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Autistic traits impact on olfactory processing in adolescent girls with Anorexia Nervosa restricting type

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ABSTRACT

The correct functioning of the chemosensory pathway is pivotal for the attitude towards feeding. In some neuropsychiatric disorders, abnormalities of the sensory processing dramatically affect feeding behavior; however, evidences for an olfactory involvement in Anorexia Nervosa (AN) are still controversial. We administered a complete olfactory testing battery, the Sniffin' Sticks Extended Test, to a cohort of 19 girls with Restrictive Anorexia Nervosa (AN-R) and 19 healthy controls. A battery of questionnaires aiming to evaluate eating attitude, psychopathologic disorders and autistic traits was also administered. No difference was found between the two groups in any of the olfactory tasks. Despite the lack of correlation between olfaction and disease severity, however, olfactory performances were related to autistic traits in anorectic girls ($r = -0.489$, $p = 0.039$). Girls with AN-R do not appear to have an impaired olfactory function with respect to controls. However, a possible correlation between olfactory ability and autistic traits was discovered. In light of such findings, the role of possible relations between social functioning-related features and olfactory processing in AN-R is discussed.

1. Introduction

Anorexia Nervosa Restricting-type (AN-R) is a severe psychiatric disorder with a typical adolescent onset, mainly affecting young females and often accompanied by other psychiatric disorders (Herpertz-Dahlmann, 2015). AN-R is characterized by a significant low body weight due to restricted food intake, a strong and persistent fear of gaining weight and becoming fat, and a disturbance in body weight and shape's perception (APA, 2013). Overall, it is clear that patients with AN-R often show an aversion to food (Bernstein and Borson, 1986; O'Hara et al., 2015), in turn leading to a reduction of the sense of taste (Cowdrey et al., 2011). Literature hypothesize a role for the poor functioning of precise brain portions, including the anterior insula, in badly recognizing hunger signals (Oberndorfer et al., 2013), but in general it is unclear whether food aversion is linked to sensory processing, or it is rather a cause or a consequence of starvation (see Garcia-Burgos et al., 2018).

On the other hand, it is also well known that the gustatory processing (along with satiety conditions) is strictly related to the olfactory pathway (see Freitas et al., 2018).

Specifically focusing on the sense of smell, it is well known that pleasant odors activate the brain circuitry supervising the reward processing that is abnormal in AN-R (Bentz et al., 2017). An altered processing of external food stimuli and of internal taste and satiety stimuli may decrease the thresholds of food flavor and pleasure in anorectic subjects (Goldzak-Kunik et al., 2012). Therefore, several studies, in past, have attempted to clarify the role of olfaction in food aversion and weight loss, with conflicting results, possibly due to different methods employed for smell assessment or to small and heterogeneous samples of participants (Aschenbrenner et al., 2008; Dazzi et al., 2013; Schecklmann et al., 2012). For example, Schecklmann et al. (2012) detected, in a sample of AN-R patients, the absence of modifications in all the olfactory sub-domains (sensitivity to 2-phenylethanol, discrimination, identification), and a significant effect of depression and medication use, without which subjects with AN might have displayed a superior olfactory identification function. Such link between olfactory abnormalities and a number of psychological/psychiatric disorders (including schizophrenia, depression, bipolar disorder and obsessive-compulsive disorder) have been reported in literature (Atanasova et al., 2008; Goldzak-Kunik et al., 2012; Kopala et al., 1995). Conversely,

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Table 1
Demographic, clinical characteristics and comparison between groups (*: statistical significance at $p < 0.05$; **: statistical significance at $p < 0.01$).

	AN-R	Healthy controls	*p* value
N	19	19	1
Age (years, mean ± SD; range)	15.4 ± 2.5 (12–21)	14.7 ± 2.9 (11–21)	0.438
Gender (M/F)	0/19	0/19	1
Body mass index (kg/m^2, mean ± SD)	17.01 ± 1.7	20.3 ± 2.6	0.001**
Eating disorder risk composite of the EDI-3 (percentile)	68.8 ± 21.3	41.2 ± 32.9	0.012*
Depression total score (CDI)	18.6 ± 11.1	7.1 ± 5.2	<0.001**
Antidepressants/atypical neuroleptics (%)	36.8	0	0.004**
Right-handedness (%)	100	84.2	0.082

Roessner et al. (2005), investigating a sample of slightly elder anorexic girls, found a decreased sensitivity to n-butanol and discrimination, with a preserved ability in identifying odors. Moreover, the impact of age on the olfactory function has already been investigated in typical subjects (Bastos et al., 2015), but it deserves closer examination in individuals with AN.

In summary, some works reported that patients with AN experience lower discrimination abilities and an impaired (either hyper- or hypo-) sensitivity, which might be normalized by weight and disorder recovery (Dazzi et al., 2013), while other authors failed to find any difference in olfactory abilities between AN patients and healthy controls, raising uncertainty about the occurrence of such disturbances in AN (see, for example, Schreder et al., 2008). The puzzle is made even more complex by the relatively frequent occurrence of autistic traits in AN patients (for a systematic review and meta-analysis see Westwood et al., 2016). In order to examine to what extent patients with AN show an autism spectrum phenotype, several recent studies used the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001). Hambrook et al. (2008) first reported that adult patients with AN, compared to healthy control individuals, scored significantly higher on total AQ scores. Anckarsäter et al. (2012) have described teenage-onset AN individuals as more autistic-like than controls on total AQ score and on social skills, attention switching, and attention-to-details subscales. A direct comparison between AQ questionnaires fulfilled by AN and ASD patients was performed by Courty et al. (2013) who found that a third of the AN participants is classified as belonging to the broader/medium autism phenotype. Also Baron-Cohen et al. (2013) confirmed the finding of higher autistic traits in female adolescents with AN compared with controls, whereas Tchanturia et al. (2013) have replicated this result through the abbreviated ten-item version, the short Autism Spectrum Quotient (AQ-10; Allison et al., 2012), and highlighted the independence of eating disorder symptoms from autistic traits. Since olfactory dysfunction in ASD subjects is a replicated finding (Fadda et al., 2018; Muratori et al., 2017; Rozenkrantz et al., 2015; Tonacci et al., 2017a), it could be questioned whether the occurrence of autistic traits in individuals with AN impacts on the olfactory processing of these patients.

Therefore, our study was aimed at:

- Investigating, with a well-grounded, validated methodology, the olfactory ability in adolescents with purely recent onset AN-R, comparing their olfactory function with that of a control group of healthy, age- and gender-matched subjects;
- Exploring possible correlations between olfactory function and age, psychopathological symptoms, autistic traits possibly associated with the eating disorder, as well as with its severity, given the replicated relationship between olfactory symptoms and ASD (see Tonacci et al., 2017a, for a recent systematic review).

2. Methods

2.1. Procedures and participants

19 female patients (mean age: 15.4 ± 2.5 years, range 12–21) with a diagnosis of AN-R (mean duration of the condition: 1.3 ± 1.4 years) according to the DSM-5 criteria were recruited for this study. The diagnosis was performed by a child psychiatrist with expertise in Feeding and Eating Disorders. A detailed history of the patient's behavior from parents, a psychiatric evaluation, a clinical observation of the eating habits, and psychological self-report testing contributed to the AN-R diagnosis.

The recruitment was performed among patients of the IRCCS Fondazione Stella Maris (Pisa, Italy) and of the National Health Service-supported Centro Arianna (Pisa) from November 2015 to September 2016. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) and informed consent was obtained by all subjects involved or their parents. For this study, exclusion criteria included: episodes of binge-eating or purging, usage of nasogastric tube, smoking habits, chronic illnesses, respiratory diseases, head injuries, psychosis, substance abuse, and borderline personality disorders. Seven out of 19 AN patients received psychopharmacological treatment with anxiolytics, antidepressants, or atypical neuroleptics, while the remaining 12 subjects were medication-naïve.

AN-R patients were compared with an age- and gender-matched control group of 19 healthy subjects (14.7 ± 2.9 years, range 11–21), enrolled from secondary schools and University of the Pisa area from April 2016 to January 2017.

The overall patients' characteristics are displayed in Table 1.

2.2. Measures

A standardized self-report clinical evaluation of symptomatology associated with eating disorders was performed in both AN-R and control groups through the administration of the *Eating Disorder Inventory-3* (EDI-3; Garner, 2004), which assesses feelings, behaviors and attitudes towards food and body shapes.

The *Child Behaviour Checklist* (CBCL) and the *Youth Self Report* (YSR) (Achenbach and Rescorla, 2001; Achenbach, 1991) were administered to assess the presence of psychopathologic disorders in both samples. The CBCL is a parent-report questionnaire, composed of 110 items, filled in by parents. The YSR is a similar to the CBCL self-report questionnaire, albeit filled in by all the girls involved in the study. Both questionnaires include items concerning potential emotional and behavioral problems, such as depression, anxiety, somatic complaints, social problems, attention problems, thought problems, rule breaking and aggressive behaviors.

In addition, we supplied a specific self-report questionnaire to assess depression traits, the *Children's Depression Inventory* (CDI) (Kovacs, 1992), which takes into account three main areas: emotional tone, sense of worthlessness, and social relationship.

The presence of autistic traits in both groups was also evaluated; the

Table 2
Psychopathologic and autistic traits: comparison between groups (*: statistical significance at $p < 0.05$; **: statistical significance at $p < 0.01$). For YSR, CBCL and SRS, the score was calculated according to the *T* value obtained).

	AN-R	Controls	*p* value
Internalizing problems (YSR)	63.6 ± 9.6	47.8 ± 15.8	0.003**
Externalizing problems (YSR)	53.6 ± 6.2	45.3 ± 8.4	0.004**
Total problems (YSR)	58.8 ± 8.4	46.8 ± 10.1	0.001**
Internalizing problems (CBCL)	66.8 ± 7.1	53.8 ± 8.1	<0.001**
Externalizing problems (CBCL)	55.9 ± 6.9	46.9 ± 6.5	<0.001**
Total problems (CBCL)	62.2 ± 7.3	48.9 ± 8.0	<0.001**
AQ total score, parent report	18.9 ± 6.4	13.4 ± 7.0	0.026*
AQ total score, self-report	19.9 ± 7.7	15.1 ± 7.1	0.071
Social responsiveness scale (SRS)	66.3 ± 13.2	48.8 ± 13.2	0.001**

administration of questionnaires assessing autistic features, including the *Autism Spectrum Quotient* (AQ) (Baron-Cohen et al., 2001), and *Social Responsiveness Scale* (SRS) (Constantino and Gruber, 2005) was performed. As far as AQ, the *Adolescent Version,* filled in both by the sample population and by parents, was used; it is composed of 50 items clustered in five areas: *social skills, attention switching, attention to details, communication* and *imagination.* A primary caregiver also completed the SRS, which is a 65-item rating scale that measures social interaction, language, and repetitive/restricted behaviours and interests in the adolescent.

2.3. Olfactory assessment

Olfactory function was evaluated in a clean, ventilated room (temperature between 18 °C and 23 °C, and humidity between 40% and 60%), using the Sniffin' Sticks Extended Test (Burghart Medizintechnik, GmbH, Wedel, Germany), a highly reliable (Hummel et al., 1997; Haehner et al., 2009) commercially available olfactory test composed of three sub-tests, namely: i) odor threshold (sensitivity) test; ii) odor discrimination test; iii) odor identification test. Each of the three sub-tests provides a score related to the subjective ability in the specific task. The three sub-scores compose a heuristic "TDI Score" (acronym of Threshold Discrimination Identification Score) ranging 0–64, related to the overall olfactory function of the subject.

In the olfactory assessment, we performed a bilateral testing, in order to avoid possible biases due to the eventual congestion of one of the two nostrils, albeit such conditions were stated among the exclusion criteria for the present study.

2.3.1. Odor threshold test

The Odor Threshold (sensitivity) Test aimed at finding the minimum concentration of a substance (here, n-butanol) that can be detected by the subject. This ability was assessed using a triple-forced choice, single staircase procedure. Three felt-tip pens were presented in a randomized order to the subject at a distance of 20–30 s each. Two of the three pens contained only solvent, while the third held one of the 16 dilutions of n-butanol (from 4% in a dilution ratio of 1:2 to 0.00012% of n-butanol in deionized water). The subject was asked to declare which of the three pens contained the odor or, equivalently, which of the three pens smelled the most. The test ended when seven reversals of the staircase, each of them triggered when the correct answer was given in two successive trials after a mistake in the previous trial, were reached. Therefore, the subjective threshold was calculated as the mean of the last four staircase reversal points by a score ranging 0–16. A lower score in this sub-test indicates a lower sensitivity (hyposensitivity trend), whereas higher points scored are related to higher olfactory sensitivity (Muratori et al., 2017).

2.3.2. Odor discrimination test

The Odor Discrimination Test evaluated the ability to discriminate between different odors. Here, the subject was presented 16 triplets of felt-tip pens, each offered 20–30 s after the previous one. In each triplet, two pens contained the same odor, while the third held a different odorant. The subject was asked which of the three pens presented smelled differently. Even in this case, the score ranged 0–16.

2.3.3. Odor identification test

The Odor Identification Test aimed at assessing the ability of a subject in identifying odors. Here, the subject was administered 32 felt-tip pens, each containing a different odor, and the subject was asked to identify the odor detected in a list with four options. The score of the sub-test ranged 0–32, indicating the number of correct answers provided.

2.4. Statistical analysis

Statistics was performed using SPSS 23 software (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was applied to evaluate whether the variables taken into account displayed a normal distribution. For the variables displaying a normal distribution, a t-Student Test for independent samples was used, whereas for variables with a non-normal distribution, a non-parametrical 1-tailed Mann-Whitney U Test was applied to compare the two groups. Regarding the correlations between olfactory scores and clinical questionnaires, they were checked through the Spearman's rho Test, separately within each group. A Bonferroni's post-hoc correction for multiple comparison was applied. In the whole analysis, statistical significance was assumed at $p < 0.05$ level.

3. Results

3.1. Questionnaire items analysis

Differences between groups on CBCL, YSR, AQ and SRS are displayed in Table 2. Higher significant scores were found in all the questionnaires except the AQ self-report total score.

3.2. Olfactory assessment

The olfactory testing was administered to all patients and controls without particular issues reported by any of the subjects involved. Overall, after controlling for comorbidities and medication use, the performances of the two groups, displayed in Table 3, were similar.

To prevent eventual modifications possibly brought by confounding factors, we evaluated the possible effect of medication use and of menstrual cycle (e.g., see Doty et al., 1981 for evidences of the effect of menstruation on smell abilities) on the olfactory performances. To

Table 3
Olfactory performances for the two groups after controlling for anxiety, depression and medications.

	AN-R (means ± SD)	Controls (means ± SD)	*p* value
Olfactory threshold score	4.1 ± 1.7	3.2 ± 1.6	0.325
Olfactory discrimination score	10.5 ± 2.8	10.9 ± 2.3	0.585
Olfactory Identification score	21.5 ± 6.5	21.9 ± 2.3	0.316
TDI score	35.3 ± 7.3	36.1 ± 4.5	0.686

accomplish this aim, we compared the performances of the 12 unmedicated subjects with AN with those of controls, displaying absence of difference between the two groups (differences: Olfactory Threshold: $p = 0.285$, Olfactory Discrimination: $p = 0.662$, Olfactory Identification: $p = 0.983$, TDI Score: $p = 0.491$). The effect of menstrual cycle was checked by comparing the olfactory performances of AN subjects with amenorrhea (primary and secondary), with those without this condition and having had their last menstruation not more than 3 months prior to the administration of the olfactory test. It is worth noting, however, that none of the girls, at the moment of the evaluation, was into menstruation. The analysis conducted between the two groups above stated revealed no significant differences between them, with the related p-values indicated as follows: Olfactory Threshold: $p = 0.606$, Olfactory Discrimination: $p = 0.093$, Olfactory Identification: $p = 0.815$, TDI Score: $p = 0.370$.

3.3. Correlations

Within the AN-R group, olfactory performances were not correlated with age, disease duration, BMI, nor with EDI-3 total score. Conversely, among controls, age was positively correlated with both threshold and identification sub-scores ($r = 0.500$, $p = 0.035$ for threshold; $r = 0.542$, $p = 0.020$ for identification), as well as with the overall TDI Score ($r = 0.505$, $p = 0.033$; Fig. 1), but not with the discrimination score ($r = 0.066$, $p = 0.795$). After Bonferroni's post-hoc correction for multiple comparison, the correlation between the overall TDI Score and the parent-reported AQ score was significant ($r = -0.489$, $p = 0.039$; Fig. 2) in AN-R, suggesting a moderate negative correlation between TDI olfactory score and AQ score that was not present in healthy controls.

Considered the length of the olfactory assessment (30′ for each patient), a particular focus was paid to attentional aspects. More specifically, correlation was studied between the “Attention Problems” sub-scale of the YSR (*T* value), as well as of the parent- (mother-) reported CBCL (*T* value) and the olfactory scores to identify possible relationships that would have impacted the olfactory performances of the subjects tested. Anyway, all the correlations between “Attention Problems” and the olfactory scores resulted not significant for both AN and controls, proving a lack of effect for this possible confounding factor on the smell capabilities of the subjects enrolled.

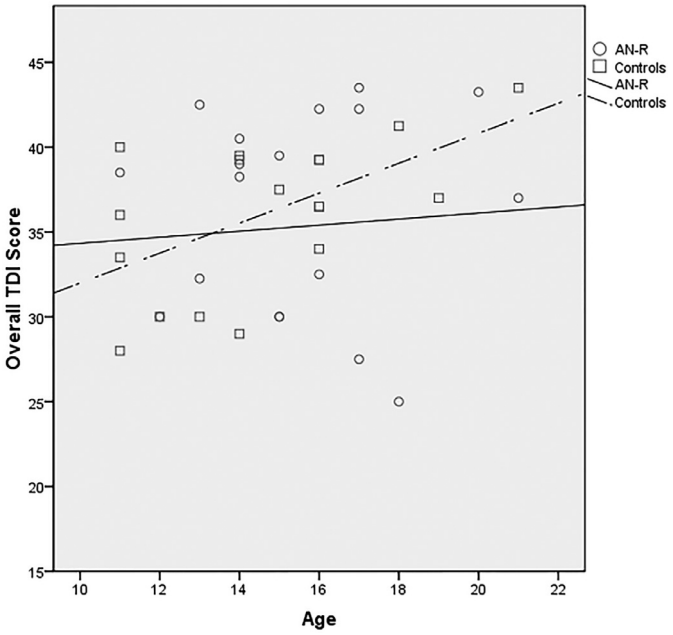

Fig. 1. Correlations between olfactory TDI score and age among AN-R and controls.

4. Discussion

The main aim of the present study was to investigate the olfactory function in young females with recent-onset AN-R. Indeed, it is well known that chemosensory channels (smell and taste) are somehow related to each other (Freitas et al., 2018), both promoting satisfaction and protection in daily life (Allis and Leopold, 2012), and involved in reward mechanisms within eating disorders (Alonso-Alonso, 2013; Soussignan et al., 2012). However, according to previous literature analyzing whether smell and taste channels are affected in eating disorders, to which AN-R is a particularly important disturbance, the olfactory function appears to be more sensitive than the gustatory function with respect to physiopathological characteristics of AN (Fernández-Aranda et al., 2016). For this reason, and taking advantage of the extreme ease of use of commercial psychophysics-based tools for assessing olfactory function, the present study was focused on smell assessment in AN-R.

Differently from other studies on this topic (see systematic review of Islam et al., 2015), our findings did not demonstrate differences between AN patients and healthy controls in any of the olfactory functions: sensitivity, discrimination, and identification, independently from comorbidities, medication use, menstrual cycle or attentional state.

However, with respect to current literature, a number of novel findings were retrieved by our work. First, the effect of anxious-depressive comorbidities, assessed according to the YSR, CBCL and CDI tests, and of menstrual cycle, was found to be negligible. Conversely, Schecklmann et al. (2012) found a significant involvement of olfactory identification, when the sample was controlled for depressivity. Indeed, in general terms, major depression problems are thought to decrease the olfactory function, especially in the odor identification subdomain (Atanasova et al., 2008); however, a number of psychotropic medications are known to positively affect the functionality of directly, or indirectly, involved olfactory areas (Benton et al., 2008; Borta and Höglinger, 2007), with the consequence of providing a superior olfactory ability in depressive subjects treated with such drugs. Therefore, considering that a significant number of our patients received psychotropic medications, we hypothesize that our result could be due to the balance between the two components, depressivity and psychotropic medications, that could have masked eventual patterns of hyper- or hypo-sensoriality. This point should be confirmed, in future, by studies on wide cohorts of subjects with AN-R controlled for medication use.

Concerning menstrual cycle, several studies reported a different olfactory functionality depending on the cycle in healthy subjects (see Doty et al., 1981, as one of the first of them). However, in our cohort of patients with AN, the effect of menstrual cycle -also considered that none of the AN girls was tested during menstruation- was negligible.

Second, while healthy controls displayed an improvement of the olfactory function with age, particularly on sensitivity and identification tasks, as already described elsewhere among typical children (Bastos et al., 2015), no effect of age was found in our AN-R group. Such a result, not detailed in literature until now, may have emerged due to one of the main strength of our study that is the homogeneity of the sample as far as diagnosis and disease severity. We could suggest that the illness have flattened the effect of age present in controls (Fedoroff et al., 1995), and that olfactory function may have stopped to a more childish level of functioning, as occurs in multiple neuroendocrine axes in many girls with AN-R (Misra and Klibanski, 2010). According to this view, if our result will be replicated in larger samples, olfactory testing could represent a possible marker of the biological alterations in AN-R.

Third, investigating the social functioning of AN-R subjects, we found a significant correlation between olfactory performances and autistic features, retrieved through the overall score of the parent-

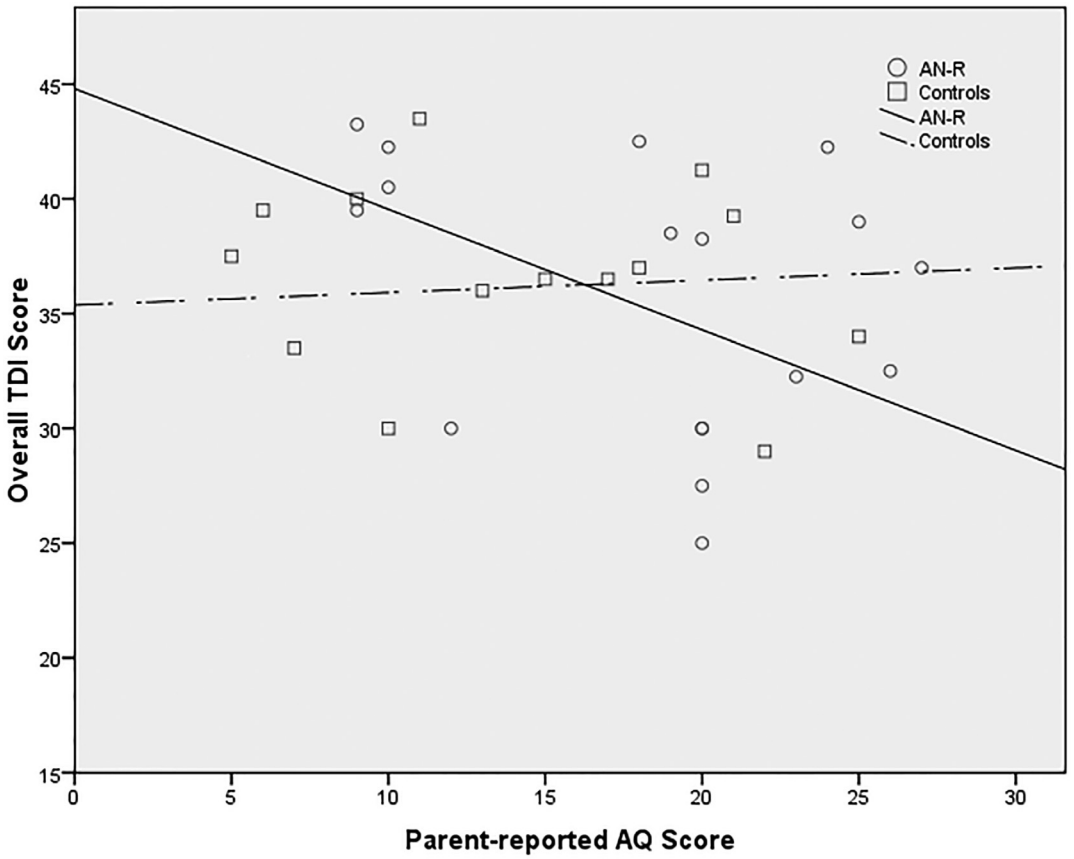

Fig. 2. Correlations between olfactory TDI score and parent-reported AQ score among AN-R and controls.

reported AQ questionnaire (Baron-Cohen et al., 2001), in the AN-R population. In fact, subjects with a lower AQ score, indicating a smaller presence of autistic traits, displayed better olfactory performance, as checked by the overall TDI Score. Conversely, Bentz et al. (2017) did not detect a correlation between olfactory functions and social functioning evaluated through the Autism Diagnostic Observation Schedule (ADOS-2; Lord et al., 2012) administration. Crucially, it is difficult to compare the two evaluations of autistic features (i.e. AQ and ADOS) with each other, as they do present substantial differences not only as to the methods of assessment (parent-report questionnaire *versus* semi-structured observation performed by an expert clinician), but also as to the focus of evaluation (autistic traits also found in the general population *versus* autistic symptoms). However, our results agree with the other findings about olfactory alterations in patients with Autism Spectrum Disorders (ASD), as recently summarized by Tonacci et al. (2017a), but represent a complete novelty within the domain of AN-R, despite elevated levels of ASD traits within the AN population with respect to controls have been previously reported (Westwood et al., 2016), and confirmed by the present study.

In fact, despite the gender and phenotypic differences, as firstly proposed by Gillberg (1983), an overlap between AN-R and ASD is strongly suggested (Westwood et al., 2016). In addition, specific similarities in cognition and social functioning have been observed in both disorders (Oldershaw et al., 2010). In fact, according to a number of evidences, some autistic traits can be revealed in female subjects with AN who could also share common neurocognitive functioning (Huke et al., 2013), including problems with the central coherence (Lang et al., 2016), theory of mind (Oldershaw et al., 2010), cognitive flexibility (Tchanturia et al., 2012), and set-shifting (Westwood et al., 2017).

Furthermore, eating disturbances, including the refusal of certain types of foods, sensitivity to texture or types of foods and unusual behaviors at mealtimes, are common in individuals with ASD (Schreck et al., 2004; Prosperi et al., 2017), and in particular in teenage girls with a diagnosis of ASD. These girls are at greater risk of experiencing eating disorder symptoms than typically developing peers, with 27% of girls with ASD who report feeding symptoms and experience an eating disorder (Kalvya, 2009).

The results obtained in the present study could reflect the presence of a complex interaction between olfactory processing, age and social functioning. Indeed, olfactory performances were seen to be correlated with age only among controls, which are less affected by social functioning issues, according to the clinical questionnaires administered. The nature of this olfactory improvement with age was both central, as demonstrated by the odor identification score, and peripheral, as seen from the odor threshold test. While the first of those two correlations can be related to odor familiarity (e.g., the more familiar an odor is for a subject, the better it is identified) (see, for example, Cornell Kärnekull et al., 2015), the relationship between odor threshold and social functioning could reflect an effective, albeit slight, hypo-sensitivity in subjects with worse social functioning, replicating the findings obtained, to a greater extent, by Muratori et al. (2017) on ASD children.

Overall, none of the portions involved in the olfactory processing appeared impaired, according to our results, starting from the olfactory bulbs (olfactory threshold), towards prefrontal and orbitofrontal cortices, until the frontal and temporal structures normally involved in the olfactory discrimination and identification processing (Kareken et al., 2003; Tonacci et al., 2014). On the other side, we could speculate that the AN-R patients of our sample did not experience the hypo-activation of frontal and temporal areas, involved in primary olfactory processing, which has been previously reported both in AN (Barbarich et al., 2003; Rask-Andersen et al., 2010) and in autism (Muratori et al., 2017). At the same time, we could hypothesize the absence of the classical hyper-activation of regions related to emotion processing, including

hippocampus and amygdala (Barbarich et al., 2003), but such findings should be confirmed by further investigations on larger samples. This fact is of particular importance, since most of the odorants of the olfactory identification test were related to food-like odors, likely to be differently processed in AN-R.

4.1. Limitations

All the findings of the present study should be taken into account in light of a number of limitations. At first, the relatively low sample size only allows partial conclusions about the involvement of olfactory function in AN-R subjects. With larger cohorts, it would be possible to better control for confounding factors, including comorbidities and medications, which could somehow affect the sense of smell. However, the number of subjects included in this study is somewhat in line with the current related literature, and also the age-range selected here is similar to that reported in the previous investigations on adolescents with AN (Bentz et al., 2017; Schecklmann et al., 2012), allowing for comparability of results across studies. Second, the cross-sectional nature of this study did not allow investigating the eventual variation in the olfactory function directly due to the recovery from the condition, nor drawing considerations about the trend of the smell capabilities throughout the various phases of the disorder. Third, restricting the olfactory assessment to female patients with AN-R does not allow us to extend conclusions to males. In fact, sex differences in olfactory abilities have been identified both in typically developing individuals (Doty et al., 2009) and in subjects with ASD (Kumazaki et al., 2015), and require a specific investigation also in patients with AN-R.

Finally, our choice to use the Sniffin' Sticks Extended Test allowed us to investigate the whole olfactory pathway, whereas the assessment of the olfactory detection threshold was performed just on a single odorous compound. This choice was determined by methodological adherence to a validated, well-grounded testing method and for time reasons. Indeed, the Sniffin' Sticks Extended Test was administered in about 30′ for each patient, time that would have dramatically increased in case of testing threshold with multiple compounds, with a consequent possible reduction in patient compliance.

4.2. Conclusions and future directions

Despite these limitations, the present study questions the presence of an olfactory impairment in AN-R, proving an intact functioning of the olfactory pathway that is responsible for sensitivity, identification and discrimination. However, a correlation between olfactory function and ASD-like traits distribution in AN-R could open new, potentially interesting speculations about clinical and physiopathological links between these two, apparently far, conditions. All these findings could be useful for reconsidering the need of olfactory assessment for clinical characterization of AN-R. For instance, the olfactory evaluation could contribute to the objective identification of subjects with AN-R *plus* ASD-like difficulties, which constitute a distinct subtype of AN-R, with specific neuroanatomical underpinnings (Björnsdotter et al., 2018), and response to treatment (Tchanturia et al., 2016).

Future investigations would employ both autonomic measurements—already used in the characterization of the complex link between odors and feeding (Tonacci et al., 2017b)—and implicit physiological measurements, jointly with verbal explicit, self-reported questionnaires about hedonic tone, arousal and intensity related to a given odor (He et al., 2016). Moreover, a deepening on the role of olfaction in human social interaction could benefit from the assessment of social chemical signals, as in the seminal study of Endevelt-Shapira et al. (2018) performed on patients with ASD. A similar protocol could be used in patients with AN-R in order to disentangle possible differences between consciously and unconsciously driven olfactory responses.

A stronger assessment of the specific response to food-related stimuli, in parallel with taste stimulation should also be part of future investigations. Further on, the use of different stimuli for the olfactory threshold assessment would increase the reliability of the absolute threshold calculation, overcoming drawbacks possibly associated with selective hyposmia or anosmia eventually occurring. To properly administer this kind of stimulation, an important upgrade of the technology for stimulation delivery with respect to the present work should be performed. For example, olfactometers like the ones described in the works of Rozenkrantz et al. (2015) and Kumazaki et al. (2016) can be employed easily in such a population, as already demonstrated by their validation on children and autistic subjects. Finally, a deeper insight into the subjective nature of the disturbance could emerge from the assessment of olfactory function in patients in the various phases of the disease, including at discharge, which could provide additional information on the trait- and state-related olfactory mechanisms.

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Declarations of interest

None.

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