Odor Identification in Aging and Dementia: Influences of Cognition and the ApoE Gene

Jonas K. Olofsson
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Cover art by artist William Utermohlen (1933-2007), who suffered from Alzheimer’s disease. From left: Self Portrait (with easel) / 1998 oil on canvas 35.5 x 25 cm; Erased Self Portrait / 1999 mixed media on canvas 45.5 x 35.5 cm; Head I / 2000 pencil on paper 40.5 x 33 cm. Illustrations courtesy of Galerie Beckel Odille Boïcos, Paris.
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Abstract

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Olfactory function is impaired in aging and dementia. The general aim of this thesis was to identify variables that predict olfactory function and dysfunction (assessed with an odor identification test) in middle-aged and elderly adults. The thesis investigated whether odor identification performance was associated with demographic variables, cognitive function, the ApoE gene, dementia, and other health-related variables. The ApoE-ε4 allele is associated with cognitive impairment and Alzheimer’s disease, the most common form of dementia. The studies included in this thesis used data from the Betula study, a large-scale, population-based prospective study on aging, memory, and health. Study 1 investigated demographic and cognitive predictors of odor identification ability in non-demented participants. The results showed that younger age, female sex, and high education contributed to better odor identification ability. Cognitive speed and vocabulary had a small additional influence. Study 2 included information about ApoE genotypes, dementia and other health-related variables. The results indicated that the ApoE-ε4 allele was associated with odor identification impairment among the elderly, but not middle-aged adults. Participants who were demented at the time of testing or became demented within five years after testing exhibited olfactory impairments. Interestingly, the age-related olfactory impairment in ε4-carriers was independent of clinical dementia within five years. In Study 3, decline in global cognitive status over a five-year test-retest interval was predicted in a sample of elderly participants. The major result was a three-way interaction reflecting that odor identification impairment, old age, in combination with the ε4 allele predicted a larger cognitive decline. However, odor identification impairment did not predict cognitive change in elderly who were non-carriers of the ε4 allele. Overall, the results indicate that odor identification impairment in elderly is related to ApoE-ε4, cognitive decline, and clinical and pre-clinical stages of dementia. Theoretical and practical implications of the results are discussed. Furthermore, it is proposed that in order to effectively predict clinical dementia or cognitive decline from olfactory assessment in the elderly, variables that mediate (e.g. neuropathology) or moderate (e.g. age) the associations between olfactory function, the ε4 allele, and dementia need to be further evaluated, preferably in studies using longitudinal assessment.

Key Words: Aging, olfaction, identification, dementia, Alzheimer, genetics, ApoE, population based, cognition, demographics
“…a complete, comprehensive understanding of odor (...) may not seem a profound enough problem to dominate all the lifesciences, but it contains, piece by piece, all the mysteries.”

*Lewis Thomas*

*To my parents*
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Stockholm, September 2008
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**List of publications**

This doctoral thesis is based on the following three studies:


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References
Introduction

The proportion of elderly increases steadily in most countries, and this demographic change entails a higher incidence of age-related diseases such as Alzheimer’s disease (AD), and increasing costs of health care (Ferri et al., 2005; Hebert, Scherr, Bienias, Bennett, & Evans, 2003; Wimo, Winblad, & Jonsson, 2007). Olfactory impairment is common in the elderly (Murphy et al., 2002). An impaired sense of smell might cause decreased appetite that leads to malnutrition and increased mortality (Schiffman & Graham, 2000; Schiffman, Moss, & Erickson, 1976). Olfactory impairment in the elderly can be caused by many factors. However, olfactory ability is particularly compromised in common dementia disorders such as AD, and it has been hypothesized that olfactory dysfunction in the elderly might constitute a sign of impending dementia (Doty & Reyes, 1987). To date, there has been little integration of findings regarding demographic, cognitive, genetic, and health-related influences on olfactory ability in the adult and elderly population. The present thesis uses data from a population-based, prospective study to investigate olfactory function, assessed with an odor identification task, in a perspective of aging. Olfactory ability is evaluated against demographic and health-related variables, cognitive functions and cognitive decline, dementia, and the apolipoprotein E (ApoE) genotypes in a population-based sample of middle-aged and elderly adults. A general aim of the thesis is to characterize how these different variables are related to olfactory function.

On the importance of the olfactory sense

The olfactory sense is among the least investigated sensory systems in humans, although the importance of olfaction in many other species is widely appreciated. The ability of an organism to respond to changes in the environment is fundamental to the organism’s survival, and the chemical senses are for many animals the primary windows to the external world (Ache, 1991). The ability to perceive chemical substances in the environment is regarded as the phylogenetically oldest perceptual ability of living organisms. From an evolutionary perspective, the basic role of the sense of smell is to direct the organism to approach and withdrawal in chemical environments that might be important for survival and reproduction. According to this view, the presence of food odors, mating
partners and kin guide approach-related behaviours, whereas toxic chemicals guide withdrawal. Even simple organisms such as bacteria have receptor mechanisms for detecting chemicals in the environment. Animals can perceive chemicals in the environment if there are receptor cells that translate the chemical environment into electrical impulses of afferent nerves. The nervous systems accomplish this in a variety of ways that differs between species, and between the chemical senses (see Doty, 2001, for review). Communication of biologically relevant information through the chemical senses is present in many species, regulating e.g. mating behaviour. It has been argued that this “pheromonal” communication is also present in humans. Although we probably lack a functional vomeronasal organ, which in other species serves a large part of pheromonal perception, it is possible that human behaviour and psychological state can be influenced by odorous steroids present in body sweat. Two structurally related odorous steroids, androstenone and androstadienone, do potentially function as pheromones in humans (Jacob, McClintock, Zelano, & Ober, 2002; Lundström, Olsson, Schaal, & Hummel, 2006). The perceived pleasantness of another person’s body odor potentially serves as a cue about this persons’ genetic setup (Jacob et al., 2002). Mothers and newborn babies identify each other from their body odors (Doucet, Soussignan, Sagot, & Schaal, 2007; Porter & Cernoch, 1983; Schaal et al., 1980). In adulthood, body odors engage brain regions that are not activated by common non-body odors, suggesting that body odors might be processed differently than non-body odors (Lundström, Boyle, Zatorre, & Jones-Gotman, 2008).

A historical perspective

The human olfactory sense was neglected by philosophers and experimental researchers for a long time. Plato and Aristotle considered olfaction as a less noble sense than vision and audition. The conception of olfaction as a primitive and crude sense is since that time evident along western intellectual history (Le Guérer, 2002). Sigmund Freud argued that the sublimation of smell was a factor of civilization (Le Guérer, 2002). Freud viewed olfaction as a primitive sense: As olfaction is associated with sexuality and emotions, Freud argued that in a modern society, olfaction needs to be suppressed (in Freuds terminology, sublimated). Le Guérer (2002) argues that the lack of psychological research on olfaction relative to several other senses is caused by a cultural hierarchy where olfaction is considered as emotional and subjective, and hence inferior to the more “intellectual” visual and auditory senses. According to a survey, university students regard the sense of olfaction as
the least important sense (VanToller, 1999). However, individuals that have lost their sense of smell reappraise its importance, as olfactory loss influences their well-being negatively (Hedén Blomqvist, Brämerson, Stjärne, & Nordin, 2004).

Given the fundamental evolutionary significance of the chemical senses, a subtle but never the less important role might be expected also in humans. Indeed, the use of perfumes and pleasant-smelling smokes (e.g. during religious ceremonies) served very important societal functions already in ancient societies (Doty, 2003). The importance of spices throughout human civilization is undeniable, and can be illustrated by the fact that during the siege of Rome in A.D. 408, a ransom of 3000 pounds of pepper was demanded for the city (Doty, 2003). A millennium later, Columbus’ journey to America and the West Indies was an attempt to find a new route to India, which would improve Spain’s position in the lucrative spice trade.

Human olfaction and its biological basis

The human sense of smell might not be as poor and insignificant as often assumed. The olfactory sensitivity in humans can detect many odors at concentration levels in the parts-per-billion range (Devos, Patte, Rouault, Laffort, & Van Gemert, 1990) which is superior to advanced technical instruments (Tagaki, 1989). Recent studies show that, in some cases, the olfactory sensitivity of primates, including humans, are comparable to the olfactory sensitivity in rats and dogs, species which are recognized for their advanced olfactory abilities (Laska, Seibt, & Weber, 2000). As noted by Zelano and Sobel (2005), “…although we all appreciate that dogs can identify humans by their odors, we don’t appreciate that, reciprocally, humans can identify dogs by their odor” (Wells & Hepper, 2000).

The human olfactory system is activated by chemical molecules reaching the olfactory neuroepithelium at the top of the nasal cavities. The olfactory neuroepithelium contains several million bipolar receptor cells. The receptor cells have cilia branching out to the outer layer of the epithelium. The cilia contain the receptors that interact with the odorant. Different receptor cells are tuned to be responsive to odors of different molecular structure. A large number of genes (~ 1000 in rodents, ~ 350 in humans) code for olfactory receptors (Buck & Axel, 1991). This enables functional specialization among receptors, and that many molecules are capable of activating the olfactory system. Cilia of the receptor neuron are depolarized by an olfactory stimulus, and summate on the receptor cell
body to evoke an action potential. The axons of the receptor cells project through the cribiform plate, a bone that separates the nasal cavity from the brain cavity, to the glomerular layer of the olfactory bulbs. For a single compound, many activated receptor cells converge on a small number of glomerular cells in the olfactory bulb, giving rise to distinct activation patterns, “images”, representing the unique odor stimuli (Mombaerts et al., 1996). Common odorants such as chocolate typically consists of hundreds of different molecules that contribute to the characteristic chocolate odor (Counet, Callemien, Ouwerx, & Collin, 2002). The molecules activate the olfactory neurons “in concert”, placing high demands of integration on the olfactory system. The receptor cells have a life span of a few weeks, and are continuously replaced through neurogenesis (Austic & Saucier, 2001). In humans, stem cells in the subventricular zone (SVZ) produce progenitor cells, which are dividing cells that have the capacity to differentiate (Curtis, Faull, & Eriksson, 2007). The progenitor cells migrate from the SVZ through the ventriculo-olfactory neurogenic system to the olfactory bulb, where they become functional neurons (Curtis, Kam et al., 2007). Through both inhibitory and excitatory processing in the olfactory bulb, activation is transduced by the olfactory nerve (CN 1) to central olfactory structures which are located primarily in the medial temporal lobe. The epithelium at the top of the nasal cavities is vulnerable to infection, and viral infection in the upper airways is a major cause of irreparable olfactory dysfunction (Doty & Mishra, 2001; Murphy, Doty, & Duncan, 2003). Furthermore, the nerve cells connecting the epithelium to the bulb reach through perforations of the cribiform plate at the base of the cranium. If the head suffers from trauma, the brain moves inside the cranium; this movement lesions peripheral nerves and often causes permanent olfactory dysfunction (Doty et al., 1997).

The reappraisal of human olfactory capabilities has led to a better appreciation of the consequences of olfactory loss. Research is accumulating regarding the consequences of olfactory dysfunction for food appreciation and nutrition, perceived life quality and subjective well-being (Hummel & Nordin, 2005; Nordin & Brämerson, 2008).

The sense of smell interacts with taste and trigeminal perception to produce complex sensory impressions during eating and drinking, engaging many brain regions (see Verhagen & Engelen, 2006, for a review). The processing is so well-integrated that we experience it as one gestalt-like percept, often denoted flavour. Individuals suffering from olfactory loss often report that their sense of taste has been impaired, which illustrates that the chemical senses are difficult to dissociate in
every-day perceptual experience (Deems et al., 1991). It has been speculated that this integration is a type of synesthesia, a cross-wiring of different sensory systems. In synesthesia, a stimulus triggers involuntary sensations in a sensory modality that is not normally engaged by this stimulus (e.g., a synesthetized person might perceive colors as tones or letters as colors). This phenomenon is more common among children than among adults since the sensory systems gradually become more differentiated during development. This differentiation might, however, be cancelled in the chemical senses since they are often activated in concert, i.e., during food perception (Verhagen & Engelen, 2006).

Accordingly, experimental studies have shown that odor quality perception is highly influenced by associative learning (Stevenson, 2001), which might explain why fruity odors such as cherry are often described as “sweet” (Stevenson, Prescott, & Boakes, 1995).

The integrative properties of the chemical senses can be exemplified by the finding that a mixture of two stimuli can be detected even though the stimuli are too weak to be detected separately. This finding has been reported in smell-smell mixtures, in taste-taste mixtures, and in smell-taste mixtures (Dalton, Doolittle, Nagata, & Breslin, 2000). In contrast, when the constituent components of a mixture are supra-threshold, the perceived intensity of the mixture is typically lower than the sum of the intensities of the components when perceived separately; this phenomenon is called hypo-addition or mixture suppression. This phenomenon occurs in both odor mixtures and taste mixtures. A weaker component in a mixture can be completely masked by a stronger component.

Assessment of human olfaction

Olfactory functions – like other sensory systems – can be assessed behaviourally in various ways. The assessments range from basic sensory processes, such as detection and discrimination thresholds, to more cognitive tasks, such as semantic and episodic odor memory. Also, the advancement in neuroimaging technologies such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) have led to a reliable mapping of the central neural substrates of human olfactory processing (Sobel, Johnson, Mainland, & Yousem, 2003). Across methods, an increasing body of research on human olfaction indicates that our knowledge of the olfactory sense is rapidly expanding. The behavioural and neurophysiological methods to study human olfaction are...
so successful that human olfaction was recently proposed to provide a model system for understanding organization of olfaction in animals (Zelano & Sobel, 2005).

Perceptual and cognitive processing of chemosensory information involves several subcortical and cortical areas, and their engagement vary with the perceptual and structural properties of the odorant, as well as the task performed by the participant (Royet & Plailly, 2004; Savic, Gulyas, Larsson, & Roland, 2000). In general, a more complex task generates a more extensive activation pattern in the brain, including several cortical regions. The epithelial neurons converge to a smaller set of neurons in the olfactory bulb. Activation patterns in the olfactory bulb reflect distinct odor qualities (Xu et al., 2003). Among the central olfactory structures, it has been proposed that the piriform cortex is responsible for encoding the unique identity of an olfactory percept (Li, Luxenberg, Parrish, & Gottfried, 2006). The piriform cortex is the primary target of projections from the olfactory bulb (Kay & Freeman, 1998). However, the activity in the piriform cortex is not only determined by stimulus-factors in olfactory perception, but is also modulated by sniffing behaviour and attention (Mainland & Sobel, 2006; Zelano et al., 2005). Furthermore, olfactory-elicited activation in piriform cortex depends on experience (Li, Howard, Parrish, & Gottfried, 2008; Li et al., 2006), suggesting that this is a key region to study the interplay between sensory-driven (“bottom-up”) and cognitive (“top-down”) factors in olfactory processing. Amygdala is also consistently activated by odors (Savic et al., 2000). This region, which receives direct projections from the piriform cortex, was initially proposed to respond to emotional components of an odorant (Zald & Pardo, 1997). Recent data, however, indicates that the amygdala responds primarily to the intensity of the odor, but only if the odor is emotional (positive or negative), and not if it is of neutral valence (Gottfried, 2006). The entorhinal cortex and hippocampus are brain regions adjacent to the amygdala in the medial temporal lobe; they are also commonly observed neural substrates of olfactory processing (Savic et al., 2000; Sobel et al., 2003). Structures in the neocortex can also be activated by odors depending on the olfactory task, the type of odorant, and factors related to stimulus presentation and control conditions (Sobel et al., 2003). Among these regions, the orbitofrontal cortex is consistently activated by odor stimuli (Gottfried & Zald, 2005). The orbitofrontal cortex is particularly engaged in processing related to odor quality discrimination and odor memory (Savic et al., 2000). However, this region also receive input from gustatory and visual centres, as well as information regarding the visceral...
state of the individual, suggesting that this region is engaged in multimodal integration and reward processing (Rolls, 2004).

Olfactory detection and discrimination ability

Olfactory detection sensitivity is typically assessed by psychophysical evaluation of detection of weak odor concentrations. These methods are often based upon Gustave Fechner’s “method of limits” or “method of constant stimuli” (described in detail in Gescheider, 1997). In the method of constant stimuli, a set of pre-determined odor concentrations are presented a pre-determined number of times, in a randomized order to the participant, and the task is to decide whether the presentation has an odor or not. In addition, blank presentations can be given randomly in the series of stimuli. The false-alarm rate can then be used to calibrate each concentration’s hit-rate for response bias. An individual graph is plotted for the proportion of correct responses at each stimulus level. The individual’s odor threshold is defined as the concentration at which it is detected at a certain proportion (often 0.50). In the method of limits, presentations of odors are given in ascending or descending magnitudes. Also here, the participant’s task is to determine whether s/he perceives the odor. The individual’s threshold is determined at the level where the participant starts to perceive the odor (ascending series) or no longer perceives it (descending series). An example of assessment of discrimination ability for similar odor qualities can be performed by means of a procedure where three sniff-bottles are presented to the participant. One of these smells is slightly different than the other two (i.e. contains a mixture of the substance present in the other bottles, and an additional, qualitatively different odorant), and the participants task is to select the odd bottle. In the discrimination test, the odd bottles vary systematically with regard to the proportion of the qualitatively different odor. This proportion measures the objective difference between the odor stimuli in the odd bottles and the other two bottles. By defining the threshold as the 50% discrimination level, a quality discrimination threshold can be established. This procedure may also be used to determine intensity discrimination thresholds; the only difference is that in intensity discrimination procedures all bottles contain the same odorant, but one is stronger/weaker than the others. Because of large individual differences in olfactory sensitivity, in combination with problems of adaptation in the olfactory sense, it is time-consuming to accurately establish odor thresholds by means of psychophysical techniques. (Doty et al., 2003)
Episodic odor memory
Episodic memory refers to memory for personally experienced events (Tulving, 1983) and provides information about the “what”, “where”, and “when” of events (temporally dated experiences) and about “how” they happened (temporal-spatial relationships). Since episodic memory tests typically employ stimuli presented visually, less is known about our ability to memorize odors. Episodic odor memory can be studied both in terms of memory for odors (when odors are presented during an encoding phase, to be recognized later), and memories evoked by odors. With regards to the latter, odorants can evoke vivid autobiographical memories from early childhood (Willander & Larsson, 2006, 2007). Since odors appear to be potent triggers of such memories, one might therefore assume that olfactory memory is more proficient than memory in other senses. Overall, there is little data to support this notion. It has also been argued that while odors might not be better encoded than visual stimuli, odor memories that are successfully encoded are more stable over time than visual memories (Engen & Ross, 1973). However, others have reported that odor memory does not have this advantage when compared with visual memory (Cain & Murphy, 1987). Olfactory stimuli are in general probably less memorized than other stimulus types when tested in a typical experimental situation, but in everyday life, some odor experiences lead to highly persistent, vivid and emotional memory traces, as indicated by the prevalence of autobiographical odor memories and flavour-learned aversions to food (Chambers & Bernstein, 2003). The ability to successfully encode and subsequently recognize an odor is influenced by semantic and emotional associations to the odor stimulus (Larsson & Bäckman, 1993). Basic detection sensitivity and discrimination ability does also influence odor memory performance (Dorty, Smith, Mckeown, & Raj, 1994).

Semantic odor memory
Semantic memory refers to our knowledge about the world. It encompasses the knowledge of e.g. word meanings, concepts, symbols and rules, and does not involve the re-experience of past events. Also, semantic memory includes the knowledge of one’s own memory processes, i.e. “metamemory”. An example of metamemory is the “tip of the loutngue” phenomenon, where a person “knows the answer” but cannot retrieve the word despite effortful attempts (Brown & Mcneill, 1966). In olfaction there is an analogous phenomenon, the “tip of the nose” effect, when the name for a common odorant cannot be retrieved without cues, although the odor is rated as highly familiar (Jonsson & Olsson, 2003; Lawless &
Engen, 1977). Odor identification refers to the ability to retrieve the correct name of a presented odor, often by means of a forced-choice procedure where several response alternatives are provided. Identifying an odor relies on semantic memory, since the task critically depends on experiential knowledge of odor-name associations and successful retrieval of these associations (Murphy, Nordin, & Acosta, 1997; Öberg, Larsson, & Bäckman, 2002). As this thesis focuses on odor identification as an index of olfactory proficiency, this type of test is discussed in more detail below.

**Odor identification**

The process of identifying an odor poses sensory and cognitive demands. A certain degree of olfactory sensitivity is obviously needed to identify an odor, as well as an ability to discriminate between odor qualities (Doty et al., 1994; Larsson, Öberg, & Bäckman, 2005). A successfully completed odor identification test thus indicates that neither olfactory detection nor discrimination ability is severely impaired in the participant. Evidence is sparse regarding what cognitive factors are required for high odor identification performance. Previous research has indicated that high semantic and verbal memory abilities are associated with better odor identification abilities (Economou, 2003; Larsson, Finkel, & Pedersen, 2000). There are several variables that influence the cognitive demands posed by an odor identification test. For example, the olfactory stimuli vary along dimensions such as familiarity, hedonics, and intensity, which influence the identifiability of the odors (Cain, 1979; Cain, de Wijk, Lulejian, Schiet, & See, 1998). Naturally, the odors used as stimuli must be familiar to the participants in order for them to make a correct identification. Therefore, it is important that odors are familiar to the investigated population. Furthermore, even a common odor such as orange might be difficult to identify at levels close to the individual's detection threshold. Strong concentrations might also lead to a mislabelling of the odor as e.g. rose might smell like perfume or soap if presented at a strong concentration. As the intensity of an odor is a stable characteristic of an odorant (in a natural context, a rose seldom smells as strong as perfumes and other artificial products), intensity provides a cue as to the source/label of the odor.

The retrieval format is important for identification performance. In cued odor identification tests, participants are provided with response alternatives; the difficult task of retrieving relevant odor names from
memory is not necessary. A cued response mode elevates the average performance rate substantially; in free response mode, young adults can only identify 22-57% of common odors (Larsson, 2002), whereas cued odor identification test performance can approach ceiling-levels in young adults (Doty, Shaman, Applebaum et al., 1984). However, while cues improve performance significantly, this does not imply that cued odor identification is easy for all participants. While a detection and discrimination deficit might be the most common cause of odor identification impairment in cued odor identification tests, the participant also has to be familiar with the odor names presented as response options. This is a further prerequisite for above chance-level performance. Labels describing familiar, well-defined and non-overlapping odor qualities will yield comparatively high performance rates. The factors described above all contribute to cognitive involvement and to overall performance on an odor identification task.

Tests of odor identification
Several different odor identification tests have been developed for use in different populations and clinical settings, including the Smell Identification Test, SIT, formerly denoted UPSIT (Doty, Shaman, & Dann, 1984), the San Diego Odor Identification Test, SDOIT (Murphy, Anderson, & Markison, 1994), the Sniffin’ Sticks (Hummel, Sekinger, Wolf, Pauli, & Kobal, 1997), and the Scandinavian Odor Identification Test, SOIT (Nordin, Brämerson, Liden, & Bende, 1998). In odor identification tests, it is necessary to adjust the odorants to the cultural context. For example, the SIT comprises the smell of root beer (a soft drink) which is less recognized by the Scandinavian population. Odor identification tests are often used clinically to determine olfactory dysfunction (Deems et al., 1991). The most commonly used olfactory test, the SIT, has both similarities and differences with the modified version of the SOIT, which is used in the studies of this thesis. The SIT is a disposable “scratch-and-sniff” test composed of forty microencapsulated items of odors which are common household odors in the USA (Doty, Shaman, & Dann, 1984). Odor identification tests, like many other tests, are more reliable if they are more extensive (Doty, McKeown, Lee, & Shaman, 1995). However, testing time is typically limited, especially in a clinical context. A shorter version was therefore developed that also had the ambition of being cross-culturally valid (Doty, Marcus, & Lee, 1996). This test, denoted the Brief Smell Identification Test (B-SIT) comprises 12 items with four verbal response options to each item. The SOIT was developed by Nordin and colleagues as a test of odor identification ability.
designed for the Scandinavian population (Nordin et al., 1998). The SOIT is inexpensive because the odors are kept in glass bottles that can be used across many participants, as opposed to the SIT that can only be used once due to the “scratch-and-sniff” format. The SIT has a well-controlled stimulus magnitude due to the microencapsulation of the odors. The SOIT uses natural oils as odor stimuli.

Demographic factors and olfactory function

Olfaction is impaired in around 20% of the general population (hyposmia), and in at least 5% of the population, olfaction is so compromised that it is referred to as anosmia – lack of a sense of smell (Brämerson, Johansson, Ek, Nordin, & Bende, 2004; Landis, Konnerth, & Hummel, 2004; Murphy et al., 2002). Prevalence of olfactory dysfunction increases rapidly with age such that at least 50% of individuals over the age of 70 are affected (Doty, Shaman, Applebaum et al., 1984; Murphy et al., 2002). Olfactory impairments are thus a common phenomenon. The high prevalence of olfactory dysfunction is in part a consequence of the fragile architecture of the peripheral olfactory system, which was described previously.

Age

A substantial decrease in general olfactory function has been reported in old age (Doty, Shaman, Applebaum et al., 1984; Stevens & Caín, 1987). However, there are large individual differences in olfactory function among elderly. The age-related deterioration of olfactory function is related to environmental (Corwin, Loury, & Gilbert, 1995), and various health-related variables such as malnutrition, infectious diseases, and medication (Schiffman, 1983a, 1983b). In elderly individuals, the surface area of the olfactory epithelium is smaller than in young individuals (Loo, Youngentob, Kent, & Schwob, 1996). The epithelial neurons are being replaced at a slower pace in the elderly compared to the young. Also, the tuning of the receptors is broader in the elderly, such that a receptor can be activated by a larger set of molecules, which might impair discrimination abilities (Rawson, Gomez, Cowart, & Restrepo, 1998).

The age-related olfactory impairment is observed across several olfactory domains, including detection sensitivity (Schiffman et al., 1976; Stevens & Cain, 1987), quality discrimination (Schiffman & Pasternak, 1979), and in more cognitively driven tasks such as odor identification (Doty, Shaman, Applebaum et al., 1984; Ship, Pearson, Cruise, Brant, & Metter, 1996; Wysocki & Gilbert, 1989), odor recognition memory (Larsson & Bäckman, 1997, 1998a, 1998b), and odor source memory (Gilbert,
The age-related impairment in episodic odor memory is shared with a naming impairment, suggesting that olfactory semantic processing is crucial for episodic odor memory (Larsson & Bäckman, 1993, 1997). The semantic and episodic olfactory functions thus seem to draw on similar processing resources. It is also possible that much of this shared variance is due to a sensory impairment in the olfactory system, which would cause broad cognitive impairments “up-stream”. Indeed, elevated olfactory detection and quality discrimination thresholds seem to account for much of the odor identification impairment associated with age (Larsson et al., 2005).

**Gender**

The status of gender differences in chemosensory function has recently been described as somewhere “between evidence and enigma” (Brand & Millot, 2001), despite many reports of a female advantage in olfactory processing. Gender-related differences in odor perception were first reported by Toulouse and Vaschide in 1899, who found lower detection thresholds (i.e., higher sensitivity) in women than in men. The female advantage has been corroborated by other studies using different odor substances for establishing detection thresholds and discrimination (Koelega, 1994; Schneider & Wolf, 1955). Although gender differences in thresholds are often not found (e.g. Öberg et al., 2002), women consistently perform better when a gender difference in olfaction is noted (Brand & Millot, 2001). Gender differences are more often reported in olfactory tasks that involve more cognitive elaboration of the olfactory information. For example, studies on gender differences in odor identification have shown higher performance in women than in men (Doty, Applebaum, Zusho, & Settle, 1985), which might be interpreted as a female advantage in verbal processing (Öberg et al., 2002). A related finding is that of Murphy and colleagues (2002), who found higher prevalence of olfactory loss in older men in a population-based study of adults aged 50 years and older, which may be indicative of an interaction between gender and age. It has also been reported that the ability to identify odors is compromised earlier in life for men than for women (Ship et al., 1996; Ship & Weiffenbach, 1993). Further support for an age by gender interaction has been documented in olfactory ERPs (Morgan, Covington, Geisler, Polich, & Murphy, 1997). However, a Swedish population-based study on odor identification ability revealed generally lower performance in men than women, and lower performance in older participants compared to younger participants, but no interactions between gender and age (Brämerson et al., 2004). Recordings of
chemosensory CSERPs have yielded higher amplitudes in women than in men in response to non-irritating odor stimulation (Becker et al., 1993; Evans, Cui, & Starr, 1995; Morgan et al., 1997) as well as for trigeminal stimuli (Hummel, Barz, Pauli, & Kobal, 1998; Olofsson & Nordin, 2004). Also, several studies report that women perform better than men in episodic memory for familiar odors, whereas no gender difference is found for unfamiliar odors; this observation suggests that females may use their documented verbal/semantic advantage to memorize odors (Choudhury, Moberg, & Doty, 2003; Larsson, Lövden, & Nilsson, 2003; Öberg et al., 2002).

Education
The influence of education on odor identification ability is sparsely investigated. Education is typically measured as the number of years that an individual has spent within the formal educational system. It is hence a very broad measure, and it is likely to covary with many cognitive abilities and sociological variables within a population. Education can be assumed to correlate positively with several cognitive abilities, such as knowledge and fluency (the principal domains of semantic memory, see e.g. Nyberg, Bäckman, Erngrund, Olofsson, & Nilsson, 1996). This makes education a variable of interest in the present thesis. Odor identification depends on semantic memory since knowledge of odor names is a prerequisite to perform well on the task. Education might correlate with odor identification performance if highly educated have more knowledge of the verbal descriptors used in the task. Larsson and colleagues found that education did not reliably influence olfactory sensory and cognitive abilities, although there was a trend indicating a positive relationship (Larsson et al., 2005). It is important to control for education level in assessment of age-related differences, since elderly individuals are typically less educated than younger individuals. Statistically adjusting for inter-individual differences in word knowledge is a method of dissociating the perceptual component from the verbal/mnemonic component of an odor identification task. If an odor identification difference is caused by differences in general knowledge rather than olfactory acuity, then this difference should manifest also as a general difference in vocabulary (a common assessment of verbal knowledge). We used vocabulary as a control task in Studies II and III to control for influences of general semantic memory in the olfactory assessment.
Olfaction in cognitive aging, and influences of dementia and genetics

Aging is associated with impairments in both cognitive and sensory functions, which might influence odor identification ability. The magnitude of age-related decline differs among cognitive domains such that episodic memory—a “fluid” ability—declines at a faster rate than semantic memory, which is a “crystallized” ability (Rönnlund, Nyberg, Bäckman, & Nilsson, 2005). Fluid abilities are considered most pertinent in cognitive aging research and have received most attention. In the cognitive aging literature, several theories have tried to explain age-related differences in cognition, with a particular emphasis on episodic memory (Luszcz & Bryan, 1999). Common among these theories is that they postulate that age-related cognitive impairments can be explained by diminished processing resources in one or a few primary cognitive domains. Working memory capacity (Craik & Byrd, 1982), general inhibitory function (Hasher & Zacks, 1988), and processing speed (Salthouse, 1996) have been proposed as such basic functions underlying age-related deficits in cognition. As one focus of the present thesis concerns the relationship between cognition and olfactory identification, rather than the relationship between different cognitive domains, the relation between sensory and cognitive function in aging will be discussed further. The effects of aging on cognition is typically shared with effects of aging on sensory acuity, which has lead to a “common cause” account of the effects of aging (Baltes & Lindenberger, 1997; Lindenberger & Baltes, 1994). It is widely acknowledged that in the elderly, sensory acuity correlates positively with cognitive performance. These correlations are larger within a sensory modality, e.g. when visual acuity is compared to a visual test of memory. However, they are often also significant across sensory modalities, even between olfactory acuity and cognitive tests assessed in vision (Dulay & Murphy, 2002). Often, sensory and cognitive impairments are conflated when comparing participants from different age groups. Cognitive testing typically requires efficient and rapid processing of sensory information that is presented on sheets of paper or computer screens. Age-differences in cognition are larger when testing is done under conditions that challenge sensory function, e.g. when the testing environment is dimly lit or noisy (Fozard & Gordon-Salant, 2001). This indicates that age-related sensory impairments might influence the perception of the stimulus material and thereby boost observed cognitive impairment in the elderly. However, when middle-aged participants underwent cognitive assessment under sensory age-simulation conditions,
cognitive performance was not reduced to the level of the elderly population (Lindenberger, Scherer, & Baltes, 2001). This finding indicates that sensory and cognitive dysfunction in the elderly is associated not merely by difficulties in perception during the cognitive assessment, but also through fundamental changes in the brain that broadly affect sensory and cognitive systems.

Although receiving much attention, the theories that postulate specific cognitive mechanisms as principal features of cognitive aging have received methodological criticism for mainly being supported by cross-sectional studies on age-heterogeneous groups, which is not an optimal research strategy to assess individual change over time (Hofer, Sliwinski, & Flaherty, 2002; Sliwinski & Hofer, 1999). The studies included in this thesis do not test the validity of these theories, but they are mentioned as to provide an overview of the current theoretical debate in cognitive aging research. Age-related differences in odor identification might be fruitfully investigated from the perspective of cognitive aging. In the discussion section, methodological aspects that are specifically relevant for the current studies will be highlighted.

**Genetic influences on cognition in aging**

In adults, around 40-50% of the individual differences in cognitive functions can be accounted for by a general factor, often denoted $g$, or intelligence (Plomin & Spinath, 2002; Spearman, 1904). This implies that people who perform well on one cognitive task tend to perform well on other cognitive tasks, also when the cognitive tasks are very different. The decline in $g$ accounts for most of the age-related decline in cognitive functions, at least when studied cross-sectionally (Salthouse & Ferrer-Caja, 2003).

In aging, genetic factors influence cognition both by influencing adult (“baseline”) function and by influencing aging-specific changes (Deary, Wright, Harris, Whalley, & Starr, 2004). In older adults, the between-person variability in cognitive performance is often larger than in young adults. As a consequence of these factors, the total genetic influences on cognition can be high in elderly samples (McClearn et al., 1997). At old age, genetic influences are especially large in general cognitive ability, compared to specific cognitive domains (Pedersen, Plomin, Nesselroade, & McClearn, 1992). Such results are in general derived from studies investigating the similarity in cognitive functions among monozygotic and dizygotic twin pairs. In such studies, a heritability measure, the general importance of genetics in the variability in a certain trait, is obtained, rather than a precise relation between a single gene and a defined cognitive
ability. It is widely acknowledged that, among the approximately 30 000 genes in the human genome, effects on cognitive functions are often caused by a combination of effects from several genes (polygenic effects), with each individual gene effect being of small or very small size (Deary et al., 2004). It can thus be expected that single genes show very small effects on cognition. Indeed, the influences of single genes on the variance on a certain trait are often very small, but the heritability coefficient is nevertheless quite large (Visscher, Hill, & Wray, 2008). In addition, multivariate genetic analysis techniques have revealed that genetic influences are often generalized across cognitive functions; a limited set of genes influences diverse cognitive functions in a similar way (Butcher, Kennedy, & Plomin, 2006). This concept of “generalist genes” is congruent with the notion of \( g \) as reflecting variability of a fundamental quality in the neurobiology of the brain. Accordingly, the generalist genes have been suggested to influence neural density or synapse plasticity, but also general psychological functions such as working memory; these might in turn influence other cognitive domains “down-stream” (Butcher et al., 2006). As of yet, however, the field is characterized by a very large number of genes that have been suggested to influence \( g \), but very few of these genes have been consistently found across studies (Plomin, Kennedy, & Craig, 2006).

**The ApoE gene in cognitive aging**

The genetic perspective in this thesis is restricted to the ApoE gene and its’ variants. ApoE is a plasma protein that is involved in the transport of lipids among various cells of the body and in the metabolism of cholesterol (Mahley, 1988). The gene for ApoE is located on chromosome 19 and carries three alleles; \( \varepsilon 2 \), \( \varepsilon 3 \), and \( \varepsilon 4 \). As each individual carry a combination of two alleles, there are six possible ApoE genotypes: \( \varepsilon 2/\varepsilon 2 \), \( \varepsilon 2/\varepsilon 3 \), \( \varepsilon 2/\varepsilon 4 \), \( \varepsilon 3/\varepsilon 3 \), \( \varepsilon 3/\varepsilon 4 \), and \( \varepsilon 4/\varepsilon 4 \). The allele frequencies vary among populations, with highest prevalence of \( \varepsilon 4 \) in northern Europe. In the Swedish population, allele frequencies has been estimated to 7.8% for \( \varepsilon 2 \), 71.9% for \( \varepsilon 3 \), and 20.3% for \( \varepsilon 4 \) (Eggertsen, Tegelman, Ericsson, Angelin, & Berglund, 1993). The ApoE gene is primarily expressed in the liver, but also in the brain. It has been reported that the \( \varepsilon 4 \) allele is associated with a higher risk of coronary disease, and that the \( \varepsilon 2 \) allele is associated with lower risk, compared to the most common \( \varepsilon 3 \) allele (Bennet et al., 2007; Song, Stampfer, & Liu, 2004). In the Swedish population, serum cholesterol and low-density lipoprotein cholesterol is associated with ApoE alleles, with highest levels found in \( \varepsilon 4 \)-carriers (Eggertsen et al., 1993).
In the last two decades, the ApoE has been established as the major genetic risk factor for AD. In 1993, it was discovered that the ε4 allele was positively linked to development of AD (Saunders et al., 1993). Many other genes have since then been suggested to be related to AD risk, however only the ApoE-ε4 has consistently produced strong and reliable influences (Bertram & Tanzi, 2004). In a recent meta-analysis, the effects of non-ApoE genetic risk factors on AD susceptibility were on average only ¼ of the estimate of the effect of ApoE-ε4 (Bertram, McQueen, Mullin, Blacker, & Tanzi, 2007).

The ApoE gene is regarded as one of the most important genetic influences on cognitive aging. However, its precise role is not clear since it is difficult to separate the effects related to pre-clinical AD from non-demented cognitive function when studying the ApoE-ε4 allele. Carriers of ApoE-ε4 show reduced glucose metabolism in temporal, parietal and frontal regions, as revealed by cross-sectional and longitudinal PET imaging (Reiman et al., 2001; Reiman et al., 1996). In non-demented elderly samples, ApoE-ε4 has been associated with volumetric and hemodynamic changes in the hippocampus that might be a cause of changes in episodic memory function (Lind, Larsson et al., 2006; Lind, Persson et al., 2006; Small, Rosnick, Fratiglioni, & Bäckman, 2004). Since ApoE-ε4 is involved in AD, and since cognitive deficits are present several years before the clinical onset of AD, it is difficult to dissociate pre-clinical effects of AD from cognitive deficits that might be unrelated to AD. It is unclear whether cognitive deficits in non-demented carriers of the ε4 can be accounted for by dementia status at a later assessment (Nilsson et al., 2006; Small, Basun, & Backman, 1998). To illustrate the different views regarding this issue, one review of genetic influences on cognitive function excluded the ApoE gene since it was difficult to exclude effects of dementia (Goldberg & Weinberger, 2004), but another review discussed the ApoE gene without attempting to disentangle demented and non-demented cognition (Deary et al., 2004).

The ApoE gene, neuropathology, and olfaction
The ApoE gene might influence age-related changes in olfactory function through mechanisms related to AD. The ApoE gene is expressed in the central nervous system, including the olfactory bulb and the olfactory epithelium (Nathan, Nannapaneni, Gairhe, Nwosu, & Struble, 2007; Struble, Short, G Hobrial, & Nathan, 1999; Yamagishi, Getchell, Takami, & Getchell, 1998). The gene has been proposed to play a role in lipid recycling during neuronal regenerative processes in the olfactory system.
The main neuropathological hallmarks of AD are neurofibrillary tangles which are aggregations of primarily tau protein, and amyloid plaques which consist of amyloid β protein. The tau protein is expressed in neurons and is regulated by phosphorylation. In AD, the tau protein is hyperphosphorylated, and it aggregates inside nerve cells to produce neurofibrillary tangles. Tau pathology accumulates in the olfactory bulbs of AD patients (Attems, Lintner, & Jellinger, 2005). ApoE-ε4 is associated with an increased amount of tau pathology in the olfactory bulbs of deceased patients with pathologically confirmed AD (Tsuboi, Wszolek, Graff-Radford, Cookson, & Dickson, 2003). However, the relationship between tangle density and ε4 in non-demented individuals is unclear (Sparks et al., 1996). AD is further characterized by amyloidosis, which is an abnormal deposition of amyloid in the brain. Amyloid plaques are extracellular aggregations of Aβ protein. Aβ is produced through a degradation of amyloid precursor protein (APP). Carriers of the ApoE-ε4 have higher levels of amyloid plaques in the brain (Ghebremedhin et al., 2001; Sparks et al., 1996). Apolipoprotein E is found in both neurofibrillary tangles and amyloid plaques in the brains of individuals diagnosed with AD (Namba, Tomonaga, Kawasaki, Otomo, & Ikeda, 1991). The statistical association between ε4 and diagnosed AD can be accounted for by the amount of neuropathology (Bennett et al., 2003). It has been proposed that neurofibrillary tangles and amyloid plaques accumulate in the transentorhinal cortex during the earliest stages of AD, and that this pathology spreads to adjacent regions in the limbic system before “higher-order” cortical regions are affected (Braak & Braak, 1991, 1995). Thus, there are clear associations between the ApoE-ε4 and AD-related neuropathological development in olfactory brain structures. However, evidence is sparse regarding the influence of ApoE status on human olfactory function in non-demented elderly and adults, and regarding the precise neurobiological function.

Olfaction in dementia
In the 1980s, independent research groups recognized that patients with AD and Parkinson’s disease (PD) had strikingly poor olfactory function (Dory, Reyes, & Gregor, 1987; Koss, Weiffenbach, Haxby, & Friedland, 1988; Serby, Corwin, Conrad, & Rotrosen, 1985). At this time, evidence had begun to accumulate that olfactory brain regions were subject to AD neuropathology, including cell loss, amyloid plaques and neurofibrillary tangles (Esiri & Wilcock, 1984). Samples of neural tissue from the
olfactory epithelium and bulb also revealed changes in morphology and immunoreactivity in AD patients (Ohm & Braak, 1987; Talamo et al., 1989). The early findings have since then been replicated and extended by findings of very early pathological changes in the olfactory bulbs due to both AD and PD (Daniel & Hawkes, 1992; Kovacs, Cairns, & Lantos, 1999, 2001). Also, temporal lobe structures that are involved in olfactory processing, most notably the entorhinal cortex, show early signs of AD pathology (Braak & Braak, 1991, 1995). Other dementia types such as Huntington’s disease and vascular dementia are sometimes associated with olfactory impairments which are considered less pronounced than those associated with AD and PD (Knupfer & Spiegel, 1986; Nordin, Paulsen, & Murphy, 1995). However, the differentiation of dementia types based on olfactory assessment have proven to be difficult, yielding mixed results (Gray, Staples, Murren, Dharriwal, & Bentham, 2001; Murphy, Razani, Nordin, Bacon, & Hamilton, 1995). The following discussion of olfactory dysfunction in dementia will be restricted to AD, since it is the most relevant dementia type in the present thesis. In the context of the current focus on the role of the ApoE gene, AD is of particular interest since it is the dementia disorder in which ApoE-ε4 constitutes a major genetic risk factor (Corder et al., 1993). Furthermore, AD is the most common dementia disorder, affecting a majority of dementia cases (Bachman et al., 1992). It is also the dementia disorder in which olfactory dysfunction is most well-studied.

In studies of AD patients, odor identification scores have been shown to correlate with MMSE, illustrating the utility of the odor identification test in detecting olfactory deficits that accompany the cognitive deficits in AD (Koss et al., 1988; Serby, Larson, & Kalkstein, 1991). Olfactory detection tests have yielded less consistent results than identification tests with regard to AD, with some studies showing impaired odor detection, and some showing odor identification impairment despite normal detection (Doty et al., 1987; Koss et al., 1988; Murphy, Gilmore, Seery, Salmon, & Lasker, 1990; Rezek, 1987; Serby et al., 1991). Odor detection often show no correlation with MMSE performance in elderly and demented participants (Larsson et al., 1999; Serby et al., 1991), although there are conflicting findings also in this regard (Murphy et al., 1990). An olfactory detection impairment in AD may contribute to the observed odor identification deficit, but may not explain it fully (Morgan, Nordin, & Murphy, 1995; Serby et al., 1991), suggesting the contribution of an additional cognitive component to the deficit in odor identification (Larsson et al., 1999). Although one study have shown that olfactory detection thresholds are impaired the year immediately
preceding AD diagnosis (Bacon, Bondi, Salmon, & Murphy, 1998), another study indicated that olfactory threshold assessment is less valuable than odor identification or other cognitive olfactory tests in detecting AD in a pre-clinical stage (Koss et al., 1988). A meta-analysis of olfactory function in AD indicated that across studies, olfactory detection, identification, and memory abilities were equally impaired in this disease (Mesholam, Moberg, Mahr, & Doty, 1998). The authors of that study argued that olfactory detection thresholds are more difficult than odor identification ability to assess reliably, and that this might explain the conflicting findings regarding impairment of olfactory detection in AD. A study that compared olfactory and gustatory sensitivity in non-demented elderly and AD patients concluded that olfactory, but not gustatory function was impaired in AD and that the difference between the groups was hence not due to a difficulty in performing the task, since both sensory modalities were studied with similar procedures (Murphy, Nordin, & Jinich, 1999).

Neurophysiological techniques have developed substantially since the initial behavioural studies, enabling the measurement of neural responses in the brain in vivo. One ERP study compared AD patients with healthy age-matched controls, using olfactory and auditory stimulation (Morgan & Murphy, 2002). Olfactory ERP measures alone correctly classified up to 92% of the participants, which was better than auditory ERP measures. Using a combination of olfactory P3 latency measures and an odor identification test, the classification rate was 100%. A study addressing the effects of the ApoE gene on olfactory ERPs indicated that olfactory ERP latency measures show higher sensitivity and specificity in classifying carriers and non-carriers of the ε4 allele, than do psychophysical measures (Wetter & Murphy, 2001). Also, deficits in olfactory sensitivity in MCI are related to a lack of ERP-responses to olfactory stimuli (Peters et al., 2003). In a study that measured haemodynamic olfactory responses, less activation in right piriform cortex were reported in AD patients (Kareken et al., 2001).

**Olfaction in groups at high risk for dementia**

Early identification of an impending dementia disorder is a major challenge in neuroscience. In particular, delaying the onset of AD was mentioned as one of the 125 most important research topics in the journal Science’s 125 years anniversary edition. To accomplish a reliable identification of individuals who will develop dementia, researchers have explored a wide variety of functions including cognitive abilities, physical health status, haemodynamic brain activity, and inventories of daily life
activities. While no single variable has been identified that will determine whether and when an individual will develop AD, a multitude of risk factors for developing the disease has been established. As was previously noted, carriers of the ApoE-ε4 are at a higher risk of developing AD than non-carriers. Mild cognitive impairment (MCI) is characterized by cognitive impairment and the MCI label is used to characterize cognitively impaired elderly that might be in a pre-clinical stage of dementia. According to a view that different types of MCI exist, impairment in episodic memory function, but otherwise normal cognitive performance, is characterized as amnestic MCI. In this definition, amnestic MCI is a major risk factor for developing AD (Gauthier et al., 2006). Given that the pre-clinical period in AD might start many years before the clinical onset (Small, Fratiglioni, Viitanen, Winblad, & Backman, 2000), identifying individuals with a pattern of cognitive impairment similar to that characterizing AD would be helpful in detecting early-stage AD. MCI is also sometimes defined by means of global dementia rating scales such as the Clinical Dementia Rating or the Global Deterioration Scale, where normal cognition, MCI and dementia are defined according to different sections on a continuum. In this MCI definition, multiple cognitive domains are slightly affected. This type of MCI classification predicts dementia more generally (Petersen et al., 2001). Not all MCI patients will develop dementia; some remain non-demented, especially in MCI where multiple domains are slightly impaired. Olfactory function is impaired in MCI as compared to healthy elderly, and the presence of an odor identification impairment in MCI predicts dementia conversion (Devanand et al., 2000; Djordjevic, Jones-Gotman, De Sousa, & Chertkow, 2007). This olfactory deficit is likely to be caused by AD-related neuropathology in olfactory structures, (Attems & Jellinger, 2006) as MCI patients often are in a stage of pre-clinical AD (Palmer et al., 2007). Also, first-degree relatives of individuals with AD exhibit olfactory deficits (Handley, Morrison, Miles, & Bayer, 2006; Serby et al., 1996).

An early study indicated that odor identification ability was impaired in a small sample of non-demented elderly ApoE-ε4-carriers (n=7) relative to a cognitively intact control group (Murphy, Bacon, Bondi, & Salmon, 1998). Since the ε4-carriers had a poor cognitive function, the difference in odor identification between the groups might have been caused by differences in cognitive, rather than olfactory function. A recent study failed to replicate the association between ε4 and odor identification in a cognitively intact sample of ε4-carriers vs. controls (Handley et al., 2006). Also, targeting episodic odor memory in the elderly, two studies reported that elderly ε4-carriers produced more false positive errors than controls,
but no main effects of ApoE-ε4 on overall odor recognition were reported (Gilbert & Murphy, 2004a, 2004b). Longitudinal data suggest that elderly ε4-carriers exhibit a larger performance decline in odor identification than controls (Calhoun-Haney & Murphy, 2005). Taken together, the findings suggest that individuals with high risk of AD might have olfactory impairments, and that such impairment might be a pre-diagnostic marker of the disease. Elderly ε4-carriers might have olfactory impairments, but the roles of cognition and pre-clinical dementia have received little attention in the studies of ApoE-ε4 and olfaction.

**Indirect associations between AD, neuropathology, and cognition**

Dementias are characterized by cognitive impairments and are most prevalent in the elderly population. However, the relationships between brain pathology, cognitive status and clinical diagnosis are not direct. Individuals with AD vary widely with respect to the load and distribution of pathology in the brain (Roth, Tomlinson, & Blessed, 1967). AD-related neuropathology is also seen in many non-demented elderly (Lippa & Morris, 2006; Tomlinson, Blessed, & Roth, 1968), and modified tau protein has even been reported in restricted areas of the brains of young, non-demented adults (Shin, Kitamoto, & Tateishi, 1991). Wilson and colleagues stated that “…not only the level of neurofibrillar pathology, but also its regional distribution contributes to individual differences in a wide range of neurobehavioural functions in old age” (Wilson, Arnold, Schneider, Tang, & Bennett, 2007). Furthermore, other non-pathological factors might moderate the associations between AD pathology and diagnosis. For example, AD patients who have had extensive education and high work complexity demands often show modest impairments, despite severe abnormalities in the brain (Stern, Alexander, Prohovnik, & Mayeux, 1992; Stern et al., 1995).
Aims of the thesis

Based on the foregoing review, the general aim of this thesis was to identify factors that predict olfactory function (assessed with an odor identification test) in the middle-aged and elderly population, and to investigate whether an odor identification deficit is associated with prospective cognitive function and dementia. Towards this goal, the thesis investigates the relationships between odor identification and cognitive, genetic (ApoE), and health-related variables that might influence cognitive aging in a large, population-based study. The main issues to be addressed in the thesis may be summarized as follows:

1. How do age and gender influence odor identification across the adult life span? (Study I)

2. Do cognitive variables influence odor identification over and above the influence of demographic variables? (Study I)

3. Do the ApoE genotypes influence odor identification in a non-demented population? (Study II)

4. Is an influence of the ApoE gene on odor identification mediated by conversion to dementia within the next five years? (Study II)

5. Does olfactory impairment, the ApoE-ε4 allele, or a combination of these factors predict a prospective cognitive decline in non-demented elderly? (Study III)
General Methods

The three separate studies include data collected within the Betula prospective cohort study: A large-scale, population-based study focusing on memory, health and aging (Nilsson et al., 2004; Nilsson et al., 1997). The Betula study is carried out in Umeå, a city of approximately 100 000 inhabitants in the north of Sweden. Persons were selected from the population registry in Umeå and contacted by mail. Participation was voluntary, and only volunteers without severe visual or auditory handicaps, or mental retardation were included in the study. A further criterion for inclusion was having Swedish as a first language. Table 1 outlines the design of the Betula study, including the test occasions (T1-4) and the included samples (S1-5). Each sample was stratified according to ten narrow age cohorts, and approximately 1000 individuals were recruited in each sample. In each sample, the goal was to recruit 100 participants aged 35, 100 participants aged 40, etc. This goal was reached in most cohorts, with exception for the oldest cohorts, where slightly fewer eligible participants could be recruited. The data used in the present thesis was primarily derived from the third wave of the Betula study (collected 1998-2000), when an odor identification test was introduced in the testing battery.

The bolded text in Table 1 marks the data used in the present studies. Study I uses cross-sectional data from S1-3 at T3. Study II uses data from S1 and S3 at T3, but also includes dementia assessments obtained at T4. Since S2 was not tested at T4, and since these data were critical to accurately establish dementia status, this sample was not included in Study II. Also, S4 did not perform the odor identification test, and was excluded for this reason. In Study III, data from S1 and S3 at T3 and T4 was used to investigate change in global cognitive function across this five-year interval. Details regarding screening criteria and participant characteristics are provided in each study of the thesis.

The odor identification test used in the Betula procedure is a modified version of the Scandinavian Odor Identification Test. The original test was developed for a Scandinavian population (Nordin et al., 1998). The original SOIT included 16 items: almond (bitter), ammonia, anise, apple, cinnamon, clove, juniper berry, lilac, lemon, orange, peppermint, pine-needle, tar, vanilla, vinegar, and violet. Three of these items (vinegar, ammonia, and peppermint) were excluded in the Betula study because they could be detected above chance level also by anosmics: Evidence that these stimuli activated the trigeminal sense (Nordin et al., 1998). The odor stimuli were natural etheric oils (Stockholm Ether and Essence

24
Table 1
Outline of the Betula design with regards to the testing waves (T) and samples (S). Bolded text indicates data used in this thesis.

<table>
<thead>
<tr>
<th>Wave</th>
<th>Year</th>
<th>Samples (age range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>1988-1990</td>
<td>S1 (35-80)</td>
</tr>
<tr>
<td>T2</td>
<td>1993-1995</td>
<td>S1 (40-85), S2 (40-85), S3 (35-80)</td>
</tr>
<tr>
<td>T3</td>
<td>1998-2000</td>
<td>S1 (45-90), S2 (45-90), S3 (40-85), S4 (35-90)</td>
</tr>
<tr>
<td>T4</td>
<td>2003-2005</td>
<td>S1 (50-95), S3 (45-90), S5 (35-95)</td>
</tr>
</tbody>
</table>

The response alternatives were changed from the original test: In order to make the test more difficult and to avoid ceiling effects, the three incorrect response alternatives for each item were replaced by alternatives that were perceptually more similar to the correct alternative (e.g., rose was one of the incorrect alternatives for violet). The items of the odor identification test used in the present studies are listed in Table 2.

Several variables were selected in Studies II and III to assess influences of health on odor identification and cognitive performance at T3 assessment. From the large number of health-related variables provided in the Betula, a limited number of major health variables were selected for inclusion in the model. In Study III, the selection of health variables was further informed by the results from Study II, excluding variables that were very infrequent in the study sample, and/or not correlated with other key variables.

In the present studies, a hierarchical regression technique was used to determine statistical relationships among the variables. Hierarchical regression, also denoted multi-level or sequential regression, is a type of...
Table 2.
Targets (bolded text) and distractors for each item in the odor identification test of the Betula study, in English and Swedish.

<table>
<thead>
<tr>
<th>English</th>
<th>Swedish</th>
<th>English</th>
<th>Swedish</th>
</tr>
</thead>
<tbody>
<tr>
<td>peppermint</td>
<td><strong>almond (bitter)</strong></td>
<td>acetone</td>
<td>ammonia</td>
</tr>
<tr>
<td>pepparmint</td>
<td><strong>bittermandel</strong></td>
<td>aceton</td>
<td>ammoniak</td>
</tr>
<tr>
<td>rose</td>
<td>cherry</td>
<td><strong>violet</strong></td>
<td>vanilla</td>
</tr>
<tr>
<td>ros</td>
<td>körsbär</td>
<td><strong>viol</strong></td>
<td>vanilj</td>
</tr>
<tr>
<td>anise</td>
<td>vinegar</td>
<td>honey</td>
<td>ginger</td>
</tr>
<tr>
<td>anis</td>
<td>vinäger</td>
<td>honung</td>
<td>ingefära</td>
</tr>
<tr>
<td>apple</td>
<td>lily</td>
<td><strong>violet</strong></td>
<td>vanilla</td>
</tr>
<tr>
<td>äpple</td>
<td>liljekonvalj</td>
<td>viol</td>
<td>vanilj</td>
</tr>
<tr>
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26
multiple regression technique and it is used to explain variation in a 
dependent variable (DV) from multiple independent variables (IV). 
Common among multiple regression techniques are that they 
compartmentalize the variance in the DV and attribute it to IVs. It is a 
highly flexible and widely used statistical technique (Tabachnick & Fidell, 
2007).

Hierarchial regressions are suitable for the type of data provided in 
non-experimental studies such as the Betula. Here, the IVs are not 
orthogonally manipulated, but correlate to a varying extent with each 
other and with the DVs. In cases of correlations among IVs, the 
hierarchical structure makes it possible to partial out the influence of an 
IV (e.g. age) before assessing the contribution of a correlated IV (e.g. 
dementia) on the DV (e.g. odor identification). Whereas standard 
multiple regression techniques are “non-theoretical” in the sense that they 
do not presuppose any given order or hierarchy among the IVs, 
hierarchical regression techniques are designed to test explicit hypotheses 
(Tabachnick & Fidell, 2007). The variables used in the thesis are 
sometimes continuous (e.g. cognitive variables) and sometimes 
dichotomous (i.e. health variables), which does not constitute a problem 
for a hierarchical regression model.
Overview of Empirical Studies

Study I

Study I was carried out to provide a description of odor identification ability in the adult and elderly Swedish population. More specifically, the major aims of Study I were to address gender- and age-related differences in odor identification, and to assess cognitive influences on identification proficiency. Effects of age and gender were well documented, although the reported evidence was only occasionally based on population-based data (Murphy et al., 2002). Although cognitive influences on odor identification in aging had been investigated previously, the large-scale Betula study with its extensive cognitive protocols provided an unusually powerful means of detecting predictors of successful odor identification in population-based samples (Larsson et al., 2000). Cognitive tests were selected to sample cognitive function in different domains.

Given that the study's aim was to investigate demographic and cognitive factors determining successful odor identification in healthy adults and elderly, anosmic and cognitively impaired participants were not of interest. Excluded from the data set were those participants who scored at chance level in the odor identification test (3 or below) in combination with reporting “worse than normal” sense of smell for weak odors (n = 35), and participants who scored below 24 on the Mini-Mental State Examination (MMSE; n = 106). The effective sample consisted of 1906 individuals who were neither cognitively impaired nor anosmic.

The results showed a linear decrease in olfactory ability with increasing age, and that women performed better than men (Figure 1). There was no significant age by gender interaction. However, because previous studies have reported of a sex by age interaction, effects of gender were analyzed at each age cohort separately. The post-hoc analyses showed that women performed significantly better at all ages except in the oldest group (85-90 years), in which women and men performed equally well. The effect size of the overall gender difference was modest, about 0.2 standard deviations. However, the gender effect was still larger in odor identification than in the other tests in this study: among the cognitive tests used (letter-digit substitution, vocabulary, letter fluency, category fluency, block design and tower of Hanoi), only block design showed a comparable correlation with gender (men performed better in this task). As expected, deficits related to higher age were observed in all non-olfactory cognitive tests.
Odor identification proficiency correlated positively with performance on all other cognitive tests. Hierarchical regression analyses were used to determine unique influences on odor identification. The regression model included the letter-digit substitution test as a measure of cognitive speed, vocabulary, letter fluency and category fluency as measures of semantic memory, and block design and tower of Hanoi as measures of executive function, visuo-spatial ability and problem-solving. In the regression, age, (block 1), demographic variables (block 2, sex and education), and cognitive variables (block 3, cognitive speed; block 4, vocabulary, letter fluency, category fluency; block 5, block design and tower of Hanoi) was entered in subsequent blocks.

Figure 1. Odor identification performance (means and S.D.) for men and women in each age group.

The demographic variables age, education, and gender were found to contribute to the variance in odor identification performance. Cognitive speed and vocabulary had small (1% and 2%, respectively) but statistically significant influences on odor identification ability over and above the effects of demographic variables. The variables included in this analysis together accounted for 17% of the total variance in odor identification.
When age was entered last in the model, it explained only 3% of the variance (unique effect), compared to 12% when it was entered first (simple effect). The other included demographic and cognitive factors were hence able to diminish the explained age-related variance by 75%.

Overall, Study I showed that odor identification ability was influenced by age, gender, and education level, and that cognitive speed and vocabulary influence odor identification ability above the contributions of demographic variables. Of the total variance, 17% could be accounted for by these factors.

Study II

In Study II, we investigated the role of the ApoE gene on olfactory processing. The main aim was to investigate potential differences in odor identification ability related to the presence of the ε4 allele in a population-based sample of adults for which health factors, cognitive ability, and dementia status were statistically controlled for. Previous research had shown that the ε4 allele of the ApoE gene is strongly associated with sporadic AD (Corder et al., 1996; Corder et al., 1993; Poirier et al., 1993). Moreover, patients with AD show severe olfactory deficits (Doty, Reyes, & Gregor, 1986; Doty et al., 1987; Larsson et al., 1999; Morgan & Murphy, 2002; Murphy et al., 1999; Nordin, Almkvist, Berglund, & Wahlund, 1997; Nordin & Murphy, 1998; Serby, 1986). Olfactory impairments was observed in cognitively impaired elderly who received an AD diagnosis within the following two years (Bacon et al., 1998; Tabert et al., 2005). Because ε4-carriers are more likely to develop AD than non-carriers, and develop AD at a younger age, follow-up assessment of dementia is pivotal to investigate whether an olfactory deficit in ε4-carriers is mediated by an influence of pre-diagnostic dementia on olfactory function, or whether an olfactory deficit can occur over and above the effect of dementia. Hence, if ε4 is associated with an olfactory impairment because of processes leading to a dementia diagnosis, the effect would be attenuated by controlling for effects of future dementia diagnoses. However, if the ε4 allele is associated with an olfactory impairment by a mechanism that is not strongly related to future dementia diagnoses, the effect of ε4 would remain after the effects of dementia are controlled.

Most previous studies on olfactory ability and the ε4 allele did not assess dementia prospectively. In contrast, this study employed a five-year
follow-up interval to address the possible effect of pre-diagnostic dementia on the association between the presence of $\varepsilon 4$ and olfactory ability. Also, we evaluated whether possible deficits in odor identification ability associated with ApoE status were olfactory specific or linked to deficits in semantic memory (i.e. vocabulary) or general cognitive status (Handley et al., 2006; Larsson, Öberg, & Bäckman, 2006; Murphy et al., 1998).

Previous studies did not investigate the role of age as a potential moderator of the association between $\varepsilon 4$ and olfactory ability. Instead, these studies compared elderly groups of $\varepsilon 4$ carriers and non-carriers (Gilbert & Murphy, 2004a; Graves et al., 1999; Murphy et al., 1998). Thus, the possible interactions between age and ApoE genotype on olfactory performance were unknown.

In Study II, we selected 1236 participants from the age cohorts 45-80 years to assess the age-related impairment in odor identification ability among carriers and non-carriers of $\varepsilon 4$. To merit inclusion, participants had to complete odor identification, vocabulary and MMSE assessment at T3, be genotyped for the ApoE gene, and provide health-related information in questionnaires and interviews conducted by the Betula staff. Information about dementia was provided from medical files of each participant at T3 and T4, to classify participants as either “currently demented” ($n=23$) or “pre-diagnostically demented” ($n=42$) at the time of testing. Similar to Study I, hierarchical regression analyses were used to investigate the contribution of several independent variables on odor identification. Block 1 included the demographic variables age, sex, education, and previous testing experience. In the second block, the genetic factors ApoE-$\varepsilon 2$ and ApoE-$\varepsilon 4$ were entered. The third block included the vocabulary test and MMSE to control for individual differences in knowledge and general cognitive status. Block four included the health-related variables; diabetes (type II), neurological disorder, psychiatric disorder, head injury, chemical exposure, allergy/asthma, ear-nose-throat disorder, cardiovascular symptoms for which the participant was receiving medication, smoking, and cold/congestion/flu at the time of testing. Current and pre-diagnostic dementia statuses were then entered in separate blocks (5 and 6). The two final blocks included the genetic interactions with age: ApoE-$\varepsilon 2$ by age, and ApoE-$\varepsilon 4$ by age (blocks 7 and 8).

The main finding was a two-way interaction of $\varepsilon 4$ and age, such that higher age was associated with more pronounced odor identification impairment in participants with $\varepsilon 4$ compared to those without the $\varepsilon 4$. Post-hoc analyses showed that in ages 75-80 years, carriers of $\varepsilon 4$
performed worse than non-carriers on the odor identification test (Figure 2). Although dementia at follow-up was associated with poor odor identification ability, the interaction effect of ε4 by age was not driven by individuals who received a dementia diagnosis within a five-year period after olfactory assessment. In total, the predictor variables accounted for 21.3% of the explanatory variance in odor identification performance. A follow-up analysis was performed to determine whether interactions between ApoE-ε4 and dementia would diminish the effect of ε4 in the ages 75-80 years, but these interactions were non-significant.

![Figure 2](image_url)

*Figure 2. Odor identification performance (mean ± S.E.) in carriers and non-carriers of the ε4 allele in each age-group. Left side: number of correctly identified odors; right side: standardized scores with the youngest cohort as reference.*

To further investigate whether the unique influence of ε4 on odor identification ability could be explained by prospective cognitive decline, a follow-up analysis is here presented on a sub-sample aged 75-80 years. Individuals from the original sample in the age-range 75-80 years were selected if they were non-demented at T3 and remained actively
participating in the Betula study by the fourth wave of testing. ANOVAs were performed in this sample of 175 participants, of which 42 were carriers of the ε4 allele. The presence of the ε4 allele was entered as a fixed factor and odor identification ability at T3 was entered as the dependent measure. Replicating the main result of Study 2, the ε4 allele was associated with an odor identification impairment, F(1,173) = 11.18; p < .01. When MMSE performance at follow-up (T4) was used as a covariate in this analysis, the effect of the ε4 allele was still significant, F(1,172) = 8.70; p < .01. The ε4 carriers performed worse than the non-carriers on the MMSE at T4, F(1,173) = 13.77; p < .001, but not at T3, F < 1. This follow-up analysis supports and extends the main findings of Study II; the ε4 allele is associated with conversion to dementia as well as with cognitive performance at a five-year follow-up, however, the effect of the ε4 allele on odor identification cannot be fully explained by these associations as the ε4-odor identification association remains after controlling for them.

Study III

In Study III, we used a longitudinal assessment of global cognitive status to investigate long-term cognitive changes in the elderly. Decline in various cognitive functions is among the first signs of dementia in the elderly, and variables that reliably predict cognitive decline might be particularly sensitive in detecting dementia at pre-clinical stages. Olfactory function has been studied in groups of non-demented elderly with an elevated risk of developing dementia, such as those with impaired cognitive function (mild cognitive impairment; MCI) and carriers of the ApoE-ε4 allele. In MCI, an olfactory deficit is often present (Djordjevic et al., 2007; Murphy et al., 1998; Wang et al., 2002) and might predict later dementia conversion (Tabert et al., 2005). Although not yet fully investigated, this association might be explained in part by the ε4-allele causing both olfactory impairment and dementia in many MCI patients (Bacon et al., 1998). However, there has been little consensus as to whether ε4 is associated with olfactory impairment in elderly without cognitive impairment (Graves et al., 1999; Murphy et al., 1998; Swan & Carmelli, 2002). Some evidence suggests that olfactory deficits predict future cognitive decline in non-demented elderly (Graves et al., 1999; Swan & Carmelli, 2002) but little is known about variables that might moderate this effect. Study II showed that the influence of the ε4 allele on olfactory function is age-dependent, and it is well known that the risk of
dementia increases with age. In study III, we investigated the predictive utility of odor identification at baseline in combination with age and the \( \varepsilon_4 \)-allele.

At the third wave of data collection, 696 individuals aged 65-90 years had completed the MMSE, the odor identification and vocabulary tests. These participants also responded to questions regarding health status, and were thus considered active participants. Among these, DNA had been extracted and ApoE had been genotyped in 675 cases (97%). Out of the genotyped participants, 41 individuals were demented at the third wave of data collection and were not considered further in this study. A high proportion of the study sample (79%) returned five years later to the fourth wave of data collection to complete the MMSE. Thus, the final sample consisted of 501 participants. The medical records of these participants indicated that 54 persons were diagnosed with dementia at the time of the fourth wave of assessment.

We decided to include the oldest age cohorts in Study III because it entailed a longitudinal assessment. Longitudinal studies of aging differ from cross-sectional studies in the expected age-cognition relationships; while the oldest cohorts might perform well in cross-sectional assessments depending on selective survival and screening criteria (Bäckman, Small, & Wahlin, 2000), they are still expected to drop substantially during the course of a longitudinal study. We included the oldest age groups because cognitive change, rather than ability, was the variable of interest. Participants were characterized as either carriers or non-carriers of the \( \varepsilon_4 \)-allele.

In the hierarchical regression, MMSE performance at T4 was used as the criterion measure. To control for global cognitive status at baseline, the MMSE performance at T3 was entered in the first block. Because MMSE at T3 was entered in the first block of the model, subsequent blocks predicted the variance in MMSE at T4 that was not accounted for by the baseline performance or by any other previous block (i.e., change). In the second block, the block of demographic variables (age, sex, and education) was entered. Genetic information regarding the ApoE gene (\( \varepsilon_4 \) allele and \( \varepsilon_2 \) allele) was entered in the third block. The fourth block comprised the following health variables: dementia, diabetes, stroke, head injury, ear-nose-throat (ENT) disorder, cardiovascular symptoms, and smoking. Sensory (odor identification) and cognitive (vocabulary) performance was entered in the fifth block. Two-way interactions involving age, ApoE-\( \varepsilon_4 \), and odor identification were entered in the sixth block. The final block consisted of a three-way interaction among age,
ApoE-ε4, and olfactory identification as potential predictors of cognitive change.

The results showed that among the demographic variables, age was a significant predictor of change, with higher age being associated with accelerated decline. With regard to the ApoE status, the results confirmed that the presence of ε4 was associated with pronounced cognitive decline, whereas ε2 was not associated with cognition. Whereas odor identification was not a significant predictor of MMSE change, per se, poor odor identification in combination with ε4 was a significant predictor of decline. The interactive effect of odor identification and ε4 was accentuated in the oldest groups, as indicated by a three-way interaction effect. The source of this interaction was that the oldest ε4 carriers with poor odor identification proficiency showed the largest drop in MMSE. In contrast, the oldest ε4 carriers with higher odor identification scores showed a mean decline in MMSE comparable to that of non-carriers of the ε4 allele. Results are shown in Figure 3.

To investigate whether vocabulary would interact with age, gender, or ε4 to predict cognitive change, a follow-up hierarchical regression was performed. This analysis was modeled similarly to the analysis of odor identification; however, vocabulary replaced odor identification in the two-way and three-way interactions. The results showed that none of the interactions including vocabulary predicted cognitive change (all ps > .10). This indicated that the interaction effect with odor identification was unrelated to a general semantic memory deficit, but rather that the effect might be olfactory-specific.
Figure 3. Change in MMSE (mean ± S.E.) as a function of ApoE-ε4 status, age (65-75 years vs. 80-90 years) and odor identification score at baseline (high, 6-13 items; low, 0-5 items).
General discussion

The general aim of this thesis was to identify variables that predict olfactory function and dysfunction (assessed with an odor identification test) in the middle-aged and elderly population. A more specific aim was to investigate whether an odor identification deficit was associated with dementia and cognitive decline. The thesis investigated the relationships among odor identification and cognitive, ApoE-genetic and health-related variables. The results from the studies presented in this thesis converge on a few themes that are discussed below.

Influences of demographic variables on odor identification

The present studies included three primary demographic variables: age, gender and education. All of these variables influenced odor identification, with the best odor identification ability generally seen in younger compared to older individuals, in women compared to men, and in individuals with more education compared to those with less education. These variables can all be conceived of as proxies for several other variables regarding life-experiences, cognition, and neurobiology. Hence, the mechanisms underlying these influences are difficult to disentangle within a non-experimental design such as that used in the present studies. A general age-related deficit in odor identification has often been reported previously (Doty, Shaman, Applebaum et al., 1984; Wysocki & Gilbert, 1989). In Study I, women performed generally better than men in odor identification. Research conducted in American samples have found larger and earlier age-related decline in odor abilities in men than in women (Doty, Shaman, Applebaum et al., 1984; Murphy et al., 2002; Ship & Weiffenbach, 1993; Wysocki & Gilbert, 1989). The present results do not conform to this pattern. In fact, when the hypothesis of a gender by age interaction was further tested in follow-up analyses men and women performed equally well only in the oldest group. Likewise, another study with Swedish participants did also not corroborate the findings of a larger female advantage at old age, showing similar age-related impairment in both sexes (Brämerson et al., 2004). It should be noted that our analyses in Study I are based on a sample that was screened for cognitive impairment and severe olfactory impairment. Murphy and colleagues reported that severe olfactory impairment in older age occurs more often
in men than in women (Murphy et al., 2002). The ambition in Study I to investigate olfactory-cognitive relationships in a healthy population, excluding anosmics and cognitively impaired, might therefore explain the different results relative to some previous studies. Furthermore, it should be noted that Wysocki and Gilbert (1989) showed a gender by age interaction pattern for detection sensitivity and self-reported olfactory acuity, whereas gender differences in odor identification performance varied greatly between odorants. Different selections of odor items in different studies might therefore be another possible source of differences among studies; given that odors and odor names may be more familiar to one gender than the other, differences in experience might influence the gender differences observed in odor identification (Cain, 1982). Further longitudinal investigations might elucidate the possible interactions of age and gender in odor identification ability within a Scandinavian population.

Education was positively associated with odor identification performance, and with performances on all other cognitive tests examined in this thesis. Also, it correlated with age, as younger participants typically had participated for a longer period in formal schooling compared to older participants. The possible role of education will be further elaborated in later sections in the context of reserve capacity as a buffer for detrimental effects of aging and dementia.

Influences of cognitive variables on odor identification

The contribution of specific cognitive tests to odor identification performance was small. However, it should be noted that cognitive tests were entered after education and age in the hierarchical regression model. Following the previous discussion regarding g and cognitive aging, this order of entry likely absorbed much of the general cognitive influences on odor identification. Remaining influences of cognition were therefore expected to be of modest size, although related to the specific processing demands of the tests. The synonyms test that was used to assess vocabulary in the present work is similar to the odor identification test in that it uses a multiple-choice format of matching a word to five synonyms, whereas the odor identification test entails the matching of an odor to four response options. Thus, vocabulary might be considered as a “non-olfactory analogue” to the odor identification test. Recent results from an American sample of elderly adults indicate that verbal retrieval difficulties
is an important factor for odor identification deficits (Dulay, Gesteland, Shear, Ritchey, & Frank, 2008). In Studies II and III, we used vocabulary as a covariate to control for the possibility that olfactory effects were mainly related to the verbal component of the odor identification test rather than the olfactory component.

The role of cognitive speed in successful odor identification was replicated by a more recent study (Larsson et al., 2005). Processing speed has been emphasized in a theory of cognitive aging (Salthouse, 1996). The theory hypothesizes that speed is an index of the processing efficiency of mental operations, and that declining speed causes cognitive decline at old age. Although the processing speed theory has been influential, it has received criticism; while speed accounts for a large proportion of cognitive differences among different age-groups, it does not account for much variance in longitudinal assessment of cognitive change (Sternäng, Wahlin, & Nilsson, 2008). Furthermore, it has been argued that the letter-digit substitution test, which is typically used as a test of processing speed, is influenced by learning; Hence processing speed is not analytically independent of the cognitive construct that it attempts to explain (Piccinin & Rabbitt, 1999). A moderate standpoint is that although letter-digit substitution might be a sensitive index of processing efficiency among elderly individuals, it might not constitute an unconfounded measure of speed, and it probably does not underlie general cognitive changes in aging. The present results, based on cross-sectional data, suggest that letter-digit substitution performance to some extent shares a common source of variance with odor identification. It might be speculated that this test is an unusually sensitive measure of fluid cognitive processing; it correlates highly with most other tests used in Study I, and this might underlie the slight unique influence on odor identification. Among the cognitive assessments, cognitive speed and vocabulary predicted the age-related differences in odor identification. In this study, 17% of the total variance was explained by the included predictors. It is a common finding that demographic (with the exception of age) and cognitive factors only account for a small portion of the variance in odor identification in large, heterogenous samples such as that used in the present studies (Larsson et al., 2000). It can be speculated that the remaining variance can be explained by variation in olfactory detection sensitivity and discrimination ability.
Influences of health-related variables on odor identification

Health-related variables were included in studies II and III to assess their relationships with the criterion measures. Results from Study II showed that, after partialling out the influences of age, sex and education, ApoE genotypes, and MMSE and vocabulary performance, health variables contributed substantially to odor identification proficiency. Among the health variables included in the analysis, diabetes was most strongly associated with poor odor identification, and neurological disorder also had a significant negative influence on odor identification. These findings correspond well with the literature. However, it is of interest to note that most health variables such as smoking were not associated with odor identification. Since the health-related variables were assessed by means of self-reports, it is possible that the current assessment was insensitive to detect subtle influences. Also, the present test of odor identification comprised fairly strong odorants that would be possible to identify even by participants with slightly elevated detection olfactory thresholds, as would be expected in smokers for example. Both current and pre-clinical dementia had significant negative influence on odor identification over and above the influence of other health variables that were entered earlier in the analysis. While it is well-known that odor identification is severely impaired in AD patients, less is known about pre-clinical deficits in dementia (Bacon et al., 1998). Most attempts to predict conversion to AD by means of olfactory assessment has focused on individuals with mild cognitive impairment (MCI), who are already identified as having a high risk of developing AD (Devanand et al., 2000; Tabert et al., 2005). The results of Study II indicate that odor identification is impaired in participants without a recognized cognitive impairment who will become demented within the next five years. This highlights the role of olfactory assessment in early detection of dementia.

Relationships between ApoE-ε4, odor identification, cognitive decline and dementia

While neither the genomic nor the cognitive assessment used in the present studies are extensive enough to address the general issues of genetic determinants of cognitive variability in aging, the present studies might provide a few contributions. The relationships between the ApoE-
ε4, odor identification, cognitive decline, and dementia are discussed below.

The findings of Study III indicate that ApoE-ε4 is a major variable for determining global cognitive decline in the elderly. The effect is to some extent attenuated by excluding participants who become demented within five years after olfactory testing, but the pattern nonetheless remains even for participants without a dementia diagnosis. These findings are congruent with previous studies, and do not exclude the possibility that the cognitive impairment associated with ApoE-ε4 is caused by pre-diagnostic dementia (Nilsson et al., 2006; Small et al., 2004). However, cognitive abilities are differentially affected by the ε4; semantic memory is relatively unaffected, whereas episodic memory is affected by the ε4 (Bondi, Salmon, Galasko, Thomas, & Thal, 1999; Bunce, Fratiglioni, Small, Winblad, & Bäckman, 2004; Small et al., 2004). The effects of ApoE-ε4 on cognitive decline might be mediated by pathology associated with pre-clinical AD (Bennett et al., 2005). It has previously been emphasized that some elderly ε4 carriers do not exhibit severe cognitive decline or dementia (J. D. Smith, 2002), and it is important to acknowledge the heterogeneity of this group. In this regard, Study III shows that olfactory impairment might be an indicator of future cognitive impairment in the ε4 group. In fact, elderly ε4-carriers that had a normal sense of smell did not display a cognitive decline above the level of elderly participants without the ε4.

The results from Study II indicated that non-demented ε4-carriers aged 75-80 years display olfactory identification impairment. The ε4 might cause olfactory impairment through an accumulation of AD-related pathology in regions engaged in olfactory processing. Since the effect of ε4 was observed in non-demented participants, a plausible explanation is that the olfactory impairment represents pre-diagnostic stages of the disease. To test this hypothesis, a prediction would be that controlling for dementia and cognitive decline would attenuate the effect of ε4 on odor identification. In Study II, the ε4 allele was related to olfactory impairment in participants aged 75-80 years, with a small difference also in the 70 year-old cohort, but there was no difference in younger cohorts. Surprisingly, this negative effect in old age was not fully accounted for by either dementia or cognitive decline within five years, suggesting that the relationships between ε4, olfactory dysfunction and dementia might not be as simple as previously assumed.

At least two plausible mechanisms might then account for the main findings of Study II. The first hypothesized mechanism involves AD-
related neuropathology that does not necessarily result in dementia diagnosis or significant cognitive impairment within five years. This proposed mechanism is theoretically possible because the pre-clinical period of AD might be longer than five years (Bäckman, Small, & Fratiglioni, 2001; Deary et al., 2002), and because not all AD-related neuropathology will eventually lead to a dementia diagnosis (Del Tredici & Braak, 2008). Recently, a study by Wilson and colleagues (Wilson et al., 2007) showed that amyloid plaques and neurofibrillary tangles, which were accumulated in the entorhinal cortex and hippocampus and assessed post-mortem, accounted for a significant portion (12%) of the variance in odor identification performance in a sample of elderly participants (odor identification was assessed on average 2.2 years before the participants died). Interestingly, the authors reported that AD diagnoses did not mediate the relationship between odor identification performance and plaques and tangles in the olfactory brain regions. The results imply that these neuropathological hallmarks of AD might be present also in participants who are not close to obtaining a dementia diagnosis, and that the plaques and tangles impair olfaction also in those participants who will not develop clinical dementia. Hence, while the mechanism responsible for the association between ε4 and olfactory identification might be the accumulation of plaques and tangles in olfactory regions, our results and those by Wilson and colleagues suggest that this mechanism might not always be a strong predictor of AD diagnosis. In elderly without a dementia diagnosis, some degree of AD-related neuropathology is present in a majority of examined cases (Davis, Schmitt, Wekstein, & Markesbery, 1999; Del Tredici & Braak, 2008). This implies that neuropathology is likely to account for behavioural differences within the population of elderly that have no clinically manifest dementia. The hypothesis that neuropathology without clinical dementia explains a part of the influence of ε4 on odor identification will be further elaborated under “Future aims and theoretical perspectives”.

A different, more speculative hypothesis is that of a regenerative failure in peripheral olfactory structures that affects elderly carriers of the ApoE-ε4 allele. ApoE has been proposed to be involved in lipid recycling, which is an important feature of neurogenesis (Maslia, et al., 1996). The peripheral olfactory system and the hippocampus are main sites of adult neurogenesis (Altman, 1969; Kaplan & Hinds, 1977). Aging is associated with a decreased neurogenesis in these structures (Enwere et al., 2004; Kuhn, Dickinson-Anson, & Gage, 1996). In non-demented elderly samples, ApoE-ε4 has been associated with volumetric and hemodynamic changes in the hippocampus that might cause changes in episodic memory
function (Lind, Larsson et al., 2006; Lind, Persson et al., 2006; Small et al., 2004). Although peripheral olfactory structures are more difficult to study than the hippocampus with current in vivo neuroimaging techniques, studies in mice have suggested that the ApoE is necessary for efficient neuronal regeneration in olfactory structures, and that the ε4 allele is associated with regenerative failure (Nathan et al., 2002; Nathan et al., 2005). While the present data do not allow us to infer the precise neurobiological mechanisms, future investigations might resolve whether regenerative failure associated with the ε4 allele might occur in the peripheral olfactory system in humans and thereby impair olfactory function in elderly participants.

The selection of different age cohorts in the different studies was motivated by the specific aims of each study. In particular, it is important to note that different age cohorts were selected for inclusion in Studies II and III. In Study II, ages from 45 up to 80 years were included. The oldest participants in the Betula sample, aged 85 and 90 years, were not included in Study II. The results from Study I indicated that odor identification in the group of oldest old might be influenced by selective survival; only in this age group did men perform as well as women in the odor identification task, possibly because men die earlier than women on average, and because men that survive until 85-90 years without cognitive impairment are a selected group of high-performing individuals. Similarly, as ApoE-ε4 is associated with shorter life expectancy (J. D. Smith, 2002), we expected effects of selective survival in the oldest old ε4-carriers. Exploratory analyses of the Betula data set indicated that up to 80 years, a linear age-related decrease was present in both ε4 carriers and non-carriers. However, while odor identification ability continued to decrease from 75-80 years to 85-90 years among the non-carriers of ε4, t (1,436) = 5.874, p = .000, there was no age-difference among the ε4-carriers, t (1,139) = -.153, p =.878. Individuals above 80 years has been described as the “oldest old” (Suzman, Willis, & Manton, 1992). Generally, very old age cohorts display different cross-sectional patterns when compared to younger cohorts, due to selective survival of high-performing individuals. Previous research has shown that the association between ε4 and mortality, cognitive impairment, and dementia is less clear in the oldest old (Juva et al., 2000; G. E. Smith et al., 1998). For this reason, ApoE-ε4 might not influence olfactory function in samples of wide age-ranges that include the oldest old (Graves et al., 1999). We decided not to include the oldest old in Study II because of the risk of selective survival that might offset the age-related functional impairment related to ε4 in oldest old. However,
longitudinal assessments of change are likely not as vulnerable to effects of selective survival as are cross-sectional measurements. The longitudinal cognitive decline typically seen in elderly participants was not attenuated at the highest cohorts, as shown in Figure 1 of Study III. Therefore, the oldest cohorts were included in the longitudinal Study III.

Future aims and theoretical perspectives

A scientific understanding of cognitive aging benefits from integrating psychological, epidemiological, biological, and genetic levels of study. The present findings might suggest future research regarding the role of olfactory processing in cognitive aging, in particular in the context of cognitive decline and dementia. As noted above, I have emphasized the role of neuropathological assessment post-mortem in elucidating the mechanisms underlying the associations between ε4, odor identification deficit, and cognitive decline in the elderly.

Reserve capacity

A hypothesis as to why AD-related neuropathology might not always predict conversion to clinically recognized dementia is that individuals to a varying degree can compensate for the damage caused by brain pathology. These pathological processes might start many years before any clinical symptoms are seen. Only after pathology has reached substantial quantity and wide cortical distribution do general cognitive functions decrease substantially. The individual vulnerability to the cognitive effects of damage to the central nervous system is determined by factors that are not fully known, but likely includes both environmental and genetic factors (Fratiglioni & Wang, 2007; Stern, 2002).

The results from Study II suggest that ε4 is associated with impaired odor identification independently of pre-clinical dementia. This can be reconciled with the notion of dementia-related neuropathology producing the odor impairment. Individuals with olfactory impairment due to early-stage neuropathology might have a varying “reserve capacity” that protects against the clinical manifestation of dementia (Satz, 1993). Education seems to be a relevant, although indirect, index of reserve capacity (Stern, Albert, Tang, & Tsai, 1999). A prediction would then be that among individuals with limited education, odor identification impairment related to ε4 would be mediated by dementia diagnoses within five years, since these individuals have a limited reserve capacity. However, in individuals
with extensive education, the ε4-related odor identification impairment would not be mediated by dementia diagnoses within five-years, since they have a reserve capacity that buffers against the effects of neuropathology.

If this testable hypothesis is true, then the findings from Studies II and III are easier to reconcile; the 75-80 year-old participants that display an odor identification deficit related to ApoE-ε4 (Study II), might to some extent be able to remain at a normal cognitive level despite the pathological burden, such that it does not result in conversion to clinical dementia within the following years. However, in oldest group of Study III (85-90 years), the ε4 is not associated with an olfactory deficit, possibly due to effects of selective survival. Nevertheless, poor odor identification ability in combination with the ApoE-ε4 allele still predicts cognitive decline in this age group.

In future studies, more consideration should be afforded to the relation between olfactory function, reserve capacity as a buffer against the clinical onset of AD and the cognitive drop that characterizes it. Since pathology develops early in peripheral olfactory structures, olfactory function might be particularly vulnerable in most cases. In contrast, dementia might only be clinically recognized in individuals where pathology has become more widely distributed, and who have insufficient reserve capacities to maintain cognitive function above clinical thresholds.

**Summary model**

The relationships between the different variables used in Studies I-III are schematically presented in Figure 4. In this figure, arrows indicate influences of one variable on another. The model does neither describe all possible inter-relations between variables, nor does it contain all influences described in previous research. Rather, the primary aim is to integrate the complex pattern of results obtained in Studies I-III into a comprehensive model.

Cognitive decline is in this model conceptualized as the consequence of the risk factors old age and ApoE-ε4, as well as their interaction with odor identification impairment (Study III). These risk factors are denoted

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1 It is difficult to exclude the possibility that dementia in highly educated individuals was detected at a later stage than in individuals with less education, since higher baseline cognitive performance might prolong the phase before an individual reaches the criteria for obtaining a dementia diagnosis. However, this possibility might not be in conflict with the concept of a reserve, since reserve is broadly defined, and might be related to environmental influences (intellectual and social stimulation, and cardiovascular exercise) that accumulate during the lifespan, as well as baseline g, and these factors all contribute to cognitive reserve capacity at high age.
primary variables. Among the primary variables, ApoE-ε4 in combination with old age is related to odor identification impairment.

The mechanisms underlying the relationships among the primary variables in the context of cognitive decline are hypothesized in a separate layer of the model. ApoE-ε4 is associated with increasing dementia-related pathology in olfactory structures (thereby the effect of ε4 on odor identification in older age). In the context of cognitive decline, the odor identification impairment is thus influenced by pre-clinical neuropathology, which later leads to cognitive decline. However, this association needs to be further qualified by taking age into consideration.

*Figure 4.* Model for predicting odor identification impairment and prospective cognitive decline, and its hypothetical mechanisms. Based on the findings of Studies I-III.

Age moderates the effects of odor identification and ε4, but also moderates the association with cognitive decline. These moderating effects of age are interpreted within a framework of a reserve capacity. This reserve denotes “the ability to tolerate the age-related changes and the brain pathology without developing clear clinical symptoms or signs” (Fratiglioni & Wang 2007). This reserve is likely to be influenced by baseline cognitive abilities, as well as fitness and many other life-style factors. Results from Study II indicate that the odor identification deficit that is related to presence of ε4 in ages 75-80 years is not explained by
prospective dementia (within five years). To explain this finding, a plausible hypothesis is that many individuals can live for many years with pathology in the brain without converting to clinical dementia. This reserve is influenced by the individual’s cognitive and education levels, (and high education and cognitive ability is also associated with better odor identification performance). Follow-up analyses that have been performed on this data-set suggest that the results in Study II might be further qualified by education level, such that ε4 and a related odor identification deficit is explained by incident dementia in individuals with limited education, but not in individuals with extensive education.

Background variables refer to variables that were not of primary interest in our investigation of cognitive decline and dementia. However, high cognitive function and extensive education is positively correlated in the study samples, and both contribute to the ability to identify odors. These variables are presumably also markers of reserve capacity (see discussion above).

### Olfactory assessment

I believe that the olfactory assessment used in these studies can be further developed in order to elucidate the relations between the key variables, as outlined in Figure 4. This development should be guided by a consideration of odor identification as a mixed sensory and cognitive test. A further discussion on this theme follows.

The ability to identify odors from a multiple-choice list is typically conceived of as mainly a sensory test of olfactory function. Indeed the variation in sensory acuity is a major determinant of odor identification performance. The present studies, in particular Study I, and to some extent Study II, related the odor identification score to performance across other cognitive psychological tests. The results indicated that non-olfactory cognitive tests contribute only marginally to odor identification performance after demographic variables are controlled for. Besides the variance shared between odor identification and non-olfactory cognitive tests, odor identification performance is presumably to a large extent dependent on olfactory sensory acuity. While the current data do not include any olfactory detection threshold assessment to address this issue further, olfactory sensitivity tests would provide valuable information of sensory vs. cognitive olfactory deficits in the elderly population. Olfactory cognition might be impaired in the elderly partly as a function of their decreased olfactory sensory acuity. In a normal elderly population, olfactory function might be more impaired than other sensory functions, at least when considering that hearing-aids and eye-glasses are widely
available to correct for auditory and visual impairments. No such aid exists for the olfactory sense, and no remedy exists for the major causes of olfactory impairment (Murphy et al., 2003). This makes it difficult to dissociate olfactory cognition from sensory function. Although the relationship between different olfactory abilities is scarcely investigated in the context of aging, a recent study indicates that individual variation in olfactory detection and discrimination abilities might be independent of each other (Cain, De Wijk, Nordin, & Nordin, 2008). If this independence would be changed by the presence of dementia disorders, this raises the possibility that there might be “olfactory profiles” that can differentiate demented from non-demented aging. A future aim is to investigate the age-trajectories of different olfactory tests that vary in sensory and cognitive load (Larsson et al., 2005). By comparing sensory and cognitive olfactory deficits in different types of aging, olfactory tests can be optimized to predict dementia and cognitive decline in the elderly.

Ethical considerations

This thesis presents data showing that olfactory identification impairment is associated with pre-clinical dementia and cognitive decline. The proportion of elderly increases in the population, and this development is associated with immense suffering and societal costs in part due to an increase in age-related dementia disorders. The quest for early markers and risk factors is highly prioritized, with the over-arching goal to prevent the disorders, or at least to delay their clinical manifestation. The possibility to predict an individuals’ risk of a future cognitive decline, which might progress to a debilitating dementia disorder, raises ethical considerations. Are the participants of a study informed about whether they belong to a particular risk group, and how would such information be perceived by the participant? In the Betula study, individuals are informed if they show a pattern of cognitive decline that might be indicative of early-stage dementia. In those cases, the participants are referred to a psychiatrist for dementia evaluation. The study thus includes an objective criterion for identifying suspected dementia cases and an established procedure for informing the participants regarding this sensitive issue.

A common notion among research participants and people in general seems to be that “ignorance is bliss”; that it is better not to know about your fate than to know that you are at risk of developing a devastating disease in several years. While this position is understandable, it should be noted that while research on risk factors for dementia has made much
progress recently, predictions are seldom very exact on an individual level. In the current case of olfactory function as a predictor of cognitive decline, effect sizes are quite small. One of the reasons is that while neurodegenerative brain disorders are hypothesized to cause an olfactory deficit, so do various medications, environmental and health-related variables that do not include pathology in the brain. Since many conditions affect olfactory function, it is difficult to predict the associated risk of cognitive impairment for an individual with olfactory impairment. There is particular need for careful evaluation of the upper respiratory system and for a review of the patient’s medical history to rule out alternative causes of olfactory decline. The present findings, as well as previous research, highlight the role of olfactory dysfunction in combination with other risk factors to predict cognitive decline and dementia (Devanand et al., 2000; Graves et al., 1999). The presence of other risk factors for dementia is likely to entail a higher proportion of olfactory-impaired participants with a neurodegenerative disorder as the primary cause, leading to better predictions.

Furthermore, it is important to note that cognitive development and dementia in aging can be influenced by medication and lifestyle factors. Regarding dementia, there is a constant development of drugs to delay the progression of the symptoms associated with neurodegeneration (Winblad et al., 2006). At present, anti-inflammatory drugs dominate the market, but treatments that might efficiently target the neurodegenerative processes by inhibiting the formation of β-amyloid and tau tangles in the brain are currently being developed. With regard to life-style factors, there is an old notion that the brain is not plastic; hence there would be nothing one could do to actively prevent a pathological deterioration at old age. However, neurophysiological research now highlight the role of neural activity in the survival and functionality of neurons in aging and dementia (Swaab et al., 2002). In a related manner, intellectual activity might have a protective role in preventing age-related cognitive decline. Although there are certainly limits to the influence of environmental stimulation in preserving cognitive function in the elderly (Salthouse, Berish, & Miles, 2002), several studies have reported that an enriched environment in adult life, assessed by level of education, work complexity, and social network reduces the risk of incident dementia (Fratiglioni & Wang, 2007; Wilson, Bennett et al., 2002; Wilson, Mendes De Leon et al., 2002). Furthermore, cognitive functioning and its underlying neural mechanisms are related to physical exercise in the elderly, and brain function can be altered within a few months of physical exercise (Colcombe et al., 2004; Kramer et al., 1999). Leisure activities that combine physical, social, and intellectual
stimulation seem most beneficial for maintaining high mental functioning in old age (Karp et al., 2006). In contrast, inactivity and social isolation increases the risk for dementia (Karp, Parker, Wang, Winblad, & Fratiglioni, 2005). Also, it should be noted that life-style and health variables interact with genetics; for example, given the negative effect of the ε4 allele on recovery after head trauma, a person with an ε4-allele could be advised to engage in sports and leisure activities with a minimal risk of head trauma (Sundström et al., 2004). Since the pattern of neurocognitive aging is modulated by environmental input during the lifespan (Kramer, Bherer, Colcombe, Dong, & Greenough, 2004), there are reasons to be optimistic about the accumulating knowledge regarding genetic and behavioural risk factors of cognitive impairment in aging.

Given the proximate nature of predictors for cognitive impairments and related disorders in the elderly, and the possibility of changing the expected cognitive trajectory by everyday activities, information about the presence of risk factors should be used responsibly. In large-scale studies such as Betula, participants are referred to a physician if they display signs of abnormal cognitive function (as observed by the testing staff), or show objective signs of cognitive dysfunction (MMSE < 23, or a decline of > 3 across two testing occasions). As one of the main aims of the Betula study is to detect signs of cognitive dysfunction in the elderly, this strategy increases also the ability of researchers in the Betula project to accurately discriminate demented from non-demented patterns of cognitive development.

Worrying about being at risk of dementia is a natural and common response when exposed to research concerning risk factors for dementia. Given that olfactory impairments are common in the elderly, and might predict cognitive decline and dementia, such information might potentially spread discomfort amongst many elderly who believe that they have a poor sense of smell. However, introspective evaluations of one's own olfactory abilities are of little, if any diagnostic value. First, in everyday life, olfactory impairments are not easily recognized. In fact, people typically have difficulty separating the sense of smell from their sense of taste. Olfactory impairments are often experienced as a loss of flavour in the food, and people misattribute this impairment to the sense of taste, and might seek medical attention for this perceived taste impairment (Deems et al., 1991). Second, the common method of assessing olfactory function by means of a cued odor naming test (odor identification) is quite remote from a layman’s notion of olfactory function. Whereas memory impairments in the elderly can be recognized by significant others through the forgetfulness and confusion it entails in
daily life, olfactory dysfunction is more associated with subtle mood changes and loss of appetite that are not typically associated with neurodegenerative disease (Nordin & Brämerson, 2008). Third, demented people are typically unaware of their olfactory deficit (Doty et al., 1987). This unawareness of dysfunction is also common among MCI patients (Devanand et al., 2000; Eibenstein et al., 2005), as well as among non-demented elderly (Nordin, Monsch, & Murphy, 1995). This suggests that progressive smell loss that occurs during aging is not readily accessible for self-reports, presumably because the loss of the ability to smell develops gradually over many years. The lack of awareness of an olfactory dysfunction might be indicative of conversion to dementia within the following two years (Devanand et al., 2000). Ironically, elderly people who are concerned about their poor sense of smell might thus actually be at a comparatively lower risk of dementia. Fourth, while several dementias (most notably AD and PD) are indeed characterized by severe olfactory impairments, pre-clinical stages of dementia are associated with less pronounced impairments. Olfactory impairment that is caused by dementia becomes more pronounced close to the clinical manifestation and after the criteria for a dementia diagnosis has been met. The magnitude of the olfactory impairment in groups that will suffer from a cognitive decline or become demented in the following years is quite modest, according to studies II and III in the present thesis, which emphasizes the need for further research directed at optimizing the sensitivity and specificity of the olfactory measurement to differentiate future dementia from non-demented cognitive development in aging (Tabert et al., 2005).

**Points of caution**

In the following section, I will highlight features of the studies included in the thesis that deserves a more thorough or critical discussion. In particular, possible limitations and shortcomings will be mentioned in order to explain the rationale behind some study features that are not obvious, and to provide suggestions for future research in the field.

**Statistical model and study design**

The present findings were obtained mainly through the use of hierarchical regression. The technique is widely used to partition different sources of variance, and its advantages were described above. In the cognitive aging literature, much effort has been devoted to identifying variables that can
explain the contribution of age to cognition, in particular episodic memory. Towards this end, results from hierarchical regression analyses have been used to support processing resource theories of cognitive aging. For example, the method has been applied to age-heterogeneous cross-sectional samples to show that most of the age-related variance in episodic memory is mediated by processing speed. These findings were taken as evidence that impaired processing speed causes poor memory in aging (Salthouse, 1996). In this context, the use of the hierarchical regression technique has received criticism. In particular, it has been shown that the analysis is influenced by the partial (age-orthogonal) correlation between the mediator variable (speed) and the dependent variable (memory) (Lindenberger & Pötter, 1998). In general, using hierarchical regression techniques on cross-sectional samples is probably insufficient to approach mechanisms underlying cognitive aging. However, the main findings reported in the present thesis were derived from a more straight-forward use of the hierarchical regression method. Rather than trying to explain the causes of age-related variance in a dependent variable, we used it mainly as a means of partitioning the total variance. In this way, we tested hypotheses regarding the additional contribution of a variable or interaction on a dependent variable after other relevant variables were accounted for. In sum, the critique regarding mediational theories of cognitive aging (e.g. Sliwinski & Hofer, 1999) has limited bearing on the present work.

A limitation of much aging research is the reliance on cross-sectional data (Hofer & Sliwinski, 2001; Hofer et al., 2002). In cross-sectional designs it is difficult to dissociate effects at the population level (i.e. the average trend) from the individual level (i.e. the deviations from this average trend). Longitudinal assessments enable stronger inferences in terms of predicting individual differences in change over time, as change is observed directly. This limitation of cross-sectional research is of some relevance to the present thesis, as it is partially based on cross-sectional data. It remains to be investigated whether analyses of longitudinal data will corroborate the present cross-sectional findings. Further longitudinal studies on the Betula data set will also be better suited to test theoretical accounts of the relation between olfactory and cognitive function in aging. In particular, it is of interest to investigate the common-cause hypothesis on olfactory and cognitive function, in both aging and dementia.

**Generalizability to the population**
A main advantage of the present studies lies in the population-based nature of the data and the large sample sizes, which provides an unusually
solid ground for making inferences to the general population. The descriptive data and statistical inferences can thus be considered quite robust and sensitive enough to detect even small effects (such as those related to genetic influences). However, it is important to note that in prospective studies, major factors that might confound results are attrition (selective drop-out) and practice effects. Both of these influences lead to higher mean-level performance, since drop-outs tend to have lower performance-levels than peers who remain in the study. As noted above, the odor identification test was introduced at the third wave of the Betula study. Thus, the olfactory data used in the present studies are hence not subject to any direct practice effects. Furthermore, odor identification performance was not likely influenced by attrition. In Study II, testing experience did not correlate with odor identification, and was not a significant predictor of odor identification in the regression analyses. Furthermore, it is noteworthy that the Betula study is characterized by a low drop-out rate (Nilsson et al., 2004; Nilsson et al., 1997). In study III, 21% of the participants dropped out of the study between the baseline assessment at T3 and the follow-up assessment at T4. Considering the baseline age of the sample in this study (65-90 years), and that a high proportion of the drop outs (67%) died during the course of the study, the drop-out rate can be considered manageable. For these reasons, I believe that the present results are stable and generalizable to the population.

**Evaluation of the odor identification test**
A further point of caution is that the psychometric properties of the odor identification test used in the present studies have not been subject to careful evaluation. As noted, the Betula version comprises odorants that are also included in the SOIT. This test has satisfactory methodological properties according to a previous evaluation (Nordin et al., 1998). The main alteration in the Betula version is that it is more difficult than the original test due to more difficult response options. This choice was motivated by an aim of avoiding ceiling effects, which would have caused statistical problems in evaluating relationships between test performance and cognitive and demographic variables. As shown in Figures 1-2, the test scores are well distributed and do not show any ceiling or floor effects for any age group. Also, the reported findings corroborate well with previous knowledge regarding declining odor identification ability across the lifespan. One result that differs from the results of studies using the SIT is that the age-related odor identification impairment in our studies follow a linear trend, whereas studies using the SIT show a more
curvilinear pattern (Doty, Shaman, Applebaum et al., 1984). This, however, might depend on the SIT being comparatively easier to perform than the present test, such that SIT performance approaches a ceiling level in adulthood. Nevertheless, a further evaluation of the psychometric properties of the test used in this thesis is warranted. This information might then be used to develop the odor items and response options so that the sensitivity and specificity in predicting cognitive impairment and dementia conversion is optimized (Tabert et al., 2005). For example, recent results indicate that age-related odor identification deficits may be selective such that aging effects are larger for pleasant than for unpleasant odors (Konstantinidis, Hummel, & Larsson, 2006). This might be due to the fact that the unpleasant odors are more distinct, and thus easier to identify in participants with decreased olfactory functions. In summary, odor item analyses have received little attention, but may serve as tools in the pursuit of developing odor identification tests designed for specific populations.

Cognitive domains
Several cognitive tests were chosen from the Betula testing battery to be included in the present studies. Given the range of possible tests available in the Betula, this selection deserves to be discussed and justified.

One aspect of the test selection that calls for particular attention is that neither of the studies included tests of episodic memory, despite several episodic memory tests being available in the Betula. Episodic memory is a domain that is sensitive to effects of aging and dementia. However, episodic memory has been the focus of many papers emanating from the Betula study. Data from the Betula project have recently been published regarding episodic memory function in the context of the ApoE gene (Nilsson et al., 2006). In Study III, we chose to focus on MMSE decline as outcome measure. MMSE is a dementia rating scale and is used to detect cognitive impairment (Folstein, Folstein, & Mchugh, 1975). The test has several shortcomings. It does not have optimal properties desired in a cognitive psychological test. In particular, the normal range approaches ceiling-level performance. Also, the different types of tasks included in the test indicates that the score might not be linearly related to the psychological construct measured (i.e. global cognitive ability). The MMSE is likely not to have ratio-scale or interval-scale properties. This should be recognized as a potential shortcoming in the present Study III. However, there were several reasons for using the MMSE; the test assesses global cognitive ability, which is impaired in pre-diagnostic AD at a similar degree as episodic memory (Bäckman, Jones, Berger, Laukka, &
Small, 2005). Several items in the MMSE assess episodic memory, and these items are most predictive of later conversion to AD (Small, Viitanen, & Bäckman, 1997). Specifically, main advantages of using MMSE in Study III were (1) that MMSE is a clinically meaningful test in the context of dementia, (2) that Betula participants with poor performance or a large drop in MMSE were referred to a psychiatrist for dementia evaluation, which ensured that incident dementia could be controlled for, and (3) that it was not clear from previous research whether olfactory impairment in non-demented elderly predicted decline in global cognitive function (Graves et al., 1999; Swan & Carmelli, 2002). A further advantage of using MMSE rather than, for example, episodic memory tests is (4) that MMSE was completed by most participants, even those who were demented or refused to undertake the full battery of cognitive tests. The MMSE was thus associated with a lower drop-out rate compared to other tests.

Regarding the use of vocabulary as a control task, it should be noted that odor identification is conceptually more similar to semantic memory (i.e. knowledge-based) tests than to episodic memory tests, which motivated a focus on semantic memory, rather than episodic memory as a non-olfactory control task.

It should be acknowledged that the cognitive tests used in Study I might in some cases not have been optimal assessments of a particular cognitive domain. In particular, block design might not have been an optimal measure of executive function as stated in Study I, but instead primarily indexing visuo-spatial ability. Furthermore, relatively unconstrained fluency tests such as “words beginning with the letter a” might not only be associated with semantic memory, but also with executive functions. If a fluency test assesses a broad category that contains many accessible items, the verbal report of these items in a speeded task presumably depends on retrieval efficiency associated with executive function. It should be noted, however, that block design and fluency had no unique influence on odor identification and was for this reason not discussed further in Study I.

Mechanisms underlying the associations between cognitive decline, dementia, and olfaction

The thesis investigated, and established, a number of associations between odor identification impairment, the ApoE-ε4 allele, cognitive impairment and dementia. As discussed previously, no neuropathological assessment was made in the Betula study. This limits the possibility to draw firm conclusions regarding the mechanisms that underlie the observed effects.
Although the main interpretation presented in this thesis is AD-related neuropathology (amyloid plaques and neurofibrillary tangles) in olfactory and cognitive brain centres, other mechanisms might also play a role. The ApoE-gene is related to cardiovascular disease in middle-age. Elderly participants with ApoE-ε4 are at higher risk for cognitive decline related to cardiovascular disease and diabetes mellitus (Haan, Shemanski, Jagust, Manolio, & Kuller, 1999). Atherosclerosis related to ApoE-ε4 might increase the risk of vascular pathology in the medial temporal lobe which instigates AD (Borenstein et al., 2005). The present data (Studies II and III) included a clinical dementia assessment, which did not permit a sensitive differentiation among dementia types such as vascular dementia (VAD) and AD. According to the clinical observations, AD was the most common type of dementia, but VAD was also prevalent. Atherosclerosis is associated with both AD and VAD and vascular pathology contributes to the cognitive impairments in these forms of dementia (Hofman et al., 1997). It is likely that many demented participants in the present studies had a combination of different pathologies.

It has been emphasized that vascular mechanisms are important factors in AD neurodegeneration (Zlokovic, 2005). Theoretically, vascular pathology might contribute to the present associations between olfactory impairment and (1) presence of ε4 and (2) current and prospective dementia (Study II), as well as (3) prospective cognitive impairment in elderly ε4-carriers (Study III). It is, however, unlikely that vascular pathology is a major source of these associations. Although few studies have directly compared olfactory impairments in AD and VAD, olfactory impairments are likely smaller in VAD than in AD, especially when cognitive status is controlled for (Gray et al., 2001; Knupfer & Spiegel, 1986; Mesholam et al., 1998). Moreover, the associations between ε4 and VAD are likely much weaker than the association between ε4 and AD (Bennet et al., 2007; Corder et al., 1993; Ji et al., 1998). The association between ε4 and dementia appears not to be mediated by vascular factors (Prince et al., 2000). In contrast, amyloid deposition mediates the association between ε4 and cognitive impairment in elderly (Bennett et al., 2005). Furthermore, plaques and tangles in the medial temporal lobe exert a significant influence on olfactory identification ability even in elderly participants without a dementia diagnosis (Wilson et al., 2007). In sum, vascular pathology might be of less direct importance than plaques and/or tangles to explain the present findings.
Concluding remarks

In this thesis, olfactory function was investigated from an aging perspective. By using regression techniques, odor identification ability was predicted by means of demographic, cognitive, clinical, health-related, and ApoE-genetic variables. As odor identification is impaired in common dementia disorders, the associations between odor identification, the ApoE gene, and future cognitive decline and dementia were of particular interest. The main results of the thesis are recapitulated below.

(1) There was a gradual and linear age-related decrease in odor identification performance. Women identified more odors than men.

(2) Individual differences in cognitive speed and vocabulary slightly influenced odor identification performance over and above the influence of demographic variables.

(3) The ApoE-ε4 allele influenced the age-related deficit in olfactory function in a non-demented population. Presence of the ε4 allele was associated with an odor identification deficit specifically in the 75-80 year age group, but not in the younger cohorts. ApoE-ε2 had no effect on odor identification.

(4) The negative influence of the ApoE-ε4 allele on olfactory proficiency in the elderly was not explained by clinical dementia within five years after olfactory testing.

(5) Olfactory impairment in combination with the ApoE-ε4 allele predicted five-year global cognitive decline in non-demented elderly participants, especially the very old (80-90 years).

In conclusion, the present findings imply that in old age, differences in olfactory identification ability might be a marker of more general differences in brain function. In particular, tests of odor identification ability in the elderly might be used as diagnostic tools to help identify individuals with a high risk of experiencing cognitive decline and potentially receiving a dementia diagnosis within the coming years. However, for odor identification impairment to be a reliable predictor of subsequent cognitive decline, other risk factors (i.e. the ApoE-ε4 allele and old age) also need to be present. This observation highlights the need for
further investigations on variables that might moderate or mediate the associations between olfactory impairment, cognitive decline, and dementia. In this thesis, I have stressed individual differences in cognition and education as potential moderators of the olfaction-dementia link remaining to be explored. Also, there is a need for theoretical work focusing on the pattern of sensory vs. cognitive olfactory impairment in aging and early-stage AD. Furthermore, an integration of longitudinal data regarding olfactory and cognitive impairment in combination with neuropathological data from key brain regions would be optimal to elucidate the mechanisms hypothesized in this thesis.

From being a “neglected” sensory system, assessment of the olfactory sense might in the future provide physicians with valuable cues to an individual’s prospective cognitive development. On a larger scale, such information might help level the rapid increase in the number of elderly individuals debilitated by dementia disorders, and the enormous costs associated with this development (Wimo et al., 2007). Hence, and contrary to the view of Sigmund Freud, who stated that olfaction needs to be suppressed in modern society, I believe that a comprehensive understanding of olfactory function and dysfunction might greatly benefit society in the decades to come.
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