	99
39	98	96	82	79	88	80	82	84	87	89	89	95	97	98	99	99	99
38	94	74	60	51	61	57	59	60	70	85	73	80	88	91	96	98	99
37	92	57	40	32	40	37	42	39	49	69	51	66	76	86	87	94	98
36	84	42	23	29	27	21	28	26	22	44	34	53	68	77	81	92	95
35	71	31	14	07	16	16	18	17	16	33	28	38	59	70	73	89	92
34	64	23	09	06	09	11	10	15	06	25	23	29	44	63	69	85	90
33	58	16	07	05	05	07	05	11	06	17	18	21	39	55	57	80	86
32	51	10	06			05		10	05	14	14	18	30	44	50	75	86
31	42	07	05					07		09	13	15	26	39	49	69	82
30	39	06						05		06	13	14	19	34	42	66	76
29	35	05								05	13	13	18	29	38	62	72
28	31										10	10	16	24	37	60	68
27	30										10	09	16	21	34	54	65
26	24										10	06	15	17	31	48	62
25	23										09	06	14	17	29	46	58
24	20										08	06	13	16	29	41	57
23	17										08	06	13	16	26	38	49
22	16												08	14	24	33	44
21	15												08	14	21	31	40
20	14												06	11	21	28	38
19	11												05	10	19	28	28
18	10												05	09	16	22	26
17	08													09	16	17	23
16	06													09	11	16	18
15	06													07	09	14	15
14	05													06	08	14	12
13														05	08	11	11
12															05	09	08
11																05	06
10																	05
9																	
8																	
7																	
6																	
5																	
4																	
3																	
2																	
1																	
0																	
N =	132	134	212	232	213	174	153	92	90	81	79	80	88	87	77	87	98

APPENDIX C. *(continued)*



APPENDIX D.

Edinburgh Handedness Inventory (adapted from Oldfield, 1971; Appendix D).

Your Initials:

Please indicate with a check (✓) your preference in using your left or right hand in the following tasks.

Where the preference is so strong you would never use the other hand, unless absolutely forced to, put two checks (✓✓).

If you are indifferent, put one check in each column (✓ | ✓).

Some of the activities require both hands. In these cases, the part of the task or object for which hand preference is wanted is indicated in parentheses.

APPENDIX D. *(continued)*

Task / Object	Left Hand	Right Hand
1. Writing		
2. Drawing		
3. Throwing		
4. Scissors		
5. Toothbrush		
6. Knife (without fork)		
7. Spoon		
8. Broom (upper hand)		
9. Striking a Match (match)		
10. Opening a Box (lid)		
Total checks:	LH =	RH =
Cumulative Total	CT = LH + RH =	
Difference	D = RH − LH =	
Result	R = (D / CT) × 100 =	
Interpretation: (Left Handed: R ‹ -40) (Ambidextrous: -40 ≤ R ≤ +40) (Right Handed: R › +40)		