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Characterisation of Physiological Responses to Odours in Autism Spectrum Disorders: A Preliminary Study

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Abstract: Abnormal sensory perception is among the earliest symptoms of autism spectrum disorders (ASD). Despite mixed findings, olfactory perception seems to be altered in ASD. There is also evidence that automatic responses to odours can serve as biomarkers of ASD. However, this potential use of odour-based biomarkers for ASD is still underexplored. In this study, we aimed to investigate whether physiological responses to social and non-social odours, measured with electrocardiography (ECG) and facial electromyography (EMG), can be used to characterise and predict ASD in adults. For that, we extracted 32 signal features from a previously collected database of 11 adults with ASD and 48 adults with typical development (TD). Firstly, non-parametric tests were performed, showing significant differences between the ASD and the TD groups in 10 features. Secondly, a k-nearest-neighbour classifier with a leave-one-out strategy was employed, obtaining an F1-score of 67%. Although caution is needed due to the small sample size, this study provides preliminary evidence supporting the use of physiological responses to social and non-social odours as a potential diagnostic tool for ASD in adults.

Keywords: autism spectrum disorders; odours; olfaction; facial EMG; ECG; machine learning

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1. Introduction

Autism spectrum disorder (ASD) is a developmental condition characterised by impaired social communication and interaction and restricted and repetitive patterns of behaviour, interests, or activities [1]. Moreover, ASD is often associated with abnormal sensory perception, as shown by evidence of hyper-reactivity (i.e., heightened sensitivity or response) and hypo-reactivity (i.e., diminished or absent sensitivity or response) to sensory stimuli in individuals with ASD compared to individuals with typical development (TD) (see [2]). For instance, 78.6% of the studies included in a systematic review found at least one abnormal physiological response to sensory stimuli in ASD [3]. Similarly, a qualitative study demonstrated that adults with ASD often report unusual physical experiences (e.g., pain, discomfort) to sensory stimuli, such as olfactory stimuli [4]. These abnormalities in sensory perception are linked to social, behavioural, and cognitive deficits in ASD (e.g., [5,6]) and are among its earliest markers, which are observed at six months of age in infants who are later diagnosed with ASD [7]. In addition, it has recently been shown that responsiveness to sensory stimuli can help to classify ASD individuals, thus overcoming some of the limitations of traditional diagnostic methods, namely their subjective assessment, lack of ecological validity, and relation to linguistic abilities (e.g., [8]).

While much attention has been paid to visual, auditory, and tactile perception over the last few decades, only recently has olfaction become a subject of interest in ASD (see [9]). Olfaction is strongly involved in various vital functions, such as the detection and identification of potential health hazards (e.g., fire, spoiled food), nutrition and eating behaviour, and social communication (see [10]). Olfaction is also closely linked to emotion processing due to its association with primary emotional brain structures (see [11]). Furthermore, odours are known to induce physiological responses, which can represent subtle changes in emotional states (see [12]). For instance, He et al. [13] found a higher averaged heart rate and skin conductance when adults with TD were exposed to unpleasant vs. pleasant non-social odours—henceforth "common odours" (CO). Additionally, Delplanque et al. [14] reported a higher percentage of activity over the facial medial frontalis muscle (involved in raising the eyebrows) and corrugator supercilii muscle (corresponding to frowning) in response to unpleasant compared with pleasant CO, with no difference between unpleasant and pleasant CO over the facial zygomaticus major muscle (involved in smiling). A similar effect was observed by Bensafi et al. [15] with a decrease in facial corrugator muscle activity as pleasantness increased.

Concerning social odours, several studies demonstrated that individuals with TD exposed to emotional body odours (BO) (e.g., samples of armpit sweat from other individuals with TD, previously collected under distinct emotional states) exhibit physiological responses that somehow mimic the emotional state of the the person who donated the BO, namely in facial muscle activity (e.g., [16,17]) and cardiac activity (e.g., [18]).

Available evidence seems to suggest that olfactory perception is altered in ASD, but results are highly inconsistent, which may reflect the dual pattern of atypical sensory perception in ASD, but also the clinical, demographic (e.g., age), and methodological heterogeneity between studies (see [19,20]). For instance, while some authors reported intact olfactory sensitivity in ASD (e.g., [21,22]), others found decreased (e.g., [23]) or even enhanced olfactory sensitivity in individuals with ASD than individuals with TD (e.g., [24]). Furthermore, it has been proposed that ASD and non-ASD neurodevelopmental disorders have distinct patterns of olfactory processing [25], and some authors argue that olfactory changes might be a relevant aspect to consider in the development of psychosocial interventions (see [9]). Nevertheless, studies on physiological responses to odour stimuli in ASD are still scarce and utterly different in terms of population and odour type, thus not allowing direct comparisons (e.g., [26–28]). For instance, Legisa et al. [27] found comparable facial muscular behaviour (measured with action units) and cardic and electrodermal activities in children with high-functioning ASD and children with TD exposed to CO. However, while children with TD showed a congruency between their facial expression and hedonic judgment of the odours, this effect was less frequent in children with ASD. The authors suggested that children with ASD might have difficulties reporting their emotional response to olfactory stimuli, while their automatic responses and facial behaviour might be preserved [27].

Of foremost relevance to the present study, Rozenkrantz et al. [28] proposed that automatic responses to odour stimuli might be a potential biomarker of ASD. In this study, 18 children with ASD (mean age = 7.0) and 18 children with ASD (mean age = 6.7) were exposed to pleasant (rose or shampoo) and unpleasant (sour milk or rotten fish) CO while they were viewing a cartoon. Sniff responses were collected as a measure of automatic olfactory perception, using a pediatric olfactometer that allowed to simultaneously deliver the odours and measure nasal airflow. Firstly, while children with TD adjusted their sniff response based on odour valence, this effect was not observed in children with ASD. Secondly, abnormal sniff response was associated with the severity of social impairment in ASD, suggesting that altered sensory coordination in sniff response is related to abnormal olfactory perception, which in turn might contribute to social impairment in ASD. Lastly, using a leave-one-out analysis with data on sniff volume and duration, the authors were able to classify 17 of the 18 children with TD and 12 of the 18 children with ASD (i.e., 81% accuracy). Later, 20 adults with ASD and 20 adults with TD were exposed to

fearful BO during two experimental tasks: the Faces task, in which participants rated the fearfulness of 27 faces, and the emotional Stroop task, in which participants indicated the colour of emotional words. Electrodermal activity (EDA), nasal airflow, and heart rate were collected [26]. While fearful BO led to a significant reduction in nasal inhalation in both groups, the authors observed a dissociation in EDA: EDA increased significantly in adults with TD but not in adults with ASD. Finally, using a discriminant-analysis classifier, 16 of the 20 adults with TD and 12 of the 20 adults with ASD were correctly classified (i.e., 70% of accuracy). These findings are particularly relevant because they provide a novel view on the use of olfaction as a potential diagnostic tool in ASD that does not require instructions, attention, and explicit perception [29]. Using functional magnetic resonance imaging, differences in olfaction between 18 adults with ASD and 18 TD controls were investigated [23]. After testing odour threshold and identification, individuals underwent a structural scan as well as functional scanning while perceiving two different odours. Multivariate analysis using the Support Vector Machine classification technique was performed to differentiate odour-evoked neural patterns in each region of interest between the two groups. Results showed that individuals with ASD had decreased function for odour thresholds and identification and decreased activation in the piriform cortex, suggesting that alterations in olfaction in ASD are already present in the primary olfactory cortex.

In the present study, we aim to extend previous findings [26,28] on odour-related biomarkers of ASD by providing preliminary evidence of the potential use of physiological responses to classify ASD in adults, using both social and non-social odours. For that, we used a database, previously collected by members of our research team, containing electrocardiogram (ECG) and facial electromyography (EMG) signals of adults with ASD and TD, while they were being exposed to positively, negatively and neutrally valenced CO and BO [30]. The ECG is the electrical response of the heart muscles to its movement. It presents a cyclic pattern with an almost standard representation which is composed of three types of waves: the P wave, responsible for atrial depolarization; the QRS complex, responsible for ventricular depolarization; and the T wave, responsible for ventricular repolarization [31]. The shape of the ECG can reveal not only heart problems but also variations in the heartbeat induced by distinct emotional states [32]. There is substantial evidence that depending on how an emotional stimulus is presented, cardiovascular activity can differ significantly based on both the valence and intensity of the stimulus [31]. EMG, on the other hand, is a technique for evaluating and recording the electrical activity generated by muscle movement [33]. Here, EMG was used to monitor facial expressions based on the activation of facial muscles associated with different emotional states [34], specifically the zygomaticus major muscle, associated with happiness, and the frontalis and corrugator supercilii muscles, associated with fear [35].

To explore physiological responses among ASD and TD during olfactory stimulation, several features were extracted from ECG and facial EMG signals, and their potential to differentiate between both groups was analysed. Moreover, although the main focus of our work is concerned with the characterisation of physiological responses to olfactory stimuli, we also attained a first approach to predict ADS based on those. Thus, two main research questions were raised: (1) Are there significant physiological differences between the the ASD and TD groups? (2) Is it possible to predict adults with ASD based on ECG and facial EMG signals collected during olfactory stimulation?

2. Dataset

Data collection was performed at the Faculty of Medicine of the University of Tübingen, Germany [30]. All procedures were approved by the Ethics Committee (956/2018BO2) and conducted under the Declaration of Helsinki and the American Psychological Association.

2.1. Sample Characterisation

The database contained data from 48 adults with TD (24 women, 50%) and 11 adults with ASD (2 women, 18.18%), aged between 19 and 59 years. Sociodemographic characteri-

sation is presented in Table 1. Only participants meeting the inclusion/exclusion criteria were included in the database: being aged between 18 and 59 years; being Caucasian; speaking fluent German; being capable of understanding and following instructions; having no illnesses problems (e.g., common flue), or regular medication intake affecting the sense of smell; not being pregnant or lactating at the time of assessment, and not having participated in the BO collection. Handedness was assessed with the Edinburgh Handedness Inventory (EHI) [36], with all but one participant being considered right-handed.

The ASD diagnosis was based on the DSM criteria (mostly 5th edition) by independent clinicians and ASD symptoms were evaluated with the German version of the Autism Diagnostic Observation Schedule (ADOS) [37]. Lastly, adults with TD had no mental illness, as confirmed with the German version of the Structured Clinical Interview (SCID-I) [38], and no first-degree relative diagnosed with psychotic disorder or ASD, as self-stated.

Table 1. Characterisation of the sample. Notes. EHI, Edinburgh Handedness Inventory. [1] Comparisons between groups were performed with Mann–Whitney Test (for continuous variables) and Fisher's Exact Test or Chi-Squared Test (for categorical variables). * $p < 0.050$; ** $p < 0.010$; *** $p < 0.001$.

		ASD ***n* = 11**	**TD** ***n* = 48**	***p*-Value [1]**
Age, *Mdn (IQR)*		36.0 (17.5)	24.50 (9.5)	0.002 **
Sex, *n* (%)	Women	2 (18.2)	24 (50.0)	0.091
	Men	9 (81.8)	24 (50.0)	
Nationality, *n* (%)	German	10 (90.9)	45 (93.8)	0.168
	Swiss	0 (0.0)	2 (4.2)	
	Hungarian	1 (9.1)	0 (0.0)	
	Bulgarian	0 (0.0)	1 (2.1)	
Working status, *n* (%)	Full-time worker	5 (45.4)	6 (12.5)	0.001 ***
	Part-time worker	2 (18.2)	3 (6.3)	
	Pensioner	2 (18.2)	0 (0.0)	
	Stick leave	1 (9.1)	0 (0.0)	
	Student	1 (9.1)	37 (77.1)	
	Working student	0 (0.0)	1 (2.1)	
	Unemployed	0 (0.0)	1 (2.1)	
Smoking habits, *n* (%)	Non-smoker	8 (72.7)	36 (75.0)	0.114
	1–5 cigarettes/week	0 (0.0)	8 (16.7)	
	5–10 cigarettes/week	0 (0.0)	0 (0.0)	
	10–15 cigarettes/week	0 (0.0)	1 (2.1)	
	Daily	3 (27.3)	3 (6.3)	
Hormonal contraceptive, *n* (%)	Yes	0 (0.0)	10 (41.7)	0.395
	No	2 (100.0)	12 (50.0)	
	Menopause	0 (0.0)	2 (8.3)	
EHI, *Mdn (IQR)*		100 (0)	100 (0)	0.894
Sniffin' Sticks, *Mdn (IQR)*	Threshold	5.2 (2.4)	7.6 (2.2)	0.008 **
	Discrimination	11.0 (1.5)	13.0 (2.0)	0.040 *
	Identification	13.0 (1.0)	14.0 (2.0)	0.176
	Total	27.7 (2.7)	33.2 (3.6)	0.002 **

Alongside the ADOS (for the ASD group) and the SCID-I (for the TD group), all participants completed a sociodemographic questionnaire and the Sniffin' Sticks Test (Burghart Medizintechnik, Germany). The Sniffin' Sticks Test is a widely used instrument to measure olfactory abilities (see [39]). The test comprises three subtests (odour threshold, identification, and discrimination) and uses odour-dispensing pens with 4 mL of odourant fluid or odourant substance dissolved in propylene glycol. In addition to scores for each subtest (maximum of 16), the Sniffin' Sticks Test contains a final score, ranging from 1 to 48. Hyposmia and functional anosmia are established, on the reference group of 21 to 30 years,

at a final score of less than 30.75 and 16 points, respectively (see [40]). Data concerning the Sniffin' Sticks Test are described in Table 1.

2.2. Odour Stimuli

BO were samples of armpit sweat from 10 healthy women and nine healthy men (henceforth "donors") collected across three individual sessions (separated by at least one week). To avoid odour contamination, donors were asked to follow a strict hygiene, dietary, and behavioural protocol in the two days before BO collection (e.g., avoid sites with intense odours, avoid eating excessive flavoured food, use odours-free hygiene products), similar to previous studies (e.g., [16–18]). Each session included a 5-min neutral film clip (Baseline), which was followed by a 30-min set of film clips from comedy movies (to induce happiness: positive BO), horror movies (to induce fear: negative BO), or documentaries about nature and space (to induce a neutral state: neutral BO). The order of film clips presented in each set was fixed, from the least intense to the most intense clip (in case of the fearful and happy sets), to keep a constant state of activation. All film clips were previously shown to induce the expected emotion (e.g., [41–43]); this was also confirmed in the current dataset based on the analysis of the donors' subjective emotional state. Donors were not aware of emotional induction (single-blind experiment), and the order of the sessions was randomised for each donor. Armpit sweat was collected during film-clips visualisation using sterilised absorbent pads (Lohmann and Rauscher, Germany), which were placed under each armpit and secured with an additional pad and a hypoallergenic tape. To avoid sweat contamination, a strict protocol for BO collection was implemented (e.g., use of steristerilizedes by the researcher handling the sweat samples, and sterilisation of reusable laboratory material). After film clips, each pad was carefully removed and saved in individual vials (Carl Roth, Germany). Vials were labelled and kept in a freezer at -21° until the day of the experimental session. At the end of their participation, each donor was fully debriefed about the purposes of the sweat collection and given 10€ per hour.

Concerning CO, positive, negative, and neutral CO were selected based on the literature (e.g., [44,45]) and experts' consensus. The positive CO was rose, 2-phenylethanol alcohol (28.16% v/v in propylene glycol, Sigma-Aldrich, Germany). The negative CO was faeces, skatole (0.04% w/v in propylene glycol, Sigma-Aldrich, Germany). The neutral CO was grass, cis-3-Hexen-1-ol (3.61% v/v in propylene glycol, Sigma-Aldrich, Germany). Volume percentage was defined through pilot testing to ensure that all COs were similar in terms of perceived intensity [30].

Before odour exposition, all stimuli were placed in individual amber vials (63 mm of height by 46 mm of diameter, Bauchblueten, Germany). In the case of BO, four pad pieces of distinct donors (same-sex and valence) were randomly combined to create a "super-donor", thus reducing inter-individual variability (e.g., [16,17]), with two coming from left armpits and two coming from right armpits. Each super-donor was prepared one hour before the first experimental session of the day. Each receiver was exposed to the same two super-donors (one female and one male) across the three emotion conditions. Concerning CO, 150 µL of each CO was applied to an absorbent pad to mimic BO presentation approximately one hour before the first experimental session of the day. Each pad was cut into four pieces, and the four pieces were placed in an amber vial. Lastly, a "no odour" condition was created, containing four pieces of an absorbent pad, which were also placed in an amber vial.

2.3. Procedures

All participants underwent an initial interview, in which they were informed about the study, including the need to follow a hygiene, dietary, and behavioural protocol three hours before the experimental session (e.g., refrain from using perfume). After giving their informed consent, participants completed a sociodemographic questionnaire, the EHI, and the Sniffin' Sticks, and they were assessed with the ADOS or the SCID-I. To avoid acute changes in olfactory perception and physiological responses, participants

were recommended to postpone the experimental session in case they were feeling sick (e.g., common flu) or had any unexpected highly emotional event the days before.

Each participant was tested individually by two researchers: one responsible for changing the vials in each trial and the other for monitoring signal collection. After checking protocol compliance, participants were seated 40 cm in front of a computer screen (LG 24MB37PM-B computer screen; 1920 by 1080 pixels; 60 Hz refresh rate), and their head was positioned on a chinrest. The chinrest included a vial holder to support the vials (2 cm below participants' nostrils). Then, the researcher cleaned the portion of the skin where the electrodes would be placed with ethyl alcohol (70%) and attached the disposable electrodes with their corresponding conductance gel. Finally, signals were checked without any reference to emotions.

Next, participants completed the Odour Perception Task while their physiological data were being recorded. The Odour Perception Task began with a 5-min Baseline, in which participants watched a 4-min neutral film clip with landscape scenes from the documentary *Deutschland von Oben*, without any odour stimulation. After the film clip, they were asked to rate how angry, calm, disgusted, fearful, and happy they were, using Visual Analog Scales (VAS) from 0 (not at all) to 100 (extremely), in a randomised order. Odours were then presented in four blocks: two blocks with CO and two blocks with BO (one female and one male). The order of the blocks was randomised for each participant. Each block was divided into two sub-blocks (of the same odour type) with four stimuli each (Figure 1). These stimuli were semi-randomly presented: each sub-block was initiated by the "no odour" stimulus, which was followed by a random presentation of the positive, negative, and neutral odour (of the same odour type). Each odour was presented for 5 s as prolonged exposure can lead to adaptation (e.g., [46]). In each trial, participants were asked to smell the odour and, after odour stimulation, to rate how intense, familiar, pleasant, and arousing the odour was, again using the VAS scales (0–100), randomly presented. Preliminary findings of the VAS are reported elsewhere [30]. Odours within the same block were separated by approximately 30 s intervals. During this period, the researcher removed the vial from the vial holder and prepared the presentation of the next vial. All vials not being used were kept closed at about 100 cm from the participant.

A 5-min washout period (three washout periods in total) was applied between each odour block. During this period, participants were exposed to a new 4-min neutral film clip from the documentary Deutschland von Oben, without any odour stimulation, and were again asked to rate how angry, calm, disgusted, fearful, and happy they were. The order of neutral film clips exhibited in baseline and washout periods was randomised for each participant. The task was concluded after the fourth odour block. At the end of the task, electrodes were detached, and participants were debriefed about the purpose of the study and were given 10€ per hour. All visual stimuli (film clips and VAS) were programmed in, and displayed with, Open Sesame 3.2.6 (Mathot et al., 2012).

2.4. Signal Recording

Physiological data were recorded with SOMNOscreenTM plus 6120 (SOMNOmedics, GmbH, Germany) using the DOMINO software (version 3.0.0.1; supplied with the SOMNOscreen), with a sampling rate of 256 Hz. For ECG, a bipolar electrode was attached to intercostal space (ICS) 4 right and ICS 2 parasternal left. For EMG, two electrodes were attached to each muscle of interest (with a distance of 1 cm), namely, zygomaticus major, medial frontalis, and corrugator supercilii. The guidelines by Fridlund and Cacioppo [47] were followed to place the electrodes. Only the left side was monitored, since right-handed individuals exhibit stronger emotional reactions on the left side of the face (e.g., [33]).

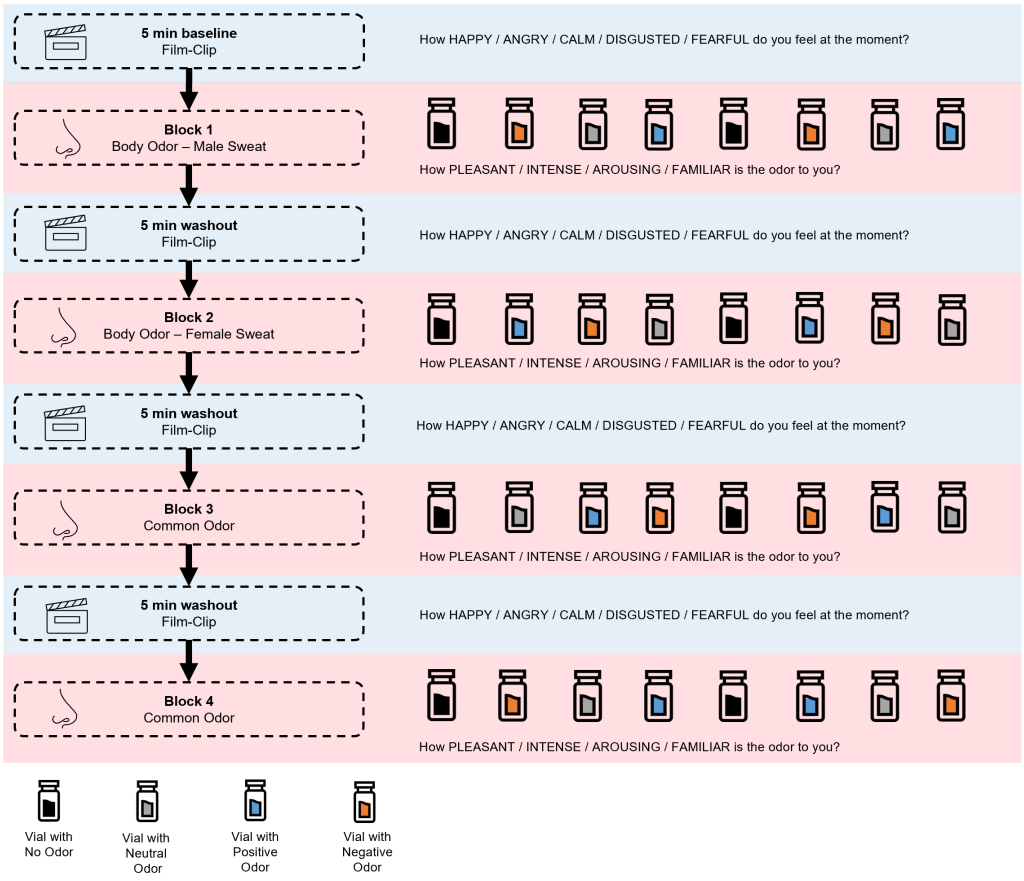

Figure 1. Odour Perception Task.

3. Methodology

Our first step consisted of analysing, reorganising, and filtering the ECG and EMG signals. Next, we used the NeuroKit2 [48] to extract 32 features from the signals. These features were separated into different groups based on the triggers defined during data collection. After these steps, we applied the sliding windows (SW) method, with windows of 5 s and 50% overlap. The relatively short windows size was due to the short period of odour exposition. Thereafter, statistical tests were applied to the medians to find which features allow distinguishing between groups. Finally, we implemented a classifier using a leave-one-out cross-validation (LOOCV) analysis, with the attributes showing a significant difference between the ASD and TD groups. Data pre-processing and processing were performed with Python version 3.8.8 using Spyder version 4.2.5 [49] and packages NeuroKit2 [48], SciPy (version 1.8.1) and scikit-learn (version 0.24.1).

3.1. Pre-Processing

After the inspection of the signal characteristics in the frequency domain, the filter parameters and type were defined accordingly to the frequencies of interest. In terms of the EMG signals, analysis of the periodograms revealed that the majority of the content of interest was above 20 Hz. Considering the absolute and relative error, coefficient of variation, and signal-to-noise ratio (SNR), the filter with the best response to the frequency of interest was the Butterworth high-pass filter of order 4, with a cutoff frequency of 20 Hz (notice that the electrical interference, at 50 Hz, was removed by the signal collection software). Analysis of the ECG signals' periodograms revealed that the content of interest was less than 40 Hz. Several filters were then applied, and based on the absolute and relative error, and coefficient of variation, we applied a Butterworth band-pass filter of order 4 for the pass band (0.5 Hz, 40 Hz).

3.2. Processing

3.2.1. Feature Extraction and Normalisation

A total of 32 features were extracted directly by the NeuroKit2 package or by further calculations, as explained in Table 2. Subsequently, the slope between each pair of successive peaks was calculated from parameter m (slope) based on linear regression. This allows us to understand the abruptness of the oscillations between peaks.

Table 2. Features extracted from the signals. Notes. EMG_front, medial frontalis muscle; EMG_zygo, zygomaticus major muscle; EMG_corr, corrugator supercilii muscle.

Feature	Meaning
ECG_Clean	Cleaned signal after filtering.
ECG_Rate	Heart rate values interpolated between the R-peaks.
ECG_P_Interval ECG_Q_Interval ECG_R_Interval ECG_S_Interval ECG_T_Interval	Distance between subsequent peaks in seconds (for P, Q, R, S, and T peaks, respectively).
ECG_P_Peaks ECG_Q_Peaks ECG_R_Peaks ECG_S_Peaks ECG_T_Peaks	Peaks amplitude (for P, Q, R, S, and T peaks, respectively).
ECG_P_Slope ECG_Q_Slope ECG_R_Slope ECG_S_Slope ECG_T_Slope	Slope between subsequent peaks (for P, Q, R, S, and T peaks, respectively).
EMG_front_Clean EMG_zygo_Clean EMG_corr_Clean	Cleaned EMG signals after filtering.
EMG_front_Amplitude EMG_zygo_Amplitude EMG_corr_Amplitude	Amplitude EMG signals (activation level).
EMG_front_IntervalActivation EMG_zygo_IntervalActivation EMG_corr_IntervalActivation	Distance between a corresponding onset and offset in seconds, for each of the EMG signals.
EMG_front_AmpOnset EMG_zygo_AmpOnset EMG_corr_AmpOnset	Onset amplitude, for each of the EMG signals.
EMG_front_AmpOffset EMG_zygo_AmpOffset EMG_corr_AmpOffset	Offset amplitude, for each of the EMG signals.

Afterwards, the feature values were normalised in order to reduce intra-subject variability and were based on the baseline block (at the beginning of the task). The calculation was based on Equation (1), where x is the non-normalised value, μ is the mean of the baseline for the participant in question, σ is the standard deviation of the baseline, and Z is the normalised value.

$$Z = \frac{x - \mu}{\sigma} \quad (1)$$

3.2.2. Triggers Application

Extracted features were segmented to divide the signal by phases (baseline, block 1, block 2, block 3, block 4). Thus, according to the respective triggers, signals were grouped by blocks of odour type, and the block corresponding to the baseline was stored in parallel.

In this regard, beyond the baseline, the signal was divided into two distinct groups, each corresponding to a different odour type: CO and BO (Figure 2). The first group corresponds to the interval covering the two blocks in which the participant was exposed to CO, and the second group covers the two blocks in which the participant was exposed to BO.

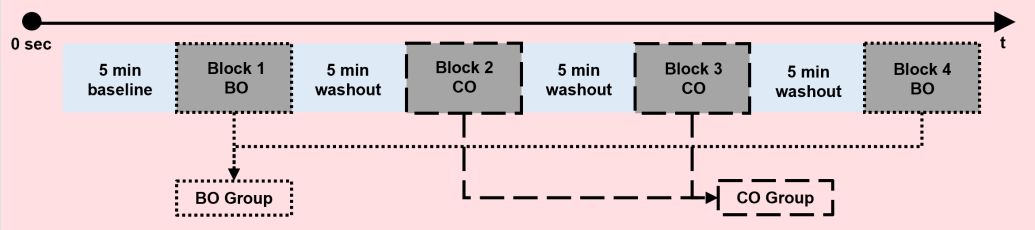

Figure 2. Division of signal into two distinct groups: CO and BO.

3.2.3. Sliding Windows

Following the application of the triggers, a 5 s SW with 50% overlap was applied. The SW approach consists of sectioning signals into fixed-size time windows, which may or may not be overlapped, thus reducing temporal complexity [50]. SW is particularly advantageous when dealing with data with short collection intervals and small sample sizes by allowing the collection of more data than non-overlapping SW (which in turn makes it less likely to miss significant events). Having this in consideration, overlapping SW were applied to all considered features, resulting in several time-series along the blocks. In the EMG activation interval calculation (EMG_front_IntervalActivation, EMG_zygo_IntervalActivation and EMG_corr_IntervalActivation), only intervals whose onset occurred within the sliding window value range were taken into account. Since the interval between odour presentation and blocks varied between participants (e.g., no time limit for the subjective assessment of the odours), the number of SW also varied. Therefore, each data segment was described by the median values of the features.

3.2.4. Data Analysis

Since the Shaprio–Wilk test showed that data were not normally distributed, we performed Mann–Whitney tests for each of the 32 features in each block to explore which physiological responses in which odour blocks presented significant differences between ASD and TD groups.

3.2.5. Physiological Based ASD Prediction

To classify ASD, we used a Machine Learning (ML) technique with a k-nearest neighbours (KNN) classifier based on a LOOCV strategy. For this purpose, we used only the features able to distinguish physiological responses from ASD and TD groups (according to the results from the Mann–Whitney tests). Regarding the prediction, we only considered the CO and BO data because the inclusion of baseline did not improve the performance of the KNN (as shown in preliminary analysis).

KNN is a supervised ML technique that can be applied to classification and regression problems. The primary objective of the KNN classification technique is to determine the class of a new case based on the classes of the k most comparable items in the database [51]. In other words, the KNN method seeks out elements with the highest degree of similarity to a query element, where the degree of similarity is determined by a distance function, to classify that point. Each database element has an associated label (class) [51].

LOOCV is a special case of k-fold cross-validation, where the model is trained on almost all data in each iteration, except for a single observation, and tested on that single observation. A precision estimate derived by LOOCV is almost impartial but has a huge variance, resulting in inaccurate estimations. Nonetheless, this technique is often used when the dataset size is small [52]. Considering the small number of participants, especially from the ASD group, this was the evaluation strategy for training and testing.

The True Positive (i.e., correct classification of a participant as belonging to the ASD group), True Negative (i.e., correct classification of a participant as belonging to the TD group), False Positive (i.e., incorrect classification of a participant as belonging to the ASD group), and False Negative (i.e., incorrect classification of a participant as belonging to the TD group) metrics were extracted from the contingency table. In a binary decision problem, a classifier assigns positive or negative labels to examples; in this case, being the class of interest, ASD are positive examples and TD are negative examples [53].

The respective precision, recall, negative predictive value, specificity, accuracy, and F1-score were also calculated:

- Precision = $TP/(TP + FP)$ is the proportion of instances predicted as Positive (ASD) that were correct; the optimal value for precision is 1 [54].
- Recall = $TP/(TP + FN)$ is the proportion of instances labelled as Positive (ASD) that were correctly predicted; the optimal value for the recall is 1 [54].
- Negative Predictive Value = $TN/(TN + FN)$ is the proportion of instances predicted as Negative (TD) that were correct; the optimal negative predictive value is 1 [54].
- Specificity = $TN/(TN + FP)$ is the proportion of instances labelled as Negative (TD) that were correctly predicted; the optimal value for specificity is 1 [54].
- Accuracy = $(TP + TN)/(TP + TN + FP + FN)$ is the ratio of the number of correctly classified samples to the total number of samples, and its optimal value is 1 [55].
- F1-score = $(2xPrecisionxRecall)/(Precision + Recall)$ is defined as the harmonic mean of precision and recall. F1-score has a range of [0, 1], with TP = 0 (i.e., when all of the positive samples are incorrectly categorised) as its lowest and FN = FP = 0 (perfect classification) as its maximum. F1 differs from accuracy in two key ways: it is independent of TN, and it is not symmetric for class switching [55].

4. Results

The Mann–Whitney test was performed on data aggregated by odour type, including the median values for each participant's response to CO, BO, and baseline. Under a significance level of 0.05, the Mann–Whitney tests revealed significant differences between the ASD and the TD groups for six ECG features and four EMG features. Table 3 displays the obtained features.

As an illustration of the distinction between features, Figure 3 represents violin plots for the ECG_P_Peaks, a feature in which the Mann–Whitney test revealed significant differences between the ASD and TD groups (ECG_P_Peaks) and for the ECG_Q_Slope, which is a feature that did not reveal significant differences between the groups.

Table 3. The features for which the Mann–Whitney test revealed a significant difference between the ASD and TD groups.

Features
ECG_Clean
ECG_P_Peaks
ECG_P_Slope
ECG_S_Slope
ECG_T_Peaks
ECG_T_Slope
EMG_corr_OnOff
EMG_corr_Amplitude
EMG_front_Amplitude
EMG_corr_Clean

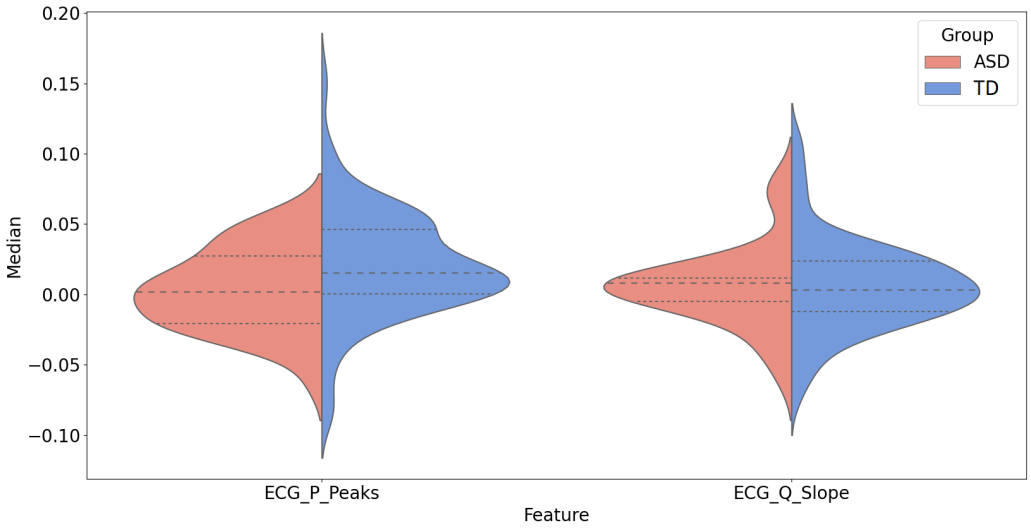

Figure 3. Violin plots of the ECG_P_Peaks and ECG_Q_Slope for the ASD and the TD groups.

Physiological-Based ASD Prediction

Using a LOOCV strategy, each participant was predicted as ASD or TD by a KNN classifier trained by other participants. Figure 4 presents the obtained contingency table for all the LOOCV and the performance metrics.

The best result achieved after testing the classifier for various values of k is shown in Figure 4, which corresponds to k = 3. It was feasible to accurately categorise 7/11 ASD subjects (TP), while the remaining four were incorrectly classified as TD (FN). In contrast, 45/48 TD participants were correctly predicted (TN), while three were misclassified as ASD (FP). In addition to these values, it is important to emphasise the obtained F1-score, which provides a global assessment of classifier performance and is usually more useful than accuracy in imbalanced class distribution, and the Recall value, which reflects the classifier's ability to correctly classify examples of the class of interest, in this case, ASD. As illustrated in Figure 4, the F1-score value was 0.67 and the Recall value was 0.64.

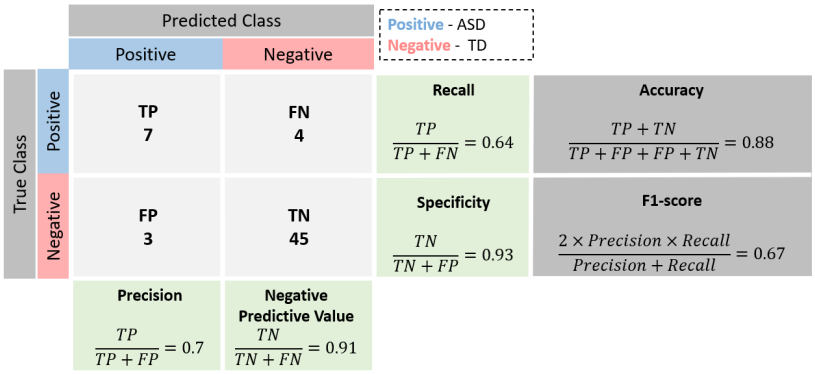

Figure 4. Contingency table and respective precision, recall, negative predictive value, specificity, accuracy, and F1-score values.

5. Discussion

In this study, we aimed to explore whether physiological responses to CO and BO can be used to distinguish and predict ASD in adult individuals. For these purposes, we analysed the ECG and facial EMG responses collected from adults with TD and adults with ASD. Considering that the potential use of autonomic responses to olfactory stimuli as biomarkers is an under-explored hypothesis [26,28], this research may provide evidence supporting their use as a potential tool to support the diagnosis of ASD in adults.

A significant difference between groups emerged for 10 out of the 32 features. Particularly, the majority of facial EMG features showing a significant difference is associated with negative emotions, namely fear: medial frontalis and corrugator supercilii [35]. This raises the possibility that stimulating negative emotions via odours may induce distinctive physiological responses in adults with ASD compared to adults with TD. In this vein, Endevelt-Shapira et al. [26] found significant differences in the EDA between adults with ASD and TD, with EDA being significantly higher in adults with TD, compared to adults with ASD, exposed to fearful BO. Therefore, the present study reveals new features with the potential to establish this distinction. Moreover, taking into account the number of features, it can be concluded that the obtained results support the hypothesis that there is a significant difference between the responses of the two groups.

When predicting ASD and TD with these discriminatory features, the best result was achieved for k = 3, where it was possible to correctly classify approximately seven out of 11 participants in the ASD group, with an F1-score of 0.67. Establishing a point of comparison with other studies in the field, according to Endevelt-Shapira et al. [26], a classifier following an LOOCV was developed based on heart rate, EDA, and nasal airflow data regarding the response of TD and ASD adult subjects to fearful BO, and the results showed the correct classification of 16 out of 20 TD subjects and 12 out of 20 ASD subjects, with an accuracy of 70%. With a similar goal, the study [28] relied on oflactometer sniff–response data to pleasant and unpleasant odours to develop a classifier, which was followed by an LOOCV analysis. In this work, 17 out of 18 children with TD were correctly classified, while 12 out of 18 children with ASD were correctly classified, for an overall accuracy of 81%.

6. Conclusions and Future Work

To sum up, despite the imbalanced data (which is known to pose difficulties in classification methods), this study supports the use of physiological responses to odours to classify ASD in a sample of adult individuals. This is consistent with previous studies relying on sniff responses and EDA [26,28] and provides preliminary data on the potential use of facial EMG data as well. Since the most significant features correspond to the activity of facial muscles associated with negative emotions, the difference between ASD and TD groups may be greater when negative emotions are induced via the sense of smell. Given that these physiological changes occur at an unconscious level and are strongly

independent of language, communication, and attention, the use of ASD classifiers could aid in the development of diagnostic tools used in both clinical and research settings.

Nevertheless, several limitations need to be considered. Firstly, caution is needed when interpreting the data due to the small and unequal sample size, which lowers the performance in predicting ASD. Additionally, the clinical manifestation of ASD is highly heterogeneous, which might be particularly critical in studies with small sample sizes due to an increased likelihood of artefacts. To overcome this limitation, future studies could focus on specific subgroups of ASD (e.g., ASD with or without Speech Onset Delay) or adopt a dimensional "autistic traits" approach (as proposed by [56]). Moreover, a profound assessment of possible comorbidities was not conducted in the ASD group. This is of foremost relevance, since approximately 70% of individuals diagnosed with ASD have co-occurring medical, developmental, or psychiatric conditions, such as intellectual disability, attention-deficit hyperactivity disorder, anxiety, and depression (see [57]). A detailed clinical (and cognitive) assessment would help to clarify if the obtained outcomes are indeed explained by the ASD diagnosis or rather by an interplay between multiple conditions. Another limitation concerns the non-inclusion of a clinical control group (e.g., individuals diagnosed with schizophrenia) and, therefore, it is not possible to assure our results are limited to ASD rather than generalised to other neurodevelopmental or psychiatric conditions. It should also be considered that groups were not matched for sex, age, and cognitive abilities. There is evidence that in general, women have better olfactory abilities than men (see [58]). Moreover, de Groot et al. [59] found a facial muscle response indicative of a fearful state in women exposed to fearful BO, whereas men failed to show any emotionally differentiated response to fearful and happy BO, suggesting that women are capable of establishing emotional synchrony via the sense of smell. Concerning a dimensional approach to ASD, Barros et al. [60] found that being a woman and reporting higher attention to detail—a subscale of the Autistic Spectrum Quotient—was associated with better odour discrimination in the general population. Despite this evidence, since ASD diagnosis is more prevalent in men than in women (see [61])—a pattern also observed in our sample—most studies addressing olfaction in ASD either recruit male individuals exclusively or fail to acknowledge sex differences. Having this in mind, we cannot fully exclude a potential role of sex differences in the obtained outcomes and urge researchers to recruit larger samples, allowing them to further explore this effect.

Furthermore, it is proposed to extend the dataset to better support the classifier and enable the application to the sub-block data, as it could not be used at this time due to the short response time associated with each sub-block (30 s). Concerning the prediction goal, other ML models should be considered so that performance comparisons can be made. Moreover, feature normalisation, such as min–max normalisation, could be a future enhancement to reduce the influence of the difference in feature scales as much as possible. On the other hand, it would be essential to implement a dimensionality reduction procedure that permits the selection of only those features from which the best results can be obtained. Lastly, applying balancing techniques to mitigate the imbalance caused by the disparity between the number of participants in the ASD group (11) and the number of participants in the TD group (48), which may be negatively impacting the classifier, may also lead to improved classification results.

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