

August 8th, 2024

Development of a Simplified Smell Test to Identify Patients with Typical Parkinson's as Informed by Multiple Cohorts, Machine Learning and External Validation

Juan Li, PhD^{1,2,4,14*}, Kelsey Grimes, MSc^{1,2}, Joseph Saade, MD¹, Julianna J. Tomlinson, PhD^{1,4,5,14,15}, Tiago A. Mestre, MD, PhD^{1,2,4,6,8}, Sebastian Schade, MD⁹, Sandrina Weber, MD¹⁰, Mohammed Dakna, PhD¹⁰, Tamara Wicke, MSc⁹, Elisabeth Lang, BSc⁹, Claudia Trenkwalder, MD⁹, Natalina Salmaso, PhD^{11,14,15}, Andrew Frank, MD^{4,12}, Tim Ramsay, PhD^{2,3,7}, Douglas Manuel, MD, MSc, FRCPC^{2,6,7}, aSCENT-PD Investigators^{14,15}, Brit Mollenhauer, MD^{9,10,13,14,15*}, Michael G. Schlossmacher, MD, FRCPC^{1,4,5,6,8,14,15*}

¹ Neuroscience Program, ² Methodological and Implementation Research Program, and ³ the Methods Centre, Ottawa Hospital Research Institute, Ottawa, ON., Canada

⁴ University of Ottawa Brain and Mind Research Institute, Ottawa, ON., Canada

⁵ Department of Cellular and Molecular Medicine, ⁶ Department of Medicine, and ⁷ School of Epidemiology and Public Health, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada

⁸ Division of Neurology, Department of Medicine, The Ottawa Hospital, Ottawa, ON., Canada

⁹ Paracelsus-Elena-Klinik, Kassel, Germany

¹⁰ Department of Neurology, University Medical Center Goettingen, Germany

¹¹ Department of Neuroscience, Carleton University, Ottawa, ON, Canada

¹² Memory Program, Bruyère Research Institute, Ottawa, ON, Canada

¹³ Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Goettingen, Germany

Li et al., Simplified Olfaction Testing to Identify Patients with Parkinson's

¹⁴ Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815

¹⁵ aSCENT-PD Investigators include: Ben Arenkiel, Zhandong Liu, Brit Mollenhauer, Josef Penninger, Max Rousseaux, Natalina Salmaso, Michael Schlossmacher, Christine Stadelmann, Julianna Tomlinson, John M. Woulfe.

* Corresponding authors

Correspondence to:

Dr. Juan Li

Ottawa Hospital Research Institute

juli@ohri.ca

Dr. Brit Mollenhauer

Paracelsus-Elena Klinik

brit.mollenhauer@med.uni-goettingen.de

Dr. Michael Schlossmacher

Ottawa Hospital Research Institute

mschlossmacher@toh.ca

ABSTRACT

Background: Reduced olfaction is a common feature of patients with typical Parkinson disease (PD). We sought to develop and validate a simplified smell test as a screening tool to help identify PD patients and explore its differentiation from other forms of parkinsonism.

Methods: We used the Sniffin' Sticks Identification Test (SST-ID) and the University of Pennsylvania Smell Identification Test (UPSIT), together with data from three case-control studies, to compare olfaction in 301 patients with PD or dementia with Lewy bodies (DLB) to 36 subjects with multiple system atrophy (MSA), 32 individuals with progressive supranuclear palsy (PSP) and 281 neurologically healthy controls. Individual SST-ID and UPSIT scents were ranked by area under the receiver operating characteristic curve (AUC) values for group classification, with 10-fold cross-validation. Additional rankings were generated by leveraging results from eight published studies, collectively including 5,853 unique participants. Lead combinations were further validated using (semi-)independent datasets. An abbreviated list of scents was generated based on those shared by SST-ID and UPSIT.

Findings: We made the following five observations: (i) PD and DLB patients generally had worse olfaction than healthy controls, as published, with scores for MSA and PSP patients ranking as intermediate. (ii) SST-ID and UPSIT scents showed distinct discriminative performances, with the top odorants (licorice, banana, clove, rose, mint, pineapple and cinnamon) confirmed by external evidence. (iii) A subset of only seven scents demonstrated a similar performance to that of the complete 16-scent SST-ID and 40-scent UPSIT kits, in both discovery and validation steps. Seven scents distinguished PD/DLB subjects from healthy controls with an AUC of 0.87 (95%CI 0.85-0.9) and PD/DLB from PSP/MSA patients with an AUC of 0.73 (95%CI 0.65-0.8) within the three cohorts (n=650). (iv) Increased age was associated with a decline in olfaction. (v) Males generally scored lower than females, although this finding was not significant across all cohorts.

Interpretation: Screening of subjects for typical Parkinson's-associated hyposmia can be carried out with a simplified scent identification test that relies on as few as seven specific

odorants. There, the discrimination of PD/DLB subjects vs. age-matched controls is more accurate than that of PD/DLB vs. PSP/MSA patients.

Funding: This work was supported by: Parkinson Research Consortium; uOttawa Brain & Mind Research Institute; and the Aligning Science Across Parkinson's Collaborative Research Network.

Key words: Parkinson disease; parkinsonism; olfaction; hyposmia; smell test; Sniffin' Stick Test; University of Pennsylvania Smell Identification Test; dementia

Research in context

Evidence before this study

Chronic hyposmia is a common feature of Parkinson disease (PD) and dementia with Lewy bodies (DLB), which often precedes motor impairment and cognitive dysfunction by several years; it is also frequently associated with α -synuclein aggregate formation in the bulb. The presence of hyposmia increases an individual's likelihood of having -what has recently been proposed as- a neuronal synucleinopathy disease, by >24-fold. Despite the strong association of PD with reduced olfaction, little is understood about it clinically, such as whether it is affected by sex and age, and whether hyposmia of PD is associated with the same scent identification difficulty seen in other conditions that present with parkinsonism. Moreover, due to its time-consuming nature and traditional administration by healthcare workers, extensive olfactory testing is not routinely performed during neurological assessments in movement disorder clinics.

Added value of this study

We analyzed the performance of both the Sniffin' Sticks Test kit and UPSIT battery to discriminate between healthy controls, patients with PD/DLB and those with MSA or PSP. Comparison to and juxtaposition with eight other published studies allowed for the generation of a markedly abbreviated smell identification test that unified both tests, as described. Group

classification performance by each scent and its distractors was further analyzed using machine learning and advanced Item Response Theory methods. Relations between each scent tested, sex and age were analyzed for the first time. Our findings suggest concrete steps to be implemented that would allow for simplified, routine olfaction testing in the future.

Implications of all the available evidence

Olfaction testing has emerged as an important neurological assessment part when examining subjects with Parkinson's and those at risk of it. A simple, validated smell test containing fewer scents than current options could facilitate rapid testing of olfaction in clinic settings and at home, without supervision by healthcare workers. The usefulness of such a non-invasive test in population health screening efforts could be further enhanced when coupled to a self-administered survey that includes questions related to other risk factors associated with PD. As such, large-scale community screening and applications to routine practice in family doctors' offices as well as in specialty clinics could be made operationally feasible and cost-effective.

INTRODUCTION

Hyposmia is a common non-motor sign of Parkinson disease (PD) and dementia. The reported prevalence of olfaction loss in PD ranges from 45% to >90% based on populations selected, testing methods, and threshold criteria.^[1] Chronic hyposmia is also described as predictive, with reduced olfaction preceding PD diagnosis by 4-20 years.^{[1]-[3]} Olfactory testing may also help in the differentiation of parkinsonian syndromes.^[4] Several screening tools and predictive models for the incidence of PD have included subjective or objective assessments of olfaction.^{[5]-[9]}

Two commonly used smell tests for evaluating olfactory function include the University of Pennsylvania Smell Identification Test (UPSIT),^[10] a battery of 40 scratch-and-sniff questions that are self-administered, and the Sniffin' Sticks Test (SST) battery,^{[11],[12]} with three subtests (for Identification; Discrimination; Threshold) comprising 16 scents each, of which the administration usually involves a trained supervisor. The SST-Identification (SST-ID) and UPSIT are comparable in that they both assess one's ability to identify a range of scents. Within SST, SST-ID is more commonly used in PD cohort studies and known to have better diagnostic performance than the SST-Discrimination subtest.^[13]

Smell test kits were initially developed to assess olfaction in the general population but have been increasingly used in research settings that study disorders of the brain. Using different cohorts and methods, some studies have ranked odorants in the UPSIT^{[14]-[17]} and SST-ID kits^{[13],[18]-[20]} by their diagnostic performances, and reported that certain subsets of scents appeared to have equal or better performance than the entire complement of 40- or 16-scents-based tests. Other studies^{[21]-[24]} have examined subsets of UPSIT but without any details on scent ranking. Moreover, external validation was frequently missing in these analyses, proposed scent combinations were found to be cohort-specific without agreement across different studies,^{[16],[25]} and the role of distractors (versus the correct scent offered) in such multi-choice settings were understudied. Furthermore, analyses of UPSIT and SST-ID kits were always conducted separately despite the similarities between the two tests. Finally, olfaction scores in patients with other, atypical forms of parkinsonism were not assessed in PD-centric studies.

In the current work, we aimed to assess olfaction performances in commonly encountered forms of parkinsonism, to assess the key features of both UPSIT and SST-ID odorants, to explain any observed differences between them, and to develop a simplified smell test to unify both kits using proper internal and external validation steps. To this end, eight published scent rankings,^{[13]-[20]} which collectively examined 5,853 participants, were incorporated into our study to make the proposed abbreviated test generalizable and to avoid overfitting. We also added Item Response Theory-based analyses when examining the behaviour of participants' responses to multiple choices provided for each scent; this, to enhance sensitivity and specificity of future test versions. Lastly, we analyzed the effects of age and sex on performance in scent identification.

METHODS

The study was conducted in adherence with STARD^[26] guideline, see **Supplemental Table 1**.

Source of data and participants

We used de-identified data from three observational, retrospective, case-control studies: the “De Novo Parkinson disease study” (DeNoPa);^[27] Ottawa (PREDIGT) Trial; and Prognostic Biomarkers in Parkinson's Disease Study (PROBE).^[28] Detailed description and inclusion/exclusion criteria of these three cohorts are provided in **Supplemental Methods**; their demographic and diagnostic characteristics are summarized in **Table 1**. Data of the cross-sectional Ottawa Trial study, baseline data of the longitudinal PROBE study, and three visits of the longitudinal DeNoPa study (baseline; 48-month; and 72-month follow-up visits) were used. Patients with PD or dementia with Lewy bodies (DLB) (DeNoPa: n=129; Ottawa Trial: n=70; PROBE: n=102), subjects with multiple system atrophy (MSA) or progressive supranuclear palsy (PSP) (DeNoPa: n= 9; Ottawa Trial: n=6; PROBE: n=53), and neurologically healthy controls (HC) (DeNoPa: n=109; Ottawa Trial: n=118; PROBE: n=54) were included. Diagnostic criteria were applied according to international guidelines;^{[27]-[30]} diagnoses were revised, as necessary, during longitudinal follow up visits or by independent raters who were blind to

olfaction test results. Most study participants with PD in the three trials were classified as Hoehn and Yahr stage II-III. No participant overlap existed between the three studies.

Analyses of these deidentified cohort data were approved by Investigational Review Boards at Paracelsus-Elena-Klinik (Kassel, Germany) in Frankfurt, Hesse (FF 89/2008), at The Ottawa Hospital (Ottawa, Canada; 20180010-01H) as well as all PROBE Study-affiliated sites, with the participants' consent.

Study assessments and statistical analyses

Sniffin' Sticks test (SST): Used in DeNoPa, the SST comprises a supervised test for one's sense of smell using pen-like odor dispensing devices, as administered in a clinic setting.^{[11],[12]} The test has three subtests: Identification (SST-ID), Discrimination (SST-DS), and Threshold (SST-TH), each with 16 odorants. In SST-ID, subjects are presented a stick and choose the scent from four options (one correct, three distractors). SST-DS is performed using triplets of odorants that are of similar intensity and hedonic tone, where subjects are required to identify which stick of the triplet has a different scent from the other two. SST-TH is performed using triplets of sticks where only one is filled with odorant at a certain dilution whereas the other two are filled with odor-free solvent. SST-TH determines at what dilution subjects can consistently identify the odorant-filled stick. The entire SST (in German) was completed by all DeNoPa participants at their baseline visit, and the SST-ID subtest was re-administered at 48-month and 72-month follow-up visits.

The University of Pennsylvania Smell Identification Test (UPSIT): UPSIT was used in the Ottawa Trial and PROBE; this self-administered kit contains 40 scratch-and-sniff questions; each question has one correct answer and three incorrect distractors.^[10]

Data preparation: Observations that had no valid response to SST-ID or UPSIT were removed from the analysis. Observations with incomplete responses were imputed with 0s, indicating incorrect responses. A dichotomous response-based transformation (0 = incorrect response; 1 = correct response) was used to calculate the sum scores and assess discriminative performances

for each scent. The exact indices of chosen options were used for Item Response Theory analysis (see below).

Demographic and diagnostic characteristics: Demographic and diagnostic characteristics of the study cohort were summarised using n (%) or median (the interquartile range (IQR)). The reported p-values represented the significance from corresponding Fisher's exact test or Kruskal-Wallis rank sum test, with false discovery rate correction for multiple testing; p-values smaller than 0.05 were considered significant.

Comparing different smell tests: Score distributions of corresponding smell tests in each subject group were illustrated using Cummings estimation plots.^[31] The raw UPSIT and SST-ID scores were also normalized into percentiles based on age and sex, and hyposmia was defined by SST-ID percentile $\leq 10\%$ ^[32] or UPSIT percentile $\leq 15\%$.^[10] Discrimination performances of these subtests were compared using area under the receiver operating characteristic (ROC) curve (AUC) values with bootstrap estimated 95% confidence interval (CI),^[33] in order to distinguish diagnostic groups. **Table 2** also reports optimal thresholds and their associated sensitivity and specificity that correspond to the maximum Youden indices.^[34]

Machine learning workflow of developing and validating the abbreviated smell test: **Figure 1** illustrates the machine learning workflow of ranking UPSIT/SST-ID scents, developing and validating respective simplified tests and the unified abbreviated test. Data of the Ottawa Trial and baseline data of DeNoPa were used as discovery cohorts, and baseline data of PROBE and follow-up data of DeNoPa were used for (semi-)independent validation.

1. *Ranking the individual scents:* Using the corresponding discovery cohorts with 10-fold cross validation (see **Supplemental Methods**), individual scents in SST-ID and UPSIT were ranked separately based on their AUC values in differentiating PD/DLB patients from healthy controls. To control over-fitting, the SST-ID and UPSIT scent rankings from our study was compared with eight external rankings,^{[13]-[20]} four for each test, and two final lists were generated by averaging internal and external rankings. Eleven scents are shared by both smell tests, and an additional "Shared" ranking was constructed using their respective positions in the averaged SST-ID and UPSIT rankings.

2. *Developing and validating the best-performing, simplified tests:* For each scent ranking, beginning with the highest-ranked odorant, subsets were constructed by adding one scent at a time in descending ranking order. A total of 95 and 220 distinct SST-ID and UPSIT subsets with various numbers of scents were compared, using their AUC values in distinguishing PD/DLB from HC, to develop the best-performing simplified tests, including one that unified both smell tests. The resulted abbreviated smell tests were also validated using (semi-)independent datasets.

Exploring observed differences in scent performance: The percentage of correct scent identification within each subject group was calculated. These percentages were further compared to examine the relationship of scent identification versus sex and age (using spline smoothing, see **Supplemental Methods**). Furthermore, Item Characteristic Curves (ICCs) for each scent within PD/DLB and HC groups from baseline DeNoPa and Ottawa Trial visits were used to analyze scent performance and the influence of distractors (see **Supplemental Methods** for more details).

Statistical analyses were performed using 'R' (version 4.3.1). Cummings estimation plots were generated using 'dabestr',^[36] and ICC curves were generated using 'TestGardener';^[37] all other plots were generated using 'ggplot2'.^[38] The package 'pROC',^[33] was used for ROC and AUC, and 'fda' was used for spline smoothing.^[39]

RESULTS

Comparison of different smell tests for classifying typical Parkinson disease

We first assessed the performance of UPSIT and SST-ID kits in each diagnostic group. As expected, across all three cohorts, PD and DLB patients generally had lower scores (*i.e.*, worse olfaction) than healthy controls (HC), whereas scores of MSA and PSP patients were intermediate; these are shown by score distributions in **Figure 2(a)-(d)**, and median scores/percentiles and percentage of hyposmia in **Table 1**. There was no significant difference in olfaction performance between MSA and PSP patients (**Figure 2(b), (d)**). UPSIT and SST-ID

kits had comparable performance in distinguishing PD/DLB patients from HC subjects (**Figure 2(e), left**) with AUC values in the three cohorts ranging between 0.89-0.93. Further, both tests showed reduced performance when distinguishing PD/DLB patients from MSA/PSP patients (**Figure 2(e), right**), but with a larger variation (0.69 to 0.92) across the three cohorts, likely due to the small sample sizes of MSA/PSP groups in the Ottawa Trial and DeNoPa cohorts. When compared to the other two SST subtests, SST-ID was found to be the most discriminative one in distinguishing PD/DLB from both HC and from MSA/PSP (**Supplemental Figure 1**), as expected.^[13] For cohort-specific thresholds and their corresponding sensitivity and specificity, see **Table 2**.

Performances of individual scents differ in discriminating PD/DLB from HC

We next sought to determine whether subsets of scents were more informative to discriminate between PD/DLB and controls compared to the complete 16-scent (SST-ID) or 40-scent (UPSIT) tests. **Figure 3(a)** shows the distribution of AUC values for each SST-ID scent across 10 folds using baseline data from the DeNoPa cohort. Clusters of scents identified included banana and mint as the two most discriminative scents (individual AUC values, ≥ 0.725), followed by anise, coffee, licorice, fish and rose in the second-most discriminative cluster. Compared with SST-ID scents, clustering was less obvious for the UPSIT scents (**Figure 3(c)**), whose AUC values ranged between 0.5 to 0.77. For the Ottawa Trial cohort, the top-7 UPSIT scents for identifying PD/DLB patients included rose, wintergreen, root beer, licorice, dill pickle, mint, and grass.

The observed differences in each scent's discriminative performance were further examined by visualizing the percentages of correct scent identification within each diagnostic group (**Figure 3(b), (d)**) and by the percentage differences between HC and PD/DLB groups (**Supplemental Figure 2**). Regardless of the study cohort and smell test used, PD/DLB patients showed significantly lower percentages of correctly identifying each scent than control subjects. Scents that were easy to identify in the HC group but difficult for the PD/DLB group (*i.e.*, larger percentage differences as in **Supplemental Figure 2**) had larger single-scent AUC values.

Scents had poorer discriminative performances when both HC and PD/DLB groups found them easy (e.g. SST-ID: orange, UPSIT: leather) or difficult (e.g. SST-ID: apple, UPSIT: lemon).

Therefore, two rankings for scents from the SST-ID and UPSIT kits were constructed. To overcome the inherent risk of overfitting due to the modestly sized cohorts used, external evidence was introduced. **Figure 4** compared the scent rankings from this study with previously published studies, and two “Average” rankings were derived. For the SST-ID kit, different studies -despite their differences in cohort design and methods applied (**Supplemental Table 1**)- showed consensus in that anise, licorice, mint, banana, coffee, fish and rose were the most discriminative scents in distinguishing PD/DLB subjects from HCs (**Figure 4(a)**). For the UPSIT battery, however, its related studies showed less agreement on their scent rankings (**Figure 4(b)**), which could be partially explained by results shown in **Figure 3(c)**; there, many UPSIT scents showing similar performances and revealed fewer clusters than did SST-ID-based scents. Nonetheless, the top-7 UPSIT scents in the final “Average” ranking were coconut, clove, wintergreen, banana, licorice, grass and cherry. With respect to a possibly common, simplified list, we noted that there are eleven scents shared by SST-ID and UPSIT, and thus, an additional “Shared” ranking was generated to construct a unified, abbreviated smell test (**Table 3**).

Item Characteristic Curves further reveal details for each scent and the influence of distractors

The findings above revealed subsets of scents that were relatively discriminative (from a PD perspective), which could suggest a disease-specific and/or scent processing-related change that is linked to *participant performance*. However, for multiple-choice tests like SST-ID and UPSIT kits, the selection of distractors paired with each scent could also influence *odorant performance*. Item Characteristic Curves (ICCs) can help address this, especially when the scent is shared by different tests.

In the current context, mint and licorice were two well-performing scents, and their ICCs (**Figure 5(1)-(4)**) showed similar characteristics in that HCs generally correctly identified them,

while PD/DLB patients had more difficulty in choosing the right option. However, there were also some noteworthy differences: when scoring on the scent for 'mint', PD/DLB patients could rule out 'chive' and 'onion' in SST-ID and 'fruit punch' in UPSIT, indicating that they detected some scent, but it was not declarative enough to choose 'mint'. However, for 'licorice', particularly in the UPSIT kit, there was strong evidence of random guessing whereby patients couldn't detect any scent to help favor or eliminate an option. Here, ICCs of scoring by HCs also eliminated the possibility of the corresponding pen (SST-ID) or encapsulated sticker (UPSIT) being defective.

The scent for 'banana' ranked 1/16 in DeNoPa but only 34/40 in the Ottawa Trial (**Figure 3**); however, these inconsistent performances were not due to differences in distractors. The option 'cherry' distracted many PD/DLB patients and HCs in the Ottawa Trial, but not in the DeNoPa study (**Figure 5(5)-(6)**). Here, cohort- or odorant (*e.g.*, its concentration or composition for the artificial scent)-related differences might be more plausible explanations.

'Orange' and 'lemon' were both ranked low in the two tests but for different reasons (**Figure 5(7)-(10)**): 'orange' in SST-ID was too easy, even for hyposmic PD/DLB patients. 'Orange' in UPSIT, however, had different distractors that were active within PD/DLB ('bubble gum') and HC ('turpentine'). For 'lemon', the distractors of 'grapefruit' in SST-ID and 'motor oil' in UPSIT confused both patients and healthy persons. Such ICC results within the normosmic control group might be evidence of a flawed odorant choice or an explanation that is rooted in chemical manufacturing of the scent. In **Supplemental Figures 3-5** we listed the ICCs for all other scents.

Development and validation of abbreviated smell tests

Based on scent rankings in **Figure 4** and **Table 3**, 95 SST-ID subsets and 220 UPSIT subsets were compared by their AUC values in distinguishing PD/DLB patients from HCs. **Figure 6** shows the results within each internal and external validation set. When using an increasing number of highly rank-ordered scents, we observed that the corresponding AUC values for

odorant subsets increased steeply for the first four; surprisingly, any improvement in performance thereafter was marginal. Compared with other published rankings, the “Average” rankings as well as their subsets appeared to be more discriminative with robust performance in all the validation sets. Considering a potential trade-off between subset performance and number of scents administered, the SST-ID subset with seven scents and UPSIT subset with ten scents, based on their corresponding “Average” rankings, emerged as the best-performing test batteries.

With respect to potentially developing a unified smell test that could be applied to large study populations (those examined here were based in North America and Europe of predominantly White ethnicity), the subset of seven scents from the “Shared” ranking (**Table 3**) with the highest performance in all validation sets comprised licorice, banana, clove, rose, mint, pineapple and cinnamon. Mean (standard deviation) scores and AUC values of this subset within each cohort are reported in **Table 4**. Note, due to inherent cohort differences, the AUC value in the diagnostic classification of PD/DLB versus HC when combining all three cohorts was 0.87 (95%CI 0.85-0.9), *i.e.*, slightly lower than those of each separate cohort. In the combined cohort, the cut-off value for distinguishing PD/DLB versus HC that corresponded to the maximum Youden index was 4.5 (score range 0-7), the resulted sensitivity and specificity were 0.76 and 0.85, respectively.

Performances of scents in discriminating PD/DLB from MSA/PSP

The same workflow was applied to the PROBE cohort to generate a subset of scents specialized in distinguishing between PD/DLB versus MSA/PSP patients. There, a subset with ten scents (clove, dill pickle, cinnamon, soap, rose, pizza, root beer, turpentine, gasoline, licorice) achieved an AUC value 0.78 in the validation set within PROBE, a promising improvement when compared with the entire 40-scent UPSIT test (AUC = 0.68; **Supplemental Figure 6**). Independent validation is needed to retest the usefulness of this subset given the small number of MSA/PSP subjects enrolled in the other two cohorts studied herein.

Assessment of age and sex on scent identification

We also investigated the influence of age and sex on scent identification. **Supplemental Table 2** shows the coefficients of the linear regression for the relationship between smell test score with age, sex, and diagnostic groups within each cohort. Not surprisingly, progression in age significantly lowered olfaction across all groups, and males generally had a worse sense of smell than their female counterparts, although the latter was not significant across the three cohorts.

When focusing just on the eleven scents shared by both tests, relationships between scent identification and sex were further evaluated by comparing the percentages of correct scent identification across groups (**Figure 7(a)**). In line with the regression results, females showed higher percentages of correct identification than males for most of the scents, except for cinnamon, turpentine, and leather. Next, we compared the probability of identifying each scent correctly across ages between the PD/DLB and HC groups (**Figure 7(b)**). As expected, older participants showed decreasing percentages for identifying specific scents correctly. The fitted lines for PD/DLB and HC groups were usually in the same direction and of similar slopes, with some exceptions, but these were not consistent across all three cohorts.

DISCUSSION

To our knowledge, this is the most comprehensive study to date describing olfactory dysfunction in late-onset, typical PD and two other neurological disorders presenting with parkinsonism using both the multimodal SST battery and UPSIT kit. The principal take home message from this study is that when probing for hyposmia in PD, the following points matter: PD/DLB patients had worse olfaction than healthy subjects, and scores of MSA/PSP patients were intermediate; there was no observed difference in olfaction between MSA and PSP patients; scent identification testing is sufficient, and threshold as well as discrimination testing could be omitted when screening populations for PD using the SST kit; fewer scents can reduce examination time and test taking fatigue without sacrificing diagnostic accuracy; the selection of specific scents should be informed by their discriminative performance in specific group

classification efforts; random guessing could lower diagnostic accuracy; and from a test design perspective, choices provided as distractors influence scent performance. Importantly, we found that a simplified smell test, with specific scents, is sufficient to identify PD/DLB-linked hyposmia. Such a test, which is now being piloted by us, holds the potential to facilitate olfactory testing in the clinical setting, used for at-home testing and population-based screening methods.

In developing and validating a simplified smell test for this purpose, we used a machine learning approach and found that only seven scents (licorice, banana, clove, rose, mint, pineapple, and cinnamon) were required to approximate the diagnostic performance of administering the 16-scent SST-ID or 40-scent UPSIT batteries and the value of adding more scents was negligible. Moreover, a test kit for rapid screening with nearly similar performance could be constructed using as few as four scents.

We demonstrated the impact distractors have on detecting specific scents using IRT analysis. We uncovered uncertainty in eliciting a choice for some scents, even for HCs with intact olfaction. This could be explained by the difficulty of biological scent discrimination or the to-be-improved selection of artificial odorants. By extension, our analyses revealed the opportunity to remove ill-performing scents, *e.g.*, orange and lemon, from currently used kits.

Unexpectedly, we found a high level of guessing among PD patients for licorice, indicating patients' difficulty in detecting this scent. SST-ID and UPSIT batteries are multiple choice-based tests, in which participants are instructed to always choose even when they cannot smell anything; such random guessing will introduce errors into data sets. Advanced IRT methods can treat missing responses as an additional option; administrators of tests would then prefer the participants to leave any uncertain questions unanswered rather than forcing a guess. However, for the future administration of olfaction tests, or for designing a new one, we would suggest adding an extra choice, such as "I cannot identify the scent" to reduce random guessing. Based on our experience in administering smell tests, the extra option would also help improve participant experience and eliminate frustration, especially for patients with severe hyposmia.

An easy-to-administer, inexpensive, sensitive and non-invasive smell test (with 4-7 scents) could have important clinical usefulness, particularly when coupled with a short, self-administered

questionnaire capturing demographic information and known risk factors of developing PD.^[9] Such a questionnaire could also explore other factors leading to hyposmia unrelated to neurodegeneration, *e.g.*, previous nasal injuries, microbial infections, seasonal allergies, and chronic exposure to air pollution, to augment specificity for PD. Upon validation, such a kit could be used as the initial step of large-scale community screening, or in routine clinic practice of a movement disorders-oriented clinic, or for early detection within a family medicine office. When it comes to screening efforts for PD, more invasive and expensive tests, *e.g.*, the α -synuclein seeding amplification assay from cerebrospinal fluid (CSF), or the administration of a dopamine transporter scan, could be administrated as an additional step to increase screening accuracy further, such as when considering enrolling PD subjects into specific, disease-modifying clinical trials.^{[40]-[44]}

Despite the findings regarding scent ranking and subset analyses, it remains unclear whether a specific PD olfaction deficit exists, rather than a global reduction in olfaction, and what the underlying mechanisms would be. We and others recently found that olfactory deficits were significantly associated with positivity on the α -synuclein seeding amplification assay in CSF samples, suggesting that patients may have an underlying disease linked to the dysregulation of SNCA expression and/or protein processing.^{[45],[46]}

Mechanistically, it remains unknown as to how chronic hyposmia arises in PD/DLB (and REM Sleep Behaviour Disorder) as well as some MSA/PSP subjects, at what age it begins, the cause and its underlying circuit-based and molecular mechanisms, and whether olfactory deficits are shared for specific scents among persons with typical PD versus those with dementia. Large scale population screening, including with a simplified testing battery derived from SST-ID and UPSIT kits, could begin to answer some of these questions.

STRENGTH

In this work, olfaction performance using SST and UPSIT batteries was studied in detail. Both internal and external validation efforts were used to test/retest performance and avoid overfitting.

Scent ranking derived from this study was also compared with eight previously published studies. By incorporating results from external studies, the unified abbreviated olfaction test kit of using just seven scents emerged as very generalizable. The observed performance differences for each scent in group classification was explained using complementary techniques and further studied considering sex and age. Our study also explored scent identification performance at the level of choices provided, highlighting the importance of distractors, which allows for future improvement in the design of test kits.

LIMITATIONS AND FUTURE WORK

Performance of the unified abbreviated smell test was estimated by simulation: responses of the related scents were partitioned from the SST-ID and UPSIT data sets, and the original distractors from SST-ID and UPSIT kits were used. Real performance of the abbreviated test, when manufactured as a stand-alone product with distractors determined for each scent, should be assessed in the same cohorts, in new studies, and in routinely run clinic settings. Further, more data are needed to validate the scent ranking and the associated subset developed here for distinguishing PD/DLB versus MSA/PSP patients.

The three cohorts used are highly homogeneous cohorts with most participants being white. Although scent rankings and the selection of a simplified smell test have been rigorously developed and validated with external information incorporated, future calibration and cultural adaption efforts will be necessary when applying them to other populations, especially those that differ from Western Europeans.

Case-control studies have an inherent potential for selection bias in their recruitment. Especially because of the age- and sex-matched design, age- and sex-effects were likely underestimated. Population studies, such as in the initial community screening effort undertaken by PARS planners and the 'PPMI hyposmia' effort, could provide complementary data sets; however, they too have potential setbacks: as the majority of participants will have a normal sense of smell, the score distribution could be highly skewed; a low percentage of PD patients will make the

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461 resulting dataset imbalanced; when smell test data are reduced to a single sum score, sub-
 462 analyses will be difficult to complete, which will limit interrogations of data between established
 463 cohorts.

464 For screening purpose, a one-time administered smell test may not be informative enough to
 465 assess a subject's sense of smell completely, because other factors, such as temporary olfaction
 466 reduction/loss due to infection, seasonal allergies, occupational exposure and/or drinking, eating,
 467 smoking before taking the test, could skew results. Retesting at appropriate time intervals will be
 468 required for even higher accuracy.

ACKNOWLEDGEMENTS

The authors acknowledge the commitment of study participants in the three cohorts and are grateful to all clinical research coordinators at all the study sites. Responsible supervisors for the:

DeNoPa Study: Paracelsus-Elena-Klinik: Brit Mollenhauer.

Ottawa Trial Study: The Ottawa Hospital: Michael Schlossmacher, Élisabeth Bruyère Hospital: Andrew Frank.

PROBE Study: PROBE Steering Committee: Voyager Therapeutics: Bernard Ravina; Brigham and Women's Hospital: Clemens Scherzer, University of Ottawa: Michael Schlossmacher, Avid Radiopharmaceuticals: Andrew Siderowf, University of Rochester: David Oakes, Arthur Watts; Institute for Neurodegenerative Disorders: Kenneth Marek; Georgetown University: Ira Shoulson.

We thank Nathalie Lengacher for help in graphic design; Nadine Mauri, Nancy MacDonald, and Yoobin Lee for help in data management/input. This work was supported by funding from Parkinson Canada (to T.M., D. M., M.G.S; 2018; to J.L.; 2019-2021), Michael J. Fox Foundation for Parkinson's Research (to T.M., D. M., M.G.S), Department of Medicine (T.M., T.R., D.M., M.G.S.), The Ottawa Hospital Foundation (Borealis Foundation to J.L.) and the Uttra & Subhash Bhargava Family (M.G.S.), the Paracelsus-Elena-Klinik Kassel, Parkinson Fonds Deutschland, and the Deutsche Parkinson Vereinigung (B.M.; C.T.). The study was also funded by the joint efforts of The Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP) initiative. MJFF administers the grant [Grant ID: ASAP-020625] on behalf of ASAP and itself.

The funders had no role in the design and execution of the study; the collection, management, analysis, and interpretation of the data; the preparation, review, or approval of the manuscript; and the decision to submit the manuscript for publication. We are grateful for the ongoing support and feedback from people with lived experiences, such as through the board of the Parkinson's Research Consortium Ottawa and members of Partners Investing in Parkinson's Research, and to Drs. P. Wells and D. Lewis for their ongoing encouragement.

496

497 **AUTHOR CONTRIBUTIONS**

498 JL and MGS contributed to the concept and design of the study; JL, KG, and MGS contributed to
 499 the acquisition of data. JL decided on the statistical methods used in this study. JL did data
 500 cleaning, data analysis, figures and tables. JL, JJT, and MGS contributed to data interpretation.
 501 SS, SW, MD, TW, EL, CT, and BM contributed to the data collection and verification of
 502 DeNoPa; KG, JS, JL, JJT, AF, and MGS contributed to the data collection and verification of
 503 Ottawa Trial. JL and MGS wrote the first draft of the manuscript. JJT, BM, NS, TAM, TR, and
 504 DM contributed to the drafting of the manuscript and revising it critically, and all authors
 505 approved the submission of its current version.

506

507 **CONFLICT OF INTEREST STATEMENT**

508 In 2024, MGS co-founded NeuroScent Inc to develop a home-based testing platform for
 509 hyposmia.

510

511 **CODE AVAILABILITY**

512 The code for data analyses and figures is publicly accessible in GitHub (link to be updated).

513

514 **DATA AVAILABILITY**

515 The datasets used in this study can be accessed via zenodo (link to be updated) upon request.

516

FIGURES

Figure 1: Machine learning workflow for developing and validating an abbreviated smell test. Details of the workflow are as indicated and described in Methods and Result sections of the main text. SST-ID = Sniffin' Sticks Identification test. UPSIT = University of Pennsylvania Smell Identification Test. DeNoPa = De Novo Parkinson Study. PROBE = Prognostic Biomarkers in Parkinson Disease. HC = healthy control. PD = Parkinson disease. DLB = dementia with Lewy bodies. MSA = multiple system atrophy. PSP = progressive supranuclear palsy. ROC = receiver operating characteristic. AUC = area under the ROC curve.

Figure 2: Distribution of olfaction scores using two established tests for different diagnostic groups with parkinsonism in three cohorts. Cummings estimation plots (a-d) were used to illustrate and compare smell test score distributions in each diagnostic group: (a) for UPSIT in the Ottawa Trial cohort, (b) for UPSIT in the PROBE cohort, (c) for SST-ID in the DeNoPa cohort, (d) UPSIT and SST-ID scores were transformed to percentiles based on age- and sex-adjusted norms in the combined cohorts. Each data point in the upper panels represents the score of one participant, and colors represent different groups and diagnosis, as shown in legends. The vertical lines in the upper panels represent the conventional mean $\pm$ standard deviation error bars. The lower panels show the mean group difference (the effect size) and its 95% confidence interval (CI) estimated by bias-corrected and accelerated bootstrap, using healthy controls as the reference group. Panels in (e) show ROC curves and AUC values with 95% confidence interval (CI) for smell tests in each cohort (indicated by different colors; individual scores shown in a-d) to distinguish PD/DLB versus HC groups (left) and PD/DLB versus MSA/PSP groups (right). Abbreviations as in **Figure 1**.

Figure 3: Individual scent performances in differentiating PD/DLB from HC groups. SST-ID scents are shown using baseline DeNoPa data (a, b) and UPSIT scents for the Ottawa Trial cohort (c, d). Panels (a) and (c) illustrate distribution of AUC values of each scent across 10-fold cross-validation using violin plots, with 25%, 50%, and 75% quantile lines. The scents are ordered in descending order of their mean single-scent AUC value; the color of each scent changes gradually from the most to the least discriminative value, as indicated by the legend. Scents shared by both tests are highlighted by bold italic font. Panels (b) and (d) show the

percentage of subjects correctly identifying each scent within both groups in each corresponding cohort. Abbreviations as in **Figure 1**.

Figure 4: Comparison of scent rankings in this study versus previously published ones. Panels (a) and (b) show scent rankings of SST-ID and UPSIT, respectively. “This study” columns show scent rankings from **Figure 3**, and the neighboring columns show corresponding rankings from other studies, as indicated at the x-axis. The “Average” column of each panel shows the scent ranking generated by averaging results from 5 separate rankings. Each scent is represented using the format “index-scent” in the “Average” ranking, and as index only in others. The lines track how each scent’s rank changes from study to study. Color of each scent changes gradually from the most to the least discriminative odorant defined by “Average”. Based on these, 7 best-performing scents in SST-ID (a) and 12 best-performing scents in UPSIT (b) are tracked by solid lines. Note, rankings by Mählknecht *et al.* and Morley *et al.* included only the top 12 scents.

Figure 5: Influence of distractors in multiple-choice smell tests for five shared scents selected. Panels with odd numbers show the Item Characteristic Curves (ICCs) of five SST-ID scents: mint, licorice, banana, orange, and lemon. Panels with even numbers show ICCs of the corresponding UPSIT scents. In each figure, panels on the left show data for PD/DLB patients, panels on the right for healthy controls. The x-axis reveals transformed score indices (percentage rank of the respective scores) within the corresponding group. The y-axis shows the probability of choosing each option at a particular score index. The correct option of each item is highlighted using thicker, blue curves. Numbers in the color legends represent option indices. The horizontal dashed lines represent 50% probability. The vertical dashed lines represent five quantiles (5%, 25%, 50%, 75%, and 95%).

Figure 6: Exploration of smaller subsets of scents tested vs. accuracy in group classification of PD/DLB vs HC. The x-axis shows the number of individual scents used for each subset examined; colors represent different scent rankings from separate studies, as indicated by the legends (see also **Figure 4** and **Table 3**). ‘Shared’ denotes scents used in both UPSIT and SST-ID; Average, all studies combined; This study, rankings derived using baseline DeNoPa and Ottawa Trail data. Individual points shown in panels (a) and (d) represent internal validation

results, averaging across 10 folds. In panels (b), (c) and (e), each point represents the AUC value of the corresponding subset using (semi-)external validation sets. The black horizontal, dashed lines indicate AUC values of the corresponding test when viewed in its entirety. Red horizontal, dashed lines indicate AUC = 0.9 as a predetermined reference line.

Figure 7: Relationships between scent identification performance, diagnosis, sex and age.

Panel (a) shows the percentage of persons that correctly identified each scent within the healthy control (HC) group (indicated by light blue region) and the PD/DLB group (indicated by pink region) in the corresponding cohorts, separated by sex (bar color). Panels in (b) show the relationship between age (x-axis), diagnostic group (HC in blue; vs PD/DLB in red) and the percentage of correctly identified scents (y-axis) for each odorant tested (columns) within each cohort (row), as indicated on the right.

586 TABLES

587 Table 1: Baseline demographic characteristics and smell test scores of adults enrolled in the three cohorts

Variable	DeNoPa					Ottawa Trial					PROBE				
	HC, N = 109 ¹	PD/DLB, N = 129 ^{1,4}	MSA/PSP, N = 9 ^{1,4}	p- value ²	q- value ³	HC, N = 118 ¹	PD/DLB, N = 70 ^{1,5}	MSA/PSP, N = 6 ^{1,5}	p- value ²	q- value ³	HC, N = 54 ¹	PD/DLB, N = 102 ^{1,6}	MSA/PSP, N = 53 ^{1,6}	p- value ²	q- value ³
Sex				0.6	0.7				0.005	0.015				0.058	0.087
Female	42 (39%)	45 (35%)	2 (22%)			74 (63%)	29 (41%)	5 (83%)			28 (52%)	34 (33%)	18 (34%)		
Male	67 (61%)	84 (65%)	7 (78%)			44 (37%)	41 (59%)	1 (17%)			26 (48%)	68 (67%)	35 (66%)		
Age	65 (60, 70)	66 (58, 72)	72 (65, 76)	0.073	0.15	68 (58, 73)	68 (60, 74)	66 (63, 70)	0.9	0.9	59 (55, 69)	64 (55, 69)	67 (61, 75)	0.002	0.007
Parkinsonism duration at baseline in months	NA	14 (9, 24)	12 (6, 33)	0.7	0.7	NA	84 (36, 132)	48 (30, 66)	0.078	0.12	NA	58 (58, 71)	58 (57, 60)	0.2	0.2
Follow-up time in months	120 (120, 120)	120 (72, 120)	72 (48, 120)	0.001	0.004	ND	ND	ND			ND	ND	ND		
Smell test score ⁷	12 (11, 14)	7 (4, 9)	10 (9, 11)	<0.001	<0.001	32 (29, 35)	17 (13, 22)	29 (26, 31)	<0.001	<0.001	35 (33, 37)	14 (14, 26)	28 (21, 32)	<0.001	<0.001
Smell test percentile ⁸	50 (25, 75)	4 (4, 10)	25 (18, 50)	<0.001	<0.001	39 (18, 66)	6 (4, 10)	23 (14, 37)	<0.001	<0.001	56 (27, 73)	4 (4, 17)	23 (9, 42)	<0.001	<0.001
Olfaction ⁹				<0.001	<0.001				<0.001	<0.001				<0.001	<0.001
Normal	94 (86%)	30 (23%)	7 (78%)			93 (79%)	7 (10%)	3 (50%)			50 (93%)	19 (28%)	34 (64%)		
Hyposmia/Anosmia	15 (14%)	99 (77%)	2 (22%)			25 (21%)	63 (90%)	3 (50%)			4 (7%)	83 (72%)	19 (36%)		

¹ n (%); Median (IQR); ² Fisher's exact test; Kruskal-Wallis rank sum test; ³ False discovery rate correction for multiple testing
⁴ PD: n = 126; DLB: n = 3; MSA: n = 4; PSP: n = 5. ⁵ PD: n = 69; DLB: n = 1; MSA: n = 5; PSP: n = 1. ⁶ PD: n = 102; MSA: n = 27; PSP: n = 26.
⁷ SST-ID scores (0-16) for DeNoPa, UPSIT scores (0-40) for Ottawa Trial and PROBE. ⁸ Age- and sex-adjusted normalized percentiles.
⁹ Hyposmia/anosmia was determined by SST-ID percentile ≤ 10%, and UPSIT percentile ≤ 15%.

588

589 DeNoPa = *De Novo* Parkinson Study. PROBE = Prognostic Biomarkers in Parkinson Disease. IQR = interquartile range. HC = healthy control. PD = Parkinson disease. DLB =
590 dementia with Lewy bodies. MSA = multiple system atrophy. PSP = progressive supranuclear palsy. NA = not applicable. ND = not documented

Table 2: Discriminative performances of complete smell tests for baseline visits in three cohorts

Cohort	Test	AUC (95% CI)	Threshold	Sensitivity	Specificity
PD/DLB vs HC					
DeNoPa	SST-ID	0.89 (0.85-0.93)	<= 10.5	0.88	0.81
	SST-TH	0.83 (0.78-0.88)	<= 3.12	0.68	0.9
	SST-DS	0.83 (0.77-0.88)	<= 10.5	0.75	0.78
Ottawa Trial	UPSIT	0.92 (0.88-0.96)	<= 28.5	0.96	0.77
PROBE	UPSIT	0.93 (0.89-0.97)	<= 29.5	0.84	0.89
PD/DLB vs MSA/PSP					
DeNoPa	SST-ID	0.8 (0.69-0.91)	<= 6.5	0.48	1
	SST-TH	0.64 (0.48-0.8)	<= 1.38	0.43	0.89
	SST-DS	0.56 (0.32-0.79)	<= 12.5	0.88	0.33
Ottawa Trial	UPSIT	0.92 (0.85-0.99)	<= 25.5	0.81	1
PROBE	UPSIT	0.69 (0.6-0.78)	<= 26.5	0.76	0.58

DeNoPa = *De Novo* Parkinson Study. PROBE = Prognostic Biomarkers in Parkinson Disease. HC = healthy control. PD = Parkinson disease. DLB = dementia with Lewy bodies. MSA = multiple system atrophy. PSP = progressive supranuclear palsy. AUC = area under the ROC curve. CI = confidence interval. SST-ID = Sniffin' Sticks Identification test. SST-TH = Sniffin' Sticks Threshold test. SST-DS = Sniffin' Sticks Discrimination test. UPSIT = University of Pennsylvania Smell Identification Test.

601 **Table 3: Ranking of scents that are shared by SST-ID and UPSIT kits**

Rank	Scent	Rank in SST-ID ¹	Rank in UPSIT ¹	Average ²
1	Licorice	2/16	5/40	0.125
2	Banana	4/16	4/40	0.175
3	Clove	8/16	2/40	0.275
4	Rose	7/16	9/40	0.33125
5	Mint	3/16	21/40	0.35625
6	Pineapple	9/16	8/40	0.38125
7	Cinnamon	10/16	16/40	0.5125
8	Lemon	12/16	23/40	0.6625
9	Turpentine	13/16	27/40	0.74375
10	Orange	15/16	29/40	0.83125
11	Leather	11/16	40/40	0.84375

¹ Based on the corresponding “Average” rankings in **Figure 4**.

² For each scent, average = (rank in SST-ID + rank in UPSIT) / 2

602

603 SST-ID = Sniffin' Sticks Identification test. UPSIT = University of Pennsylvania Smell

604 Identification Test.

605

Table 4: Performance of the unified abbreviated smell test with 7 scents

	PD/DLB	HC	MSA/PSP
DeNoPa			
Mean (sd) ¹	3.17 (1.72)	5.69 (1.15)	4.67 (1.00)
AUC (95% CI) ²		0.88 (0.83-0.92)	0.76 (0.65-0.87)
Ottawa Trial			
Mean (sd) ¹	2.81 (1.56)	5.58 (1.53)	5.00 (1.79)
AUC (95% CI) ²		0.89 (0.84-0.93)	0.82 (0.62-1)
PROBE			
Mean (sd) ¹	3.49 (1.74)	6.37 (0.90)	4.68 (2.06)
AUC (95% CI) ²		0.91 (0.87-0.96)	0.68 (0.58-0.77)
Combined			
Mean (sd) ¹	3.20 (1.71)	5.77 (1.31)	4.71 (1.92)
AUC (95% CI) ²		0.87 (0.85-0.9)	0.73 (0.65-0.8)

¹ For the unified abbreviated smell test with 7 scents, score range is 0-7.

² AUC values were for distinguishing patients with PD/DLB from healthy controls or patients with MSA/PSP.

DeNoPa = *De Novo* Parkinson Study. PROBE = Prognostic Biomarkers in Parkinson Disease.

HC = healthy control. PD = Parkinson disease. DLB = dementia with Lewy bodies. MSA = multiple system atrophy. PSP = progressive supranuclear palsy. AUC = area under the ROC curve. CI = confidence interval. sd = standard deviation.

SUPPLEMENTAL METHODS

Detailed description of the study cohorts

De Novo Parkinson disease study (DeNoPa): The DeNoPa cohort^[27] is an ongoing, single-center study based in Kassel, Germany. The DeNoPa cohort is an observational, longitudinal study of patients with a newly established diagnosis of PD (UK Brain Bank Criteria^[29]), who were naïve to L-DOPA therapy at baseline, and of age- and sex- and education-matched, neurologically healthy controls (HC). Details of inclusion/exclusion criteria have been described elsewhere.^[27] Diagnostic accuracy was ensured by ongoing follow-up visits every two years (as of 2023, 10-year follow up visits were underway). Data used were received on May 16th, 2023.

Ottawa (PREDIGT) Trial: The Ottawa Trial is a pilot study to evaluate the performance of a 2-step screening tool that combines the PREDIGT questionnaire^{[8],[9]} and the UPSIT test to distinguish patients with PD/DLB from age-matched neurologically healthy controls and patients with various other neurological diseases. Enrolment and assessment of this cross-sectional, case-control study was completed in March 2024. A manuscript that describes this cohort is in preparation. Diagnostic accuracy was ensured by independent chart review by three subspecialty-trained neurologists (JS, MGS, and AF) according to UK Brain Bank Criteria and MDS Criteria.^[30]

Prognostic Biomarkers in Parkinson Disease (PROBE): PROBE^[28] is a longitudinal, case-control study to test biomarkers in PD subjects and controls to determine their feasibility and potential utility as markers of risk and prognosis for PD. Details of inclusion/exclusion criteria have been described elsewhere.^[28] Participants were enrolled from August, 2007 to December, 2008. Diagnosis of PD, probable MSA, and probable PSP met UK Brain Bank criteria, Consensus Criteria, and NINDS-PSP Criteria, respectively.

Supplemental methods

10-fold cross validation (CV): For each smell test, the discovery dataset was randomly partitioned into 10 parts, where the case-control ratio was maintained in each part. In each fold, 9/10 parts were used as the development set and the remaining 1 part was used for internal

641 validation. This procedure was repeated for 10 times, and results were showed either in
642 distribution or average across 10 folds.

643 *Relationship between scent identification and age:* Within each cohort, participants' ages were
644 segregated into four bins with similar sample size. For each scent, the proportion of correctly
645 identification was calculated for each bin, and spline smoothing was then used to represent the
646 relationship between the proportions and age.

647 *Item characteristic curves (ICCs):* The version of ICCs used in this study differed from
648 traditional parametric ICCs in two aspects: 1) the x-axis was the score percentage rank in [0,100],
649 not the latent trait on the whole real line; and 2) ICCs represented spline smoothing lines that fit
650 response data, rather than being fitted to any pre-defined parametric model.^[35]

651

SUPPLEMENTAL FIGURES

Supplemental Figure 1: Distribution of Sniffin' Sticks Threshold (SST-TH) and Discrimination (SST-DS) scores for each subject group in the DeNoPa Study at baseline and their ROC curves for group classification. The Cummings estimation plots (a, b) were used to illustrate and compare smell test score distributions in each group: (a) SST-TH, (b) SST-DS. Each data point in the upper panels represents the score of one participant, and colors represent different groups and diagnosis as shown in the legend. The vertical lines in the upper panels represent the conventional mean $\pm$ standard deviation error bars. The lower panels show the mean group difference (the effect size) and its 95% confidence interval (CI) estimated by bias-corrected and accelerated bootstrap, using HC as the reference group. Panel (c) shows the ROC curves of each smell test (indicated by color) to distinguish PD/DLB versus HC (left) and PD versus OND (right). HC = healthy control. PD = Parkinson disease. DLB = dementia with Lewy bodies. MSA = multiple system atrophy. PSP = progressive supranuclear palsy. ROC = receiver operating characteristic. AUC = area under the ROC curve.

Supplemental Figure 2: Percentage differences of correct scent identification between HC and PD/DLB groups (% HC - % PD/DLB) in the DeNoPa (a) and Ottawa Trial (b) cohorts. The scents are ordered in descending orders of their mean single-scent AUC value (see Figure 3 (a) and (c)); the color of each scent changes gradually from the most discriminative to the least discriminative odorant, as indicated by the legend.

Supplemental Figure 3: Influence of distractors in the multiple-choice smell tests: the remaining 6 scents shared by UPSIT and SST-ID. Panels with odd numbers show the Item Characteristic Curves (ICCs) of six SST-ID scents, and panels with even numbers show the ICCs of the corresponding UPSIT scents. In each figure, the left panels are the ICCs using data of the PD/DLB patients, and the right panels are corresponding to healthy controls. The x-axis is transformed score indices (percentage rank of the SST-ID score) within the corresponding group. The y-axis is the probability of choosing each option at a particular score index. The correct option of each item is highlighted using the thicker blue curves. Numbers in the color legends are the option indices. The horizontal dashed lines represent 50% probability. The vertical dashed lines represent five quantiles (5%, 25%, 50%, 75%, and 95%).

Supplemental Figure 4: Item Characteristic Curves (ICCs) of the remaining SST-ID scents using baseline data from the DeNoPa Cohort. In each panel, the left panels are the ICCs using data of the PD/DLB patients, and the right panels are corresponding to healthy controls. The x-axis is transformed score indices (percentage rank of the SST-ID score) within PD and HC group, respectively. The y-axis is the probability of choosing each option at a particular score index. The correct option of each item is highlighted using the thicker blue curves. Numbers in the color legends are the option indices. The horizontal dashed lines represent 50% probability. The vertical dashed lines represent five quantiles (5%, 25%, 50%, 75%, and 95%).

Supplemental Figure 5: Item Characteristic Curves (ICCs) of the remaining UPSIT scents using the Ottawa Trial cohort. In each panel, the left panels are the ICCs using data of the PD/DLB patients, and the right panels are corresponding to healthy controls. The x-axis is transformed score indices (percentage rank of the SST-ID score) within PD and HC group, respectively. The y-axis is the probability of choosing each option at a particular score index. The correct option of each item is highlighted using the thicker blue curves. Numbers in the color legends are the option indices. The horizontal dashed lines represent 50% probability. The vertical dashed lines represent five quantiles (5%, 25%, 50%, 75%, and 95%).

Supplemental Figure 6: Range of performances for UPSIT scents in differentiating PD/DLB from MSA/PSP subjects in the PROBE cohort and AUC values for numerical subsets of odorants in group classification. Panel (a) illustrates distribution of AUC values of each scent across 10-fold cross-validation using violin plots, with 25%, 50%, and 75% quantile lines. The scents are ordered in descending order of their mean single-scent AUC value. The color of each scent changes gradually from the most to the least discriminative odorant, as indicated by the legend. Panel (b) shows the percentage of subjects correctly identifying each scent within the MSA/PSP and PD/DLB groups, and panel (c) shows the percentage differences between the two groups. Scents in panels (b) and (c) follow the same rank order as in panel (a). In panel (d), the x-axis is the number of scents included for each subset, with individual points representing average AUC values for the validation set across ten CV folds gathered in PROBE. The black horizontal, dashed line indicates the corresponding AUC values of the whole test (= 40 scents). The red horizontal, dashed line indicates AUC = 0.8 as a predetermined reference line.

710 SUPPLEMENTAL TABLES

711 Supplemental Table 1: STARD checklist.

Section & Topic	No	Item	Reported on page #
TITLE OR ABSTRACT			
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)	P1
ABSTRACT	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)	P3
INTRODUCTION	3	Scientific and clinical background, including the intended use and clinical role of the index test	Research in context: P4-5 Introduction: P6-7
	4	Study objectives and hypotheses	P7
METHODS			
Study design	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)	P7
Participants	6	Eligibility criteria	P7, Supplemental methods: P2
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)	P7, Supplemental methods: P2
	8	Where and when potentially eligible participants were identified (setting, location and dates)	Supplemental methods: P2
	9	Whether participants formed a consecutive, random or convenience series	P7, Supplemental methods: P2
Test methods	10a	Index test, in sufficient detail to allow replication	Description of SST and UPSIT: P8 The abbreviated smell test: P13
	10b	Reference standard, in sufficient detail to allow replication	Supplemental methods: P2
	11	Rationale for choosing the reference standard (if alternatives exist)	Supplemental methods: P2
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory	P9
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory	Supplemental methods: P2
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test	P7
	13b	Whether clinical information and index test results were available to the assessors of the reference standard	P7
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy	P8-10
	15	How indeterminate index test or reference standard results were handled	Supplemental methods: P2
	16	How missing data on the index test and reference standard were handled	Index test: P8. No missing data on the reference standard.
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory	P9
	18	Intended sample size and how it was determined	P7
RESULTS			
Participants	19	Flow of participants, using a diagram	P7, Supplemental methods: P2
	20	Baseline demographic and clinical characteristics of participants	Table 1
	21a	Distribution of severity of disease in those with the target condition	P7, Table 1
	21b	Distribution of alternative diagnoses in those without the target condition	Table 1
	22	Time interval and any clinical interventions between index test and reference standard	P7, Supplemental methods: P2
Test results	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard	Figure 2; Supplemental Figure 1; Tables 1, 2, 4; P10-11, P13-14
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)	Figures 2, 6; Supplemental Figures 1, 6; Tables 2, 4; P10-11, P13-14
	25	Any adverse events from performing the index test or the reference standard	The smell tests are non-invasive and there was no adverse event from performing them. P16
DISCUSSION			
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability	P17-18
	27	Implications for practice, including the intended use and clinical role of the index test	P5, P15-17

OTHER INFORMATION			
	28	Registration number and name of registry	Supplemental methods: P2
	29	Where the full study protocol can be accessed	Supplemental methods: P2
	30	Sources of funding and other support; role of funders	P4, P19

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712 Supplemental Table 2: Comparison of cohorts and methods of this study and eight published studies.

	UPSIT					SST-ID				
	<i>This study</i>	<i>Bohnen et al.</i>	<i>Hawkes et al.</i>	<i>Joseph et al.</i> ⁸	<i>Morley et al.</i>	<i>This study</i>	<i>Boesveldt et al.</i>	<i>Casjens et al.</i>	<i>Lo et al.</i> ⁹	<i>Mahlknecht et al.</i>
<i>Study cohorts</i>										
<u>Discovery</u>										
Cohort name/location	Ottawa Trial	University of Pittsburgh	Ipswich	PREDICT-PD	Michael J. Crescenzo VA Medical Center in Philadelphia and the University of Pennsylvania	DeNoPa	VUMC; LUMC	ParkCHIP	Oxford Discovery	Innsbruck and Bruneck
PD	70 (37.2) ^{1,6}	27 (50) ¹	96 (50) ¹	40 (4.3) ¹	314 (50) ¹	129 (54.2) ^{1,7}	404 (72.9) ¹	148 (50) ¹	890 (74) ¹	134 (28.5) ¹
Male	41 (59) ¹	20 (74.1) ¹	49 (51) ¹	30 (75) ¹	261 (83) ¹	84 (65) ¹	253 (62.6) ¹	78 (52.7) ¹	569 (64) ¹	84 (62.7) ¹
Age in years	68 (60, 74) ²	60 (11.1) ³	57 (27-81) ⁴	63.8 (9.6) ³	67.4 (10.0) ³	66 (58, 72) ²	61.5 (40-90) ⁴	67 (14) ²	66.5 (9.6) ³	68.0 (8.8) ³
PD duration in years	7 (3, 11) ²	2.5 (2.7) ³	unknown	unknown	unknown	1.2 (0.75, 2) ²	0-44 ⁵	n.a.	1.2 (0.9) ³	6.2 (4.8) ³
HC	118 (62.8) ¹	27 (50) ¹	96 (50) ¹	891 (95.7) ¹	314 (50) ¹	109 (45.8) ¹	150 (27.1) ¹	148 (50) ¹	313 (26) ¹	336 (71.5) ¹
Male	44 (37) ¹	20 (74.1) ¹	39 (40.6) ¹	343 (38.5) ¹	261 (83) ¹	67 (61) ¹	87 (58) ¹	81 (54.7) ¹	165 (53) ¹	156 (46.4) ¹
Age in years	68 (58, 73) ²	60 (7) ³	41.7 (18-78) ⁴	67.3 (4.8) ³	67.4 (10.0) ³	65 (60, 70) ²	59.2 (45-78) ⁴	62 (16) ²	64.4 (9.8) ³	68.8 (8.3) ³
<u>(Semi-)External Validation</u>	PROBE: PD = 102, HC = 54	n.a.	n.a.	PREDICT-PD: HC = 191, who have completed UPSIT in only year 3.	UCL: PD = 167, HC = 130 Barts: PD = 176, HC = 177	DeNoPa at 48 months: PD=114, HC=101 DeNoPa at 72 months: PD=91, HC=93	n.a.	n.a.	Tracking cohort: 452	VUMC; LUMC: PD = 400, HC = 150 Vienna: PD = 112, HC = 120
<i>Methods</i>										
Internal validation	10-fold cross-validation	n.a.	n.a.	n.a.	n.a.	10-fold cross-validation	n.a.	10-fold cross-validation	Data balance with leave-one-out cross-validation (LOOCV)	n.a.

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Ranking scents	AUC values of each question in distinguishing PD/DLB from HC	Difference of percentages responding correctly in each group (%HC - %PD)	Difference of percentages responding correctly in each group (%HC - %PD)	Difference of percentages responding correctly in each group (%HC - %PD)	40 scents were ranked using 5 methods and only the top-12 of each ranking were reported: 1) the absolute difference in percentage of PD and control subjects answering incorrectly (Difference), 2) odds ratio, 3) discriminant function analysis (Discriminant), 4) logistic regression (Regression), 5) a weighted average combining the first four methods (Combined).	AUC values of each question in distinguishing PD from HC	Difference of percentages responding correctly in each group (%HC - %PD)	Random forest with permutation accuracy	Random forest with predictor importance (Gini diversity index)	L1-regularized logistic regression implementing the least absolute shrinkage and selection operator (the LASSO)
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Values are ¹n (%), ²median (Inter-quartile range (IQR)), ³mean (standard deviation (SD)), ⁴mean (range), ⁵range,

⁶Also includes one patient with dementia with Lewy bodies (DLB). ⁷Also includes three patients with DLB.

⁸The paper assessed all combinations of 1–7 smells from UPSIT and identified 28 “winning” smell combinations that had highest combined sensitivity and specificity to define hyposmia within 1001 healthy controls in PREDIGT-PD. The ranking below was not directly used to develop the optimal subset of scents.

⁹The discovery cohort was 267 HC with good (normosmia/super-smeller) sense of smell and 721 PD with poor (functional anosmia/hyposmia) sense of smell as defined by age- and sex-specific percentiles: functional anosmia: SST-ID scores ≤ 8; hyposmia: < 10th percentile; super-smeller: > 90th percentile. The outcome was to classify participants with poor vs normal smell, not to classify PD vs HC, nor conversion to PD in the PD cohort.

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714 **Supplemental Table 3: Relationship between the whole SST-ID score (DeNoPa) and the**
715 **whole UPSIT score (Ottawa Trial and PROBE) with age, sex, and diagnostic groups.**

	DeNoPa			Ottawa Trial			PROBE		
Variable	Beta	95% CI ¹	p-value	Beta	95% CI ¹	p-value	Beta	95% CI ¹	p-value
Age	-0.05	-0.09, -0.01	0.022	-0.22	-0.31, -0.14	<0.001	-0.21	-0.31, -0.12	<0.001
Sex									
Female	—	—		—	—		—	—	
Male	-0.66	-1.4, 0.06	0.073	-2.9	-4.6, -1.2	<0.001	-1.9	-3.9, 0.07	0.06
Group									
HC	—	—		—	—		—	—	
PD/DLB	-5	-5.7, -4.3	<0.001	-13	-14, -11	<0.001	-13	-15, -11	<0.001
MSA/PSP	-1.4	-3.3, 0.51	0.2	-1.8	-6.5, 2.9	0.5	-6.4	-9.2, -3.7	<0.001

¹ CI = Confidence Interval

716

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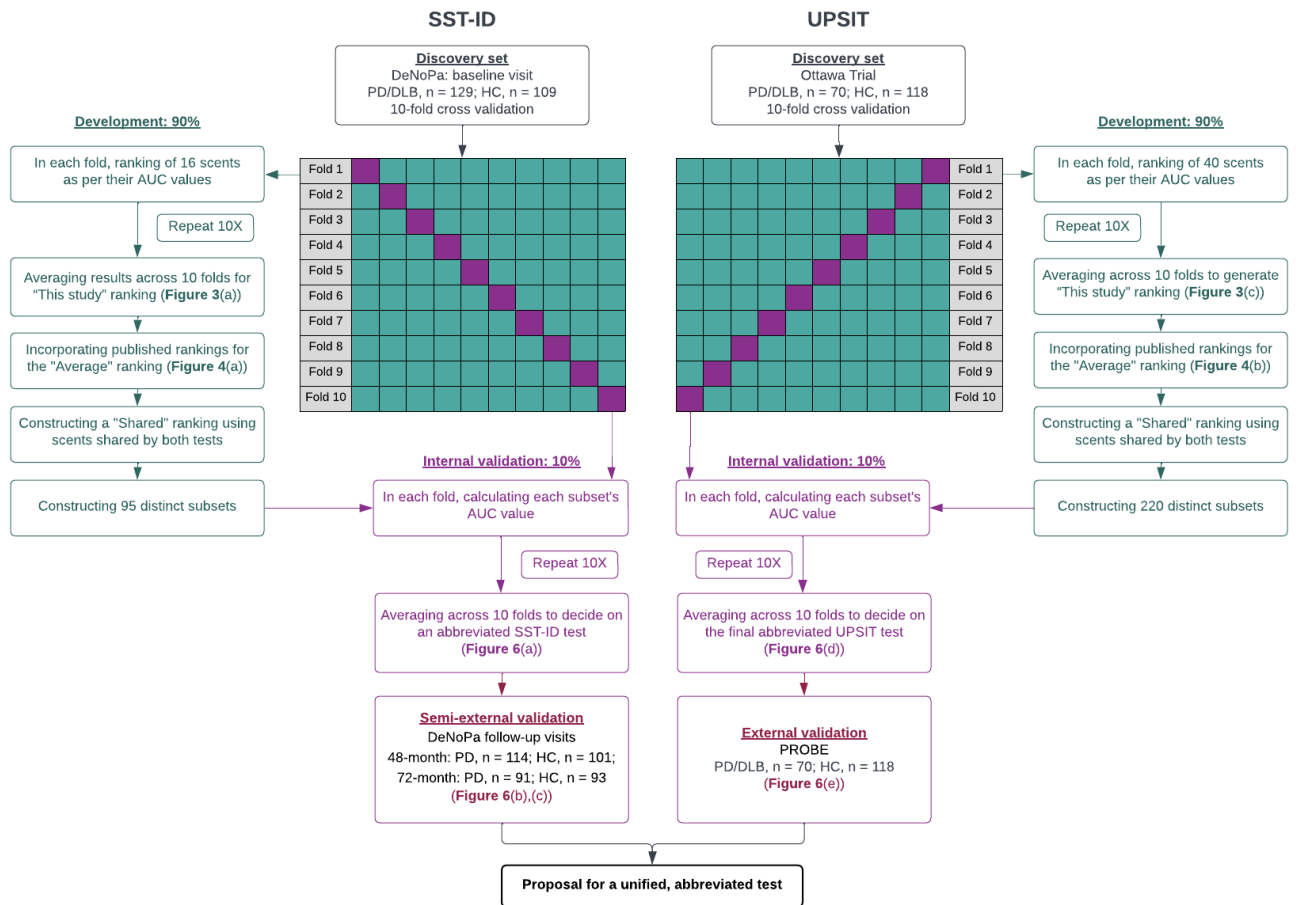


Figure 1: Machine learning workflow for developing and validating an abbreviated smell test.

Details of the workflow are as indicated and described in Methods and Result sections of the main text. SST-ID = Sniffin' Sticks Identification test. UPSIT = University of Pennsylvania Smell Identification Test. DeNoPa = *De Novo* Parkinson Study. PROBE = Prognostic Biomarkers in Parkinson Disease. HC = healthy control. PD = Parkinson disease. DLB = dementia with Lewy bodies. MSA = multiple system atrophy. PSP = progressive supranuclear palsy. ROC = receiver operating characteristic. AUC = area under the ROC curve.

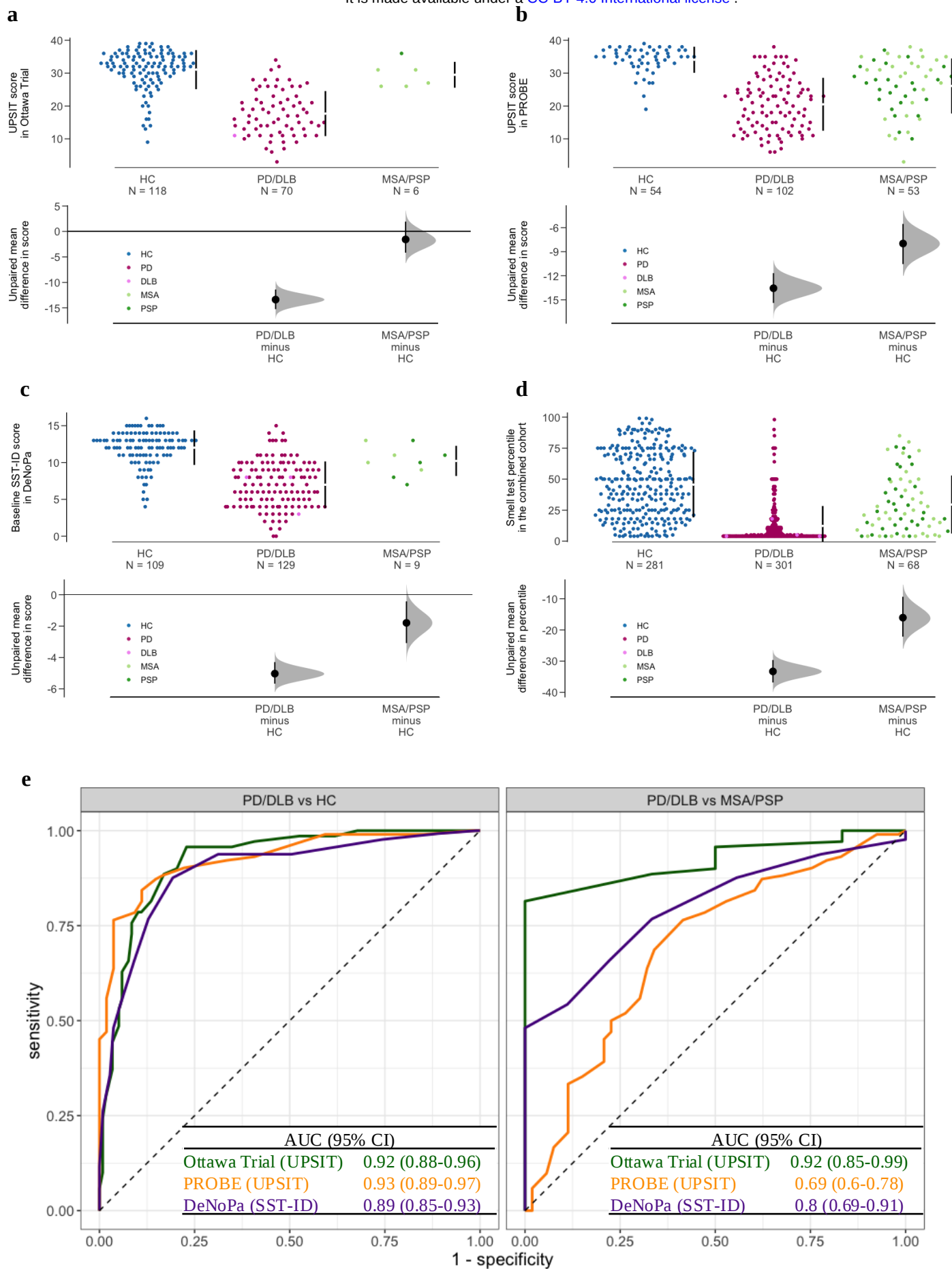


Figure 2: Distribution of olfaction scores using two established tests for different diagnostic groups with parkinsonism in three cohorts.

For figure caption, see the next slide.

Figure 2: Distribution of olfaction scores using two established tests for different diagnostic groups with parkinsonism in three cohorts.

Cummings estimation plots (a-d) were used to illustrate and compare smell test score distributions in each diagnostic group: (a) for UPSIT in the Ottawa Trial cohort, (b) for UPSIT in the PROBE cohort, (c) for SST-ID in the DeNoPa cohort, (d) UPSIT and SST-ID scores were transformed to percentiles based on age- and sex-adjusted norms in the combined cohorts. Each data point in the upper panels represents the score of one participant, and colors represent different groups and diagnosis, as shown in legends. The vertical lines in the upper panels represent the conventional mean $\pm$ standard deviation error bars. The lower panels show the mean group difference (the effect size) and its 95% confidence interval (CI) estimated by bias-corrected and accelerated bootstrap, using healthy controls as the reference group. Panels in (e) show ROC curves and AUC values with 95% confidence interval (CI) for smell tests in each cohort (indicated by different colors; individual scores shown in a-d) to distinguish PD/DLB versus HC groups (left) and PD/DLB versus MSA/PSP groups (right). Abbreviations as in Figure 1.

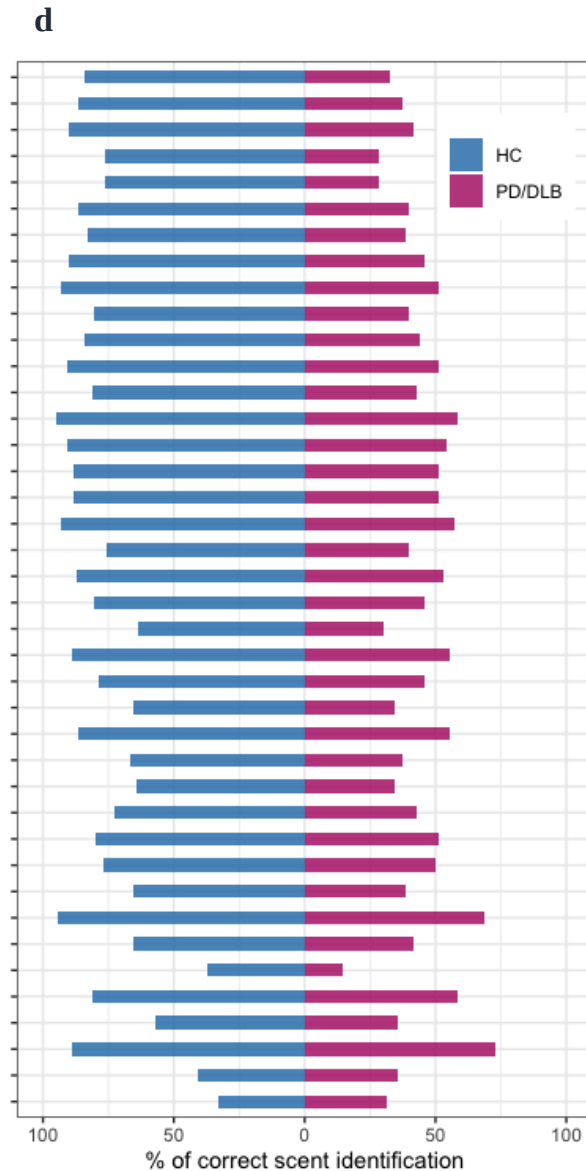
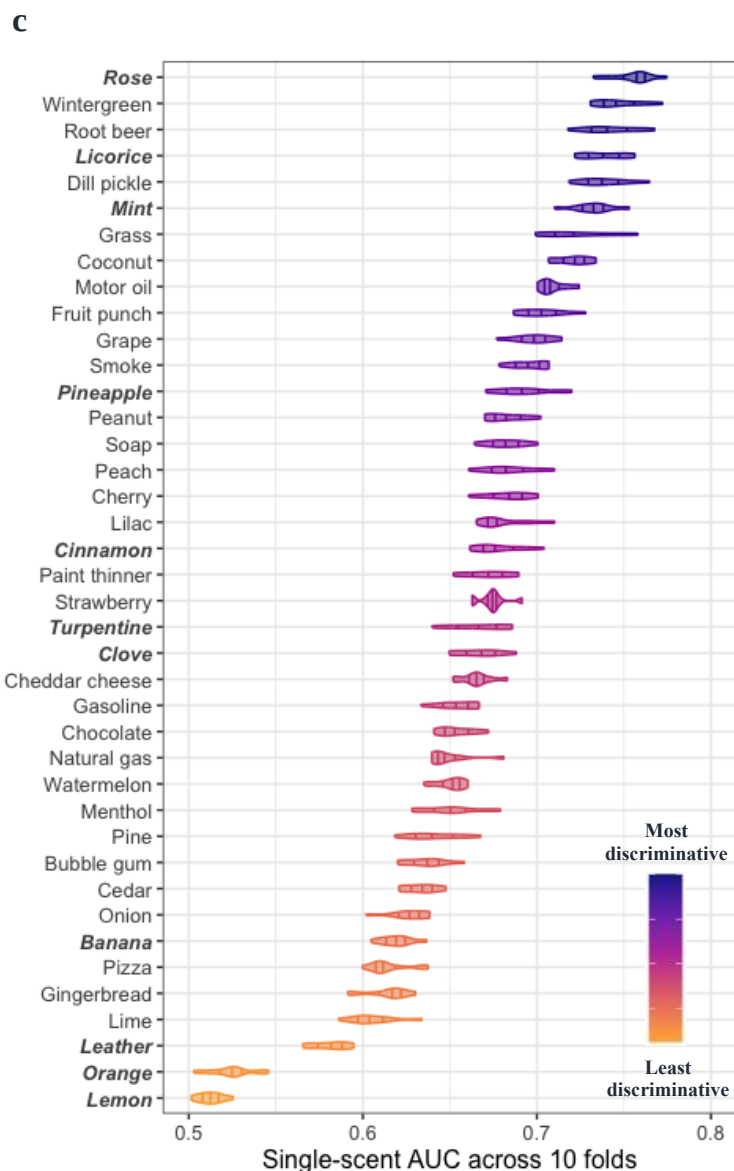
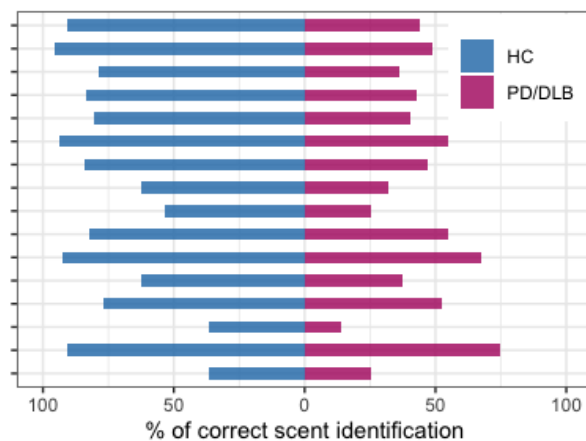
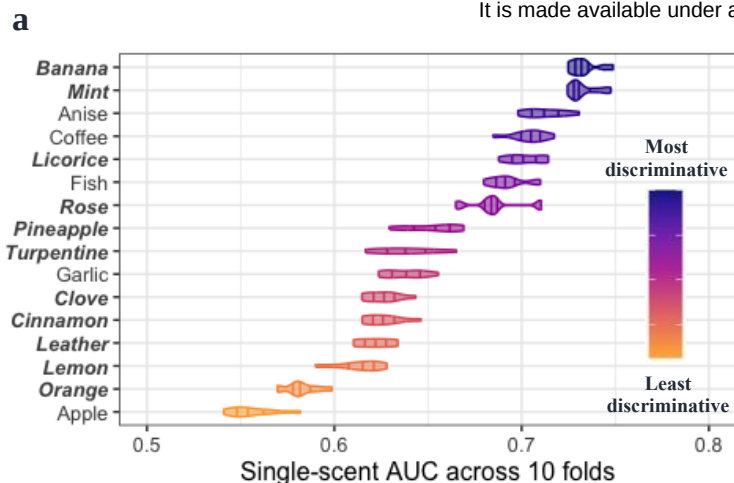


Figure 3: Individual scent performances in differentiating PD/DLB from HC groups.

SST-ID scents are shown using baseline DeNoPa data (a, b) and UPSIT scents for the Ottawa Trial cohort (c, d). Panels (a) and (c) illustrate distribution of AUC values of each scent across 10-fold cross-validation using violin plots, with 25%, 50%, and 75% quantile lines. The scents are ordered in descending order of their mean single-scent AUC value; the color of each scent changes gradually from the most to the least discriminative value, as indicated by the legend. Scents shared by both tests are highlighted by bold italic font. Panels (b) and (d) show the percentage of subjects correctly identifying each scent within both groups in each corresponding cohort. Abbreviations as in Figure 1.

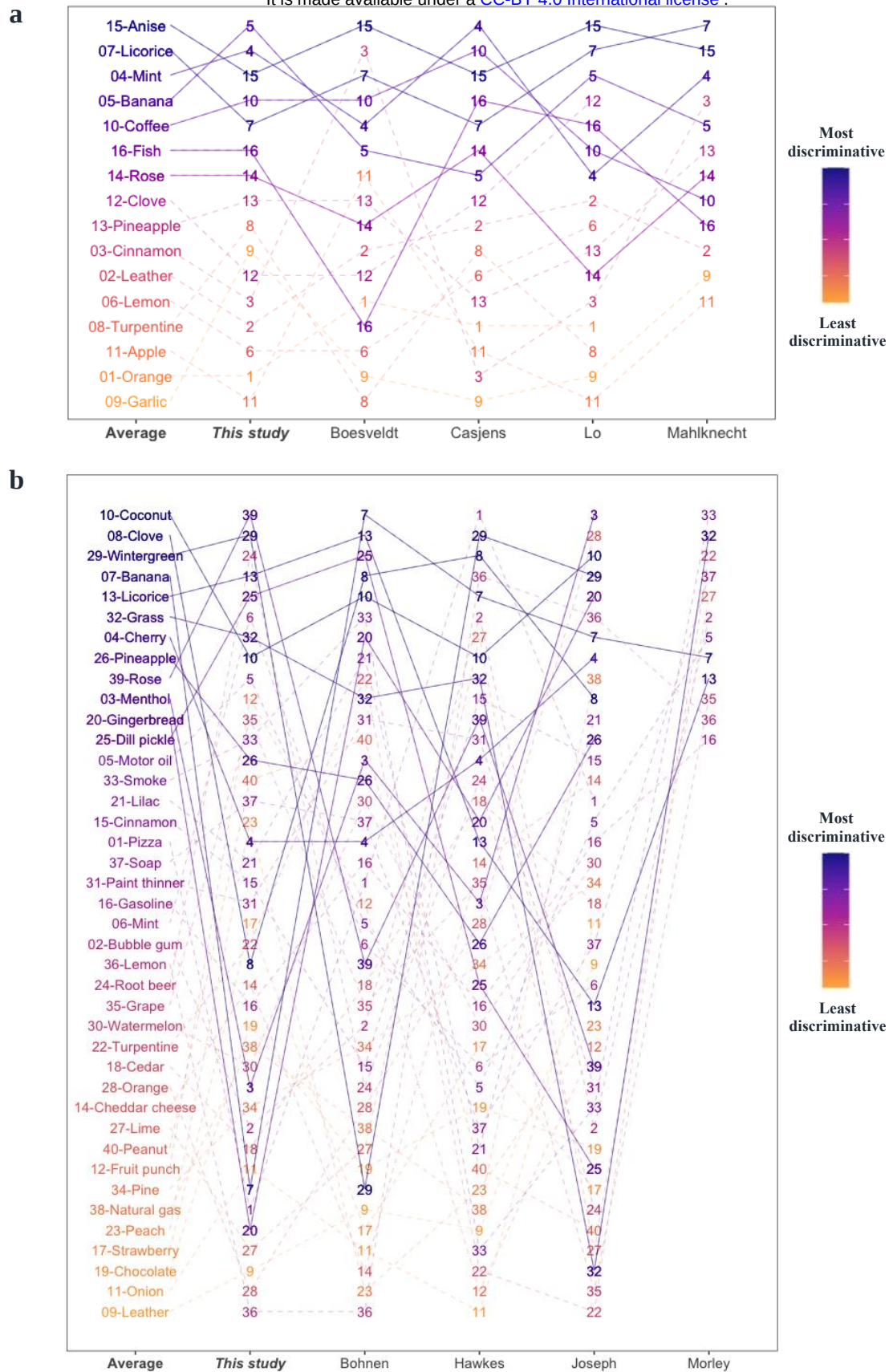


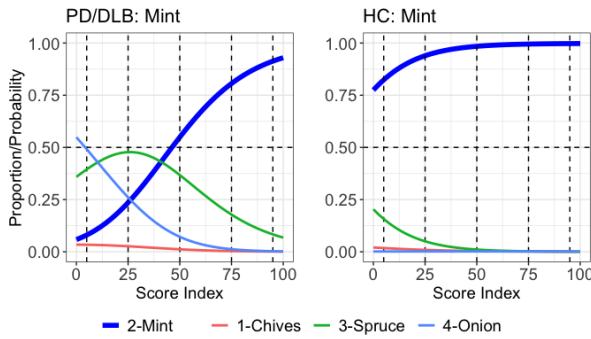
Figure 4: Comparison of scent rankings in this study versus previously published ones.

Panels (a) and (b) show scent rankings of SST-ID and UPSIT, respectively. “This study” columns show scent rankings from Figure 3, and the neighboring columns show corresponding rankings from other studies, as indicated at the x-axis. The “Average” column of each panel shows the scent ranking generated by averaging results from 5 separate rankings. Each scent is represented using the format “index-scent” in the “Average” ranking, and as index only in others. The lines track how each scent’s rank changes from study to study. Color of each scent changes gradually from the most to the least discriminative odorant defined by “Average”. Based on these, 7 best-performing scents in SST-ID (a) and 12 best-performing scents in UPSIT (b) are tracked by solid lines. Note, rankings by Mahlkecht *et al.* and Morley *et al.* included only the top 12 scents.

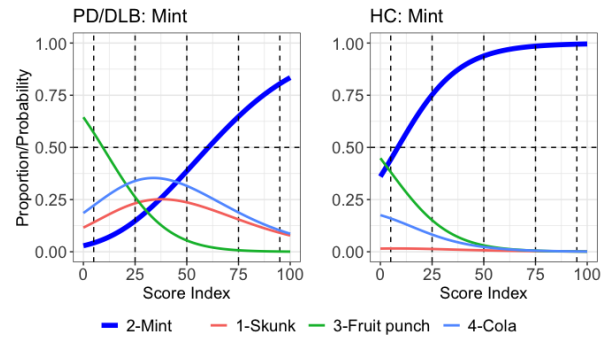
SST-ID, DeNoPa, baseline

UPSIT, Ottawa Trial

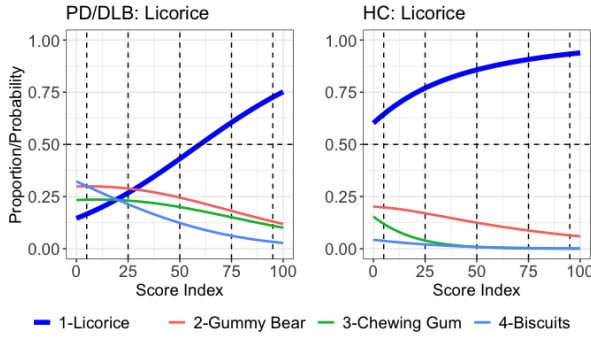
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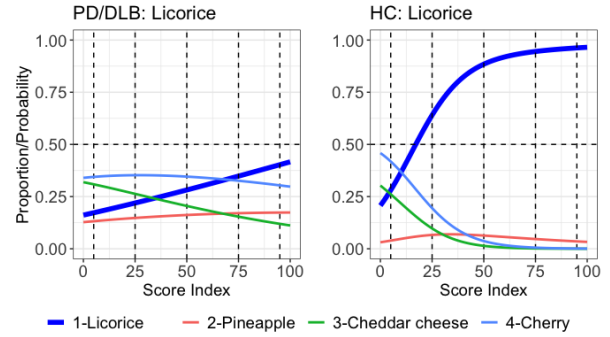
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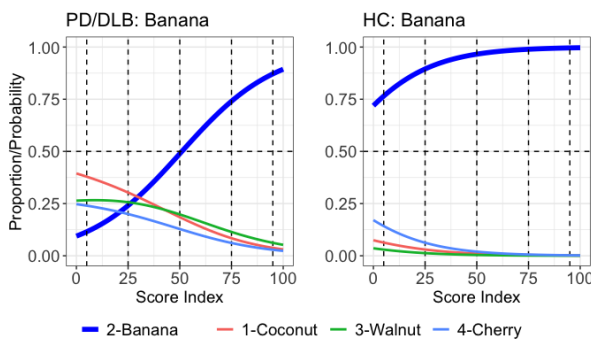
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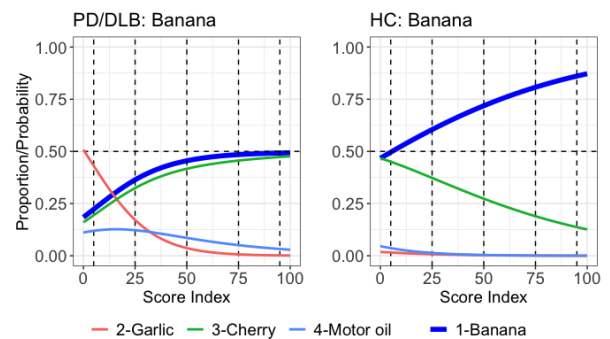
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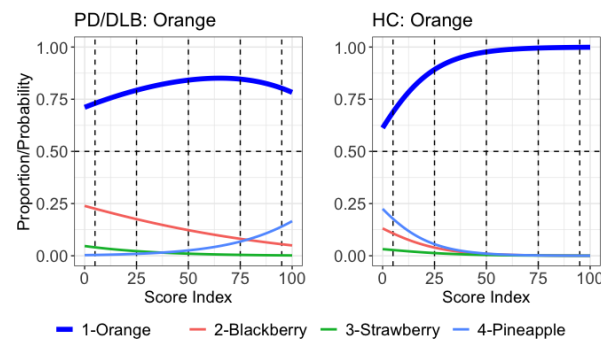
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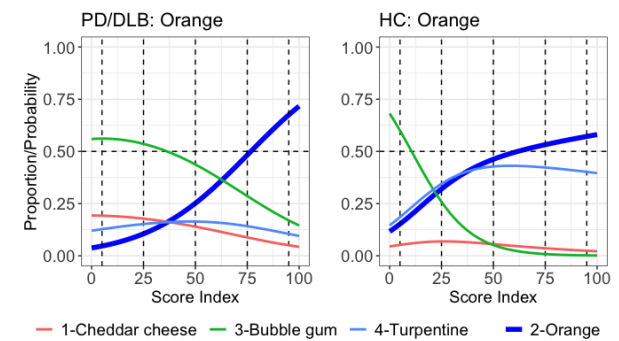
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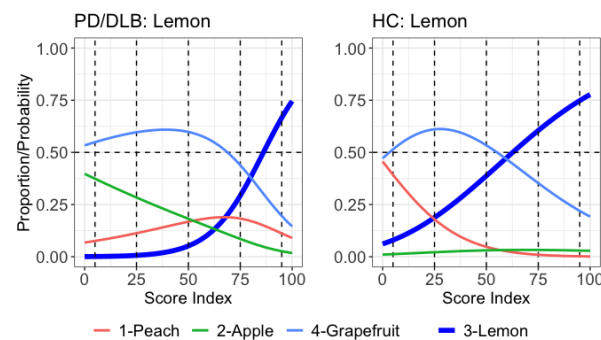
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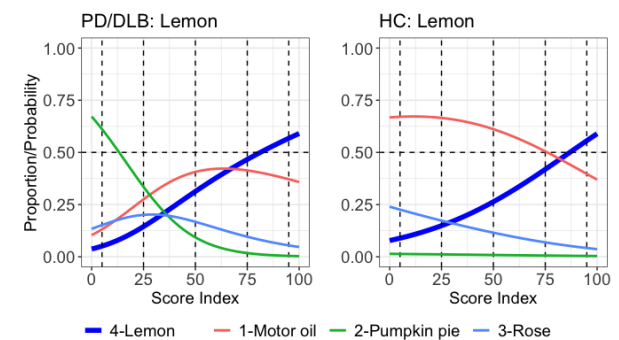


Figure 5: Influence of distractors in multiple-choice smell tests for five shared scents selected.

For figure caption, see the next slide.

Figure 5: Influence of distractors in multiple-choice smell tests for five shared scents selected. Panels with odd numbers show the Item Characteristic Curves (ICCs) of five SST-ID scents: mint, licorice, banana, orange, and lemon. Panels with even numbers show ICCs of the corresponding UPSIT scents. In each figure, panels on the left show data for PD/DLB patients, panels on the right for healthy controls. The x-axis reveals transformed score indices (percentage rank of the respective scores) within the corresponding group. The y-axis shows the probability of choosing each option at a particular score index. The correct option of each item is highlighted using thicker, blue curves. Numbers in the color legends represent option indices. The horizontal dashed lines represent 50% probability. The vertical dashed lines represent five quantiles (5%, 25%, 50%, 75%, and 95%).

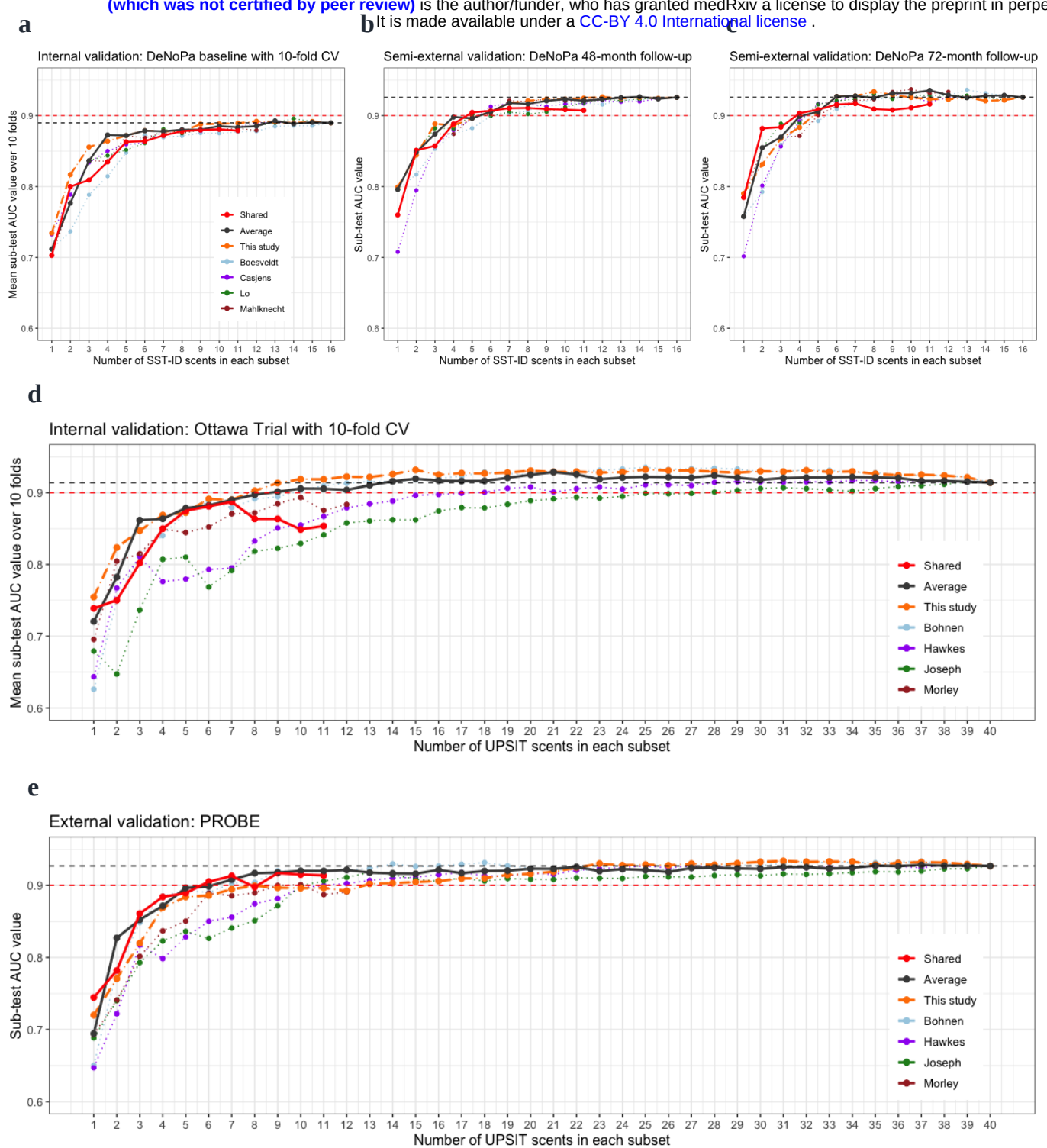
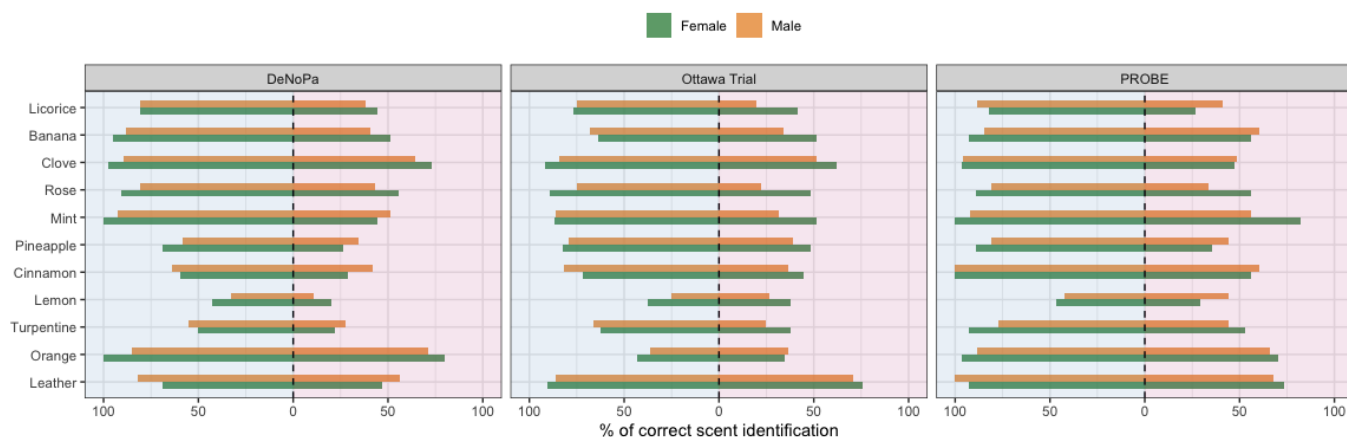


Figure 6: Exploration of smaller subsets of scents tested vs. accuracy in group classification of PD/DLB vs HC.

The x-axis shows the number of individual scents used for each subset examined; colors represent different scent rankings from separate studies, as indicated by the legends (see also Figure 4 and Table 3). ‘Shared’ denotes scents used in both UPSIT and SST-ID; Average, all studies combined; This study, rankings derived using baseline DeNoPa and Ottawa Trail data. Individual points shown in panels (a) and (d) represent internal validation results, averaging across 10 folds. In panels (b), (c) and (e), each point represents the AUC value of the corresponding subset using (semi-)external validation sets. The black horizontal, dashed lines indicate AUC values of the corresponding test when viewed in its entirety. Red horizontal, dashed lines indicate AUC = 0.9 as a predetermined reference line.

a



b

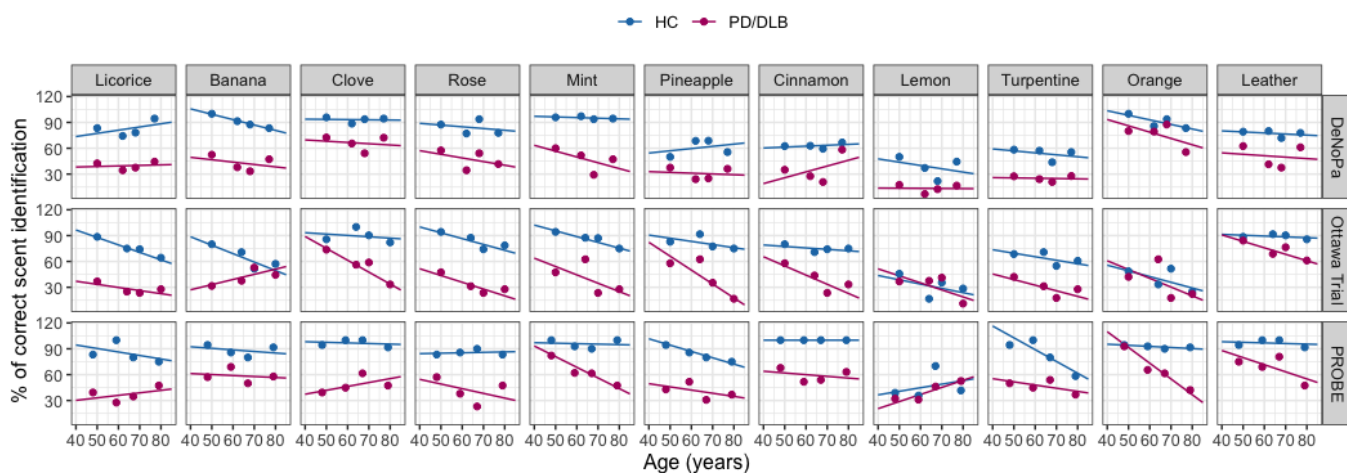


Figure 7: Relationships between scent identification performance, diagnosis, sex and age.

Panel (a) shows the percentage of persons that correctly identified each scent within the healthy control (HC) group (indicated by light blue region) and the PD/DLB group (indicated by pink region) in the corresponding cohorts, separated by sex (bar color). Panels in (b) show the relationship between age (x-axis), diagnostic group (HC in blue; vs PD/DLB in red) and the percentage of correctly identified scents (y-axis) for each odorant tested (columns) within each cohort (row), as indicated on the right.