Molecular triggers of symptoms of parosmia and insights into the underlying mechanism

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Article

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Abstract

The molecular stimuli that trigger a parosmic response have been identified. Parosmia is a debilitating condition in which familiar smells become distorted and unpleasant, frequently leading to clinical depression. Often a result of post infectious smell loss, incidences are increasing as COVID-19 cases escalate worldwide. Until now, there was little understanding of its pathophysiology, and the prevailing hypothesis for the underlying mechanism is a mis-wiring of olfactory sensory neurons. However, with novel application of flavour chemistry techniques as a relatively rapid screening tool for assessment of both quantitative and qualitative olfactory loss, we identified 15 different molecular triggers in coffee. This provides evidence for peripheral causation, but places constraints on the mis-wiring theory. Furthermore, it provides the basis for development of a practical diagnostic tool and treatment strategies.

Introduction

Prior to the COVID-19 pandemic, olfactory dysfunction was largely unrecognised, and often underestimated by health care professionals. Since the spread of SARS-CoV-2, and the realisation that 50-65% of cases result in anosmia (the loss of sense of smell)[1], there is a greater awareness of the debilitating effect of olfactory disorders[2]. Typically, in cases of COVID-19, normal olfactory function returns within a few weeks, but one study estimates 12% of all cases result in long term smell dysfunction[3]. With >170 million confirmed cases of COVID-19 worldwide[4], this is a significant problem facing the global population today and, as a persistent neurological deficit resulting from COVID-19 infection, it should be considered part of the Long COVID syndrome.

Parosmia often occurs in the early stages of recovery from anosmia, typically 2-3 months after onset[1], particularly in those whose anosmia was either acquired post-infection or post-traumatic brain injury[5]. It is a triggered, qualitative olfactory disorder in which familiar everyday smells become altered and unpleasant, to the extent that they become almost unrecognisable. Those severely affected find their quality of life deteriorates as everyday activities such as eating, showering and social interactions become a challenge. They report being distressed and anxious about their future[6] and, with many food aromas being intolerable, they start to reject food, leading to significant changes in weight[7], a decline in mental health and, in severe cases, to clinical depression[8-9].

The aim of this work was to gain insight into the mechanisms involved in parosmia. In 2013, coffee and chocolate were found to elicit distorted olfactory experiences in parosmia[10] and more recently, coffee, meat, onion, garlic, egg, mint/toothpaste were identified in a thematic analysis of group posts on social media[6]. These foods contain aroma compounds with some of the lowest odour-thresholds known, and we suggest that these compounds may be involved in triggering olfactory distortions. Our original hypothesis was based on that of Leopold[11] who proposed that parosmia was a result of incomplete characterisation of the odorant. As olfactory sensory neurons (OSN) regenerate from basal stem cells, selective detection of just the pungent highly odour-active compounds might result in an incomplete, and

therefore distorted perception of certain foods and beverages. Whether this would be sufficient to cause the strong sense of disgust, often reported with parosmia, was not clear.

Our approach is novel in that we use GC-Olfactometry (GC-O) to determine which of the aroma compounds present in the headspace of coffee are responsible for distortions and the sense of disgust experienced by those with parosmic. Gas chromatography separates the hundreds of volatile components present in the sample headspace which, when coupled to an odour-port, allows subjects to sniff and assess each component as it elutes from the column, thereby allowing participants to assess many single aroma compounds in a short time. The participants recorded the descriptor, intensity and parosmic character of each of the aromas as they eluted, and we recorded the total number of aromas detected and the number of aromas which triggered the distortions associated with parosmia.

Results And Observations

Participants. Table 1 shows demographic data for all participants (N = 44) which includes 29 participants reporting post-viral parosmia (PAR) and 15 without parosmia (NONPAR) (further details provided in Supplementary Table S1). All participants were non-smokers and self-reported that they could taste the difference between salt and sugar. Pre-COVID-19 PAR and NONPAR were age-matched with mean ages of 56 and 49 y respectively and there was no significant difference between the two groups ($p=0.12$), whereas post-COVID-19 PAR were significantly younger (mean age 37 y) than their pre-COVID-19 counterparts or the NONPAR group ($p<0.000$, $p=0.008$ respectively).

Olfactory Function. Bilateral olfactory function was assessed using the complete validated orthonasal psychophysical Sniffin' Sticks test (Burghart, Wedel, Germany)[12] based on the threshold of 2-phenylethanol (T), discrimination (D) and identification (I) tests which gives a TDI score (range 0-48) (Table S1 in supporting information). TDI scores were significantly lower in pre- and post-COVID-19 participants (mean 28 and 27 respectively) compared to the NONPAR group (mean 37) (ANOVA, $p<0.0001$ respectively) but there was no significant difference between pre- and post-COVID-19 groups ($p=0.71$). The TDI scores of the combined parosmic groups ranged from anosmic to normosmic (10-38). Ten of this group were classified as normosmic on raw TDI score, increasing to 17 (more than half the group) when age adjustment was applied[13], whereas three scored <16 and were classified as functionally anosmic. We demonstrate here that although on average most parosmic participants had a low olfactory function, parosmia also occurs in those with a normal olfactory function.

Gas Chromatography-Olfactometry. PAR (pre- or post-COVID-19) detected significantly fewer aromas at the GC-odour port than NONPAR ($p<0.0001$) (means 19.9, 18.5 and 37.3 respectively). The number of GC-O aromas correlated well with the TDI score ($R^2=0.66$, Fig. 1A) as both are quantitative indicators of olfactory function. However, the mean number of aromas which triggered the sense of distortion in PAR was only 6.4 (range 0-13) indicating that on average they detected three times more "normal" aroma molecules than trigger molecules – one of our most important findings that demonstrates that not all aroma compounds are triggers (Supplementary Table S1). The role of individual molecules in triggering

parosmic distortions has never been demonstrated before and suggests that, in those presenting with parosmia, it is only specific molecules which trigger the altered perception of food and the sense of disgust. There was no strong correlation between the number of molecular triggers reported and TDI score (R^2=0.16, Fig. 1B) suggesting that although quantitative and qualitative olfactory disorders may occur together, their mechanism may be quite different.

Molecular triggers. Over 30 different molecules were detected by PAR as a group. The 20 most frequently detected are shown in Table 2 (see Fig.2 for structures). Of these, 18 were reported to trigger the sense of distortion. The most frequently reported trigger is 2-furanmethanethiol (**T1**) which has an exceptionally low odour threshold in water (0.004 ug/kg[14]). Whereas NONPAR used a range of food-related terms to describe it ("coffee", "roasty", "popcorn", "smoky"), PAR often struggled to find suitable descriptors, as they were unable to relate it to anything they had smelled before. PAR typically used words describing its hedonic quality ("disgusting", "repulsive" and "dirty") or "new coffee" (relating to the altered smell of coffee since onset of parosmia). Four PAR described it in the same way as NONPAR ("biscuit", "toasty" or "roasty") indicating that it is not universally parosmic, but certainly an important and frequent molecular trigger of parosmia. All NONPAR except one detected this compound.

The equally potent 2-methyl-3-furanthiol **(T2)** (threshold 0.0004 ug/kg in water[15]) and its corresponding methyl disulfide **(D2)** were also detected but reported less frequently as distorted. They are character impact compounds in meat, and we confirmed in four parosmic participants who assessed grilled chicken by GC-O that these compounds also triggered parosmic responses to meat.

2-Ethyl-3,6-dimethylpyrazine **(P1)** was the second most frequent trigger in coffee, described with a variety of food terms by NONPAR, but by "new coffee", "unpleasant" and "distorted" by PAR. Some could distinguish it from **T1**, but others could not. Other trisubstituted pyrazines (2,3-diethyl-5-methylpyrazine **(P2)**, 2-ethyl-3,5-dimethylpyrazine **(P3)** and trimethylpyrazine **(P4)**) were common triggers. These pyrazines are highly odour-active compounds in roasted, fried and baked goods, and we confirmed by GC-O that these compounds also triggered a parosmic response to cocoa (N=4), grilled chicken (N=4) and peanut butter [N=3]. 2-Ethyl-3-methoxypyrazine **(M1)**, 2-isobutyl-3-methoxypyrazine **(M2)** and 2-isopropyl-3-methoxyprazine **(M3)** were common triggers in coffee, and we confirmed that these also contributed to the parosmic character of bell peppers (N=5), where they are character impact compounds.

Another thiol, 3-methyl-2-butene-1-thiol **(T3)**, with a pungent weedy character and low threshold (0.0002 ug/L[16]), was reported as a trigger 9/29 times. Although not heterocyclic like the others, it contains the same α,β-unsaturated thiol moiety as **T1**. The polyfunctional thiol, 3-mercapto-3-methylbutanol (**T4**) and its formyl ester (**T5**) are potent aroma compounds in coffee[17] and were detected in half the cases, but only reported as distorted 5 or 6 times.

Although thiols and disulfides seem to effectively trigger a parosmic response, there are two notable exceptions. Methanethiol (odour threshold 0.02 ug/L[18]), which was detected by some NONPAR, was not

detected by any PAR. Likewise, dimethyl trisulfide (0.01 ug/L[14]) is an exceptionally potent compound detected by 12/15 NONPAR but only by 4 PAR, and only once reported as a trigger.

Furthermore, a few compounds were detected but never reported as triggers. 4-Ethylguaiacol (**NT1**) was detected by 7 PAR and always described as spicy, sweet and smoky, but never parosmic. Similarly, (*E*)-β-Damascenone (**NT2**), a key odour-active compounds in coffee with a low odour threshold (0.01 ug/kg[14]), was detected by 6 PAR and always described as jammy and fruity.

Principle component analysis. Principal component analysis was carried out on the intensity data (Supplementary Table 2) for PAR for the 20 most frequently detected compounds (Fig. 3). The compounds scored with the greatest intensity tended to have a greater component on PC1, whereas PC2 separated the three most frequently detected thiols (**T1, T2, T3**) from the three most frequently detected pyrazines **(P1, P2, P3**). Furthermore, the two disulfides are together **(D1, D2**) and close to their parent thiols, and the two branched methoxypyrazines (**M2, M3**) are also close together. There is some evidence of a structure activity relationship emerging suggesting, for example, that some participants might perceive thiols more intensely and others may perceive pyrazines more intensely.

Faecal odours. Volatiles such as skatole and indole are perceived by most people as among the most objectionable of odours and are present in faeces[19]. Those suffering from parosmia often comment that the smell of faeces is never as unpleasant as before, often smelling like other distorted foods, or even more pleasant and biscuity[6], presenting the interesting corollary that foods smell of faeces yet faeces smell of food. Two parosmic researchers who carried out GC-O on the headspace of a 50% faecal slurry in water did not detect these compounds and were unaware of any foul smells. However, they detected several other compounds, many of which they had also detected in coffee, and only some of which triggered a parosmic distortion. In comparison, a normosmic scored the intensity of indole and skatole as close to strongest imaginable. This provides a neat explanation as to why the changes in valence for faecal samples is reversed. In the absence of signals from the compounds usually associated with disgust in faecal odour, PAR detect other potent volatiles in the sample, normally masked by the stench of the faecal compounds. For some, these other compounds may have a positive valence, for others they may be distorted.

Correlation between ligand structure and odour receptor (OR)? Identifying a small number of common molecular triggers for parosmic distortions raised the obvious question of an olfactory receptor similarity. To determine whether the clusters are associated with any of the known ligand odour receptor pairs, we searched the ODORactor database[20]. We found no obvious segregation of triggers by olfactory receptor (Fig. 3). Most of the triggers activated (with > 50% probability) either OR1G1 or OR52D1. We also compared molecules never reported as triggers such as disubstituted pyrazines, indole, skatole, cresol and found these to activate the same ORs, making it unlikely that these olfactory receptors are the source of the parosmic signal. OR1G1 is known to be very broadly tuned and bind odorants of different chemical classes[21]. However, more data is required to propose any relationship between structure, OR and

parosmic distortions. Only a fraction of the known ORs have been de-orphaned, and further identification of ligand-OR pairs is required.

Participant observations.

1. A case of parosmia with no reported decrease in olfactory function: One participant identified 45 aromas, of which just 2 were triggers. The more intense one was 2-ethyl-3,5-dimethylpyrazine (**P3**) and the second a low intensity unidentified compound. They had a TDI score of 37, had tested positive for COVID-19 antibodies but reported no loss of sense of smell.
2. A case of parosmia improving with no concomitant increase in olfactory function: After 4 months one participant showed no improvement in threshold score, a decrease in the number of GC-O aromas detected (22 to 16), and a 5-fold decrease in GC-O intensity scores (750 to 147) and reported an improvement in parosmia. Further work on the temporal aspect of parosmia is in progress.
3. A case of excellent recovery from parosmia with significant improvement of olfactory function: After several years, one participant scoring 35 on the TDI test reported a “new normal” olfactory function where for example coffee was still perceived as different, but acceptable. This indicates regrowth of a broad range of healthy OSNs, yet two of the 52 aromas detected were still identified as triggers of parosmia: 2-methyl-3-furanthiol (**T2**) and one unidentified compound.
4. A case of a functional ansomia with parosmia: At the other extreme, one participant was within 3 months of onset and had a low TDI score (15) indicating functional anosmia. Only five aromas were detected, all scored as barely detectable, and only one, 2-furanmethanethiol (**T1**), had parosmic character. Further investigations showed their parosmia was triggered more by different compounds present in onion and garlic.
5. A case of two distinct parosmic characters: one participant reported several distinct parosmic smells. One, which was described as plastic, chemical and burning rubber, was associated with sulfur compounds, and a second, described as sickly sweet, smoky and woody, was associated with pyrazines. Further investigation showed that onion and garlic gave a third parosmic character. These groupings are consistent with the PCA and a sub-theme emerging from the “AbScent Parosmia and Phantosmia Support” group on Facebook[6].
6. A case of sulfur triggers only: one participant attended just 4 weeks after onset of parosmia and was our most “fresh” participant. Their reactions at the GC-O were quite extreme when a trigger was encountered, with three thiols scoring 90-100 on the intensity scale. With a TDI score of 27, 20 GC-aromas were detected but only five of these were triggers, either thiols or disulfides. Six pyrazines were detected but not reported as triggers.

Summary. In summary, we have identified for the first time, specific molecules which trigger the distortions reported by those suffering from parosmia. We demonstrate that there is a common set of molecular triggers causing the perception of distortions and a sense of disgust in coffee, and they also trigger distorted perceptions of other chemically related foods. However, not all molecules in this set are triggers for all PAR. These molecules tend to be potent, have very low olfactory detection thresholds and,

in isolation, are neither distorted nor unpleasant for NONPAR. However, odour activity is not the defining factor since (*E*)-β-damascenone, which has an exceptionally low odour threshold[14], was always perceived as jammy and fruity by both PAR and NONPAR. Most of the trigger molecules found in coffee belong to one of four distinct groups: thiols, pyrazines, disulfides, methoxypyrazines but there are no known odour receptors which are specific for the described trigger molecules. In addition, individual case studies suggest that parosmia symptoms are independent of olfactory function and parosmia may occur in patients with objectively normal olfactory function. Recovery from parosmia can be associated with either improvement or stasis of olfactory function. Although incomplete odorant characterisation (a lack of contribution from other more desirable and less potent aroma compounds) may increase the parosmic experience for those with poor olfactory function, we found that the molecular triggers alone are key drivers of distortions and individually elicit the perception of disgust.

Discussion

Parosmia is a tetrapartite symptom: it is a triggered, short lived, altered smell sensation which almost universally elicits the basic emotion of disgust, but little is known of its pathophysiology. Our finding that the sense of distortion is reliably triggered by a common group of low threshold odorants, advances our understanding of this debilitating condition and constrains the pathophysiological hypothesis space. Like the aetiologies of smell loss, several mechanisms have been proposed[11] and can broadly be thought of as the central theory, the ephaptic theory and the peripheral mis-wiring theory, recent findings not withstanding[22].

The central theory is based on the changes occurring in the integrative centres in the brain. A decrease in olfactory bulb volume [23, 24] and a significant loss of grey matter volume has been demonstrated in parosmic patients[25]. Further evidence has been published recently showing different fMRI activation patterns in parosmic patients compared to those with hyposmia[26]. Increased activation in the thalamus and the putamen was observed in the parosmic patients, the latter being of relevance since it is connected to the olfactory cortical networks and has been associated with the perception of disgust. Also, stronger activation was observed in the ventral striatum which is associated with odour valence. Whilst there is good evidence in humans for the central theory of parosmia, a purely central causation seems unlikely based on our evidence that parosmia is triggered by a group of highly specific molecules at the periphery. We suggest that the changes in activity in central areas are more likely to be a consequence and not a cause of parosmia.

The “mis-wiring” theory posits aberrant targeting of OSN to the glomerulus during regeneration following insult. This has been observed in mice with impaired olfactory function induced by ciliopathies[27-28], physical lesioning[29-30], or chemical degeneration[31] but not yet in humans. However, it has been adopted as the likely mechanism for the perception of distorted olfactory percepts in parosmia. It is further suggested that the change in hedonic valence is due to broad activation of the olfactory bulb sending a disordered and unmoderated array of signals to the central neural processing system which invokes a

strong sense of disgust. Our data neither support nor refute the mis-wiring hypothesis, but certainly place constraints on it. We have demonstrated the requirement to account for the non-stochastic nature of the OSNs involved in any proposed mis-wiring theory. Whilst mis-wiring is attributed to a loss of axonal pathfinding mechanisms[27], in light of our results, this theory needs to explain why some OSNs are relaying the “correct” undistorted signal, whereas others are not.

The ephaptic theory summarised by Hawkes[32] suggests that demyelination of the OSNs allow the activation of other, non-stimulated OSNs adjacent to the activated OSN by current flow in the extracellular fluid: “a form of short circuiting". This too would result in a broader activation of the olfactory bulb but does not readily account for the non-random nature of the OSNs involved. Of course, these theories are not mutually exclusive.

These proposed mechanisms have to explain four characteristics: that parosmia arises almost uniquely in settings of widespread synchronous neuronal destruction either post infection or post traumatic brain injury, is triggered by one of a number of common odorants, is of novel odour character, and that this character is almost always unpleasant. Whereas the mis-wiring theory is consistent with the first, and the central theory may explain the novel odour character and the change in valence, it remains for us to determine why only a few potent molecules elicit such a strong parosmic response. These trigger molecules share the trait of having an extremely low threshold in human olfaction, so they are detectable at very low concentrations. The common molecular structures, low odour thresholds and physiochemical grouping of the molecular triggers of parosmia strongly suggest that this is related to changes in the olfactory epithelium with downstream consequences, although we are unable to identify any specific olfactory receptors responsible from publicly available databases. The fact that some parosmic participants only report distortion with some of the groups suggests that there may be several olfactory receptors involved, especially when we consider other food and household items that elicit distortions. So why then these specific groups of OSNs? Several mechanisms could account for the role of specific receptors and their neurons:

1. Specific OSNs are regenerating because they are selectively damaged by the insult and others are preserved, but to date there is no evidence of selective damage.
2. The specific OR is predominant within the regenerating OSN population. Instead of a purely stochastic OR selection process in the olfactory mucosa, these “parosmic ORs” are preferentially selected for expression in OSNs either normally or just in the post-insult olfactory mucosa, thus increasing the number of these neurons in the olfactory mucosa as a whole.
3. Alternatively, there is evidence in mice that activated OSNs have a longer lifespan[33]. If the trigger OSNs are more readily activated, they may survive longer and make up a greater proportion of the overall OSN population.
4. The specific OR are not over-represented but merely more easily activated. Although many OSNs regenerate and aberrantly innervate the glomeruli, only a few afferent neurons pass to each

individual glomerulus and these few molecules are powerful enough to activate many glomeruli simultaneously at physiological odorant concentrations.

5. Since axon guidance is at least partially OR-dependant[34] these specific OR-expressing OSNs could be more likely to demonstrate aberrant targeting of glomeruli in an as-yet unknown way.

What these mechanisms do not explain is the presumably hypothalamic disgust response to the particular altered odour, so why the change in valence?

1. It is possible that parosmia does not arise from the activation of the glomeruli per se, but from the disruption of the network of interneurons, mitral, glial and tufted cells which is thought to act as a habituation and modulatory network in the bulb. Disruption of input innervation and sporadic re-innervation could cause feedback loops to interfere with the previous web of inhibition and promotion of signal at this level, and this disordered activation is experienced as unpleasant.
2. The miswiring hypothesis posits that broader, unregulated patterns of glomerular activation are de novo perceived as unpleasant and disgusting, but this has not been demonstrated. Certainly, in the normal nose, novel smell percepts are not usually automatically disgusting, so the mere novelty of the percept is unlikely to be enough to explain this.
3. There exists a glomerulus or small groups of glomeruli which are "hardwired", developmentally or otherwise, to elicit a disgust component from activation. In the regeneration from insult this "disgust glomerulus" is aberrantly innervated and activated by low threshold odorants, resulting in the altered valence.

In this paper we identify the first common molecular triggers of parosmic responses, characterised by their physiochemical properties and sharing a low odour threshold for human perception. We demonstrate that parosmia is an olfactory dysfunction only partially correlated with olfactory loss and provide evidence to support its arising in the periphery of the olfactory system.

Whilst the novel use of flavour chemistry techniques has led us to a better understanding of the aetiology and pathophysiology of an increasingly relevant syndrome, our findings also have implications for the development of practical diagnostic tools and design of therapies to combat the primary effects and common sequelae (including anxiety, loss of quality of life and clinical depression) of qualitative olfactory dysfunction. An understanding of trigger molecules allows bespoke development of objective tests for parosmia, which are much sought after by patients and clinicians alike, and lays the ground for further development of treatment strategies. An understanding of trigger foods, on a molecular basis, allows us to provide informative and scientifically sound advice around dietary choices and meal planning for those with post-infectious olfactory disorder, and the clinicians, health professionals and families who care for them. This study represents a significant advance in the understanding of this increasingly widespread condition, considered an important part of long COVID syndrome, and will guide further research and future therapies.

Methods

Participants. This study (No 22/19) was approved by the University of Reading Research Ethics Committee. All parosmic participants were recruited via Facebook support groups or local ENT consultants, and non-parosmic participants from within the Department of Food and Nutritional Sciences at the University of Reading, or through private Facebook pages. The initial study was carried out with pre-COVID-19 parosmic participants (N=14) and non-parosmic participants (N=15) between October 2019 and March 2020. This was supplemented with post-COVID-19 parosmic participants (N=15) between July and September 2020. All volunteers completed a screening questionnaire (Supplementary Table S3) before attending a study day in the Olfaction Laboratory at the University of Reading. Selection was based on the participants listing coffee as a key trigger, and answering "often" at least once to two key questions which discriminate most efficiently between parosmic participants and those with quantitative olfactory disorders[35]:

1. Are odours that are pleasant to others, unpleasant to you? Never/rarely/often/always
2. Is the taste of food different to what you expect? Never/rarely/often/always

Olfactory function. The bilateral olfactory function of all participants was assessed at the beginning of the day using the well-established and validated orthonasal psychophysical Sniffin' Sticks test (Burghart, Wedel, Germany)[12]. Involving threshold (T), discrimination (D) and identification (I) tests, the resulting TDI score ranges from 0 to 48 with those scoring >30.3 classified as normosmic.

Materials. The following authentic aroma standards were purchased: 2-furanmethanethiol (**T1**), 2-methyl-3-furanthiol (**T2**), and 2-isopropyl-3-methoxyprazine (**M3**) from TCI (Oxford, UK); 2-ethyl-3,6-dimethylpyrazine (**P1**), 2,3-diethyl-5-methylpyrazine (**P2**), and 2-ethyl-3,5-dimethylpyrazine (**P3**) from Oxford Chemicals (Hartlepool, UK); 2-furanmethyl methyl disulfide (**D1**), 2-methyl-3-furyl methyl disulfide (**D2**), 2-ethyl-3-methoxypyrazine (**M1**), 2-isobutyl-3-methoxypyrazine (**M2**), 3-mercapto-3-methylbutanol (**T4**), 3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolone) (**X1**), trimethylpyrazine (**P4**), 2-methoxyphenol (guaiacol) (**X2**), 2,3-butanedione (**X4**), 4-ethylguaiacol (**NT1**), (E)-β-damascenone (**NT2**) and the C6-C25 alkane standard from Sigma (Poole, UK); 3-methyl-2-butene-1-thiol (**T3**) in a capsule from Aroxa (Leatherhead, UK) and 3-mercapto-3-methylbutyl formate (**T5**) from Fluorochem (Hadfield, UK). Nescafé Original instant coffee sachets were purchased from office Depot (Hounslow, UK). One box of instant Nescafé sachets (use by date August 2021) was purchased in September 2019 for use with the subjects between October 2019 and March 2020. A second box (use by date Oct 2022) was purchased in October 2020 to cross check the stability of the sachets over one year. Cocoa powder (Bournville, Cadbury, Bourneville, UK), skinless chicken breast fillet, smooth peanut butter (Tesco, Cheshunt, UK) and red bell peppers were purchased from a local supermarket.

Faecal samples were kindly prepared under Class 2 conditions by members of the food and microbial science group at the University of Reading. A faecal sample was collected from a healthy donor who had not consumed antibiotics within the previous six months. For transportation to the laboratory the sample was held under anaerobic conditions, using an Oxoid Anaerogen sachet (Oxoid, Hampshire, UK), for up to 2h before being frozen at -20 °C.

Extraction of coffee aroma. Fresh deionised water from a MilliQ system at 18.2 MΩ/cm resistivity was boiled in a kettle and 300 mL was added to the contents of the sachet (2.15 ± 0.05 g) in a 500 mL Duran bottle. The bottle was sealed, stirred for 2 min and an aliquot (3.0 ± 0.05 g) was transferred into an SPME vial. More concentrated extracts (contents of 1 sachet in 3 g boiling water) were also prepared for expert GC-O analysis and a detailed GC-MS analysis to aid identification of compounds. In both cases, the vial was equilibrated at 55 °C for 20 min and a preconditioned triple phase solid phase microextraction (SPME) fibre (50/30 µm divinylbenzene/carboxen on polydimethylsiloxane (Supelco, Poole, UK)) was exposed to the headspace at 55 °C for 20 min prior to analysis by GC-O.

Gas Chromatography-Olfactometry (GC-O). After extraction, the SPME device was inserted into the injection port of an HP7890 GC from Agilent Technologies (Santa Clara, CA, USA) coupled to a Series II ODO 2 GC-O system (SGE, Ringwood, Victoria, Australia). The SPME fibre was desorbed in a split/splitless injection port held at 280 °C. The columns employed were either an Agilent HP-5 MSUi capillary (30 m, 0.25 mm i.d., 1.0 µm df) non-polar column or a Stabilwax®-DA (30 m, 0.25 mm i.d., 0.25 µm df) polar column (Restek, Bellefonte, PA, USA). The temperature gradients were set as follows: 40 °C for 2 min, then a rise of 5 °C/min up to 200 °C and 15 °C/min from 200 °C to 300 °C (or 250 °C for the polar column), and the final temperature held for a further 19 min. Helium was used as carrier gas (2 mL/min). At the end of the column, the flow was split 1:1 between a flame ionisation detector (kept at 250 °C) and a sniffing port using 2 untreated silica-fused capillaries of the same dimensions (1 m, 0.32 mm i.d.). The flow to the odour-port was diluted with a moist make up gas.

Procedure at the odour-port. Subjects were familiarised with the instrument, instructed to breathe normally during the run, and advised that they could stop at any time, particularly if they felt dizzy or light-headed. As the aromas eluted from the column, 3 bits of information were requested from the subjects: an odour description, an odour intensity and an indication of whether the odour elicited a parosmic response. Since the description and identification of aromas in the absence of any other cues is difficult, all participants were presented with a flavour wheel (Supplementary Figure S1) before they started which they could use as a reference during the GC-O run. It had been developed by 2 experts who sniffed samples of the same coffee (both at regular strength and concentrated) by GC-O. The words were categorised into food and non-food, and colour coded for quick reference. This was of more use to non-parosmic participants, as parosmic participants found it hard to describe many of the aromas, even with the help of the flavour wheel. Many resorted to using the terms “new coffee”, “that parosmia smell”, “trigger number 1” or “trigger number 2”. As each aroma eluted, parosmic participants were prompted to highlight anything that had a parosmic character or trigger. Intensity was scored on a 158 mm horizontal general labelled magnitude scale (gLMS) with anchors at “barely detectable”, “weak”, “medium”, “strong”, “very strong” and “strongest imaginable” corresponding to intensity scores of 1.4, 6, 17, 35, 51 and 100 respectively. This was chosen over the more common visual analogue scale to allow for instances where parosmic participants in particular wanted to extend upwards the range of scores. It is a logarithmic scale which better relates the psychophysics of perception to the concentration of the stimulus (Stevens’s Law). Time of elution was recorded manually by the researcher. All subjects carried out the GC-O of

coffee twice, once before lunch and once after a 45 min lunch break. During the second run, the focus was on refining the descriptors as well as obtaining a duplicate intensity rating. During the first run, the subjects recorded the descriptors in their own words, prompted only by the flavour wheel, whereas during the second run, there was more discussion between the researcher and the subject, to verify the odour character and identity of the compound eluting.

Gas chromatography-mass spectrometry (GC-MS). An extract from a coffee prepared with one sachet in 3 mL of boiling water was extracted as above and analysed by GC-MS to aid identification of aroma compounds detected by GC-O and confirm their presence in the coffee extract. A7890A Gas Chromatograph coupled to a 5975C series GC/MSD from Agilent was used, equipped with either of the columns described above. The oven was held at 40 °C for 2 min, increased from 40 °C to 250 °C at a rate of 4 °C/min and then kept constant at 250 °C for 5 min. Helium was the carrier gas at a flow rate of 1.2 mL/min. Mass spectra were recorded in electron impact mode at an ionisation voltage of 70 eV and source temperature of 220 °C. A scan range of m/z 20-300 with a scan time of 0.69 s was employed and the data were controlled and stored by the ChemStation software (Agilent, Santa Clara, CA).

Identification of odour-active compounds. Linear retention indices were calculated by comparison with the retention times of C_6-C_{25} n-alkane series analysed on the same day using the same conditions as for sample analyses (Supplementary Table S4). Aromas eluting from the GC-O were identified by comparing their LRIs, mass spectra and the odour as described by the experts with those of authentic compounds on two columns of different polarity. Mass spectral libraries, such as NIST 2011 and Inramass (INRA, France), were used for primary identification of compounds in the coffee extract using ChemStation software (Agilent, Santa Clara, CA). In most cases, authentic compounds were analysed using the same chromatographic method to confirm their identity by comparison of their mass spectra, LRI, and odour quality. Identification was confirmed by GC-MS on a Stabilwax column. For compounds at concentrations below the detection limit of the GC-MS, odour character and LRI were used.

Confirmation of identity of the trigger molecules. Three parosmic participants returned to assess coffee on a polar column to confirm the identity of trigger compounds. Once identified, selected trigger compounds were also presented to two parosmic participants in dilute form to verify their parosmic character, using the sample preparation protocol described for the European test of olfactory capabilities[36]. Aroma chemicals were diluted in mineral oil or propylene glycol and applied to small discs (5 mm diameter) of absorbent paper in vials which were presented to the participants. They were asked to sniff the vial and indicate whether each compound released “that parosmia smell” which they had described previously.

Additional samples. All additional samples were prepared as for coffee with the following modifications. Cocoa: 3g of cocoa powder was dissolved in 10 g boiling water, stirred and a 3 g aliquot was used for extraction. Meat: a lean breast fillet, thickness 1 cm was grilled for 3 min on either side using a Cuisinart grill (Stamford, CT) set on high. Finely chopped meat (3 g) was used for extraction. A 50:50 slurry of peanut butter (3 g) was used for extraction. Finely diced red pepper (3 g) was extracted at 40 °C prior to

desorption. The faecal sample was thawed, mixed with an equal weight of water, and 3 g transferred to an SPME vial. Chromatography conditions for all samples remained the same as for coffee.

Statistics. The age of the pre-COVID-19 parosmic participants, post-COVID-19 parosmic participants and non-parosmic participants was analysed using Kruskal-Wallis followed by pairwise comparison using Steel-Dwas-Critchlow-Fligner (significance set at 0.05) to determine significant differences between the groups, whereas Anova followed by Tukey HSD (p=0.05) was used for TDI scores. Principal component analysis was carried out on intensity data. All statistical analyses were carried out using XLSTAT version 20201.1.1 statistical and data analysis solution (Addinsoft 2020).

Data availability. All data are supplied in the supplementary information**.**

Declarations

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Authorship contribution

JP contributed to conception, acquisition, analysis, data interpretation, manuscript draft and review; CK contributed to conception, participant management, data acquisition and review; SG contributed to conception, data interpretation, manuscript draft and review (SG).

Conflict of interest

The authors declare no conflict of interest.

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Tables

Table 1 Summary of participant demographics

Participant demographic data	No	Male	Female	White	Age (mean)	Age (range)	Age (SD)	CRS
All Participants	44	12	32	42	47	19-73	14	3
Pre-COVID-19 parosmics	14	3	11	14	56	33-73	9.6	1
Post-COVID-19 parosmics	15	3	12	14	37	19-60	12.2	1
Non-parosmics	15	6	9	14	49	33-71	13.2	2

CRS = chronic rhinosinusitis, na = not applicable

Table 2 Compounds most frequently detected by parosmic participants.

Molecular triggers	Code	Odour threshold ug/L	Number times detected by parosmic	Number times reported as trigger
2-furanmethanethiol	**T1**	0.005[14]	24	20
2-ethyl-3,6-dimethylpyrazine	**P1**	0.01	18	15
2,3-diethyl-5-methylpyrazine	**P2**	0.05[14]	20	13
2-furanmethyl methyl disulfide	**D1**	0.04[17]	18	11
2-methyl-3-furanthiol	**T2**	0.0004[15]	19	10
2-methyl-3-furyl methyl disulfide	**D2**	0.004[15]	18	10
2-ethyl-3,5-dimethylpyrazine	**P3**	1[37]	17	10
3-methyl-2-butene-1-thiol	**T3**	0.01[16]	21	9
2-ethyl-3-methoxypyrazine	**M1**	0.4[18]	12	9
2-isobutyl-3-methoxypyrazine	**M2**	0.002[14]	17	7
3-mercapto-3-methylbutanol	**T4**		13	6
3-hydroxy-4,5-dimethylfuran-2(5*H*)-one (sotolone)	**X1**	0.5[14]	10	6
3-mercapto-3-methylbutyl formate	**T5**		15	5
2-methoxyphenol (guaiacol)	**X2**	12[14]	14	5
trimethylpyrazine	**P4**	9.6[18]	10	5
unknown LRI 981	**X3**		12	4
2-isopropyl-3-methoxyprazine	**M3**	0.001[14]	15	3
2,3-butanedione	**X4**	1[14]	15	2
4-ethylguaiacol	**NT1**	4[14]	10	0
(E)-β-damascenone	**NT2**	1[14]	9	0

T = thiol, P = pyrazine (trisubstituted), D = disulfide, M = methoxypyrazine, X = unclassified, NT = non-trigger

Figures

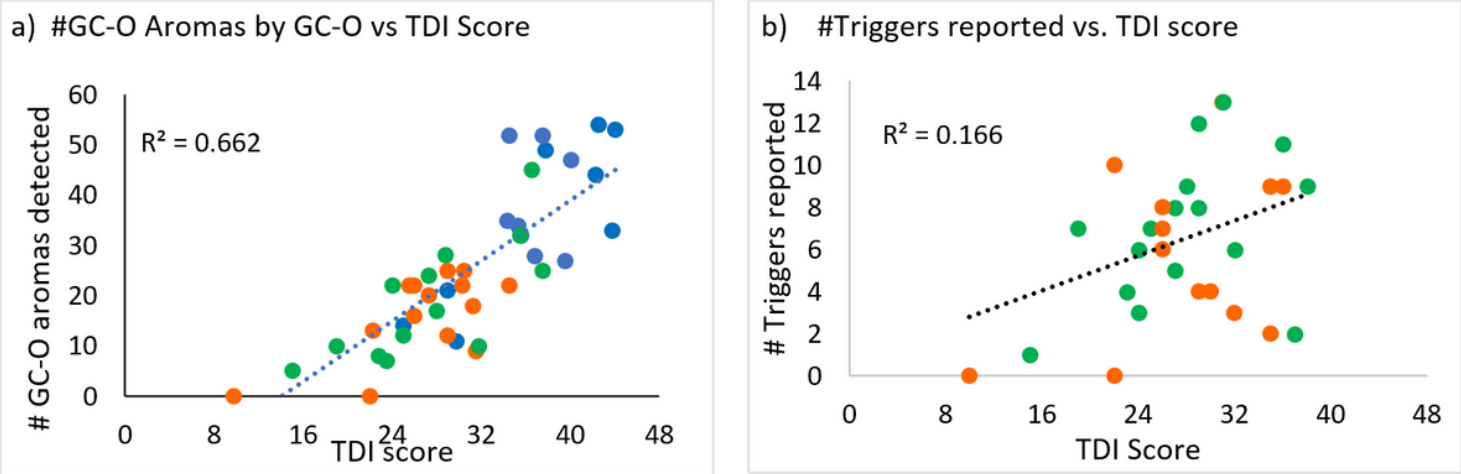

Figure 1

Correlations between olfactory function and GC-O. a): Correlation between TDI score and number of aromas detected at the GC odour-port. b): Relationship between number of triggers detected in the coffee extract and TDI score. In both figures, non-parosmic participants = blue, pre-COVID-19 parosmic participants = orange, post-COVID-19 parosmic participants = green.

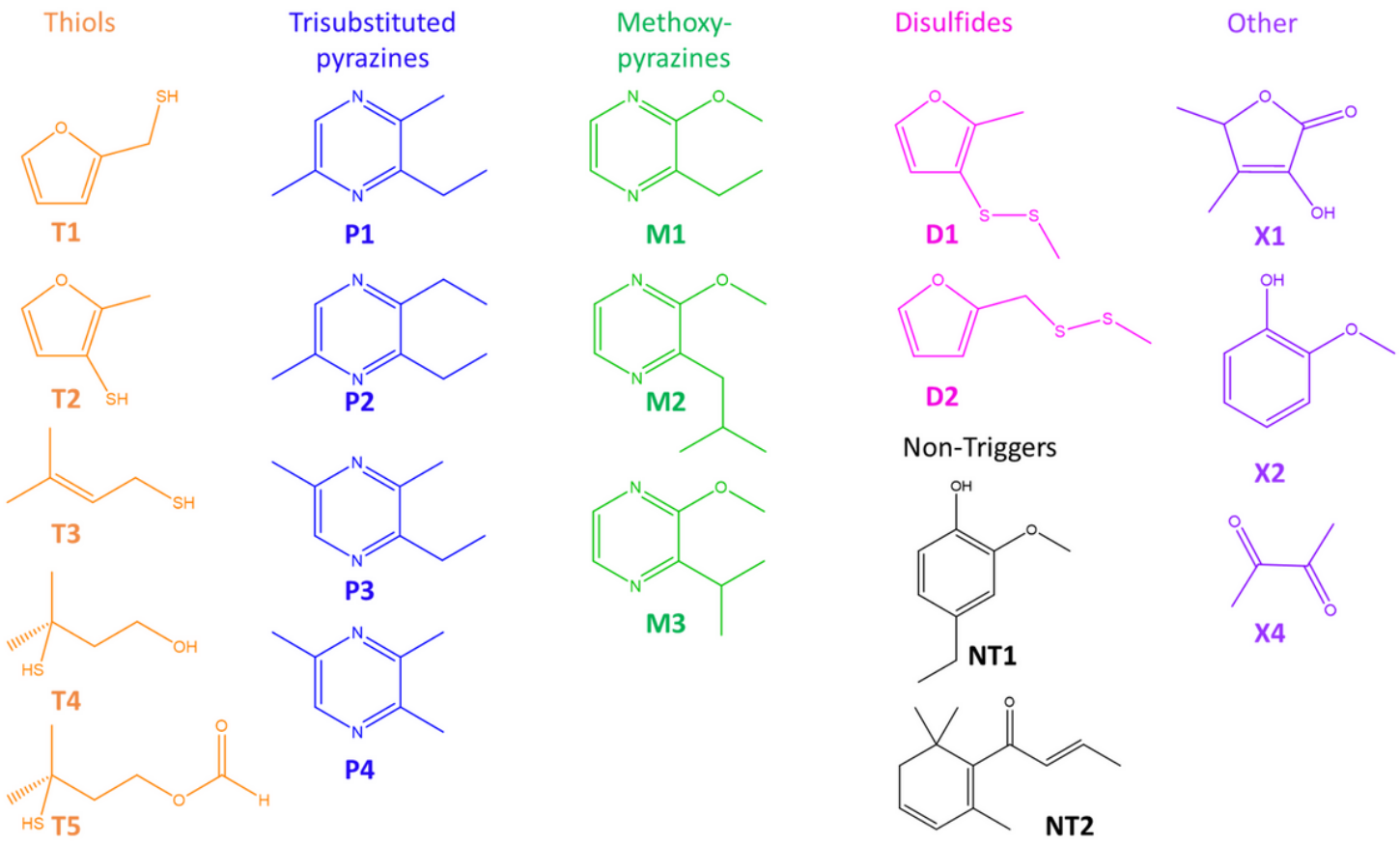

Figure 2

Structures of the most frequently detected compounds.

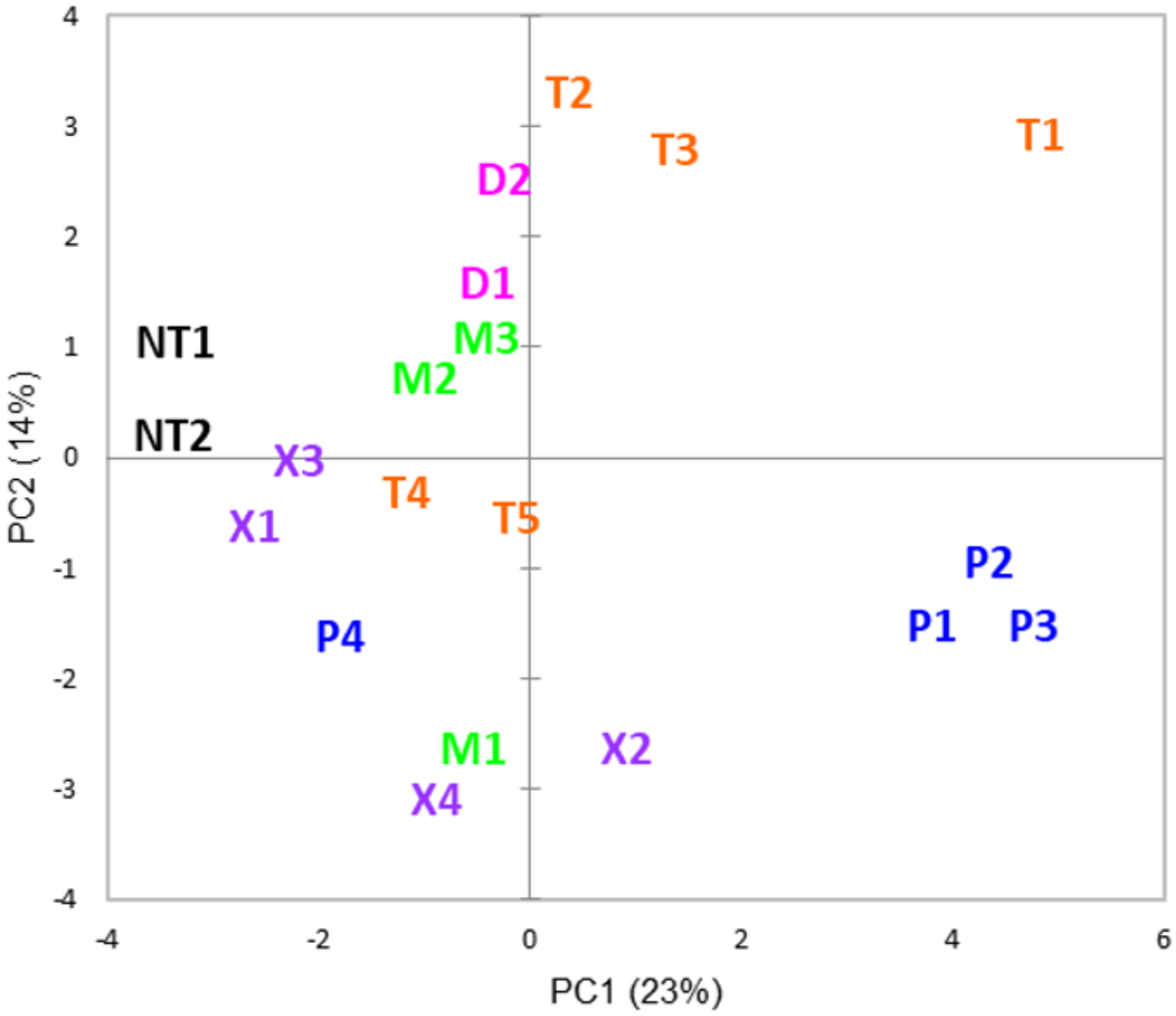

Figure 3

Principal component plot (PC1 vs. PC2) for intensity of 20 most frequently detected compounds.

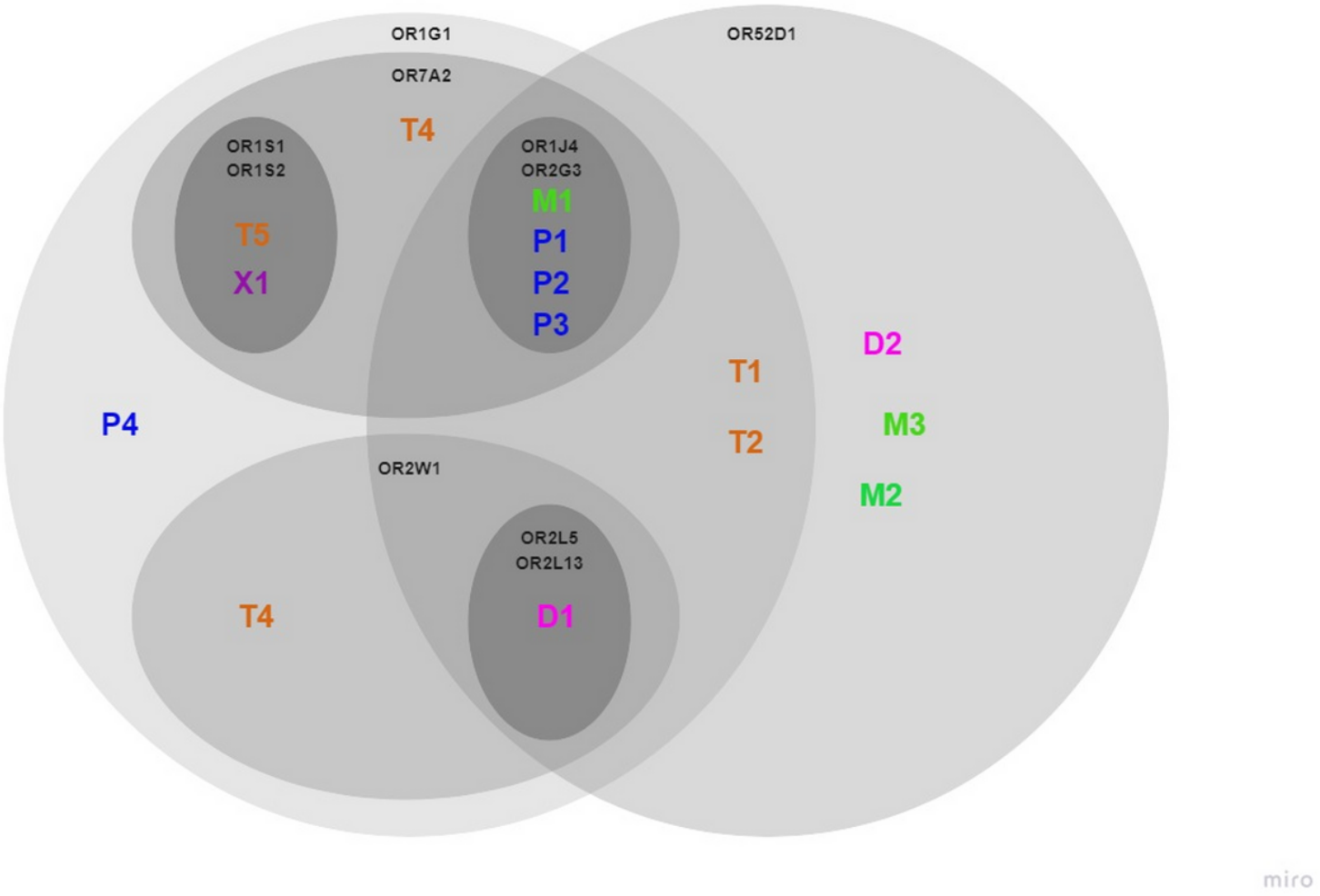

Figure 4

Venn diagram showing molecular triggers and their known odour receptors if known (from ODOractor20). Compounds are colour coded according to Figure 2. Triggers within OR1J4 and OR2G3 from top to bottom are 3-ethyl-2-methoxypyrazine (M1), 2-ethyl-3,6-dimethylpyrazine (P1), 2,3-diethyl-3-methylpyrazine (P2), 2-ethyl-3,5-dimethylpyrazine (P3). In addition, the following ligand OR pairs were retrieved: butanedione (X4): OR6Y1; guaiacol (X2): OR5L2, OR1L8, OR5AS1, OR8G2, OR4K15, OR5D18, OR10R2, OR5V1, OR8J1, OR6C75, OR1F1, OR8H2, OR1J2, OR7G1, OR1E3; 3-methyl-2-butene-1-thiol (T3): OR1L3.

Supplementary Files

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