Universidade de Aveiro
2023

FILIPA MANUELA GONÇALVES BARROS

RELAÇÕES ENTRE CARACTERÍSTICAS DO AUTISMO, VARIÁVEIS EMOCIONAIS E O PROCESSAMENTO OLFATIVO NA POPULAÇÃO GERAL

RELATIONSHIPS BETWEEN AUTISM CHARACTERISTICS, EMOTION-RELATED VARIABLES, AND OLFACTORY PROCESSING IN THE GENERAL POPULATION

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Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Psicologia, realizada sob a orientação científica da Doutora Sandra Cristina de Oliveira Soares, Professora Auxiliar com Agregação do Departamento de Educação e Psicologia da Universidade de Aveiro, da Doutora Valentina Parma, Professora Auxiliar do Monell Chemical Senses Center (Filadélfia, Estados Unidos da América), e do Doutor Gün Refik Semin, Professor Catedrático do Departamento de Psicologia do ISPA – Instituto Universitário.

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Aos meus pais e irmãos, pelo seu amor infinito, confiança, compreensão e orientação em todos os momentos da minha vida.

o júri

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palavras-chave

Autismo; Fenótipo Ampliado do Autismo; Ansiedade traço; Ansiedade cognitiva; Ansiedade somática; Alexitimia; Olfato; Processamento sensorial

Resumo

Embora alterações no processamento sensorial sejam uma característica-chave da Perturbação do Espetro do Autismo (daqui em diante “autismo”), o funcionamento olfativo ainda é pouco compreendido nesta condição. Considerando o papel do olfato na comunicação, interação social e bem-estar, é crucial investigar que variáveis estão relacionadas com os resultados inconsistentes frequentemente observados no âmbito do processamento olfativo no autismo. Estudar a expressão de traços de autismo na população geral, bem como a expressão multidimensional de outras variáveis relacionadas, pode ser útil para compreender que dimensões estão relacionadas com os sintomas, alterações e heterogeneidade frequentemente observados no autismo, incluindo no domínio olfativo. O presente trabalho pretendeu contribuir para a avaliação multidimensional da ansiedade e de traços de autismo em adultos da população geral, bem como para uma melhor compreensão da relação multivariada entre as características do autismo, processamento olfativo, ansiedade e alexitimia. O Estudo 1 e o Estudo 2 tiveram como objetivo estender a evidência disponível sobre as propriedades psicométricas do State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA) e do Autism Spectrum Quotient (AQ). Os resultados suportaram a adequação de ambos os instrumentos para medir ansiedade e traços de autismo, respetivamente, numa perspetiva multidimensional. Em linha com a literatura, o Estudo 1 providenciou suporte para uma estrutura de quatro fatores, bem como para uma estrutura de dois fatores dentro das dimensões de ansiedade traço e estado do STICSA. Observou-se ainda invariância fatorial considerando a variável sexo, assim como boa validade nomológica. Os resultados também sugeriram que as dimensões cognitivas e somáticas da ansiedade traço, medidas pelo STICSA, estão relacionadas de forma distinta com as respostas subjetiva e psicofisiológica em diferentes contextos emocionais. Os resultados do Estudo 2, de modo consistente com estudos anteriores, suportaram uma estrutura de três fatores do AQ, bem como o papel da alexitimia, particularmente das dificuldades em identificar sentimentos e emoções, como mediadora da relação entre traços de autismo e ansiedade traço. O Estudo 3 analisou o impacto das dimensões de traços de autismo relacionadas com as capacidades sociais e atenção para os detalhes, e da ansiedade traço cognitiva/somática, nas capacidades olfativas da população geral. Os resultados evidenciaram o papel das variáveis sexo, atenção para os detalhes e ansiedade traço somática como preditores significativos da capacidade de discriminação olfativa. Por fim, o Estudo 4 apresentou uma revisão integrativa sobre o processamento olfativo no autismo, e como o avanço da investigação nesta área pode beneficiar o conhecimento e a prática no âmbito da cognição e comportamento social. Os resultados desta investigação destacam a importância de explorar as diferentes dimensões das variáveis relacionadas com o autismo para melhor compreender a complexidade das suas relações e impacto no funcionamento do espetro, incluindo no que diz respeito ao funcionamento olfativo.

keywords

Autism; Broader Autism Phenotype; Trait anxiety; Cognitive anxiety; Somatic anxiety; Alexithymia; Olfaction; Sensory processing

abstract

Although altered sensory processing is recognized as a key-feature of Autism Spectrum Disorder (henceforth “autism”), olfactory functioning is still poorly understood in this condition. Considering the role of olfaction in human social communication and well-being, it is crucial to investigate which variables are related to the often-observed inconsistent results concerning olfactory functioning in autism. Study of the expression of autism traits and other autism-related variables in the general population may be useful to understand which specific dimensions are related to the often-observed symptoms, alterations, and heterogeneity in the autism spectrum, including in the olfactory domain. The present work sought to contribute to the multidimensional assessment of anxiety and autism traits in adults of the general population, as well as to the understanding of the multivariate relationships between autism characteristics, olfactory processing, anxiety, and alexithymia. Study 1 and Study 2 aimed to extend the available evidence about the psychometric properties of the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA) and the Autism Spectrum Quotient (AQ). Results supported the adequacy of both instruments to measure anxiety and autism traits, respectively, in a multidimensional perspective. Consistent with the literature, Study 1 found support for a four-factor, as well as a two-factor structure within the state and traits forms of the STICSA. Moreover, measurement invariance across sex groups, and good nomological validity were also supported for the STICSA. Results also suggested that the cognitive and somatic dimensions of trait anxiety, as measured by the STICSA, are differently related with the subjective and psychophysiological responses in distinct emotional contexts. Results of Study 2 further supported a three-factor structure of the AQ, consistent with previous studies, as well as the role of alexithymia, particularly difficulties in identifying feelings, as a mediator of the relationship between autism traits and trait anxiety. Study 3 analyzed the impact of the social skills and attention to detail dimensions of autism traits, and cognitive/somatic trait anxiety, on the olfactory abilities of the general population. Results emphasized the roles of sex, attention to detail and trait-somatic anxiety as significant predictors of odor discrimination abilities. Finally, Study 4 provided an integrative review about olfactory processing in autism and how advancing research in this area may benefit the knowledge and practice regarding social cognition and behavior in autism. The findings of this research highlight the need to explore the distinct dimensions of autism-related variables to better understand their complex relationships and impact in the functioning of the spectrum, including in olfactory functioning.

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LIST OF ABBREVIATIONS

ADOS	Autism Diagnostic Observation Schedule
ADOS-2	Autism Diagnostic Observation Schedule, Version 2
ANOVA	Analysis of Variance
ANS	Autonomic Nervous System
AQ	Autism Spectrum Quotient
ASD	Autism Spectrum Disorder
AT	Autism Traits
BAP	Broader Autism Phenotype
BO	Body Odor
CFA	Confirmatory Factor analysis
CFI	Comparative Fit Index
COs	Common Odors
DASS-D	Depression, Anxiety and Stress Scales – Depression Scale
DDF	Difficulties in Describing Feelings (Alexithymia)
DIF	Difficulties in Identifying Feelings (Alexithymia)
DSM	Diagnostic and Statistical Manual of Mental Disorders
EFA	Exploratory Factor Analysis
EOT	Externally Oriented Thinking (Alexithymia)
HF	High Frequency Band of Heart Rate Variability
HighCG	Group with High Trait-cognitive Anxiety
HighSG	Group with High Trait-somatic Anxiety
HRV	Heart Rate Variability
LF	Low Frequency Band of Heart Rate Variability
LF/HF	Ratio of LF to HF Power of Heart Rate Variability
LowCG	Group with Low Trait-cognitive Anxiety
LowSG	Group with Low Trait-somatic Anxiety
ML	Maximum Likelihood
OC	Oral Contraceptives
PAF	Principal Axis Factoring
PANAS	Positive and Negative Affect Schedule
PEA	Phenylethanol

RIDO	Restricted Interests and Detail Orientation
RMSEA	Root Mean Square Error of Approximation
SI	Supporting Information
SRMR	Standardized Root Mean Residual
SRS-A	Social Responsiveness Scale for adults
SRS-2	Social Responsiveness Scale, second edition
STAI	State-Trait Anxiety Inventory
STAI-Y	State-Trait Anxiety Inventory – Form Y
STAI-Y1	State anxiety subscale of the STAI-Y
STAI-Y2	Trait anxiety subscale of the STAI-Y
STICSA	State-Trait Inventory for Cognitive and Somatic Anxiety
STICSA-Cognitive	Cognitive dimension of the STICSA-Trait form
STICSA-Somatic	Somatic dimension of the STICSA-Trait form
STICSA-State	State form of the STICSA
STICSA-Trait	Trait form of the STICSA
TANX	Trait anxiety
TAS-20	Toronto Alexithymia Scale of 20 items
TD	Typically Developing (individuals)
TLI	Tucker and Lewis Index
UPSIT	University of Pennsylvania Smell Identification Test
VAS	Visual Analogue Scales
WLSMV	Weighted Least Square Estimator

GENERAL INTRODUCTION

Autism Spectrum Disorder is a complex and fascinating neurodevelopmental condition characterized by significant alterations across several domains, including in social, emotional, cognitive, and sensory functioning (American Psychiatric Association, 2013; Lord et al., 2022; C. E. Robertson & Baron-Cohen, 2017; Uljarevic & Hamilton, 2013; Velikonja et al., 2019). This condition is marked by substantial heterogeneity in the presentation of symptoms, which often complicates assessment, diagnosis, and intervention but, above all, hinders the understanding about autism. Taking into account its lifelong nature and the associated costs, suffering, and general impact on daily life (Cakir et al., 2020; Leigh & Du, 2015; Rogge & Janssen, 2019), it is crucial to invest in research that contributes to an improved assessment of autism-related characteristics, as well as to a better understanding of the mechanisms underlying the heterogeneity and comorbidities observed in the spectrum. For this purpose, studying autism considering a dimensional perspective, and specifically the expression of autism traits in the general population, may be useful (Landry & Chouinard, 2016).

Altered sensory processing is currently considered a key-feature of autism and previous research suggested that it is observed across multiple sensory modalities (e.g., Ben-Sasson, Carter, et al., 2009; Leekam et al., 2007). Yet, notwithstanding previous evidence supporting altered olfactory processing in autism (Larsson et al., 2017; Tonacci et al., 2015), the nature of these alterations is poorly understood, and their impact on the social, emotional and behavioral functioning of people with a marked expression of autism characteristics is still underexplored. The main aim of this thesis was to contribute to the understanding of the relationships between autism characteristics, olfactory processing, anxiety, and alexithymia in the general population. For this, four studies were conducted, aiming to (1) contribute to the improvement of the multidimensional measurement of anxiety (**Study 1**) and autism traits in the general population (**Study 2**); (2) explore the multivariate relationship between the dimensions of autism traits, alexithymia and anxiety in the general population (**Study 2**); (3) analyze the impact of the social skills and attention to detail dimensions of autism traits and cognitive/somatic trait anxiety on the olfactory

abilities of the general population (**Study 3**); and (4) review the current evidence about olfactory processing in autism and outline how advancing research in this area may benefit knowledge and practice regarding social cognition and behavior in autism (**Study 4**).

The present thesis is divided into five chapters. In **Chapter 1**, a theoretical background supporting our aims is provided, involving an elucidation about (1) the main features of autism, including its dimensional model and the research in the context of the Broader Autism Phenotype; (2) the role of olfaction in our daily life and the main consequences of olfactory dysfunction; and (3) the status of the literature regarding olfactory processing in autism, including the potential role of anxiety in the olfactory processing of the spectrum, and the role of alexithymia in the relationship between autism and anxiety. Finally, the specific aims – and the corresponding studies – are also outlined.

In **Chapter 2**, a brief description of the methodology used to conduct the research is provided, including a summary of the characteristics of the samples, the inclusion and exclusion criteria, the material used, the procedure – including the analytical strategies employed – and, finally, the ethical procedures that were followed.

Chapter 3 comprises four original studies presented in the format of scientific articles. These studies were conducted in order to attain the objectives proposed for this thesis; all the studies are already published in international scientific journals.

Chapter 4 consists of a general and integrative discussion of the results provided by the four aforementioned studies. Firstly, the main results are outlined, followed by the research and clinical implications of the present work, the strengths and limitations and, finally, the proposed suggestions for future research. The chapter ends with a brief conclusion.

Finally, in **Chapter 5** the bibliography of the thesis (including the bibliography specific to each one of the studies) is presented.

CHAPTER 1 - THEORETICAL BACKGROUND

"Individuals with autism and other neurodevelopmental disorders are a valued part of society and represent a prototype of neurodiversity."

(Lord et al., 2022, p. 2)

1.1 Autism

1.1.1 Definition and main characteristics

Autism Spectrum Disorder (henceforth "autism") is described by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, Text Revision (DSM-5-TR; American Psychiatric Association, 2022) as a neurodevelopmental condition characterized by pervasive, persistent, and clinically significant difficulties in reciprocal social interaction and communication across contexts, as wells as by the presence of patterns of restrictive and stereotypical behaviors, activities, and interests. These alterations are associated with clinically significant impairments in social and interpersonal functioning, daily life activities, and/or other areas of functioning (American Psychiatric Association, 2022). Together with the core features, several cognitive (Velikonja et al., 2019), emotional (Harms et al., 2010; Uljarevic & Hamilton, 2013), sensory (C. E. Robertson & Baron-Cohen, 2017), as well as neuroanatomical and neurophysiological alterations (Amaral et al., 2008; Peng et al., 2020; Philip et al., 2012; Via et al., 2011) are often observed in people with the diagnosis, contributing, among others, for the observed social difficulties (e.g., Bishop-Fitzpatrick et al., 2017; Thye et al., 2018). Finally, people with autism often present intellectual and language impairments, learning difficulties, and behavioral alterations (American Psychiatric Association, 2022; Lord et al., 2022).

Autism is a highly heritable neurobiological condition (Tick et al., 2016), with both genetic and environmental influences (e.g., Modabbernia et al., 2017; M. J. Taylor et al., 2020; Thapar & Rutter, 2021). Furthermore, the "spectrum" nature of autism reflects the considerable variation in the presentation and severity of symptoms across individuals, who can also have distinct comorbidities and, consequently, differing levels of functioning

(American Psychiatric Association, 2022). Notwithstanding the frequently observed impairments and difficulties across contexts, many people with autism present normal or even above average intelligence and language (e.g., Charman et al., 2011), as well as superior skills (e.g., memory and computation skills; Meilleur et al., 2015) and increased performance in several tasks (e.g., visual-attentional tasks; Kaldy et al., 2016; see also Mottron et al., 2006). Thus, the complexity and heterogeneity associated with the spectrum make it as fascinating as it is challenging, explaining in part the exponential growth in autism research during the last decades.

Autism worldwide prevalence is estimated to be around 1-2% (see Lord et al., 2022), although it is variable across the world (Chiarotti & Venerosi, 2020). Furthermore, as autism persists throughout life, these people face a multiplicity of challenges across the distinct developmental stages (e.g., regarding education, employment and independent living); this implies that they often require lifelong informal care, family support, medical care, therapy and special education, while also having significant productivity losses (individual and parental) and residential accommodation costs, for instance (see Cakir et al., 2020; Rogge & Janssen, 2019). This significantly increases the personal, family, social and financial costs associated with the condition (Cakir et al., 2020; Leigh & Du, 2015). Importantly, variables such as autism severity, intellectual functioning, and comorbidities (e.g., psychiatric disorders) greatly influence the social outcomes in adulthood (Howlin & Magiati, 2017), and are also likely associated with higher costs and burden throughout life (see Rogge & Janssen, 2019 for a review). Yet, despite being a relatively common, complex and lifelong condition, the majority of people with autism may not always have access to appropriate assessment and intervention (Lord et al., 2022). Altogether, this highlights the urgent need to improve not only the access to proper educational, social, and healthcare services throughout life, but also to develop adequate, multidisciplinary, and cost-effective assessment and intervention protocols which target the unique pattern of strengths and needs of people with autism and their families/caregivers.

Although the first signs of autism can be observed from very early in development, missed diagnosis, misdiagnosis or delayed diagnosis are often observed (Hus & Segal, 2021). On the one hand, autism diagnosis does not depend on a medical test nor are there reliable biomarkers that can assist a more objective assessment; it requires a thorough clinical assessment which involves collecting the developmental and behavioral history of

the individual, and using standardized observations, interviews and instruments (Hus & Segal, 2021). Also, the first signs of the condition may be unclear and can substantially vary across individuals, with many presenting more subtle alterations (Lord et al., 2022). Autism symptoms can also change with age (Lord et al., 2022) and developmental regressions may occur (Hus & Segal, 2021). The comorbidities associated with the disorder can differ across individuals, and frequently mask autism symptoms due to overlapping or similar features (Hus & Segal, 2021; Lord et al., 2022). Lastly, many people with autism, especially those with milder symptoms and less severe comorbidities at an early age, may learn and develop strategies to compensate for their difficulties throughout life, until the challenges of life eventually exhaust their resources (American Psychiatric Association, 2022).

Other obstacles to diagnosis include sociocultural issues, such as difficulties in the access to healthcare, increased stigma in certain cultures, and sex differences (Hus & Segal, 2021; Lord et al., 2022). In fact, prevalence studies have been consistent in showing that autism is more prevalent in men in comparison with women (see Chiarotti & Venerosi, 2020 for a meta-analysis). A recent meta-analysis of the sex ratio in autism suggested an overall 4.20:1 male-to-female ratio (Loomes et al., 2017). Yet, this seems to depend on several factors. For instance, when considering studies that recruited individuals already diagnosed with autism, the ratio is 4.56:1, while studies actively searching for people with an autism diagnosis result in a ratio of 3.25:1. Also, Loomes et al. (2017) pointed out that, when considering studies with higher methodological quality, the ratio is estimated to be inferior – 3.32:1. Furthermore, intellectual functioning seems to influence the male-to-female ratio, since higher intellectual functioning has been shown to be associated with a higher imbalanced sex ratio (e.g., see Werling & Geschwind, 2013). Many studies have been exploring the mechanisms behind these sex differences, although the phenomenon is still poorly understood (Ferri et al., 2018). It is likely that a combination of factors, rather than one single explanation, drive the often-observed male bias (Ferri et al., 2018; Werling & Geschwind, 2013). These include: genetic, hormonal, and neurobiological factors, which may confer higher protection for females and/or higher risk for males of developing autism symptoms (e.g., Ferri et al., 2018; Werling & Geschwind, 2013); as well as methodological and environmental factors, including biases in assessment and/or diagnostic processes, sociocultural norms, and sex and gender-related expectation biases and stereotypes (e.g.,

de Giambattista et al., 2021; Ferri et al., 2018; Kreiser & White, 2014; Lai & Szatmari, 2020). Moreover, sex and gender-related factors may influence the behavioral manifestation of autism symptoms (Lai & Szatmari, 2020); in fact, females seem to have a distinct behavioral presentation of autism symptoms and are more likely to camouflage their difficulties, which may catalyze underdiagnosis in this group of individuals (for reviews see Ferri et al., 2018; Halladay et al., 2015; Hus & Segal, 2021; Kreiser & White, 2014; Lai & Szatmari, 2020; Werling & Geschwind, 2013). Altogether, these factors often hinder an early and accurate diagnosis, and, consequently, the mobilization of resources and services for an adequate intervention.

1.1.2. The evolution of the autism concept

The concept of autism and the underlying diagnostic criteria have undergone great evolution over the last decades, informed by important developments and increasing interest in autism research and clinical practice. Before autism was recognized as a distinct disorder as we know it today, it was thought to be a manifestation of psychosis or childhood schizophrenia (see Evans, 2013; Gyawali & Patra, 2019). In fact, the word "autism" was firstly used by Bleuler, in 1911, to address symptoms of severe cases of schizophrenia (Evans, 2013). Yet, in 1943, the psychiatrist Leo Kanner published his seminal work about "Autistic disturbance of affective contact", where he described 11 children "whose condition differs so markedly and uniquely from anything reported so far" (Kanner, 1943, p. 217). Although he observed and described several individual differences between the cases, he reported that the children he observed presented a set of characteristics including alterations in the way they related to people and their environment, as well as an insistence in sameness and "extreme aloneness" observed since the beginning of their lives. In addition, he reported other alterations regarding, for instance, language, communication, and sensory responsiveness (Kanner, 1943). Shortly after, in 1944, Hans Asperger added an important contribution with his work "'Autistic psychopathy' in childhood", where he described four boys with persistent and severe impairments in social contact and interaction, as well as with restrictive and stereotyped behaviors and interests similar to those described by Kanner (Asperger, 1991). The work of Asperger also emphasized the heterogeneity observed in the abilities shown by people

with autism, and the fact that some of these individuals seem to have increased and unique abilities, which may lead to "exceptional achievements" in life (Asperger, 1991, p. 74).

The works of these psychiatrists were pioneer and crucial for autism conceptualization and, consequently, affected autism classification. However, the recognition of autism as a diagnostic category distinct from psychosis was only observed several years later, in the third edition of the DSM (American Psychiatric Association, 1980), supported by critical developments and several lines of research that emerged during the end of the 1960s, and during the 1970s (see Rosen et al., 2021 for an overview). The diagnostic criteria of the DSM-III "infantile autism" required an early onset of the symptoms (before 30 months of age), a "pervasive lack of responsiveness" to people, language impairments, and abnormal speech patterns and response to several aspects of the environment (such as bizarre interests). Therefore, in this edition, autism diagnosis was still marked by rigid criteria and was fundamentally focused on the childhood (Rosen et al., 2021). Since then, with the evolution of the knowledge about autism, adjustments were being made to correspond to the particularities, heterogeneity and comorbidities associated with the spectrum, reflecting advances in research and practice and resulting in wider and more developmentally-oriented diagnostic criteria (see Rosen et al., 2021). Examples of this evolution are the reformulation of "infantile autism" to "autistic disorder" in the revision of DSM-III (American Psychiatric Association, 1987), and the emergence of the "Asperger Disorder" in DSM-IV, to include the cases where clinically significant impairments in language and cognitive development were absent (American Psychiatric Association, 1994).

The DSM-5 (American Psychiatric Association, 2013) brought an important change in autism classification, by presenting a single dimensionally-based rather than a multi-categorical diagnosis (Rosen et al., 2021). Autism is now referred to as "Autism Spectrum Disorder", reflecting the behavioral heterogeneity observed in the condition. Diagnostic criteria and specifiers were also added or reformulated to better reflect the heterogeneity observed in core and other autism-related symptoms, and the criteria could be met currently or by history. These changes were motivated by previous difficulties in categorizing autism variability, as well as by the absence of strong evidence concerning distinct autism subcategories (J. N. Miller & Ozonoff, 2000; see also Rosen et al., 2021 for this discussion). As pointed out by Rosen et al. (2021), through this dimensional

classification model it is possible to identify the core characteristics of autism while also allowing for the variation in their manifestation.

Therefore, there were major changes in the autism concept that included, as previously described, the widening of the diagnostic criteria to account for the variability and specificities of the condition, the notion that autism is a lifelong condition (and not only a childhood condition), and the acknowledgement of its complexity as a condition that often co-occurs with other mental and physical diagnoses and symptoms (see Happé & Frith, 2020 for a review). As also noted, another major change in the understanding of autism includes its evolution from a discrete diagnosis to a dimensional approach. Importantly to this work, although autism was originally assumed to be a condition clearly differentiated from the typical development and from other disorders, recent studies have been suggesting that the characteristics that define autism extend to the general population, expressing in a continuum from more severe and clinically relevant traits observed in the autism diagnosis, to milder and subclinical traits in the general population (Constantino & Todd, 2003; Happé & Frith, 2020; Ingersoll & Wainer, 2014).

1.1.3. The Broader Autism Phenotype

In their seminal work, both Leo Kanner (1943) and Hans Asperger (1991) noted that some of the relatives of the children they observed presented characteristics that somewhat resembled autism symptoms; for instance, Kanner reported that the relatives of these children were “persons strongly preoccupied with abstractions of a scientific, literary, or artistic nature, and limited in genuine interest in people” (Kanner, 1943, p. 250). Asperger also noted that “many of the fathers of our autistic children occupy high positions, despite their noticeable peculiarities” (Asperger, 1991, p. 84), and that the behavior of many of the mothers “had decidedly autistic features” (Asperger, 1991, p. 85). With the first classical twin studies (e.g., Folstein & Rutter, 1977), empirical support for the genetic component and highly heritability of autism started to emerge (see Ingersoll & Wainer, 2014). Subsequent family studies also observed that many of the relatives of people with autism exhibited subthreshold autism-related features, including social and communication abilities, stereotyped behaviors, and psychiatric problems (Bailey et al.,

1998; Bolton et al., 1994; Piven et al., 1997). The subclinical manifestation of autism traits has been addressed as the Broader Autism Phenotype (BAP; Piven et al., 1997).

Later, the BAP was also explored in the general population (see Ingersoll & Wainer, 2014 for a review). Evidence suggested that autism traits are continuously distributed in the general population (e.g., Baron-Cohen et al., 2001; Broadbent et al., 2013; Constantino & Todd, 2003; R. M. Hurst, Mitchell, et al., 2007), are heritable (e.g., Constantino & Todd, 2003, 2005; Hoekstra et al., 2007; E. B. Robinson, Koenen, et al., 2011; for a review see Ronald & Hoekstra, 2011), and that there is shared etiology between normal variation (general population) and quantitative extreme groups (e.g., E. B. Robinson, Koenen, et al., 2011). Additionally, these traits seem to be persistent, as suggested by studies observing high stability in childhood (e.g., Haraguchi et al., 2019; Holmboe et al., 2014; E. B. Robinson, Munir, et al., 2011) and modest stability across development (Whitehouse et al., 2011).

Furthermore, several studies have been showing that, similarly to the observed in people with autism and in their relatives, a high expression of autism traits in the general population is associated with, for instance: psychosocial difficulties, including self-reported interpersonal problems (Kanne et al., 2009; Wainer et al., 2011), loneliness and less friendships (e.g., Jobe & White, 2007); higher emotional reactivity (e.g., Pisula et al., 2015); more self-reported sensory processing problems (e.g., Horder et al., 2014; A. E. Robertson & Simmons, 2013); higher neuroticism and low extraversion (e.g., Austin, 2005; Wainer et al., 2011; Wakabayashi et al., 2006); psychiatric conditions and symptoms, such as depression and anxiety (Kanne et al., 2009; Kunihira et al., 2006; Wainer et al., 2011), as well as schizotypy (e.g., R. M. Hurst, Nelson-Gray, et al., 2007; Wainer et al., 2011); altered cognitive processing (Bayliss & Tipper, 2005; Dunn et al., 2016; Grinter et al., 2009); altered attention orienting to emotional faces and atypical facial emotion processing (Bothe et al., 2019; English et al., 2017; Lassalle & Itier, 2015; Poljac et al., 2013); altered social attention (e.g., Lin et al., 2020) and joint attention (e.g., Zhao et al., 2015); and structural and functional alterations (e.g., Di Martino et al., 2009; Gebauer et al., 2015; von dem Hagen et al., 2011). Therefore, and as noted by Happé (2020), a dimensional nature of autism seems to be supported at the behavioral, cognitive, genetic, and neuroanatomical levels. Nevertheless, it is worth noting that the literature is not consistent and is also scarce for many of these relationships, with several studies failing to

observe an association between self-reported autism traits and the variables of interest in the general population (e.g., Koolschijn et al., 2015; Kunihira et al., 2006). At least in part, mixed findings may be due to methodological differences across the studies, namely differences in the instruments used to measure autism traits.

As interest and research on autism traits grew, so did the need to develop instruments capable of an adequate measurement of these characteristics. Indeed, having instruments capable of reliably measuring autism characteristics in the general population is crucial to understand the nature of these traits, as well as their relationship with variables of interest in the spectrum. Throughout the last decades, several instruments have been developed and/or used to measure autism traits; these include screening tools used in clinical diagnosis, such as the Social Responsiveness Scale for adults (SRS-A; Constantino et al., 2000; Constantino & Todd, 2005); instruments specifically developed to assess autism traits in relatives of people with autism, such as the Broader Autism Phenotype Questionnaire (Hurley et al., 2007); and instruments developed to assess autism traits in adults of the general population, such as the Autism Spectrum Quotient (AQ; Baron-Cohen et al., 2001), the Subthreshold Autism Trait Questionnaire (Kanne et al., 2012), the Adult Autism Subthreshold Spectrum (Dell'Osso et al., 2017), and the Comprehensive Autistic Trait Inventory (English et al., 2021). The first instrument specifically developed to measure autism traits in adults of the general population with normal intelligence was the AQ (Baron-Cohen et al., 2001). This self-report instrument is probably the most widely used to measure autism traits in adults, having been used across studies with clinical and nonclinical samples (English et al., 2020; Ruzich et al., 2015). However, the fact that past studies have observed inconsistent results regarding its factor structure and the reliability of its dimensions (see English et al., 2020 for an overview) highlights the need to further extend research about this instrument to improve the measurement of autism traits. Furthermore, although this instrument was already validated in distinct countries and cultures, research exploring its suitability for certain contexts, namely in the Portuguese population, is still scarce.

1.1.3.1. How can the BAP help to understand autism?

As noted before, early research with family members and the subsequent studies with the general population have been contributing to a better understanding of autism, and gathered support for a dimensional model that conceptualizes autism as the extreme of a continuum of characteristics (e.g., Constantino & Todd, 2003). However, this does not necessarily imply that a dimensional and a categorical conception are mutually exclusive, with evidence supporting a mixed categorical and dimensional nature of autism (e.g., Abu-Akel et al., 2019). Measuring autism traits also informs about the contribution of distinct autism dimensions to the social, sensory, cognitive, emotional, behavioral and other alterations observed within the spectrum, which can be critical to understand autism heterogeneity and developmental trajectories, for instance (Happé & Frith, 2020; Landry & Chouinard, 2016).

As stated before, autism is characterized by variability in the nature and severity of symptoms (American Psychiatric Association, 2022). This variability is also observed in the general population, since distinct people can present different combinations of autism traits (e.g., Palmer et al., 2015). This means that two people with a similar quantitative total score of autism traits can have a distinct behavioral presentation of autism characteristics, since it is possible to have a higher expression of traits in specific dimensions, and a lower expression in other dimensions. Related to this, there is evidence suggesting that not all dimensions of autism traits significantly correlate with each other (e.g., Bothe et al., 2019; English et al., 2020; Russell-Smith et al., 2011). Importantly, social and non-social autism traits seem to be genetically dissociable (Warrier et al., 2019), and variations in social and non-social dimensions of autism traits seem to be associated with distinct outcomes. For instance, distinct dimensions differently predict social cognitive processes (J. Davis et al., 2017), are differently associated with socioemotional processing (Bothe et al., 2019), and also relate distinctively to sensory processing in typically developing adults (Yaguchi & Hidaka, 2020). This is in line with the evidence reviewed by Happé and Ronald (2008), which supported the dissociation and independence of the distinct autism dimensions (i.e., social skills, communication, and restricted and repetitive behaviors and interests) at the cognitive, behavioral, genetic, and neural levels. This suggests that a unitary approach to autism may not be suitable or helpful since autism

seems to be characterized by "multiple impairments" – or rather multiple particularities – each with likely distinct explanations and associated with different outcomes (Happé & Ronald, 2008).

Studying the BAP in the general population yields great potential in terms of understanding autism in a broader perspective. First, since all the key criteria must co-occur to have an autism diagnosis (i.e., social-communicative alterations, as well as repetitive and restrictive interests and behaviors), studying the expression of autism traits in the general population may allow more variability in the presentation of symptoms, since some people may only present certain characteristics of autism. This can be helpful to understand the role of certain dimensions and combinations of symptoms in the functioning of people, for instance. Therefore, studying the impact of the several dimensions of autism can shed additional light on many dysfunctional or altered processes observed in the autism spectrum, while also helping to understand previously observed mixed results and guide future research. Also, with samples of the general population it is also easier to control for some confounding variables (e.g., comorbid conditions) that are often very hard to handle in clinical samples (for reviews see Ingersoll & Wainer, 2014; Landry & Chouinard, 2016).

An area where extending research about the BAP could be very promising corresponds to sensory processing and, particularly, olfactory processing in autism. Impaired sensory processing across sensory domains is a well-known characteristic of autism (e.g., Baum et al., 2015; C. E. Robertson & Baron-Cohen, 2017). Yet, some sensory modalities, such as olfaction, are still underexplored and poorly understood in this condition (Baum et al., 2015; Marco et al., 2011). For instance, although altered social cognition is a key feature of autism, most studies in the field are focused on the processing of social information conveyed by visual stimuli, such as faces (e.g., Griffin et al., 2021; Harms et al., 2010; Uljarevic & Hamilton, 2013) and, in a less extent, on information conveyed by auditory stimuli (e.g., Haesen et al., 2011; O'Connor, 2012). The focus on vision is not surprising, since research in general has heavily explored this sensory modality in comparison with others (Hutmacher, 2019), perhaps due to the view that vision is our dominant sense and due to its critical role in the way we perceive and interact with the world (e.g., Pazzaglia, 2015; Shams & Kim, 2010). Also, another explanation may be the more obvious connection between vision (as well as audition) and social behavior and

communication (e.g., Baum et al., 2015). However, social communication was pointed as a major function of olfaction (R. J. Stevenson, 2010), and dysfunctions on this sensory modality may yield important consequences not only for social behavior, but also for other areas of functioning that are often impaired in autism (such as eating behaviour; Nimbley et al., 2022), as we will further discuss in the next sections.

1.2. Olfaction and its role in daily life

1.2.1. The unique sense of olfaction

In the past, it was commonly assumed that humans have a poor sense of smell, although the evidence accumulated throughout the years has been supporting that humans have good olfactory abilities, with an important impact on human behavior (McGann, 2017; G. M. Shepherd, 2004). Yet, as previously stated, olfaction was often neglected and was underrepresented in research in comparison to other sensory modalities, perhaps due to the assumption that it was not as relevant or dominant as other senses (such as vision; Boesveldt & Parma, 2021; Hutmacher, 2019; Perl et al., 2020). Furthermore, the impact of olfaction in our daily life often goes unnoticed because most of this information is processed without requiring conscious awareness (Lundström et al., 2011; Lundström & Olsson, 2010). In fact, the subliminal nature of olfaction is, undoubtedly, one of its most notable particularities (e.g., Smeets & Dijksterhuis, 2014).

Olfaction possesses several features that distinguish it from other senses; first, its anatomical "simplicity", which includes, for instance, the ability to directly reach the olfactory cortex without a thalamic relay (e.g., Gottfried, 2006; Merrick et al., 2014). Second, its anatomical and functional closeness with the limbic system (Kontaris et al., 2020; Soudry et al., 2011), which creates a privileged link between olfaction, emotion and memory. In fact, olfaction is well-known for its ability to strongly modulate mood and emotions (e.g., Smeets & Dijksterhuis, 2014) even when odors are subliminally present (e.g., Lundström & Olsson, 2005) or masked (e.g., Cecchetto et al., 2019; for a review see Kontaris et al., 2020). Conversely, emotions and emotion-related traits, such as anxiety, neuroticism and stress (Bombail, 2019; Kontaris et al., 2020) also strongly influence olfactory perception. For instance, high levels of anxiety and neuroticism were previously

shown to be associated with enhanced (e.g., Larsson et al., 2000) and decreased odor identification performance (e.g., Takahashi et al., 2015), as well as with enhanced odor detection abilities (D. Chen & Dalton, 2005; Havlíček et al., 2012; La Buissonniere-Ariza et al., 2013; Pause et al., 1998; but see also Rovee et al., 1973). Odors are also able to strongly trigger autobiographical memories, in a more "emotional" way than the memories triggered by other senses (see Herz, 2016). Finally, odors also act as powerful contextual cues, interacting with the information provided by other senses to help make sense of the world. For instance, odors were shown to influence and/or facilitate the processing of visual (e.g., S. Cook et al., 2017; Rocha et al., 2018; Seigneuric et al., 2010), auditory (e.g., La Buissonnière-Ariza et al., 2012), taste (e.g., Djordjevic et al., 2004), and touch stimuli (e.g., Croy, D'Angelo, et al., 2014).

According to Stevenson (2010), olfaction has three major aims: food ingestion, detection and protection from danger, and social communication (see also Lübke & Pause, 2015; Parma et al., 2017). In fact, through olfaction, we are able to detect, discriminate, and respond to a wide range of odors around us, which signal available resources and threats, such as the availability and quality of food and the presence of toxins (Spehr, 2017a). Importantly, chemical signals also communicate information about ourselves, as well as about the strangers, relatives, friends, potential mates, and competitors around us, providing cues thought to be critical for social interaction and behavior (Blomkvist & Hofer, 2021; Boesveldt & Parma, 2021; Perl et al., 2020; Smeets & Dijksterhuis, 2014; Spehr, 2017a). Each human has an "odorprint" (Natsch, 2017, p. 942), i.e., a unique body odor influenced by both genetics (e.g., Penn et al., 2007) and the environment (e.g., Havlicek & Lenochova, 2008). Importantly, unlike non-social odors, body odors activate brain structures related to the processing of social and emotional stimuli, attentional regulation, visual processing, and the creation of a basic perception of a human body (e.g., Lundström et al., 2008; Prehn-Kristensen et al., 2009); this differential processing is likely due to the role of body odors in social communication and behavior, and their biological and evolutive relevance (Lundström et al., 2008; Pause, 2012).

Human body odors are highly informative about us and our conspecifics, as corroborated by studies suggesting, for instance, the encoding of age (e.g., Mitro et al., 2012), gender (e.g., Penn et al., 2007), kinship (e.g., Porter, 1998), personality traits (e.g., neuroticism; Sorokowska et al., 2012), menstrual cycle variations (e.g., S. L. Miller &

Maner, 2011), disease (e.g., Olsson et al., 2014), and even emotional state (e.g., de Groot et al., 2012; see Boesveldt & Parma, 2021 for a review). Salient social and emotional information is perceived by others, eliciting congruent emotional and behavioral changes which possibly foster adaptive responses to environmental demands (e.g., de Groot et al., 2017). For instance, body odors collected in fear-inducing situations have demonstrated the ability to induce a congruent fear facial expression and to increase the inhalation magnitude and eye-scanning behavior, both related to sensory acquisition mechanisms (de Groot et al., 2012). Importantly, olfaction, and especially the information conveyed by human body odors, seem to be critical for the development and maintenance of appropriate and stable relationships with others (Boesveldt & Parma, 2021; Lübke & Pause, 2015). Examples of social processes in which olfaction seem to play such a relevant role include mother-child bonding (e.g., Schaal, 1988), kin recognition (e.g., Lundström et al., 2009), mate-choice (e.g., Havlicek & Roberts, 2009), and sexual behavior (e.g., Alves-Oliveira et al., 2018; see Boesveldt & Parma, 2021; Blomkvist & Hofer, 2021 for reviews).

1.2.2. Olfactory dysfunction

A myriad of individual differences influence how we perceive and process odors. These include, for instance, genetics (Hasin-Brumshtein et al., 2009), sex (Sorokowski et al., 2019), consumption of alcohol and tobacco (e.g., J. E. Hayes & Jinks, 2012; Liu et al., 2016; Rupp et al., 2003), environmental factors (e.g., pollution; Sorokowska et al., 2015), personality dimensions (e.g., D. Shepherd et al., 2017), and affective symptoms (e.g., anxiety; Takahashi et al., 2015). Other factors such as age, respiratory infections, sinunasal disorders, or neurodegenerative disorders are often associated with olfactory dysfunction (see Boesveldt, Postma, et al., 2017; Hummel et al., 2017). Significant deviations from a normal sense of smell (i.e., normosmia) may include hyposmia, when an individual has lower olfactory ability, and anosmia, when there is a complete loss of olfactory function. Furthermore, olfactory dysfunction can also involve alterations in odor perception, triggered in the presence (parosmia) or the absence (phantosmia) of an odor (see Hummel et al., 2017; Koçak et al., 2020). To evaluate olfactory functioning, several reliable and valid procedures can be used (Han et al., 2021; Hummel et al., 2017; Hummel & Podlesek, 2021; Su et al., 2021); these include otolaryngology examination, electrophysiological

methods, questionnaires, and psychophysical methods, such as the Sniffin' Sticks Extended Test (Hummel et al., 1997) or the University of Pennsylvania Smell Identification Test (UPSIT; Doty et al., 1984). Through psychophysical methods, it is possible to assess, among others, the abilities corresponding to odor detection, odor discrimination and odor identification. Tests of odor detection assess the lowest concentration of a stimulus that an individual can detect; tests of odor discrimination evaluate if the individual is able to distinguish between odors with different properties; finally, tests of odor identification are the most commonly used and evaluate the ability to identify a set of common odors (Doty, 2017; Hummel et al., 1997, 2017). A recent systematic review and meta-analysis reported an estimated prevalence of 22% for olfactory dysfunction in the general population (Desiato et al., 2021). Furthermore, these authors observed a prevalence of 29% when olfactory abilities were assessed through psychophysical tests, with self-report methods leading to a much lower estimated prevalence of 9.5%. Unfortunately, many times olfactory dysfunction is not early identified or is not even noticed at all, especially in older adults (e.g., D. R. Adams et al., 2017; see also Oleszkiewicz & Hummel, 2019; Schäfer et al., 2021). In fact, Desiato et al. (2021) observed that less than one-third of the people presenting olfactory dysfunction (assessed through psychophysical tests) were aware of their olfactory problems, highlighting the importance of using more objective measures in this context.

Since olfaction plays a significant role in human safety, well-being, and social relationships, olfactory dysfunction may entail critical consequences at several levels (Boesveldt, Postma, et al., 2017; Hummel et al., 2017; Schäfer et al., 2021). In fact, poor olfactory abilities seem to affect behaviors related to food enjoyment, selection and ingestion, including difficulties in preparing meals and an increase in the ingestion of spoiled food (Blomkvist & Hofer, 2021; Boesveldt & Parma, 2021; D. V. Santos et al., 2004; Schäfer et al., 2021). Furthermore, it can increase the risk of toxin ingestion, the risk of injuries and can also increase concerns about the ability to detect danger (Hummel et al., 2017; D. V. Santos et al., 2004; Schäfer et al., 2021; R. J. Stevenson, 2010). Finally, it can increase occupational problems and social insecurities, and compromise social/sexual functioning and relationships with others (Blomkvist & Hofer, 2021; Mahmut & Croy, 2019; Schäfer et al., 2021). Olfactory dysfunction is also a strong predictor of 5-year

mortality in older adults (J. M. Pinto et al., 2014) and has a negative impact on quality of life (Croy, Nordin, et al., 2014; Schäfer et al., 2021).

Olfactory dysfunction has already been proposed as a key-symptom or indicator of some medical, psychiatric, and neurodegenerative conditions. Examples of these include Parkinson's and Alzheimer's disease (e.g., Haehner et al., 2009; Marin et al., 2018; Murphy, 2019), SARS-CoV-2 infection (e.g., Lao et al., 2020; Rocke et al., 2020), schizophrenia and depression (e.g., Atanasova et al., 2008; Croy & Hummel, 2017). In the last decade, the study of olfactory processing in autism has also gained increased interest (e.g., Larsson et al., 2017; Martin & Daniel, 2014; Tonacci et al., 2015), perhaps due to the potential applications of this research. In this regard, olfaction has been pointed as a candidate for a reliable marker of this condition (e.g., Hrdlicka et al., 2011; Muratori et al., 2017; Tonacci et al., 2015), as well as a potential aid to mitigate social and eating difficulties, for instance (e.g., Luisier, Petitpierre, Clerc Bérod, et al., 2019; Parma et al., 2013). However, the nature and impact of olfactory (dys)function in autism are still poorly understood, as we will discuss in more detail in the next sections.

1.3. Olfactory processing in autism

1.3.1. Sensory processing alterations in autism

Altered sensory processing or unusual interests in sensory aspects of the environment were recognized as part of the restricted and repetitive behaviors and interests criterion of the autism diagnosis contemplated in the 5th edition of the DSM (American Psychiatric Association, 2013; Rosen et al., 2021). These alterations often involve multiple sensory modalities (e.g., Ben-Sasson, Carter, et al., 2009; Leekam et al., 2007) and are present since early in the development (e.g., Baranek et al., 2013), persisting throughout life (e.g., Crane et al., 2009; Leekam et al., 2007) and impacting the life of these individuals (American Psychiatric Association, 2013; Baranek et al., 2014; Ben-Sasson, Hen, et al., 2009; see C. E. Robertson & Baron-Cohen, 2017). Altered sensory processing may include hypersensitivity, when there is a faster, more intense and/or longer response towards a sensory stimulus; hyposensitivity, when there is a weaker or inexistent response towards a sensory stimulus; and sensory seeking, when there is an active and energetic

seek for sensory input (L. J. Miller et al., 2007). All these response patterns may be present in people with autism (e.g., A. E. Lane et al., 2010) and can even co-occur (e.g., Baranek et al., 2006; A. E. Lane et al., 2010; Leekam et al., 2007; Schoen et al., 2008, 2009), reflecting a complex relationship between sensory abnormalities and several individual characteristics such as mental (e.g., Baranek et al., 2006) and chronological age (see Ben-Sasson, Hen, et al., 2009), intellectual abilities (e.g., Crane et al., 2009; Leekam et al., 2007) and severity of symptoms (Ben-Sasson, Hen, et al., 2009; but see also Crane et al., 2009). Importantly, these sensory alterations were shown to be associated with autism symptoms (e.g., Hilton et al., 2007; Liss et al., 2006), abnormal social cognitive abilities and socioemotional functioning (Baranek et al., 2013; Ben-Sasson, Carter, et al., 2009; Kojovic et al., 2019; Thye et al., 2018), impaired adaptive behavior (Dellapiazza et al., 2020; Kojovic et al., 2019; A. E. Lane et al., 2010), behavioral problems (e.g., Mazurek et al., 2013), and affective symptoms (e.g., Green et al., 2012; S. J. Lane et al., 2012) in individuals with autism (see Glod et al., 2015 for a review). Nevertheless, some sensory modalities have been more investigated in comparison with others, and olfactory processing in particular seems to be still underexplored (e.g., Galle et al., 2013; Tonacci et al., 2015).

1.3.2. Olfactory processing in autism

1.3.2.1. Olfactory abilities

Olfactory abilities, including olfactory detection, discrimination, and identification, have been probably the most studied olfactory dimensions in autism, with evidence reflecting inconsistent results. Children with autism seem to present normal (Sweigert et al., 2020) or decreased olfactory detection abilities (Dudova et al., 2011; Kumazaki et al., 2016; Muratori et al., 2017), while adults evidenced decreased (Fadda et al., 2018; Koehler et al., 2018), normal (Galle et al., 2013; Okumura et al., 2019; Suzuki et al., 2003; Tavassoli & Baron-Cohen, 2012) or enhanced abilities (Ashwin et al., 2014). Xu et al. (2020) also observed high variability in odor thresholds displayed by adults with autism, contrary to the observed in typically developing adults. Interestingly, Sweigert et al. (2020) observed that odor detection scores were significantly and positively correlated with odor identification abilities in children with autism, but not in typically developing children.

Therefore, odor detection seems to be altered in autism, although this is especially observed in children; in fact, age was pointed out as a potential modulator of olfactory functioning in autism (Larsson et al., 2017; Tonacci et al., 2015). Heterogeneous results across and even within studies (see Xu et al., 2020, where there were patterns of hyposensitivity and hypersensitivity in individuals with autism) were also suggested to reflect potential developmental differences in olfactory functioning among people with autism (May et al., 2011; Sweigert et al., 2020), as well as differences in symptomatology (see Sweigert et al., 2020).

With regard to olfactory identification, results suggest impaired abilities both in children (Bennetto et al., 2007; Legisa et al., 2013; May et al., 2011 [Study 2]; Muratori et al., 2017; Sweigert et al., 2020) and adults with autism (Galle et al., 2013; Koehler et al., 2018; Suzuki et al., 2003; Wicker et al., 2016), although there is also evidence suggesting no differences between children (Brewer et al., 2008; Dudova et al., 2011; May et al., 2011 [Study 1]), and adults (Fadda et al., 2018; Okumura et al., 2019; Xu et al., 2020) with and without autism. Interestingly, Dudova et al. (2011) observed a significant positive correlation between age and odor identification ability, but only in the typically developing group of children. Conversely, Brewer et al. (2008) observed a negative significant relationship between olfactory identification and age only in the autism group, suggesting an abnormal developmental trajectory of olfactory abilities in this group. However, in a 5-year follow-up (May et al., 2011), this research group found that the previously observed relationship between odor identification and age was no longer significant, although the association remained negative and moderate in the autism group. Importantly, they observed that two individuals with autism got worse in odor identification ability, while the remaining individuals with autism and all the typically developing individuals improved in this ability. This may suggest a heterogeneous development of olfactory identification in autism, although more studies are needed to understand olfactory development in this condition. Furthermore, these studies have important differences; for instance, the studies that observed no odor identification impairments recruited younger children in comparison with some of the studies observing impairments (e.g., Bennetto et al., 2007). Also, the task and stimuli used to assess odor identification differed across studies, which limits direct comparisons. Once more, most of the studies suggest impaired odor identification in autism, although this dysfunction is more evident in children than in adults. Finally,

olfactory discrimination studies, which are the most scarce regarding olfactory abilities, suggest normal abilities in both children (Muratori et al., 2017) and adults with autism (Fadda et al., 2018; Galle et al., 2013).

1.3.2.2. Processing of common and social odors

In regard to the processing of common odors, studies suggest altered subjective responses regarding the pleasantness of odors in children (Hrdlicka et al., 2011; Legisa et al., 2013) and adults with autism (Wicker et al., 2016). Xu et al. (2020) also observed that adults with autism with lower odor detection abilities displayed lower self-reported pleasantness of common odors (namely rose and mint). However, Sweigert et al. (2020) and Galle et al. (2013) did not observe group differences regarding the perceived pleasantness of common odors in both children and adults with autism, respectively. Furthermore, Sweigert et al. (2020) observed a significant and moderate negative correlation between odor identification abilities and the self-reported pleasantness of rose and vanillin odors in children with autism; in typically developing children, the same relationship was observed but only for the rose odor. Regarding perceived intensity, studies observed either intact (Addo et al., 2017; Galle et al., 2013) or heightened perception in adults with autism (e.g., Wicker et al., 2016). Galle et al. (2013) further failed to observe group differences regarding perceived familiarity in adults. Interestingly, Legisa et al. (2013) further observed that children with autism had relatively similar facial and psychophysiological responses to common odors, in comparison with their typically developing peers. However, children with autism seemed to have difficulties expressing their emotional response towards olfactory stimuli, considering the low agreement between their facial response and verbal subjective rating of the odors. Finally, Rozenkrantz et al. (2015) also observed that children with autism do not adjust their sniffing response in relation to odor valence, unlike typically developing peers. This suggests a possible dysfunction in the mechanisms of sensory acquisition and sensory rejection, which are important to regulate the quantity of information received in the presence of a beneficial (e.g., a pleasant odor) or harmful (e.g., an unpleasant odor) chemical signal (de Groot et al., 2012).

Considering the processing of social odors in autism, the picture is even more unclear. The study of Endevelt-Shapira (2018) reported that adults with autism are as capable as typically developing adults of spontaneously sampling body odors, as well as of detecting and discriminating between distinct body odors. However, evidence also suggested altered electrodermal responses (Endevelt-Shapira et al., 2018; Haigh et al., 2020) and behavioral responses to fear body odors, in adults with autism (Endevelt-Shapira et al., 2018). Reduced trial-to-trial variability in electrodermal response to fear body odors was also observed to be significantly reduced in people with a higher total score of autism traits, as measured by the AQ, but only for individuals diagnosed with autism (Haigh et al., 2020). Apart from these results, there is also evidence suggesting that maternal body odors facilitate automatic imitation behavior in children with autism (Parma et al., 2013). Individuals with autism often show deficits in imitation behavior (Edwards, 2014), which involves the ability to mimic others' actions (Brass & Heyes, 2005). In the study of Parma et al. (2013), children with and without autism observed a model (which could be their mother or a stranger) performing (or not) a reach-to-grasp action towards an object, that could have their mother's body odor, the body odor of other person or no odor at all. After the model's action, children were invited to carry out the same action. A reduction of total movement time – suggesting automatic imitation effects – was observed in children with autism only when the object had their own mother's body odor, independently of the identity of the model performing the action. In a subsequent study performed with the same sample, Parma et al. (2014) observed that maternal body odor also facilitated action planning in children with autism, reflected by a reduction in the initiation time of the child's movement subsequent to the presentation of the maternal body odor. These results suggest that odors, particularly socioemotionally relevant odors, such as maternal body odor, may facilitate social behavior in children with autism. Nevertheless, more research is necessary to replicate these findings and understand if they generalize to other social contexts, other types of olfactory stimuli (e.g., common odors or body odors collected in emotional situations), and if odors facilitate social behavior across development, for instance.

1.3.2.3. Relationship between autism traits and olfactory processing across the autism spectrum

The relationship between the expression of autism traits and olfactory processing in the general population is also still underexplored. Studies exploring the association between sensory abnormalities and autism traits in typically developing adults observed a significant correlation between autism traits and sensory problems/abnormalities in general (e.g., Amos et al., 2019; Horder et al., 2014; Mayer, 2017; A. E. Robertson & Simmons, 2013; Tavassoli et al., 2014). Interestingly, while all dimensions of the AQ were found to be significantly correlated with sensory alterations in the study of Robertson and Simmons (2013), Mayer et al. (2017) observed that the dimensions of Attention to Detail and Imagination were significantly correlated only with specific sensory abnormalities (such as sensory sensitivity) in the group of typically developing adults. Weiland et al. (2020) also observed that the Imagination dimension of the AQ – 10 items (Hoekstra et al., 2011) was not significantly correlated with sensory alterations. However, it is important to note that the instruments used to measure sensory problems and autism traits differed across studies, which may limit comparisons and may have led to slightly different results. Furthermore, although the instruments used in the aforementioned studies contain items regarding olfactory problems, these results only provide information about how autism traits relate to self-reported sensory problems in general and, thus, the way in which distinct dimensions of autism are related to olfactory functioning in particular is still yet to be explored.

In fact, some authors specifically assessed olfactory processing to explore the relationship with autism traits, despite not having explored the distinct relationships with the dimensions of autism traits. For instance, Robertson (2012) explored the relationship between autism traits, as measured by the AQ, and olfactory abilities, as measured by the Sniffin' Sticks, in typically developing adults. However, no association between these variables was observed. Nevertheless, only a total score of autism traits was used, which does not allow us to understand if specific dimensions of autism are associated with distinct olfactory abilities. Lastly, Stafford et al. (2017) failed to observe a relationship between olfactory sensitivity and autism traits in a sample of university students. However, these authors used a specific food-related odor in the odor detection task and, once more, they only used a total score of autism traits, as measured by the AQ.

Regarding the relationship between autism traits and olfactory abilities in clinical populations, Ashwin et al. (2014) observed a positive correlation between autism traits and olfactory detection in adults with autism; the relationship in the control group was not explored, because autism traits were assessed only in the clinical group. Other studies with clinical population also measured autism traits with the AQ; however, AQ's scores were used only to distinguish groups, i.e., to confirm that the autism group had significantly more autism traits than the control group (e.g., Addo et al., 2017; Tavassoli & Baron-Cohen, 2012). The study conducted by Sweigert et al. (2020) did not measure autism traits through the AQ, but explored the relationship between the Autism Diagnostic Observation Schedule, Version 2 (ADOS-2; Lord et al., 2012), and olfactory abilities of children with autism. The ADOS-2 is considered a "gold-standard" assessment tool in autism, that measures communication, social interaction, play/imagination, and restricted and/or repetitive behaviors (Kanne et al., 2008; McCrimmon & Rostad, 2014). Results suggested a significant, moderate, and negative correlation between odor identification abilities, as measured by the UPSIT, and the total score of ADOS-2, in children with autism. Similarly, a significant negative correlation was observed with the social affect dimension of the ADOS-2, but not with the restrictive/repetitive behavior dimension. The authors suggest that these results may reflect shared mechanisms between impaired olfactory processing and social deficits (Sweigert et al., 2020), although these mechanisms should be further explored in future research.

1.3.2.4. Neural correlates of olfactory functioning in autism

Research exploring the neural correlates of olfactory processing contributes to a better understanding about the mechanisms underlying the often-observed differences between people with and without autism in this regard. For instance, there is evidence suggesting no differences in the olfactory bulb volume between adults with and without autism (Koehler et al., 2018), contrary to the observed in other conditions associated with olfactory impairment (e.g., schizophrenia; Turetsky et al., 2000). However, in an fMRI task, adults with autism evidenced reduced activation of the piriform cortex when smelling common odors (namely peach and coffee; Koehler et al., 2018). The authors argued that the olfactory alterations observed in autism may be, then, related to abnormalities in the

primary olfactory cortex. On the other hand, during an odor detection task using 2-phenylethyl alcohol, adults with autism evidenced altered event-related potentials in the late stages of olfactory processing, in comparison with the control group (Okumura et al., 2019). These group differences were observed in the bilateral cuneus and the posterior cingulate cortex. As these areas were previously shown to be activated during olfactory tasks involving, for instance, memory and emotion components (e.g., Cerf-Ducastel & Murphy, 2006; Wintermann et al., 2013), these differences may reflect abnormalities in higher level olfactory processing (see Okumura et al., 2019). Finally, Xu et al. (2020) observed reduced activity in the dorsolateral prefrontal cortex in the group of adults with autism that displayed higher detection thresholds (i.e., lower odor detection abilities) while the odors of phenylethyl alcohol and mint were presented. Also, the strength of activation in this area was negatively correlated with odor thresholds in general. Considering that the odor detection task employed in this study required some attention and working memory abilities to successfully detect the target odor, decreased activation in the dorsolateral prefrontal cortex was argued to possibly reflect the often-observed deficits in attention and working memory in autism (e.g., Velikonja et al., 2019; see Xu et al., 2020 for this discussion). By looking at these results together, the mechanisms associated with olfactory alterations in autism are unclear; nevertheless, it is important to note that these studies involved different tasks, measures, and samples, which precludes direct comparisons and stresses the need to further investigate the mechanisms underlying abnormal olfactory processing in autism.

1.3.3. Which variables may be associated with mixed results and altered olfactory functioning in autism?

By analyzing the literature reviewed in the last subsection, we can observe distinct patterns of olfactory processing in autism, across olfactory domains. The meta-analysis conducted by Larsson et al. (2017) supports this conclusion, and shows that the effect sizes varied substantially across studies for both odor detection and odor identification abilities. Although these heterogeneous findings can reflect a dual pattern of sensory processing in autism (Larsson et al., 2017), they can also be the result of important methodological and sample differences across studies (Larsson et al., 2017; Tonacci et al., 2015). For instance,

some studies have been using forced-choice staircase procedures (e.g., Galle et al., 2013; Sweigert et al., 2020) to measure odor threshold, while others use other procedures consisting, for instance, in varying the distance between the odors and the individual (e.g., Ashwin et al., 2014). Some of these tasks could pose more demands compared to others, which could influence results across studies (Tavassoli & Baron-Cohen, 2012). Also, the target odors used in these tasks were different (e.g., isopropyl alcohol, Ashwin et al., 2014; isoamyl acetate and allyl caproate, Kumazaki et al., 2016; phenylethyl alcohol and pure vanillin, Sweigert et al., 2020; n-butanol, Tavassoli & Baron-Cohen, 2012), which, combined with distinct procedures, could also have led to distinct results (Zernecke et al., 2010). The tasks used for odor identification (e.g., UPSIT vs. Sniffin' Sticks), as well as the number and nature of odors evaluated in these tasks and tasks consisting of odor ratings (e.g., pleasantness) are also often distinct across studies, which limits comparisons. As some authors perform adaptations to the response format of the identification tests (e.g., Bennetto et al., 2007), this can also account for differences across studies. Finally, other methodological aspects regarding the olfactory testing, such as not using parfum in the day of the evaluation, sometimes are not reported by researchers (e.g., Addo et al., 2017).

Aspects related to the recruited samples can also be critical for the observed heterogeneous results. In the first place, several studies recruited relatively small samples (Addo et al., 2017; Galle et al., 2013; Suzuki et al., 2003), ranging from around 5 to 44 individuals per group. Studies with lower sample sizes may have lacked statistical power and may lead to inaccurate conclusions. As recommended by Larsson et al. (2017), recruiting large sample sizes would help to reduce uncertainty regarding results. Another important aspect is the fact that the characteristics of participants may differ across studies. First, distinct studies target different age groups and age has been pointed as a potential modulator in olfactory processing in autism (Larsson et al., 2017; Tonacci et al., 2015). By analyzing results across studies, we could observe that most studies supported odor detection impairment in children with autism, while in adults there is more evidence supporting normal abilities. Regarding odor identification, the pattern is similar, with most studies supporting impairments in children with autism, while in adults the pattern of results is unclear (studies support both normal and decreased abilities). Apart from the role of age in olfactory functioning in autism, potential distinct development trajectories in

people with autism may also explain these heterogeneous results (May et al., 2011; Sweigert et al., 2020; Xu et al., 2020), although this should be further explored.

In their meta-analysis, Larsson et al. (2017) found that lower mean age and lower intelligence quotient (below 30 and 113, respectively) were associated with lower odor detection ability, while higher mean age and higher IQ (above 35 and 113, respectively) were associated with higher detection abilities. Interestingly, for odor identification, higher IQ was associated with impaired performance. Therefore, IQ may also be a potential modulator of olfactory functioning in autism. Some researchers did not assess participants' IQ (e.g., Addo et al., 2017) or measured this variable only to discard intellectual disability and/or to match groups regarding their scores (e.g., Galle et al., 2013; Sweigert et al., 2020). Sex may be also an important variable to consider in this regard; as previously described, autism sex ratio seems to be close to 3:1, but many studies do not reflect this proportion – and vary greatly in the proportion of males and females recruited for their studies. In fact, many studies recruited male individuals exclusively (e.g., Ashwin et al., 2014; Galle et al., 2013; Muratori et al., 2017; Suzuki et al., 2003), while others have samples mostly involving males (e.g., Fadda et al., 2018; Wicker et al., 2016) and one study recruited a sample predominantly composed of women (Addo et al., 2017). Studies exploring the BAP in the general population, on the other hand, seem to have samples mostly composed of women (A. E. Robertson, 2012; A. E. Robertson & Simmons, 2013; Stafford et al., 2017). As olfactory processing is associated with sex, with women generally showing better olfactory abilities in comparison to men (e.g., Oleszkiewicz et al., 2019; Sorokowski et al., 2019), more research is needed to further understand the role of sex in olfactory processing in autism.

In their systematic review, Tonacci et al., (2015) also highlighted the potential role of the severity of autism symptoms as a modulator of olfactory impairment. In fact, previous evidence, although scarce, points out that having more autism symptoms is related to poorer olfactory abilities (Ashwin et al., 2014; Sweigert et al., 2020). Nevertheless, most studies did not explore the relationship between autism severity or autism symptoms, and olfactory performance, thereby the mechanisms underlying this relationship remain unclear. Importantly, as explained in previous sections, autism symptomatology can greatly vary in nature and severity across individuals, and the social and non-social dimensions seem to be independent of each other (Happé & Ronald, 2008;

Warrier et al., 2019). Therefore, it is possible that this variation in symptomatology within the groups of individuals with autism explains, at least in part, the dual pattern of hypo- and hypersensitivity often observed across olfactory domains. The study of Sweigert et al. (2020) suggests that the social affect dimension, as measured by the ADOS-2, is associated with olfactory impairment in children with autism, contrary to the observed for the restrictive/repetitive behaviors dimension of ADOS-2. Future studies should further evaluate the contribution of distinct autism dimensions to different olfactory abilities in autism, across the development and considering other variables with potential impact on olfactory functioning. For this purpose, a useful way to start would be to measure the expression of autism traits in the general population and assess the contribution of specific dimensions on the olfactory functioning across domains. On the one hand, while individuals in clinical samples must meet all diagnostic criteria to a certain extent, individuals in the general population may present more variability in the presentation of symptoms. On the other hand, recruiting larger samples and controlling for certain variables, such as medication intake and physical/mental disorders, may be easier to achieve in the general population. Nevertheless, as the results obtained in this context cannot be generalized for the clinical side of the spectrum, future studies must explore these relationships in distinct groups and more controlled samples.

Finally, and of relevance in the context of the present thesis, studies often do not control for other individual differences that can have a critical impact on olfactory functioning. For instance, although some studies control for respiratory, neurological, and medical conditions (Galle et al., 2013; Kumazaki et al., 2016; Muratori et al., 2017), most psychiatric disorders and symptomatology are often (if not always) discarded from exclusion criteria and, most importantly, from the analysis. Sweigert et al. (2020) further reported that, while psychotic disorders were part of the exclusion criteria, mood, anxiety, and other developmental disorders were not, due to the high rate of psychiatric disorders in autism. Therefore, the influence of individual differences in psychiatric problems, including anxiety symptomatology, on the olfactory performance of the autism spectrum is still little understood, despite the high prevalence of anxiety in autism (e.g., Hollocks et al., 2019; van Steensel & Heeman, 2017) and the role of anxiety, and emotions in general, in olfactory processing (e.g., D. Chen & Dalton, 2005; La Buissonniere-Ariza et al., 2013).

1.3.3.1. Anxiety

Emotions, when appropriate in type, frequency, intensity, and duration, are adaptive because they help us to deal effectively with daily challenges, including in the social context (e.g., Ekman, 1992; Frijda, 2016; Keltner & Gross, 1999; Lazarus, 1991; Nesse, 1990; Tooby & Cosmides, 1990). Depending on the specific situation and how it is evaluated, the several emotion components change accordingly to prepare the body for action (e.g., Ekman, 1992; Matsumoto et al., 2007; Nesse & Ellsworth, 2009; Tooby & Cosmides, 1990). Yet, the emotional response is not always necessarily triggered by the immediate presence of a stimulus/situation; for instance, when considering a situation which can be potentially harmful for us, it would be adaptive to anticipate it and adequately prepare ourselves beforehand to successfully deal with the imposed demands. This is what happens in an anxiety response, which is triggered by the anticipation of a stimulus or a situation appraised as potentially threatening or highly demanding (American Psychiatric Association, 2022; Lang et al., 2000). Anxiety is, then, a subjective, behavioral and biological phenomenon characterized by marked psychophysiological activation, and cognitive symptoms such as worry or intrusive thoughts, which prepare the body for action and facilitate adaptive behavior (Lang et al., 2000; Öhman, 2008; Tooby & Cosmides, 2008). In many cases, the manifestation of anxiety is not clinically significant to receive a formal diagnosis; nevertheless, it is still frequently accompanied by substantial suffering, or even difficulties across several areas of functioning (e.g., social, occupational), which has a critical impact in people's lives (Turner & Michelson, 1984).

Anxiety is often associated with clinically significant distress, autonomic dysfunction and both physical and mental disorders when recurrent, persistent and/or significantly intense (American Psychiatric Association, 2022; Comer & Olfson, 2010; Miu et al., 2009). Anxiety disorders seem to be the most prevalent mental health problem in the general population (Castaldelli-Maia & Bhugra, 2022; Kessler et al., 2009; Steel et al., 2014), estimated to affect one in 15 persons per year (6.7%; 6.1–7.9%), and having an estimated lifetime prevalence of 12.9% (Steel et al., 2014). Women seem to present significantly higher period and lifetime prevalence rates of anxiety disorders than men (Steel et al., 2014). Anxiety disorders can develop throughout life, with differences in their epidemiology and presentation with ageing (e.g., Lenze & Wetherell, 2011). Importantly,

they are often comorbid with each other (Goldstein-Piekarski et al., 2016) and are frequently associated with other psychiatric disorders such as depression (Lamers et al., 2011), neurodevelopmental disorders such as autism (Hollocks et al., 2019), as well as with other health problems, such as cardiovascular disease, hypertension and gastrointestinal alterations (e.g., Celano et al., 2016; Härter et al., 2003; Johnson, 2019; Vogelzangs et al., 2010). Anxiety disorders, as well as anxiety comorbid with other medical conditions, are also associated with poorer quality of life (e.g., Olatunji et al., 2007; Sareen et al., 2006; Sherbourne et al., 1996; Wilmer et al., 2021) and impaired social functioning (e.g., Saris et al., 2017), and may also impact the prognosis of the comorbid conditions (e.g., El-Mallakh & Hollifield, 2008).

High levels of anxiety have been associated with autonomic dysfunction, as suggested by the relationship with Heart Rate Variability (HRV; e.g., Bleil et al., 2008; Miu et al., 2009; see Chalmers et al., 2014 for a review of HRV in anxiety disorders). The HRV involves the variations inherent to the cardiac inter-beat intervals, reflecting the Autonomic Nervous System (ANS) activity and the balance between its sympathetic and parasympathetic branches (Berntson et al., 2007; Task Force, 1996). Adequate levels of autonomic variability are pivotal for flexibility, resilience, and the adaptation of the organism to environmental changes and challenges (Berntson et al., 2008; Shaffer et al., 2014). The HRV is also associated with emotion regulation abilities (e.g., D. P. Williams et al., 2015) and can be used as an objective measure of the emotional response, being also an indicator of well-being and health (e.g., Chalmers et al., 2014; Kemp & Quintana, 2013; Shaffer et al., 2014). High levels of trait anxiety, i.e., the proneness to experience anxiety symptoms in daily life (Spielberger & Reheiser, 2009) were previously observed to be associated with reduced HRV in healthy individuals (in resting conditions), particularly concerning the vagally-mediated indexes of HRV (Bleil et al., 2008; Miu et al., 2009; D. Shepherd et al., 2015; L. L. Watkins et al., 1998). Similarly, reduced HRV was observed in individuals with anxiety disorders (Chalmers et al., 2014; H. Cohen & Benjamin, 2006). Reduced HRV and, therefore, decreased flexibility and regulatory abilities, have been associated with several health problems, some of which are also frequently associated with anxiety disorders, such as cardiovascular disease (e.g., Task Force, 1996). Therefore, an early assessment and treatment of mal-adaptive anxiety is critical for the prevention of

complex medical conditions and to provide adequate treatment, improving people's health and quality of life.

As an adequate intervention should be preceded by a comprehensive and accurate assessment, it is crucial to properly measure anxiety symptoms, using valid instruments capable of reliably assessing symptoms across contexts. For this purpose, it may be useful to obtain information about changes in specific dimensions (Elwood et al., 2012), to allow a more complete characterization of an individual's anxiety profile. In this regard, anxiety can be addressed as a transient emotional state experienced in a specific moment, or as a more stable trait that reflects the vulnerability or tendency to experience anxiety symptoms; these dimensions correspond to state and trait anxiety, respectively (Spielberger et al., 1983; Spielberger & Reheiser, 2009). Cognitive and somatic dimensions of anxiety have been also explored in the context of anxiety measurement (Schwartz et al., 1978), and correspond to the cognitive symptoms (e.g., difficulties in concentrating, obsessive thoughts) and physiological activation (e.g., dizziness, trembling, heart beating fast) observed in the anxiety response, respectively. Measuring these distinct anxiety dimensions can be an advantage in research and clinical practice, since different people can present different anxiety profiles (e.g., Schwartz et al., 1978), which can be, in turn, related to different outcomes (e.g., Belem da Silva et al., 2014).

Although many assessment instruments have been developed and explored regarding their ability to provide an adequate evaluation of the symptoms, only a few are capable of distinguishing between several distinct dimensions. In this regard, the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA; Ree et al., 2008) was developed to measure both state, trait, cognitive and somatic dimensions of anxiety in the general population. Although STICSA seems to be an adequate instrument to measure self-reported anxiety symptoms across dimensions (e.g., Carlucci et al., 2018; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021), further characterization of STICSA's factor structure, validity and adequacy across cultures and contexts is still lacking, including in the Portuguese context. Importantly, considering the multidimensionality of the emotional response, it would be useful to understand not only people's pattern of self-reported anxiety symptoms, but also the relationship between the information provided by instruments like STICSA and people's response in certain contexts of their daily life. In this regard, predictive validity, i.e., the ability of an instrument to predict other measures

conceptually associated with the construct (Field, 2013), is an analysis that is generally lacking in the literature concerning the validation of psychological instruments and that can add critical information about the usefulness of an instrument in specific contexts. In sum, a multidimensional and multimodal assessment of anxiety could foster a better characterization of people's anxiety profile, which may have benefits for a more comprehensive assessment, accurate diagnosis, psychopathology prevention and intervention planning. On the other hand, in research settings, distinguishing between anxiety dimensions may provide a better understanding of the mechanisms underlying psychological and other normative or dysfunctional processes. In the context of this thesis, this multidimensional assessment could, for instance, help to shed some light on the complexity of the relationship between different variables commonly associated with autism, as well as the complexity of olfactory processing.

1.3.3.2. Anxiety on the autism spectrum

Apart from the core features, autism is often associated with other physical and mental disorders. Anxiety disorders seem to be one of the most common psychiatric comorbidities in autism (Hossain et al., 2020; Kent & Simonoff, 2017; Lai et al., 2019; Simonoff et al., 2008). Prevalence estimates greatly vary across studies; an umbrella review of systematic reviews and meta-analyses observed that the reported prevalence of anxiety disorders in autism varied between 1.47% and 54%. Children with autism seem to have higher anxiety levels in comparison with their typically developing peers and children with externalizing and developmental problems (van Steensel & Heeman, 2017), and evidence also suggests that 39.6% of children with autism have at least one comorbid anxiety disorder, as evaluated through DSM-IV criteria (van Steensel et al., 2011). Regarding adults with autism, Hollocks et al. (2019) observed a pooled prevalence of 27% for any current anxiety disorder, as well as a pooled lifetime prevalence of 42%. In their study, Nimmo-Smith et al. (2020) also observed that 20.1% of adults with autism had an anxiety disorder, compared with only 8.7% of typically developing adults.

These results indicate that anxiety disorders are likely to be more prevalent in autism than in the general population (Kent & Simonoff, 2017). Likewise, people with

autism seem to present higher levels of trait anxiety in comparison with their typically developing peers, although most of these studies do not include older adults and people with intellectual disability (for a review see Jolliffe et al., 2022). Having more autism traits seems to be also associated with higher levels of anxiety in adults with autism (e.g., MacLennan et al., 2020; Maras & Bowler, 2012), although some authors argued that this can be due to a high symptom overlap between autism and anxiety (e.g., South et al., 2017). A positive significant association between anxiety, including trait anxiety (e.g., Rosbrook & Whittingham, 2010), and autism traits was also previously observed in the general population (Bothe et al., 2019; Kanne et al., 2009; Liss et al., 2008). Importantly, anxiety disorders and high levels of trait anxiety may have a negative impact on the overall functioning, quality of life and well-being of people with autism and people with higher levels of autism traits. For instance, anxiety was found to be associated with poorer social skills and social functioning (e.g., Bellini, 2004; Chang et al., 2012), reduced adaptive behavior (e.g., Zukerman et al., 2019), sleep problems (e.g., Mazurek & Petroski, 2015), gastrointestinal problems (e.g., Mazurek et al., 2013) and lower quality of life and/or poor life outcomes in adults (e.g., I. C. Smith et al., 2019) and children with autism (e.g., D. Adams et al., 2020; den Houting et al., 2022; van Steensel et al., 2012), as well as their parents (e.g., D. Adams et al., 2020; den Houting et al., 2022). Higher levels of trait anxiety may also be associated with negative outcomes for people with autism, as suggested by studies observing a relationship between high trait anxiety and higher levels of depressive symptoms (Zukerman et al., 2019), as well as autonomic nervous system dysfunction (E. C. Taylor et al., 2021).

Anxiety has been also associated with differences in sensory processing in autism (e.g., Ben-Sasson et al., 2008; MacLennan et al., 2020; Mazurek et al., 2013; Uljarević et al., 2016). Particularly, higher anxiety seems to be associated with sensory hypersensitivity (Black et al., 2017; Hwang et al., 2020; MacLennan et al., 2020, 2021; Mazurek et al., 2013; Wigham et al., 2015), although there is also evidence supporting a significant association between anxiety and hyposensitivity in autism (e.g., higher anxiety related to hyposensitivity: Glod et al., 2019; Hwang et al., 2020; lower anxiety related to hyposensitivity: MacLennan et al., 2020). Nevertheless, while anxiety has been suggested to influence sensory hypersensitivity, this relationship may be bidirectional, with the hypersensitivity also contributing to the development of anxiety symptomatology in autism

(Carpenter et al., 2019; Green et al., 2012; MacLennan et al., 2021). Furthermore, it was also suggested that a third variable or overlapping diagnostic criteria may influence the causal relationship between sensory hypersensitivity and anxiety (for an overview of the models conceptualizing the relationship between anxiety and sensory hypersensitivity, please see Green & Ben-Sasson, 2010). Of relevance for the current work, the role of self-reported anxiety in specific sensory processing domains, such as olfactory processing, has not been explored yet. It is possible that, considering the impact of anxiety symptomatology in olfactory processing (e.g., Burón & Bulbena, 2013), previous mixed results regarding olfactory processing in autism may be explained, at least in part, by differences in anxiety symptomatology.

1.3.3.3. The role of alexithymia in anxiety symptomatology in autism

The correlates of anxiety symptomatology in autism have been explored by several researchers. These include autism severity and level of social impairment (Kent & Simonoff, 2017; White et al., 2009), as well as age and level of intellectual functioning (Mingins et al., 2021; Nimmo-Smith et al., 2020; White et al., 2009). Several emotional, cognitive, and sensory factors, such as emotion regulation, intolerance of uncertainty and sensory sensitivity, were also identified as variables influencing anxiety symptomatology in autism (Conner et al., 2020; Hwang et al., 2020; Normansell-Mossa et al., 2021; South & Rodgers, 2017; Vasa et al., 2020; Wigham et al., 2015). Alexithymia, a construct reflecting persistent difficulties in emotional functioning and expression (Sifneos, 1973; G. J. Taylor, 1984), was also pointed out as a critical factor underlying anxiety symptomatology in autism (e.g., Maisel et al., 2016; Morie et al., 2019).

Alexithymia involves multiple components, which include difficulties in identifying and verbalizing one's own emotional states and feelings, as well as externally-oriented thinking (Bagby, Parker, et al., 1994). The set of cognitive and emotional difficulties associated with high levels of alexithymia include, for instance, impaired emotion recognition (e.g., R. D. Lane et al., 1996), difficulties in emotion regulation (e.g., Swart et al., 2009) and impaired interoception (e.g., Herbert et al., 2011; but see also Zamariola et al., 2018). Importantly, alexithymia has a significant impact on mental and

physical health; for instance, high levels of alexithymia are associated with depression (e.g., Honkalampi et al., 2000), eating disorders (e.g., Cochrane et al., 1993), personality disorders (Nicolò et al., 2011) and anxiety symptoms (e.g., Fietz et al., 2018; Karukivi et al., 2010). Similarly, it is associated with health conditions such as hypertension (e.g., Grabe et al., 2010) and gastrointestinal problems (e.g., Carrozzino & Porcelli, 2018). Moreover, alexithymia is often associated with other negative outcomes, such as higher self-reported somatic symptoms (e.g., Mattila et al., 2008), interpersonal difficulties (e.g., Vanheule et al., 2007), suicide ideation (e.g., Hemming et al., 2019) and lower health-related quality of life (Mattila et al., 2009). Thus, considering its impact on emotional functioning and health, alexithymia is an important variable to consider in emotion assessment and intervention, either in clinical or research contexts.

Alexithymia has been receiving plenty of attention in the context of autism, due to the high association between the two conditions (Kinnaird et al., 2019; Poquérusse et al., 2018); in fact, alexithymia is more prevalent in autism than in the typically developing population, with an estimated prevalence of 49.93% (against only 4.89% in typical development; Kinnaird et al., 2019). Importantly, alexithymia has been suggested to be a critical factor in the socioemotional difficulties often observed in autism (Bird & Cook, 2013; R. Cook et al., 2013; Oakley et al., 2020; Ola & Gullon-Scott, 2020; Trevisan et al., 2016). In this regard, alexithymia has been suggested to influence the relationship between autism symptoms and anxiety symptomatology (Maisel et al., 2016; Morie et al., 2019). For instance, Milosavljevic et al. (2016) observed significantly higher alexithymia rates in adolescents with autism in comparison with their typically developing peers (55% versus 16%); furthermore, the group of adolescents with autism and co-occurring higher levels of alexithymia also presented significantly higher levels of self-reported anxiety in comparison with the group with autism but lower levels of alexithymia. Through structural equation modelling with mediation analyses, Maisel et al. (2016) explored the role of emotional acceptance, intolerance of uncertainty and alexithymia in the relationship between autism traits and anxiety, in a sample of adults with and without an autism diagnosis. They observed that alexithymia and emotional acceptance explained 64% of the effect between autism traits and anxiety symptoms. Specifically, alexithymia independently explained 36% of the effect of autism traits on anxiety symptoms, while emotional acceptance explained 28% of the effect. When conducting the analyses

separately by group (autism diagnosis vs. typical development), the pattern of results was similar. In a sample of adults with autism, Morie et al. (2019) also observed a strong relationship between alexithymia and emotion regulation, which serially mediated the relationship between autism symptoms and anxiety. Finally, in a sample of the general population, Brett and Maybery (2022) performed a serial mediation model, where they observed that autism characteristics related to social difficulties and restricted and repetitive behaviors were associated with both higher alexithymia and trait anxiety, as measured through the Toronto Alexithymia Scale of 20 items (TAS-20; Bagby, Parker, et al., 1994) and the STICSA, respectively. Higher levels of alexithymia were also related with higher trait anxiety.

Although the literature supports the role of alexithymia as a mediator of the relationship between autism traits or symptoms, and anxiety, these studies are highly heterogeneous regarding several variables (e.g., participants characteristics, instruments, outcome variables and mediators, performed analyses) and did not explore the specific associations between the distinct dimensions of the constructs of autism, alexithymia, and anxiety. As previously argued, the distinct dimensions of autism traits seem to be distinctively associated with each other (e.g., Bothe et al., 2019; English et al., 2020). Likewise, the literature has been suggesting that autism symptoms, alexithymia and anxiety dimensions are distinctly associated between them. For instance, concerning the association between autism traits and alexithymia in the general population, Bothe et al. (2019) observed a strong and positive correlation between the social dimensions of the AQ and a total score of alexithymia, as measured by the TAS-20, while the non-social dimension of the AQ was only weakly and negatively correlated with alexithymia. This is in line with the results of Liss et al. (2008), who also observed that all dimensions of alexithymia (G. J. Taylor et al., 1992) were significantly associated with the social dimensions of the AQ, but not with attention to detail (non-social dimension). However, Brett and Maybery (2022) recently found that both social and non-social dimensions of autism were related to alexithymia in a sample of the general population. Yet, these authors used a distinct instrument to measure the non-social dimension of autism traits and used a total score for alexithymia; therefore, direct comparisons are limited. On the other hand, in both a mixed sample of adolescents and adults with and without autism and a sub-sample composed of only adolescents and adults with autism, Oakley et al. (2020) observed that

higher levels of total alexithymia (measured through TAS-20), as well as of each one of the TAS-20 subscales, were associated with higher self-reported social-communication difficulties as measured by the SRS, second edition (SRS-2; Constantino & Gruber, 2012). Yet, the association was stronger for the dimension of alexithymia corresponding to describing feelings, and weaker for the dimension corresponding to externally oriented behavior (Oakley et al., 2020). In this study, only the social-communication dimension of autism symptoms was inspected, precluding conclusions about the relationships regarding the non-social dimension of autism.

Anxiety symptoms were also previously shown to be differentially correlated with autism traits. For instance, Liss et al. (2008) observed statistically significant positive relationships between anxiety, as measured by the Beck Anxiety Inventory (Beck & Steer, 1990), and the social skills and attention to detail dimensions of the AQ, but not the communication dimension, in a sample of typically developing adults. Consistently with these results, Brett and Maybery (2022) found that both the social skills dimension of the AQ, and repetitive and restrictive behaviors as measured by the 20-item Adult Repetitive Behaviours Questionnaire-2 (Barrett et al., 2015) were positively associated with trait anxiety (as measured by the STICSA). Concerning the relationship between anxiety and alexithymia, Liss et al. (2008) also observed that, while anxiety was positively correlated with the dimensions concerning the ability to identify and describe feelings of the revised version of TAS, it was not associated with the dimension concerning externally-oriented thinking. On the other hand, in a mixed sample composed of individuals with and without an autism diagnosis, as well as in a clinical subsample, Oakley et al. (2020) observed that only the dimension corresponding to difficulties in identifying feelings of the TAS-20 was significantly associated with anxiety symptomatology.

In sum, while previous studies have provided important knowledge about the relationships between autism characteristics, alexithymia, and anxiety, they still do not provide a clear picture of the multivariate relationship between them, either in the general population or in clinical samples. Considering the multidimensional nature of these variables, as well as the clinical relevance of anxiety and alexithymia in autism, investigating which specific dimensions are related to each other (and with which magnitude) could help to better understand the heterogeneity often observed in autism and help to head assessment and intervention. For instance, taking into account the role of

alexithymia in anxiety symptomatology in autism, targeting alexithymia in the assessment and intervention planning – particularly the specific dimensions that may be more directly impacting symptomatology – could be critical to prevent psychopathology and mitigate some of the difficulties observed in the spectrum (Albantakis et al., 2020).

1.3.4. What is the role of (altered) olfaction in autism?

As referred in a previous section, olfaction plays an important role in nutrition well-being, and safety, as well as in social functioning. Considering this, the following questions arises: how do olfaction and potential olfactory alterations impact functioning and behavior in autism, especially social difficulties, which are perhaps the most salient core feature of the condition? Olfactory dysfunction seems to negatively impact social functioning and relationships (see Blomkvist & Hofer, 2021; Boesveldt & Parma, 2021; R. J. Stevenson, 2010). Furthermore, the evidence outside of the (clinical) autism spectrum suggested, for instance, a relationship between odor identification performance and social cognition measures in schizophrenic (Kohler et al., 2007) and euthymic bipolar patients (e.g., Lahera et al., 2013); an association between odor discrimination and social cognitive abilities in patients with first-episode psychosis (Etyemez et al., 2022); a relationship between odor identification performance and measures of social life (e.g., number of friends) in older women (Boesveldt, Yee, et al., 2017); a relationship between odor detection performance and social network size in typically developing adults (Zou et al., 2016); and an association between social cognitive skills (mentalizing) and odor discrimination performance in typically developing women (Lübke et al., 2022). Therefore, it seems that olfactory alterations impact important aspects of social processing, although the pattern of results is not consistent – which is expected, due to the differences in the samples and methods used to assess olfactory and social variables.

Considering the results in autism, Bennetto et al. (2007) found a marginal relationship between odor identification and the ability to engage in social chatting, as well as a significant association between odor identification abilities and reciprocal conversation skills in children with autism. Furthermore, Del Valle Rubido et al. (2020) observed that adults with autism and odor identification dysfunction presented decreased emotion recognition abilities, higher irritability and a lower full-scale intelligence quotient

in comparison with adults with autism but with normal olfactory abilities. Rozenkrantz et al. (2015) also observed a strong association between alterations in the sniff response (e.g., longer sniff duration towards unpleasant odors) and the severity of symptoms. Interestingly, the link was specific to the social affect component of the ADOS (Gotham et al., 2007; Lord et al., 2000). Finally, and in line with these results, Sweigert et al. (2020) also observed a relationship between autism severity in general, as well as autism severity in relation to the social affect dimension, and odor identification abilities in children with autism. Altogether, this suggests that worse odor identification may be associated with worse social, emotional and communication skills in autism, although caution is needed when interpreting these results, since the studies cannot be directly compared due to considerable methodological differences.

Although it seems plausible to hypothesize that olfactory alterations in autism may be related to impaired functioning at several levels, that does not necessarily mean that olfaction – and especially meaningful olfactory stimuli – cannot be successfully used to foster appropriate emotional and social behaviors in autism, for instance, as indicated by the results obtained by Parma et al. (2013). This, then, poses another question: Can olfaction be a social and emotional facilitator in autism? If yes, how? Does this facilitation occur across development and social cognitive processes? The literature still does not offer a clear picture of how these questions can be addressed, and in which dysfunctional processes olfaction could act as an aid. For instance, Parma et al. (2013) observed the role of maternal body odors in automatic imitation; does maternal body odors also facilitate other dysfunctional processes in autism, such as face processing and social attention? How could olfaction do it and how could it be integrated into real-life interventions? Further investigation is necessary to understand how olfactory alterations are connected to social cognitive and behavioral processes in autism. Yet, it is as important to tackle this question, as it is to clarify the nature and extent of olfactory alterations in autism. As we observed throughout this section, evidence about olfactory processing in autism is unclear, with studies suggesting either decreased, increased or normal olfactory abilities, as well as normal or altered subjective, behavioral, and physiological responses to odors across the explored dimensions. These results motivated the search for variables that can influence this mixed pattern of results, and that can help to deconstruct the complexity of olfactory processing in autism.

1.4. Aims and outline of the thesis

Grounded in the previously presented theoretical background, the present work sought to contribute to the understanding of autism, olfactory processing, and the multivariate relationship between autism characteristics and emotion-related variables that are also often associated with the spectrum, namely anxiety and alexithymia. The following specific aims were considered:

1. To evaluate the dimensionality and psychometric properties of the STICSA and its utility to measure anxiety in the general population **(Study 1)**. In line with previous studies, empirical support for a 4-factor (Balsamo et al., 2018; Grös et al., 2007; Roberts et al., 2016) and/or a 2-factor structure (cognitive/somatic) for both state and trait forms (e.g., Carlucci et al., 2018; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021) was expected. Regarding the relationship between STICSA-Trait scores and the emotional response in distinct emotional conditions, an overall reduced HRV in individuals with higher levels of trait anxiety, in comparison with individuals with lower levels of trait anxiety, was expected (Miu et al., 2009).
2. To evaluate the factor structure of the AQ in the Portuguese general population **(Study 2 – part 1)**. Similarly to several previous studies, we expected to observe a poor adjustment of the originally proposed 5-factor model, and to find a 3- or 4-factor structure similar to that observed by previous research (e.g., English et al., 2020).
3. To further explore the relationship between autism traits, alexithymia, and anxiety **(Study 2 – part 2)**. A pattern of results compatible with the previous literature was expected, including a significant association between autism traits and alexithymia (e.g., Liss et al., 2008; Maisel et al., 2016), especially considering the social dimension(s) of the AQ and the identification/description of feelings dimensions of the TAS-20 (e.g., Liss et al., 2008; Oakley et al., 2020). In addition, a significant relationship between alexithymia and trait anxiety was hypothesized, consistently with previous studies (e.g., Fietz et al., 2018; Oakley et al., 2020). We also expected to find a significant relationship between autism traits and trait anxiety, especially concerning the social dimension of the AQ (e.g., Bothe et al., 2019; Liss

et al., 2008). Lastly, a statistically significant indirect effect of autism traits in trait anxiety through alexithymia (Maisel et al., 2016) was also hypothesized.

4. To evaluate the role of distinct dimensions of autism traits and trait anxiety in the olfactory abilities of the general population **(Study 3)**. Although the previous research does not provide support for strong hypotheses, we expected to observe a significant association between a higher expression of autism traits and olfactory functioning in general, considering that olfactory alterations have been frequently reported in autism, although in an inconsistent fashion (Larsson et al., 2017; Tonacci et al., 2015). A significant relationship between olfactory performance, especially regarding odor detection, and trait anxiety was expected (e.g., Takahashi et al., 2015). Lastly, since a more marked expression of autism traits seems to exist in men (e.g., Baron-Cohen et al., 2001; Loomes et al., 2017) and taking into account that women often present superior olfactory abilities (see Sorokowski et al., 2019), the variable sex was added to the analyses to understand its impact on the models.
5. To review current knowledge about olfaction in autism and present arguments supporting how and why olfactory processing is a priority research area, and how advancing this line of investigation can help to improve knowledge and practice in the context of social cognition and social functioning in autism **(Study 4)**.

CHAPTER 2 - METHODOLOGY OVERVIEW

In this section, an overview of the general method used to conduct the four studies will be outlined. Table 1 summarizes the characteristics of the recruited samples, the inclusion and exclusion criteria used to select participants, as well as the materials and procedures used in each study. Throughout this section, we will also provide a brief characterization of the materials used to operationalize the proposed aims, as well as the general analytical strategies and ethical procedures followed throughout the studies. The specific methodology associated with each study is outlined in detail in Chapter 3, in the sections corresponding to materials and methods (within each study).

2.1. Materials

2.1.1. Sociodemographic questionnaires

In Study 1-3, sociodemographic questionnaires were used to characterize the samples, as well as to confirm inclusion/exclusion criteria. Questions such as age, sex, nationality, literacy, professional situation, medical/psychiatric problems, and medication intake were common in all studies. For Study 1 (psychometric study), questions about the city of residence, marital status, number of children and the existence of traumatic events were also included. Still in relation to Study 1 (relationship between trait anxiety and emotional response), visual acuity was also questioned. For Studies 2 and 3, the questionnaire also included questions about the ethnic origin, native language, relevant psychiatric/neurodevelopmental problems of first-degree relatives and smoking habits.

Table 1. Summary of the main characteristics of the samples, inclusion and exclusion criteria of the participants, materials, and procedures used in the empirical studies.

	Study 1.1	**Study 1.2**	**Study 2.1**	**Study 2.2**	**Study 3**
Aim	Analyze the dimensionality, reliability, measurement invariance and nomological validity of the STICSA	Analyze the relationship between the scores in STICSA-Trait and the subjective and psychophysiological response in distinct emotional situations	Analyze the dimensionality of the AQ	Analyze the multivariate relationship between the dimensions of autism traits, alexithymia and trait anxiety	Analyze the multivariate relationship between autism traits, trait anxiety and olfactory abilities
N	1153	74	292	244	116
Mean age	29.47 (SD=13.70)	21.41 (SD=3.09)	24.22 (SD=4.45)	24.18 (SD=4.31)	24.30 (SD=4.24)
Age range	18-78	18-31	18-42	18-36	
Male/Female	400/753	25/49	114/178	103/144	48/68
Inclusion criteria	- Age ≥ 18 years - Portuguese nationality - Currently residing in Portugal	- Age ≥ 18 years - Portuguese nationality - Normal or corrected-to-normal visual acuity	- Age ≥ 18 years - Portuguese nationality	- Age ≥ 18 years - Portuguese nationality	- Age ≥ 18 years - Portuguese nationality - Caucasian
Exclusion criteria	-	- Any medication or condition with an impact on cardiac functioning - Any psychiatric diagnosis or neurological disease	- Language other than Portuguese as the native language - Autism diagnosis - First-degree relative with an autism diagnosis	- Language other than Portuguese as the native language - Autism diagnosis - First-degree relative with an autism diagnosis	- Any psychiatric, neurological, endocrine, respiratory or immunological disorders, or any condition with a

		Study 1.1	Study 1.2	Study 2.1	Study 2.2	Study 3
					- Self-reported psychological or psychiatric disorders (anxiety)	significant impact on olfactory functioning - Any medication with an impact on olfactory functioning (except oral contraceptives) -Autism diagnosis -First-degree relative with an autism diagnosis
Materials		- STICSA - STAI-Y - DASS-D - PANAS	- STICSA-Trait - Emotional videos - Visual Analogue Scales - Material for cardiac signal recording	- AQ	- AQ - STICSA-Trait - TAS-20	- AQ - STICSA-Trait - Sniffin' Sticks
Procedure		- Translation of STICSA - Filling out questionnaires (online and paper)	- Filling out questionnaires (online) - Experimental task + cardiac signal recording	- Translation of AQ - Filling out questionnaires (online)	- Filling out questionnaires (online)	- Filling out questionnaires (online) - Olfactory evaluation
	Analytical procedure	- Missing data analysis - CFA (WLSMV estimator) - Reliability analysis	- Cardiac signal processing - HRV calculation - Repeated measures ANOVA	- CFA (WLSMV estimator) - EFA (PAF estimator) - Reliability analysis	- Path analysis (ML estimator) - Pearson correlations	-Preliminary analyses (impact of respiratory disorders, oral contraceptives)

	Study 1.1	Study 1.2	Study 2.1	Study 2.2	Study 3
	- Pearson correlation (convergent and concurrent validity)				- Hierarchical multiple linear regression

Note. STICSA: State-Trait Inventory for Cognitive and somatic anxiety; STAI-Y: State-Trait anxiety Inventory – Form Y; DASS-D: Depression, anxiety and Stress Scales – Depression Scale; PANAS: Positive and Negative Affect Schedule; CFA: Confirmatory Factor analysis; WLSMV: Weighted Least Square Estimator; HRV: Heart Rate Variability; ANOVA: Analysis of variance; AQ: Autism-Spectrum Quotient; EFA: Exploratory Factor Analysis; PAF: Principal Axis Factoring; TAS-20: Toronto Alexithymia Scale of 20 items; ML: Maximum Likelihood

2.1.2. State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA)

The STICSA is a self-report instrument developed to measure anxiety in four dimensions: trait-cognitive, trait-somatic, state-cognitive and state-somatic anxiety (Ree et al., 2008). It has two forms – STICSA-State and STICSA-Trait – assessing state and trait anxiety, respectively. Each form is composed of 21 questions evaluating cognitive and somatic symptoms. Items are measured on a 4-point scale ranging from 1 ("not at all") to 4 ("very much so"; Grös et al., 2007). Although Ree et al. (2008) have proposed distinct response scales for state and trait forms (ranging from not at all to very much so in the state form, and from almost never to almost always in the trait form), other studies have been using the labels of the state form for both state and trait forms (Balsamo et al., 2015; Roberts et al., 2016; Styck et al., 2020), as indicated by Grös et al. (2007). As the latter was the first psychometric study of the STICSA, in Studies 1-3 the indications of Grös et al. (2007) were followed. The Portuguese version of the STICSA was developed according to the procedure described in Study 1 (Barros, Figueiredo, Brás, et al., 2022) and the trait form was, then, used in the second part of Study 1, Study 2 and Study 3.

In the first part of Study 1, a psychometric study of the STICSA was performed; previous research on the STICSA has found support for the four-factor model (Balsamo et al., 2018; Grös et al., 2007; Roberts et al., 2016), and a hierarchical model with a global anxiety factor and four factors (Roberts et al., 2016). Separate models for state and trait anxiety have been also explored, with evidence supporting a two-factor model (cognitive/somatic) for state and trait forms (e.g., Carlucci et al., 2018; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021). In general, studies have been also suggesting good psychometric properties regarding convergent and nomological validity (Carlucci et al., 2018; Grös et al., 2007; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021), despite some concerns regarding STICSA's ability to differentiate anxiety from depression (Grös et al., 2007; e.g., Tindall et al., 2021).

Regarding STICSA's predictive validity, Ree et al. (2008) evaluated how cognitive and somatic dimensions of STICSA-Trait predicted self-reported state anxiety response in a stressful event (as measured by STICSA-State). They observed that the STICSA-Trait cognitive dimension was associated with both state-cognitive and state-somatic anxiety under cognitive stress, while the STICSA-Trait somatic dimension was associated with state-cognitive and state-somatic anxiety under a somatic stressor, providing support for the utility

of STICSA to predict the anxiety response towards different types of stressors. Nevertheless, the usefulness of STICSA-Trait to predict the emotional response across other daily contexts, as well as how STICSA-Trait relates to other dimensions of the anxiety response (e.g., psychophysiological response), remains an underexplored area that was investigated in the second part of Study 1. Apart from Study 1, the Portuguese version of the STICSA was used in Study 2 and Study 3.

2.1.3. Autism-Spectrum Quotient (AQ)

The AQ is a self-report questionnaire of 50 items developed by Baron-Cohen et al. (2001) to measure autism characteristics in adults of normal intelligence. A five-domain structure was proposed for the original version of the AQ, covering critical areas of autism-related alterations, namely social skills (e.g., "I prefer to do things with others rather than on my own"), attention switching (e.g., "I frequently get so strongly absorbed in one thing that I lose sight of other things"), attention to detail (e.g., "I often notice small sounds when others do not"), communication (e.g., "When I talk, it isn't always easy for others to get a word in edgeways") and imagination (e.g., "If I try to imagine something, I find it very easy to create a picture in my mind"). For each one of the 50 items that compose the questionnaire (10 items per dimension), individuals must select one of four possible answers: "definitely agree", "slightly agree", "slightly disagree" and "definitely disagree". According to the proposed in the original work, the "definitely agree" and "slightly agree" response categories are scored with 0 points, while the "slightly disagree" and "definitely disagree" response categories are scored with 1 point for half of the items; for the other half of the items, the scoring is reversed, since the items were counterbalanced to avoid a response bias (Ruzich et al., 2015). Nevertheless, many subsequent studies have been scoring AQ's items considering a 4-point Likert-type scale, to account for all the response variability (e.g., Austin, 2005; English et al., 2020; Hoekstra et al., 2008; Stewart & Austin, 2009). Furthermore, the Likert scoring method seems to be associated with higher internal consistency (J. L. Stevenson & Hart, 2017). Lastly, AQ's scores can be calculated for each dimension or globally. The total score reflects the sum of the points obtained in all items and has been also calculated across studies to represent the number of autism traits presented by an individual.

Regarding the psychometric properties of the AQ, studies observed both adequate and problematic results. For instance, in AQ's original work, results suggested acceptable-to-good internal consistency across dimensions and the total score ($.63 < \alpha < .77$; Baron-Cohen et al., 2001). Although subsequent studies found satisfactory internal consistency for the total score in non-clinical samples, some of the original subscales of the AQ presented poor internal consistency, especially the Attention-Switching, Imagination and Communication subscales (e.g., Broadbent et al., 2013; English et al., 2020; J. L. Stevenson & Hart, 2017). Studies exploring the dimensionality of the AQ across non-clinical samples and distinct cultures also found inconsistent results, with only a few studies supporting a 5-factor structure (and even those retained fewer items than the original solution; e.g., Kloosterman et al., 2011; Leth-Steensen et al., 2021; Sierro et al., 2016). In fact, several studies observed a three-factor (e.g., Austin, 2005; do Egito et al., 2017; English et al., 2020; R. M. Hurst, Mitchell, et al., 2007; Ingersoll et al., 2011; Palmer et al., 2015; Russell-Smith et al., 2011) or a four-factor model (e.g., Freeth, Sheppard, et al., 2013; Russell-Smith et al., 2011; Stewart & Austin, 2009) of AQ instead of the originally proposed 5-factor model. In addition, as noted by English et al. (2020), which performed a comprehensive psychometric analysis of the AQ, only a few items consistently loaded in the same factor across distinct studies, and many items were excluded from the final solutions. Yet, it is important to note that some of these studies differ in certain aspects that can influence results and preclude direct comparisons; for instance, the sample sizes, participants' characteristics (e.g., university students vs. general population samples), analytical procedures and method of scoring (binary vs. Likert). Although the literature about the factor structure of the AQ is not clear, posing difficulties to the assessment, interpretation and understanding of autism traits scores, the AQ is still one of the most used instruments to measure these traits, possibly due to its multidimensional nature, as well as easy access and administration. This stresses the need to further investigate this instrument's properties and adequacy to reliably measure autism characteristics across cultures and settings. This was the purpose of Study 2, which sought to investigate the factor structure of the AQ in a sample of the Portuguese population. The Portuguese version of the AQ was, then, developed according to the procedure described in Study 2 (Barros, Figueiredo, & Soares, 2022). After its development, this version was used in the second part of Study 2, as well as in Study 3.

2.1.4. Toronto Alexithymia Scale of 20 items (TAS-20)

The TAS-20 (Bagby, Parker, et al., 1994) is a self-report questionnaire that assesses alexithymia, a multidimensional personality construct characterized by alterations in the experience and expression of emotions (e.g., Bagby et al., 2020; Sifneos, 1973; G. J. Taylor, 1984). The instrument comprises three dimensions: (1) difficulties in identifying feelings (DIF); (2) difficulties in describing feelings and emotions (DDF); (3) externally oriented thinking (EOT; Bagby et al., 2020; Bagby, Parker, et al., 1994). A fantasizing facet was assessed in a previous version of the TAS ("reduced daydreaming"), but the items composing this dimension were later removed because they presented low item-total correlations and/or high correlations with social desirability (Bagby et al., 2020; Bagby, Parker, et al., 1994; G. J. Taylor et al., 1985). Due to the exclusion of this dimension, many authors have suggested that the TAS-20 does not assess alexithymia as it was originally conceptualized (e.g., De Gucht & Heiser, 2003; Vorst & Bermond, 2001; see also Bagby et al., 2020 for this discussion). Nevertheless, it was also suggested that the EOT factor of the TAS-20 may be able to measure this dimension of alexithymia indirectly (see Bagby et al., 2020); for instance, EOT was previously found to correlate negatively with the fantasy dimension of the NEO-Personality Inventory (Bagby, Taylor, et al., 1994). Each item of the TAS-20 is answered on a 5-point Likert scale, varying from 1 "strongly disagree" to 5 "strongly agree". The Portuguese adaptation of TAS-20, developed in the study of Prazeres et al., (2000), was used in Study 2.

2.1.5. State-Trait Anxiety Inventory – Form Y (STAI-Y)

The STAI - Form Y (Spielberger et al., 1983) is a widely used self-report questionnaire that assesses anxiety in two subscales: the state anxiety subscale (STAI-Y1; 20 items), and the trait anxiety subscale (STAI-Y2; 20 items). This instrument follows the conceptualization of anxiety as a transitory emotional state (state anxiety), or as a stable proneness to experience anxiety symptomatology (trait anxiety; Spielberger et al., 1983; Spielberger & Reheiser, 2009). Items are answered on a 4-point scale varying from 1 ("nothing at all") to 4 ("very much so") in the state subscale, and from 1 ("almost never") to 4 ("almost always") in the trait subscale. A Portuguese adaptation and validation of the STAI-Y was provided by Santos and Silva (S. C. Santos & Silva, 1997) and was used in Study 1.

2.1.6. Depression, Anxiety and Stress Scales – Depression Scale (DASS-D)

The DASS of 21 items (Lovibond & Lovibond, 1995) is a self-report questionnaire that evaluates anxiety, depression and stress in three distinct subscales. The DASS-D corresponds to the subscale assessing depression symptoms and encompasses seven items. The 4-point response scale ranges from 0 ("did not apply to me at all") to 3 ("applied to me very much or most of the time"), taking into account the frequency or severity of emotional symptoms experienced in the last week (Lovibond & Lovibond, 1995). The Portuguese adaptation of the DASS-D, which was developed by Pais-Ribeiro et al. (2004), was used in Study 1.

2.1.7. Positive and Negative Affect Schedule (PANAS)

The PANAS (Watson et al., 1988) is a self-report questionnaire that measures positive (10 items) and negative affect (10 items). In each item, a word corresponding to an emotion is presented and the individual must indicate how much he/she felt that emotion in the last weeks, considering a scale varying from 1 ("very slightly or not at all") to 5 ("extremely"). The Portuguese adaptation of the PANAS was provided by Galinha and Pais-Ribeiro (2005) and was used in Study 1.

2.1.8. Emotional videos

Three sets of videos (each with 8-12 film clips) were used to induce emotions in the laboratory (McGinley & Friedman, 2017). Each set had an approximate duration of 30 minutes and aimed to induce fear, happiness, or nothing in particular (neutral condition). For this purpose, film clips of horror movies, comedy movies and documentaries were selected, respectively. In each set, the order of visualization of the film clips was fixed, from the least intense clip to the most intense clip, in order to keep a constant state of activation. Three additional emotionally neutral documentary clips, of approximately five minutes each, were presented before each set of emotional videos to provide baseline psychophysiological data (baseline videos). All the film-clips were previously analyzed regarding their ability to

convey the corresponding emotions and were used in previous studies with a similar protocol (e.g., Barros et al., 2019; Gouveia et al., 2020; G. Pinto et al., 2020). Three Visual Analogue Scales (VAS) of 100 points were presented together with the videos to assess the emotional state of participants. These VASs evaluated how happy and fearful participants felt at the moment (before and after the visualization of each emotional set), as well as how much aroused/activated they felt during the visualization of the videos (question asked after the baseline videos and after the emotional set of videos). The experimental task was programmed with the software OpenSesame (version 3.2.1; OpenSesame Inc.), using Python language (version 2.7.13). The task was performed using a Dell OptiPlex 7040 and a 17-inch Dell digital monitor (Model: E178FP), with a refresh rate of 60 Hz.

2.1.9. Sniffin' Sticks

The Sniffin' Sticks Extended Test (Hummel et al., 1997) is psychophysical method to quantify olfactory evaluation, consisting of a battery of tests assessing odor detection, discrimination and identification (see also Doty, 2017). This test considers the response of the individuals towards the presentation of felt tip pens with distinct odors. The sum of the scores obtained by individuals in each subtest (Threshold + Discrimination + Identification; TDI) can be interpreted as their general olfactory ability. Separated scores for each olfactory dimension can also be obtained, reflecting specific olfactory abilities. The odor threshold test assesses the minimal concentration that an odor must have to be detected by an individual (Hummel et al., 1997). The test consists of 16 triplets of pens, with only one pen of each triplet presenting a target-odor in a certain concentration; the remaining pens contain only the solvent used to dilute the target-odor. The target-odor can be n-butanol or phenylethanol (PEA); the latter was used as target odor in Study 3. Each trial corresponds to the presentation of a triplet, starting with the triplet with the smallest concentration of the target odor. Each pen of the triplet is presented for 3 seconds. Between the presentation of each pen, there is a 5-second gap and between the presentation of each triplet there is an interval of 30 seconds. The individual is asked to identify the pen of the triplet that contains the target odor (forced-choice paradigm). Following a staircase procedure, when the pen with the target odor is correctly identified twice in a row, a staircase reversion occurs and the next triplet with the lower concentration is presented. When the participant gives an incorrect answer, a new

reversion occurs and the next triplet with the higher concentration is presented. The odor threshold score is calculated through to the average of the last four reversals (Hummel et al., 1997).

The odor discrimination test also involves the presentation of 16 triplets of pens, all imbued with common odors; two have the same odor and one has a different odor. Each trial corresponds to the presentation of a triplet similarly to the one described for the odor threshold. The individual must identify the pen that contains the odor that is different from the remaining two pens. The final score is calculated through the sum of the corrects answers. Finally, the odor identification test encompasses 16 pens with different common odors. Each pen is presented separately for 3 seconds, with an interval of at least 30 seconds between pens. A card with four options is presented before the presentation of each pen. Following the presentation of the pen, the individual is asked to choose the word that represents the odor. The score is calculated through the sum of correct answers. The literature suggests that the identification and discrimination tests relate to central olfactory processing, while threshold tests are more associated with peripheral olfactory function (e.g., Hedner et al., 2010; see Hummel et al., 2017). To characterize olfactory (dys)function and distinguish between normosmic, hyposmic and anosmic individuals, normative data is necessary. According to the normative data for the Portuguese population, provided by Ribeiro et al. (2016), the means for the 18-35 years old group are: (1) TDI: M=36.9 (SD=3.96); (2) Threshold test: M=10.83 (SD=2.31); (3) Discrimination test: M=12.05 (SD=2.16); (4) Identification test: M=14.10 (SD=1.21). The percentile 10, which is an indicator of hyposmia, corresponds to: (1) TDI: 31.75; (2) Threshold test: 7; (3) Discrimination test: 8; (4) Identification test: 13.

2.2. General analytical strategies

2.2.1. Psychometric analyses

In studies 1 and 2, psychometric analyses were carried out. A Confirmatory Factor Analysis (CFA) was performed to analyze the factor structure of the STICSA and of the AQ. The Weighted Least Square Estimator (WLSMV) was used for these analyses since both the instruments have a 4-point Likert response scale. The assessment of the model fit was performed considering a robust chi-square statistic, and was also supported in a combination of different fit indexes (see Brown, 2006; Schermelleh-Engel et al., 2003): (1) Comparative

Fit Index (CFI) higher than .95; (2) Tucker and Lewis Index (TLI) higher than .95; (3) Root Mean Square Error of Approximation (RMSEA) equal or less than .07; and (4) Standardized Root Mean Residual (SRMR) equal or less than .07.

A measurement invariance analysis was also performed in Study 1 to provide evidence that the same factor structure corresponded to the same constructs in different groups of individuals (Brown, 2006; van de Schoot et al., 2012). For this purpose, a multi-group CFA was conducted to analyze the measurement invariance of the STICSA in relation to groups of women/men, using the estimator chosen for the previously performed CFA (Barros, Figueiredo, Brás, et al., 2022). The assessment of measurement invariance was performed for the STICSA-Trait and the STICSA-State separately and tested several constrained models. Three increasingly constrained models were tested for each group separately, in order to compare them regarding configural, metric, and scalar invariance (Brown, 2006; van de Schoot et al., 2012). Model fit assessment considered the parameters previously mentioned. Furthermore, the comparison between the constrained models considered the difference in robust chi-square (chi-square difftest), which is the most recommended procedure when using the WLSMV estimator (Sass et al., 2014).

In Study 2, an Exploratory Factor Analysis (EFA) was carried out, with the Principal Axis Factoring (PAF) method of parameter estimation. An oblique rotation (direct oblimin) was used assuming that the dimensions of the AQ were correlated.

Finally, reliability analysis was conducted in Studies 1 and 2, using McDonald's Omega, which accounts for the effective results of the CFA (A. F. Hayes & Coutts, 2020; Viladrich et al., 2017) and Cronbach alpha, a widely used measure of reliability (DeVellis, 2012; A. F. Hayes & Coutts, 2020).

2.2.2. Relationships between the variables of interest

In order to explore the multivariate relationship between autism traits, alexithymia and anxiety (Study 2), as well as between autism traits, anxiety and olfactory processing (Study 3), a path analysis and hierarchical multiple regression analyses were performed, respectively. The path analysis performed in Study 2 considered the relationship between the observed variables and used the Maximum Likelihood (ML) estimator. The model fit was evaluated considering the same indexes described above (subsection 2.2.1.). Three hierarchical multiple regression analyses were performed in Study 3 to assess the multivariate

impact of autism traits and trait anxiety on the distinct olfactory dimensions. In the first step, sex was added as a control variable. In the second step, attention to detail and social skills (autism traits), as well as trait-cognitive and trait-somatic anxiety were added as predictors. The multicollinearity of the predictors was assessed (less than .70) and values of tolerance higher than .20 were considered. Influent outliers were also inspected, and the goodness-of-fit and the magnitude of improvement of the model (as well as of the individual steps) were assessed through R^2 and F-Ratio. Finally, standardized beta coefficients were interpreted (Howell, 2006; Tabachnick & Fidell, 2007).

Pearson correlations were also used to analyze the level of association between the variables, namely between the dimensions of autism traits, alexithymia, and anxiety (Study 2) and between different olfactory dimensions (Study 3). They were also performed to explore the convergent and concurrent validity of the STICSA, in Study 1 (Howell, 2006). The interpretation of the correlations considered the following indicators: small effect if [.10 - .30[; medium effect if [.30 - .50[; large effect if [.50 – 1] (J. Cohen et al., 2002).

2.2.3. Group differences

A repeated measures ANOVA was performed in the second part of Study 1 considering:

- LF, HF, LF/HF, self-reported happiness, fear and arousal as dependent variables (separated ANOVAs);
- Condition (fear/happy/neutral emotional induction) and Moment (baseline/emotional induction) as within-subjects variables;
- Group (high/low anxiety) as a between-subjects variable.

All the analyses were performed for groups of trait-cognitive and trait-somatic anxiety separately. The main effects were reported using the Greenhouse-Geisser correction when sphericity was not assumed. Simple-effect analyses were performed for all the significant interactions, and the significance levels of the comparisons were corrected using the Bonferroni correction (Howell, 2006).

Finally, additional ANOVAs were performed to test differences between the baselines across emotional conditions, as well as to test differences of groups of cognitive and somatic anxiety in the baseline (pre-test), across conditions and measures.

In Study 3, preliminary analyses were conducted to evaluate if there were mean differences between groups of women taking oral contraceptives vs. women free of oral contraceptive (considering the menstrual phase), as well as between groups with respiratory problems vs. without respiratory problems. For these purposes, an independent-samples t-test, a non-parametric Mann-Whitney U test, and a one-way ANOVA were performed.

2.3. Ethical procedures

The studies were performed considering the principles of the Declaration of Helsinki and the American Psychological Association. All procedures were further approved by the Ethics and Deontology Committee of the University of Aveiro. Participants were always informed about the voluntary nature of their participation, as well as about the possibility of withdrawing at any time. Informed consent was collected prior to their participation, in paper and/or online, depending on the specific study. Participants were also informed about the confidentiality of the data. To ensure proper data management and the privacy of participants, data collected was the strictly necessary for the research purpose (according to the data minimization principle), an appropriate place was used for data collection, and the data was always coded and anonymized after collection. In addition, data was only processed by the researchers of the team authorized for this purpose (obliged to professional secrecy). All processed data is currently preserved in its anonymous format, considering the transparency policy adopted by scientific journals and the eventual need to re-analyze data at a later stage of the research. The necessary organizational measures were adopted for data protection. Personal information was used solely for the purpose of recruiting and scheduling sessions (Study 1 and Study 3) and does not appear in data files. The instruments and techniques adopted (questionnaires, emotional induction, psychophysiological measures, Sniffin' Sticks) were painless and effortless, having already been used in similar contexts, and, to our knowledge, no harm was previously reported. Therefore, we believe that the possibility of discomfort was not greater than what can be expected from any other daily activity. Nevertheless, the comfort of the participants was monitored whenever possible. A draw of three vouchers of 20 euros each was done for the participants of the second part of Study 1, as a way to compensate them for their time and availability during the whole procedure.

CHAPTER 3 – STUDIES

Study 1

Multidimensional assessment of anxiety through the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA): From dimensionality to response prediction across emotional contexts

This study was published online by Public Library of Science in PLOS ONE, on 25 January 2022. The reference list of the manuscript has been integrated in the bibliography of the thesis – please see Chapter 5. The necessary structural adaptations were performed to incorporate the content of the article in the present thesis.

Reference:

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Abstract

The assessment of mal-adaptive anxiety is crucial, considering the associated personal, economic, and societal burden. The State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA) is a self-report instrument developed to provide multidimensional anxiety assessment in four dimensions: trait-cognitive, trait-somatic, state-cognitive and state-somatic. This research aimed to extend STICSA's psychometric studies through the assessment of its dimensionality, reliability, measurement invariance and nomological validity in the Portuguese population. Additionally, the predictive validity of STICSA-Trait was also evaluated, through the analysis of the relationship between self-reported trait anxiety and both the subjective and the psychophysiological response across distinct emotional situations. Similarly to previous studies, results supported both a four-factor and two separated bi-factor structures. Measurement invariance across sex groups was also supported, and good nomological validity was observed. Moreover, STICSA trait-cognitive dimension was associated with differences in self-reported arousal between groups of high/low anxiety, whereas STICSA trait-somatic dimension was related to differences in both the subjective and psychophysiological response. Together, these results support STICSA as a useful instrument for a broader anxiety assessment, crucial for an informed diagnosis and practice.

Keywords

STICSA; trait anxiety; cognitive anxiety; somatic anxiety; psychometric study

Introduction

Anxiety is an emotional response following the anticipation of a threatening stimulus, either real or perceived (American Psychiatric Association, 2013). It encompasses a complex and multidimensional phenomenon associated with muscular tension, subjective distress, enhanced vigilance concerning future threat and avoidance behaviors, which prepare the body for action and adaptive behavior (American Psychiatric Association, 2013; Öhman, 2008). Nevertheless, anxiety is often associated with great distress and poor health outcomes when persistent and/or significantly intense (American Psychiatric Association, 2013; Comer & Olfson, 2010). In fact, anxiety disorders are one of the most observed groups of psychopathologies, with an estimated prevalence of 7.3% globally (4.8 – 10.9%; see Baxter et al., 2012), and are often associated with other mental disorders (Lamers et al., 2011), and health problems, such as cardiovascular diseases (Celano et al., 2016). Furthermore, anxiety symptomatology is characterized by great inter and intra-individual heterogeneity, along with variable symptom trajectories over time (Nandi et al., 2009). Altogether, these factors complexify the assessment, diagnosis and treatment of non-adaptive anxiety, catalyzing the associated societal, personal and health care costs and burden (Comer & Olfson, 2010; Konnopka & König, 2020).

Self-report instruments have been extensively used as a simple, brief and non-invasive method of assessing anxiety across contexts (Balsamo et al., 2018). Although many present satisfactory psychometric properties (Beck & Steer, 1991; Spielberger et al., 1983), most present several limitations regarding, for instance, the ability to discriminate anxiety from depression (Kennedy et al., 2001), and the ability to embrace multiple anxiety dimensions. To surpass some of these difficulties and to provide a multidimensional assessment of anxiety, Ree and colleagues (2008) developed the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA). This instrument encompasses 42 items divided in two forms of 21-items each: the state anxiety (STICSA-State) and the trait anxiety (STICSA-Trait) form. State anxiety is conceptualized as the anxiety response experienced at the moment, i.e., in a limited period; in turn, trait anxiety corresponds to individual differences regarding anxiety as an emotional response relatively stable in time (Spielberger et al., 1983; Spielberger & Reheiser, 2009). STICSA also addresses the cognitive and somatic components of

anxiety within each one of its forms; the cognitive dimension encompasses symptoms such as worry, intrusive thoughts and rumination (e.g., "Feel agonized over problems"), while the somatic component refers to psychophysiological activation, including palpitations or excessive sweating (e.g., "Heart beats fast"). Discerning between these four dimensions can be critical to characterize anxiety profiles and to predict people's response in certain contexts. In fact, people can present distinct cognitive and somatic symptomatology (Schwartz et al., 1978).

STICSA has been supported as an adequate measure of anxiety across healthy and clinical samples, demonstrating good nomological validity (Grös et al., 2007; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021). Furthermore, most psychometric studies of STICSA support the construct validity of the two-factor structure within STICSA-Trait and STICSA-State forms (Carlucci et al., 2018; Gros et al., 2010; Ree et al., 2008; Roberts et al., 2016; Styck et al., 2020; Tindall et al., 2021), as well as the four-dimension structure (Balsamo et al., 2015; Grös et al., 2007; Roberts et al., 2016). Yet, past studies still evidence mixed findings and have left open questions regarding STICSA's dimensionality (see Styck et al., 2020). Therefore, it is crucial to extend STICSA's psychometric studies to other settings and cultures, as well as to confirm its adequacy and utility across contexts. Also, although STICSA provides important information about self-reported anxiety symptomatology, the relationship between the reported symptoms and other dimensions of the emotional response of the individuals in certain contexts remains poorly understood. The accordance between the multiple components of the emotional response (e.g., subjective and psychophysiological responses) is often low (Mauss et al., 2005), with each component providing unique and complementary information (Mauss & Robinson, 2009). Nevertheless, in the absence of more objective measures and considering the frequent time and financial constraints observed across contexts, self-report is often one of the few and more reliable tools to assess anxiety. Therefore, studying how scores in the four dimensions of STICSA are able to predict individual's emotional response beyond the subjective domain is crucial to obtain more accurate information about people's anxiety profile, health and well-being.

Considering this mindset, we propose two main aims for our research. First, we sought to extend the validation studies of STICSA, while adapting this instrument for the Portuguese population. Second, we aim to assess the relationship between STICSA-

Trait scores and the individual's subjective and psychophysiological responses to emotional stimulation. Since trait anxiety reflects relatively stable differences between people in the proneness to experience symptoms of anxiety (Spielberger & Reheiser, 2009), it may be especially useful to predict an individual's emotional response across contexts. With these aims, we intend not only to support the construct validity of STICSA, but also to maximize and integrate information about different dimensions of anxiety across emotional contexts. Moreover, this research seeks to provide a better comprehension of how distinct dimensions of self-reported trait anxiety predicts an individual's emotional response in different emotional contexts, including how these scores relate to a biomarker of autonomic dysfunction and psychopathology, namely Heart Rate Variability (HRV). This can be very critical knowledge, not only considering research settings but also clinical practice, given that self-report is often the most accessible and cost-effective tool to support assessment, diagnosis and monitoring of anxiety.

Study 1: Psychometric study of STICSA

Previous psychometric studies of STICSA assessed and supported factorial validity of the state-trait and cognitive-somatic distinction by computing full scale confirmatory factorial models and observing, for instance, a four-factor correlated model (Balsamo et al., 2015; Grös et al., 2007; Roberts et al., 2016), or a hierarchical model with a global anxiety factor supported in the four factors (state-cognitive, state-somatic, trait-cognitive and trait-somatic anxiety; Roberts et al., 2016). Dimensionality was also established by estimating separate models, namely a two-factor model for state (Carlucci et al., 2018; Ree et al., 2008; Roberts et al., 2016; Styck et al., 2020; Tindall et al., 2021) and trait forms (Carlucci et al., 2018; Gros et al., 2010; Ree et al., 2008; Roberts et al., 2016; Styck et al., 2020; Tindall et al., 2021). Furthermore, studies of measurement invariance across gender and age groups (Carlucci et al., 2018; Tindall et al., 2021), as well as a multi-method approach with an informant within friendship dyads of trait anxiety (Gros et al., 2010), gave further support for the quality of the STICSA measurement. Previous studies have also revealed good convergent and nomological validity (Balsamo et al., 2015; Carlucci et al., 2018; Grös et al., 2007; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021). Nevertheless, STICSA evidenced

medium to high correlations with depression measures (r= .42 - .67), especially when considering the correlation with cognitive anxiety (r > .48; Balsamo et al., 2015; Grös et al., 2007; Tindall et al., 2021), despite of being better at discriminating anxiety from depression in comparison with, for instance, the State-Trait Anxiety Inventory (STAI; Spielberger et al., 1983), an instrument broadly used to measure anxiety in its state and trait dimensions (e.g., Grös et al., 2007). STICSA has been proven to be, therefore, a suitable instrument to measure anxiety in general and clinical populations, but it is necessary to extend its psychometric studies to better characterize the instruments' factor structure, validity, and its adequacy across cultures and settings. In order to assess STICSA's psychometric properties in the Portuguese population, its dimensionality, reliability, measurement invariance and nomological validity were assessed in Study 1.

Materials and methods

Participants

The inclusion criteria included: 1) Age ≥18 years old; 2) Portuguese nationality; 3) Currently residing in Portugal. A final sample of 1153 Portuguese adults (753 females; 65.3%) from different regions of Portugal was collected. Age ranged from 18 to 78 years old (M=29.47; SD=13.70). Most of the participants were higher education students (n=658; 57.2%) or active employed individuals (n=338; 29.4%). Most of the participants were single (n=825; 71.7%) and did not have children (n=872; 75.7%). Furthermore, 13.4% of the participants (n=154) reported having a psychological/psychiatric problem, and 6.2% (n=71) reported being monitored/receiving treatment regarding those problems. For further information about sample's characteristics please refer to Table 1. All participants were informed about the voluntary nature of their participation and the anonymity of collected data. The study was approved by the Ethics and Deontology Committee of the University of Aveiro (ref. 08/2018) and was performed according to the guidelines of the Declaration of Helsinki and the American Psychological Association.

Table 1. Sample's demographic information.

		Total sample (n=1153)		Women (n=753)		Men (n=400)	
Age		M= 29.47	SD= 13.70	M=29.65	SD=13.39	M=29.15	SD=14.28
		N	%	N	%	N	%
Sex	Female	753	65.3	753	100.0	-	-
	Male	400	34.7	-	-	400	100.0
Education	Basic education	44	3.8	30	4.0	14	3.5
	Secondary education	555	48.2	331	44.0	224	56.0
	Higher education	536	46.5	387	51.5	149	37.3
	Other	17	1.5	4	0.5	13	3.3
Occupation	Students	658	57.2	412	54.8	246	61.7
	Active employment	338	29.4	235	31.2	103	25.9
	Both student and employee	47	4.1	29	3.9	18	4.5
	Domestic	6	0.5	6	0.8	-	-
	Retired	38	3.3	21	2.8	17	4.3
	Unemployed	29	2.5	24	3.2	5	1.3
	Other	35	3.0	25	3.3	10	2.5
Marital status	Single	825	71.7	527	70.1	298	74.7
	Married	235	20.4	158	21.0	77	19.3
	Cohabiting	47	4.1	35	4.7	12	3.0
	Divorced	37	3.2	26	3.5	11	2.8
	Widower	7	0.6	6	0.8	1	0.3
Have children?	Yes	280	24.3	192	25.5	88	22.0
	No	872	75.7	560	74.5	312	78.0
Psychiatric problems	Yes	154	13.4	120	16.0	34	8.5
	No	997	86.6	631	84.0	366	91.5
Currently monitored/ receiving treatment for psychiatric problems	Yes	71	6.2	55	7.3	16	4.0
	No	1075	93.8	694	92.7	381	96.0

Note. Considering the measurement invariance analyses, descriptive data are also presented for groups divided by sex (women/men). Whenever the total value of cases does not correspond to 1153, it is due to missing values. Only valid answers are presented in this table.

Instruments

In a first step, the 42 items of STICSA were translated to the Portuguese language by the research team, followed by a retroversion conducted by an English native speaker. The two versions were then discussed to obtain a preliminary version of the scale. This version was tested in a group of individuals to assess clarity and to identify possible misinterpretations associated with the items wording and instructions, as well as to evaluate the average response time of the overall protocol. After collecting their feedback, the research team made minor adjustments, finalizing the process of translation and adaptation of the 42 items (21 items per subscale).

Considering the procedure followed by one of the original psychometric studies of STICSA (Grös et al., 2007), STAI (Spielberger et al., 1983), the Depression, Anxiety and Stress Scales – Depression scale (DASS-D; Lovibond & Lovibond, 1995) and the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) were selected to evaluate nomological validity. These instruments have been widely used to measure anxiety, depressive symptomatology, as well as positive and negative affect, respectively, aspects that were on the basis of STICSA's development. In fact, STICSA was developed considering a state/trait distinction similar to STAI, and it sought to surpass one of STAI's greatest limitations, related to its high associations with depression and low positive affect (Bados et al., 2010; Caci et al., 2003). The protocol was then firstly constituted by a sociodemographic questionnaire, with relevant information for the sample's characterization, followed by STICSA (42 items), STAI (40 items), PANAS (20 items) and DASS-D (7 items).

State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA). The STICSA (Ree et al., 2008) is a self-report instrument that measures anxiety considering four dimensions: state, trait, cognitive and somatic anxiety. It is divided in two forms, each one with the same 21 items: one evaluating anxiety in its state dimension ("how do you feel right now"; STICSA-State) and the other evaluating its trait dimension ("how often this is true for you"; STICSA-Trait). Both forms evaluate cognitive and somatic components of anxiety, with items being rated in a 4-point scale that ranges from 1 (Not at all) to 4 (Very much; see Grös et al., 2007).

State-Trait Anxiety Inventory (STAI - Form Y). The STAI - Form Y (Spielberger et al., 1983) is a psychological assessment instrument that measures anxiety. This scale encompasses two self-report scales of 20 items each: the state anxiety scale (STAI-Y1), which assesses how the individual feels at the moment; and the trait anxiety scale (STAI-Y2), assessing how the individual generally feels. Participants are asked to respond to each item using a four-point scale that ranges from 1 (Nothing at all) to 4 (Very much so) in the state scale, and from 1 (Almost never) to 4 (Almost always) in the trait form. This instrument was adapted and validated for the Portuguese population by Santos and Silva (1997) and further psychometric studies supported its adequacy as a measure of anxiety (D. R. Silva & Campos, 1998).

Positive and Negative Affect Schedule (PANAS). The PANAS (Watson et al., 1988) is a self-report instrument that measures positive and negative affect. This scale encompasses 10 items evaluating positive affect and 10 items evaluating negative affect. Each item corresponds to an emotion and the individual is asked to indicate how much he/she felt that emotion in the last weeks, using a scale ranging from 1 (Very slightly or not at all) to 5 (Extremely). PANAS was adapted for the Portuguese population by Galinha and Pais-Ribeiro (Galinha & Pais-Ribeiro, 2005) confirming the original factor structure of the instrument, and its adequate psychometric properties.

Depression, Anxiety and Stress Scales – Depression scale (DASS-D). The DASS-21 is a self-report instrument comprising three scales evaluating anxiety, depression and stress, with 21 items distributed equally in each dimension (Lovibond & Lovibond, 1995). Response scale encompasses a four-point scale that ranges from 0 (Did not apply to me at all) to 3 (Applied to me very much or most of the time), considering the frequency or severity of the negative emotional symptoms experienced in the last week (Lovibond & Lovibond, 1995). This instrument was adapted for the Portuguese population by Pais-Ribeiro and colleagues (2004), revealing adequate psychometric properties for the three subscales in an adult sample. In the current study, only the depression subscale was used.

Procedure

The participants' recruitment and data collection followed a mixed-mode survey procedure (Dillman et al., 2014), namely: 1) data collection in paper, mostly carried out in classroom context (targeting higher education students) and with older people of the general population; and 2) data collection through a digital platform (targeting the general population). In both modes, participants were informed about the study mainly through e-mail, class visits, flyer's distribution, and social networks. All participants received detailed information about the study and gave their informed consent before their participation (by consenting and proceeding with the protocol in the digital platform, or by providing written informed consent when data collection was performed in paper). There was no compensation for their participation in the study. The protocol took, on average, between 15 and 20 minutes to complete.

Analytic procedures

The Mplus 8 version (Muthén & Muthén, 2017) was used to conduct Confirmatory Factorial Analysis (CFA), both in one group and multi-group (measurement invariance). The IBM SPSS Statistics (version 23) was employed for descriptive and reliability analyses. Additionally, R package's Amelia II (Honaker et al., 2018) was used to handle missing data. The missing data analysis was performed considering a sample higher than 1000, through a multiple imputation procedure. The used algorithm first created a bootstrapped version of the original data, through which estimated the sufficient statistics by Expected Maximization (EM) on a bootstrapped sample, and then imputed the missing values of the original data using the estimated sufficient statistics. This procedure was carried out by producing five dataset that were aggregated afterwards, assuming data as ordinal; in fact, three of the four measurement instruments with imputed missing data had 4-point answer scales, and the remaining instrument had a 5-point scale that ought to be considered ordinal as well. The imputation procedures were performed separately for each instrument. All participants presenting more than 10% of missing values in an instrument were excluded from the final sample (Allison, 2002). The maximum imputed by variable was 1.2%, and the overall imputation encompassed less than 0.2% of total responses in the dataset.

The CFA were computed using a categorical robust Weighted Least Square Estimator (WLSMV) developed by Muthén and Muthén (2017). This estimator has been suggested as the one that best performs when testing categorical data (Brown, 2006; Flora & Curran, 2004). The assessment of model fit adjustment was based on robust chi-square statistic, as well as in the following fit indexes: Comparative Fit Index (CFI), Tucker and Lewis Index (TLI), Root Mean Square Error of Approximation (RMSEA), and Standardized Root Mean Residual (SRMR). Given the sample size of the present study, the interpretation of chi-square statistic was less helpful, since a non-significant result would not be expected (R. Kline, 2005). Therefore, the model assessment assumed the following cut-off points: 1) CFI and TLI values higher than .95; and 2) RMSEA and SRMR values equal or less than .07 (Brown, 2006; Schermelleh-Engel et al., 2003).

A relevant dimension in psychometric validation studies is the equivalence of the measurement scale across different groups, i.e., to provide evidence that the same items are composing the same constructs in different groups of individuals, a condition that supports the accuracy of the group statistics (Brown, 2006; van de Schoot et al., 2012). In this line, several studies suggest that anxiety disorders are more prevalent in women than in men (e.g., McLean et al., 2011). Therefore, sex invariance was tested in our sample, considering both state and trait models separately. Measurement invariance was assessed by testing several constrained models. First, it was assessed for each group separately, following three increasingly constrained models to compare the two groups regarding configural, metric, and scalar invariance (Brown, 2006; van de Schoot et al., 2012). Model fit decision was assessed considering the parameters previously mentioned, and the comparison between the constrained models was done considering the difference in robust chi-square (chi-square difftest), being this option the most recommended when using the WLSMV estimator (Sass et al., 2014).

Reliability analysis was supported in McDonald's Omega, which accounts for the effective results from the CFA analysis conducted (A. F. Hayes & Coutts, 2020; Viladrich et al., 2017). Cronbach alpha was also computed, as it is the most common internal consistency coefficient with Likert-type response scales, allowing the comparison between different studies (DeVellis, 2012). Lastly, convergent and concurrent validity were observed through Pearson correlation (Howell, 2006). The interpretation of the correlation was supported considering Cohen´s suggestion: small

effect [.10- .30[, medium effect [.30 - .50[, and large effect: [.50 -1] (J. Cohen et al., 2002).

Results

Dimensionality analysis

According to the procedures used to develop STICSA, as well as to the constructs it is meant to reflect, the dimensionality analysis can approach the full model or divide the analysis by trait and state forms. The full models are expected to express an extensive degree of multicollinearity since the items of state and trait have the same wording. Nevertheless, the dimensionality analysis assumed and explored both options. The CFA of the full model of STICSA scale (42 items) was assessed through five concurrent models:

- (MF1) a unidimensional model with all items loading in one factor;
- (MF2) a four correlated factor model with the state-cognitive, state-somatic, trait-cognitive and trait-somatic dimensions;
- (MF3) a four-factor model including state-cognitive, state-somatic, trait-cognitive and trait-somatic factors, as well as a second-order anxiety factor;
- (MF4) a four-factor model including state-cognitive, state-somatic, trait-cognitive and trait-somatic factors, as well as second-order state and trait factors;
- (MF5) a four-factor model including state-cognitive, state-somatic, trait-cognitive and trait-somatic factors, as well as a second-order state and trait factors and a third-order anxiety factor.

The MF4 and MF5 models were tested in the present study since they reflect the most complex models underlying the instrument's structure, considering the somatic and cognitive dimensions within higher-order trait and state dimensions (MF4), as well as a third higher-order anxiety factor (MF5). Considering that all items of state and trait forms are exactly the same, with differences only in the scales' instructions (Grös et al., 2007), the error terms were allowed to correlate between the mirror items.

All five models showed acceptable to good fit indexes. The decision about model adequacy was based on the results of the selected fit indexes (CFI, TLI, RMSEA and SRMR), since the results for the chi-square test were statistically significant, as expected due to the sample size (R. Kline, 2005). Results showed that the four-factor

model with second-order state and trait factors (MF4), as well as the three-order factor model (MF5) presented good adjustment. The model with four-correlated factors (MF2) was the one with the best overall adjustment within the full models (Table 2).

Table 2. Goodness of fit statistics for all concurrent models (n=1153).

Models	χ^2	df	CFI	TLI	SRMR	RMSEA (95% CI)
Full models [a]						
MF1 - One factor	5878.459*	798	.916	.909	.089	.074* (.073 - .076)
MF2- Four correlated factors	**2448.328***	**792**	**.973**	**.970**	**.050**	**.045* (.043 - .045)**
MF3- Four correlated factors and one second order anxiety factor [b]	6427.093*	795	.907	.899	.097	.078* (.077-.080)
MF4 - Four factors and two 2nd order correlated factors (State-Trait)[b]	3622.462*	794	.953	.949	.067	.056* (.054 - .058)
MF5 - Three order factors (4 first order-2 second order - 1 third order) [b]	3643.984*	793	.953	.949	.067	.054* (.054 – 0.58)
State models						
MS1 - One factor	1850.142*	189	.877	.863	.084	.087* (.084 - .091)
MS2 - Two factors	**817.260***	**188**	**.953**	**.948**	**.051**	**.054 (.050 - .058)**
MS3 - One second order factor	4410.885*	189	.687	.652	.141	.139* (.136 - .143)
Trait models						
MT1 - One factor	2769.272*	189	.889	.877	.082	.109* (.105 - .112)
MT2 - Two factors	**1032.127***	**188**	**.964**	**.960**	**.044**	**.062* (.059 - .066)**
MT3 - One second order factor	6979.225*	189	.709	.677	.146	.177* (.173 - .180)

Note. Statistics: Chi-square (χ^2); Comparative Fit Index (CFI); Tucker and Lewis Index (TLI); Standardized Root Mean Squared Residual (SRMR); Root Mean Square Error of Approximation (RMSEA).

[a] all full models have correlated error terms between similar state and trait items; [b] models with latent variable covariance matrix not positive definite due to linear dependency between two or more latent variables.

* p<.001.

The three models with good fit indexes were theoretically sound, with these results supporting the factorial structure proposed in the development of STICSA. However, these models presented very high correlations between the symmetric latent variables in state and trait forms. For instance, the MF2 model showed a correlation of .817 between the two dimensions of somatic anxiety and .916 between the two dimensions of cognitive anxiety. In general, the trait and state dimensions were highly

associated in all models, suggesting that the respondents did not completely separate these two forms of anxiety.

Considering these findings, as well as the complexity of the models when all the mirror items were allowed to have their error terms correlated, separated models for STICSA-State and STICSA-Trait were tested. This option enables further assessment of the hypothesized models and their validity to be conducted in simpler models, respecting the assumption of local independence (Baghaei & Tabatabaee Yazdi, 2016), as well as assuring the parsimony of the models by, for instance, not increasing the parameters to be estimated (R. Kline, 2005). Different concurrent models were compared for each form. Three models were computed both for state and trait forms: (MS1 and MT1) a unidimensional model with all items loading in one factor; (MS2 and MT2) a two-factor model including somatic and cognitive factors within state or trait anxiety; and (MS3 and MT3) a two-factor model for somatic and cognitive dimensions explaining a second-order factor for state or trait anxiety. Once more, the MS3 and MT3 models were not tested in previous studies but were tested in the present study since they reflect a more complex model of the instrument's structure. Results of the CFA performed for both trait and state models showed that the best overall adjustment was observed for the models with two factors (somatic and cognitive anxiety; MS2 and MT2). The results from the selected fit indexes for the unidimensional and the second-order factor model were below .95 for CFI and TLI and above .07 for SRMR and RMSEA, being these the suggested cut-off points for the models to be considered acceptable (Table 2).

The factor loadings for all items in both state and trait models were significant ($p<.001$). Standardized loadings for the state anxiety model ranged from .486 to .787 regarding the somatic factor and from .404 to .821 in the cognitive factor. Accordingly, on average, a square multiple correlation of .443 was observed for the somatic factor ($.236 \leq SMC \leq .620$), and of .516 for the cognitive factor ($.163 \leq SMC \leq .675$). Standardized loadings for the trait models ranged between .584 till .832 for somatic factor and between .447 till .876 for the cognitive factor. Square multiple correlation for somatic dimension of trait anxiety corresponded to, on average, .506 ($.341 \leq SMC \leq .692$), and .607 for cognitive dimension ($.200 \leq SMC \leq .767$). These results suggested that the factors were explaining, on average, at least half of the variance of the indicators (Table 3). The correlation between somatic and cognitive latent factors was of large magnitude (greater

than .50; J. Cohen et al., 2002), specifically .674 for state model and .705 for trait model.

Table 3. Standardized (Unstardardized) factor loadings: State and trait models of STICSA (MS2 and MT2).

Factor	Item	State model	Trait model
Somatic	My heart beats fast	.614 (1.000)	.711 (1.000)
	My muscles are tense	.652 (1.063)	.676 (0.950)
	I feel dizzy	.736 (1.200)	.721 (1.015)
	My muscles feel weak	.692 (1.127)	.713 (1.003)
	I feel trembly and shaky	.787 (1.283)	.832 (1.170)
	My face feels hot	.546 (0.890)	.630 (0.886)
	My arms and legs feel stiff	.770 (1.256)	.793 (1.116)
	My throat feels dry	.538 (0.877)	.636 (0.895)
	My breathing is fast and shallow	.744 (1.212)	.799 (1.124)
	I have butterflies in the stomach	.676 (1.101)	.685 (0.963)
	My palms feel clammy	.486 (0.793)	.584 (0.821)
Cognitive	I feel agonized over my problems	.753 (1.000)	.828 (1.000)
	I think that others won't approve of me	.722 (0.958)	.754 (0.911)
	I feel like I'm missing out on things because I can't make up my mind soon enough	.710 (0.943)	.753 (0.910)
	I picture some future misfortune	.762 (1.012)	.834 (1.008)
	I can't get some thought out of my mind	.764 (1.014)	.828 (1.001)
	I have trouble remembering things	.404 (0.536)	.447 (0.540)
	I think that the worst will happen	.814 (1.080)	.876 (1.058)
	I keep busy to avoid uncomfortable thoughts	.575 (0.763)	.700 (0.846)
	I cannot concentrate without irrelevant thoughts intruding	.756 (1.003)	.810 (0.979)
	I worry that I cannot control my thoughts as well as I would like to	.821 (1.090)	.866 (1.046)

Reliability analysis

The reliability of the factors was assessed by computing Omega for categorical data, with results supporting a very good reliability level. In the state dimension, the

Omega was .895 for the somatic factor and .912 for the cognitive factor. In the trait dimension, the index presented values of .918 for the somatic dimension and .945 for the cognitive dimension. Cronbach alpha was also computed, allowing to compare our results with other studies. Results also supported a very good level of internal consistency. The state dimensions showed alphas of .803 for the somatic factor, and .869 for the cognitive factor, with item-total correlations of at least .35 for all the items of both factors. The trait dimensions presented an alpha of .869 for the somatic factor and .857 for the cognitive factor, with strong item-total correlations of at least .45.

Measurement invariance

Sex invariance was tested considering both state and trait models separately, aligned with the chosen strategy to further explore the psychometric models. In a first step, the models for male and female participants were tested separately, evidencing very good fit indexes. Although chi-square tests were significant, it was possible to assume the structure as equivalent in the two groups. The nested models were then tested sequentially adding restrictive constrains: (1) configurational, to assure that the factorial structure is equivalent across the sex groups; (2) metric, to test if the loadings are equivalent in the two groups; and (3) scalar, to test if the loadings and the intercepts are considered equal and, therefore, the scores on the two dimensions (somatic and cognitive) can be compared across sex groups. The results showed a very good level of overall adjustment, with CFI and TLI values above the cut-off of .95, and RMSEA lower than .07 in all the tested models. Although the individual results of the models were good, and even considering the slight improvements in the models with the increase in the constrains, the chi-square differences revealed that it was possible to assume configural and metric invariance, but not scalar invariance, in both trait and state forms (Table 4). The overall findings suggest that both male and females interpreted the underlying factorial structure of STICSA-State and STICSA-Trait, their loadings, but not intercepts, in an equivalent way.

Table 4. Fit indexes for sex invariant models (n_{male}=400; n_{female}=753).

Invariant Models	χ^2	df	$\Delta\chi^2$	CFI	ΔCFI	TLI	RMSEA (90%CI)	ΔRMSEA
State								
Male	296.826	188		.968		.964	.038 (.030-.046)	
Female	684.423*	188		.953		.947	.059 (.054-.064)	
Configurational	951.702*	376		.959		.954	.052 (.047-.05)	
Metric	975.866*	395	42.221*	.958	.001	.956	.051 (.047-.055)	-.001
Scalar	937.195*	435	38.945	.964	.006	.965	.045 (.041-.049)	-.006
Trait								
Male	452.880*	188		.955		.949	.059 (.052-.066)	
Female	768.620*	188		.966		.962	.064 (.059-.069)	
Configurational	1200.481*	376		.964		.960	.062 (.058-.066)	
Metric	1245.004*	395	64.774*	.963	-.001	.961	.061 (.057-.065)	-.001
Scalar	1185.691*	435	46.939	.968	.005	.969	.055 (.051-.058)	-.006

Note. Statistics: Chi-square (χ^2); Comparative fit index (CFI); Tucker and Lewis Index (TLI); Standardized Root Mean Squared Residual (SRMR); Root Mean Square Error of Approximation (RMSEA).

* p<.001.

Concurrent and nomological validity

Regarding concurrent validity, STICSA was highly associated with a measure of anxiety (STAI), specifically regarding its cognitive dimensions in both state and trait forms. The highest associations were observed between STICSA trait-cognitive dimension and STAI trait (r=.796), followed by the relationship between STICSA state-cognitive dimension and STAI state (r=.764), suggesting that STAI covers largely the cognitive aspects of anxiety. These correlation values are in line with the cut-off suggested by Kline (2000) for concurrent validity (r<.75). On the other hand, the association between the somatic dimensions of STICSA and the dimensions of anxiety measured by STAI was lower (between .452 and .508), highlighting the distinction between cognitive and somatic dimensions.

Correlations with depression (DASS-D) were lower compared to the ones with anxiety (STAI), regarding the four dimensions of STICSA. Still, the level of association between the depression measure's score and STICSA's state-cognitive (r=.652) and trait-cognitive (r=.634) dimensions presented a large effect (>.50; J. Cohen et al., 2002).

When comparing the correlation between depression and both STAI state (r= .643) and STAI trait (r=.722) scores, STICSA dimensions evidenced lower levels of association with depression in all dimensions, with the exception of state-cognitive dimension. The difference in the correlations of the four STICSA factors and the positive and negative affect (PANAS) was about twice the level of association, being the association with positive affect small (.10≤r<.30) and with negative affect moderated (.30≤r<.50) in somatic dimensions and large in cognitive dimensions (>.50; Table 5). On the other hand, STAI evidenced moderate correlations with positive affect (STAI State: r=-.468; Trait: r=-.517), and strong correlations with negative affect (STAI State: r=.694; Trait: r=.723).

Table 5. Cronbach's α and Pearson correlation between STICSA factors and anxiety (STAI), depression (DASS-D), positive affect (PA) and negative affect (NA).

	Nº items	α	STICSA State Somatic	STICSA State Cognitive	STICSA Trait Somatic	STICSA Trait Cognitive
STAI state	20	.50	.508	.696	.459	.657
STAI trait	20	.45	.452	.764	.500	.796
DASS-D	7	.87	.426	.652	.393	.634
PANAS PA	10	.89	-.188	-.339	-.181	-.320
PANAS NA	10	.89	.436	.655	.466	.677

Note. Ns range from 1136 and 1139. All correlations are significant at p<.001.

Discussion

The present study aimed to extend STICSA psychometric studies by exploring its dimensionality, measurement invariance, reliability and nomological validity. Our results replicated previous findings, providing strong evidence for STICSA's construct validity, including the distinction between the four dimensions, in non-clinical samples (Balsamo et al., 2015; Ree et al., 2008; Roberts et al., 2016). The theoretical structure was reflected in the underlying factorial structure considering either the test with the total of items and the four dimensions (full factorial model), and the models separated

by state and trait dimensions. This further corroborates the psychometric properties and validity qualities of this new anxiety measure in the Portuguese population.

Several full and state/trait factor models were tested in the present study, considering the theoretical structure of STICSA. Regarding the full model with the 42 items, in the present study, the best overall adjustment was observed for the model with four correlated factors, thus replicating the previous findings provided by studies testing the full model in non-clinical samples (Balsamo et al., 2015; Roberts et al., 2016). Yet, considering the high multicollinearity observed between the state-cognitive and the trait-cognitive, as well as between the state-somatic and trait-somatic dimensions, several models with the state and trait forms analyzed separately were tested. These analyses revealed that the model with the best adjustment encompassed two correlated factors (cognitive and somatic) within each form. The correlations between somatic and cognitive dimensions were higher than .65 across state and trait forms, suggesting that, despite of being independent measures, they are closely associated (Ree et al., 2008). Similar results were also found for non-clinical samples (Carlucci et al., 2018; Ree et al., 2008; Roberts et al., 2016; Styck et al., 2020; Tindall et al., 2021) which, altogether, corroborates this instruments' construct validity and the distinction between the four dimensions of anxiety (trait, state, as well as cognitive and somatic anxiety). The reliability analyses conducted in the present study also support STICSA as a reliable measure of anxiety, extending the previous literature (Balsamo et al., 2015; Carlucci et al., 2018; Grös et al., 2007; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021).

In order to be accurate in group comparisons, individuals of different groups should interpret the scale and its components in an equivalent way. The multi-group CFA performed in the present study supported the equivalence of the underlying structures between groups regarding sex (males vs. females) for STICSA's scale interpretation, at the level of metric invariance, which adds further strength to the quality of this measure and reinforces the confidence in comparing the latent variable scores across groups. A similar result regarding measurement invariance across sex was reported by past studies (Carlucci et al., 2018; Tindall et al., 2021). The intercepts (the means) differ between the groups, which reinforces the expected sex differences (McLean et al., 2011), maintaining the good characteristics of the STICSA regarding the factorial structure and its loadings, although not its intercepts.

The level of association between the dimensions of STICSA and the two subscales of STAI gave further evidence of convergent validity, particularly regarding the cognitive dimensions. The difference in the levels of association between both cognitive (>.75) and somatic (.45 - .51) dimensions and the STAI state and trait scores highlights the newness and relevance of the somatic subscales of STICSA. Our results, in line with the previous literature (Roberts et al., 2016; Tindall et al., 2021), thus suggested that STAI is a more specific measure of cognitive anxiety, while STICSA opens the possibility of evaluating anxiety in a more comprehensive fashion, i.e., in a multidimensional perspective. Nevertheless, although STICSA aims to provide a better differentiation between anxiety and depression, our results do not fully support the achievement of this goal. In fact, the Portuguese version of STICSA was shown to be strongly correlated with depression, particularly its cognitive dimensions ($r>.50$). These results are in line with previous psychometric studies of STICSA (Carlucci et al., 2018; Roberts et al., 2016), are also expected in light of the tripartite model of anxiety and depression (L. A. Clark & Watson, 1991). This model explains the frequent overlap and comorbidity between anxiety and depression, and highlights a component specific to anxiety (high physiological arousal), as well as a component specific to depression (low positive affect), and a third component shared by both of them, corresponding to negative affect.

Hereupon, it is expected that anxiety and depression overlap and correlate at a certain extent, in particular considering the cognitive dimensions of STICSA, which seem to greatly reflect negative affect in comparison with the somatic dimensions which, in turn, reflects physiological arousal (Grös et al., 2007). Furthermore, according to the tripartite model, it is not expected that a measure of anxiety encompasses items reflecting low levels of positive affect, which is exclusive to depression. Our results, once more, support both the tripartite model assumptions and STICSA as measure of anxiety, by showing that its cognitive dimensions are more associated with high negative affect ($r\geq.30$ for somatic dimensions and $r>.50$ for cognitive dimensions) than with low positive affect ($.10\leq r<.30$). Therefore, STICSA seems to better discriminate anxiety from depression in comparison with STAI, given STICSA's weaker correlations with low positive affect, as well as the lower levels of association with the depression measure. Nevertheless, the lower associations with depression were observed for all dimensions of STICSA, except for the state-cognitive; this result is intriguing and new,

and possibly suggests that the state dimension reflects negative affect in a higher extension than the trait-cognitive dimension. Importantly, these results highlight the relevance of the somatic subscales' scores to differentiate anxiety from depression (Roberts et al., 2016; Tindall et al., 2021).

Study 2: Relationship between trait anxiety and the subjective and psychophysiological emotional response towards distinct emotional situations

Given the multidimensional nature of anxiety, it is of utmost relevance to consider, in self-report validations, how the scores are associated with such dimensions, as well as to the emotional experiences under distinct situations. Ree and colleagues (2008) explored how the STICSA's cognitive and somatic dimensions of STICSA-Trait predicted the state anxiety response in a stressful event. They observed that trait-cognitive anxiety predicted a significant part of the variance in the self-reported state-cognitive and state-somatic anxiety under a cognitive stressor. Conversely, trait-somatic anxiety was associated with the self-reported state-cognitive and state-somatic anxiety in response to a somatic stressor. These results support trait-cognitive and trait-somatic anxiety as independent constructs, as well as the usefulness of STICSA to predict the anxiety response towards different types of stressors (Ree et al., 2008). Yet, it is critical to extend this knowledge to other emotional contexts (e.g., such as under fear or happiness emotional states), which can be related to different demands, and, therefore, with distinct subjective, cognitive, behavioral, emotional and physiological responses (for reviews see Kreibig, 2010; Mauss & Robinson, 2009).

On the other hand, it would be equally helpful to understand how trait-cognitive and trait-somatic anxiety are differently associated not only with the subjective, but also with the psychophysiological dimension of the emotional response. In fact, the anxiety response is multidimensional, and is often characterized by differences in the psychophysiological domain. For instance, high self-reported trait anxiety has been associated with autonomic dysfunction, specifically with a decrease in vagally-mediated indexes of HRV (Bleil et al., 2008; Miu et al., 2009). Reduced HRV, in turn, reflects decreased flexibility and adaptability, influencing general well-being and health (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). To date, no study has explored this relationship considering self-reported trait anxiety as measured by STICSA; its

differentiation between the cognitive and somatic dimensions of anxiety can yield important implications, considering that many anxiety instruments, such as STAI, possibly cover items that mainly assess the cognitive dimensions of anxiety (as supported by our results of Study 1).

Adopting a multimodal perspective to assess the predictive validity of STICSA-Trait can add to the conceptualization of trait-cognitive and trait-somatic anxiety as distinct constructs (Ree et al., 2008), which have been shown to distinctively predict behavior (Belem da Silva et al., 2014). Also, given the inter-individual variability found in the emotional response associated with the different components of emotions (Mauss et al., 2005), a multimodal approach can contribute to better understand people's emotional responses (Amstadter, 2008). The current study sought to explore how STICSA-Trait explains differences in psychophysiological and self-report measures. We designed an emotional induction procedure, during which the subjective and psychophysiological responses of the participants were collected. We expected to observe an overall reduced HRV in individuals with high trait anxiety in comparison with individuals with low trait anxiety (Miu et al., 2009).

Materials and method

Participants

Seventy-six participants from a Portuguese university volunteered to participate in the study. Only participants with normal or corrected-to-normal visual acuity and free of any medication or disease that could influence the cardiac functioning (e.g., tricyclic antidepressants or cardiac arrhythmia) were included. Also, participants did not report any diagnosis of mental or neurological illness. All participants completed a sociodemographic questionnaire online with relevant information for the study, as well as the STICSA-Trait developed in Study 1. Two participants were later excluded for being extreme cases regarding the Low Frequency (LF) and High Frequency (HF) power values. The final sample was constituted by 74 participants, 49 females (66.2%) and 25 males (33.8%), aged between 18 and 31 years old (M=21.41; SD=3.09). The study was approved by the Ethics and Deontology Committee of the University of Aveiro (ref. 10/2017) and followed the same ethical procedures as in Study 1. Participants were rewarded for their participation through a draw of three 20€ vouchers.

Materials

Visual Stimuli. An emotional induction paradigm involving the visualization of film clips was used (e.g., McGinley & Friedman, 2017). Stimuli consisted in three sets of 8-12 film clips, with approximately 30 minutes of duration (per set). These clips were taken from horror movies, comedy movies and documentaries, to induce fear, happiness and a neutral emotional state, respectively. Moreover, three documentary clips, of approximately 5 minutes, were also selected and presented before each set of emotional videos, to provide baseline psychophysiological data. All film clips demonstrated previously their ability to induce the expected emotions (Barros et al., 2019; Gouveia et al., 2020; G. Pinto et al., 2020).

Evaluation of the subjective emotional response. Three Visual Analogue Scales (VAS) of 100 points were used to assess participants' emotional state before and after the emotional induction, covering the measurement of subjective Happiness and Fear (Emotion VAS; "How do you feel right now?"), as well as of self-reported arousal (Arousal VAS; "How much aroused did you feel during the visualizations of the clips?") after the baseline videos and after the emotional videos. The experimental task, which included the presentation of the VAS and the videos, was programmed with the software OpenSesame (version 3.2.1; OpenSesame Inc.), using Python language (version 2.7.13). The task was performed using a Dell OptiPlex 7040 and a 17-inch Dell digital monitor (Model: E178FP), with a refresh rate of 60 Hz.

Cardiac signal recording. The cardiac signal was recorded using BIOPAC MP160 data acquisition system and AcqKnowledge 5 software (BIOPAC Systems, Inc.), with a sampling rate of 1000 Hz (Berntson et al., 2007). Ag/AgCl disposable vinil electrodes (EL503; BIOPAC Systems, Inc.) and conductive gel were used to acquire the signal, following a *Lead II* configuration (Berntson et al., 2007).

Procedure

Before data collection, all participants received information about the study's procedure and provided written informed consent. Furthermore, the experimenter asked participants beforehand if they had been through any emotionally intense situation in the past days, to assure that the sessions would be scheduled in a "emotionally neutral" period. The three sessions were spaced for at least one week, to avoid emotional contagion between sessions. The order of presentation of each emotional condition was counterbalanced between participants. Each session started with the electrodes' placement. Following this step and a 10-minute interval to allow signal stabilization, participants were instructed to seat in a chair and place their chin on a chin rest located at approximately 60 cm from a computer monitor. Participants started the task by answering the Emotion VAS, presented in a randomized order. Then, the baseline video was presented, followed by the Arousal VAS and, finally, by the film clips (presented consecutively). In the end of the video set, a new Arousal VAS was presented, followed by the Emotion VAS. Each experimental session lasted approximately 70 minutes. The study was conducted in a quiet and well-ventilated room.

Design and Statistical Analyses

HRV was calculated considering the RR or inter-beat intervals and, in this case, several spectral components may be used to interpret the signal (Shaffer & Ginsberg, 2017). Three HRV indexes were considered: The High Frequency (HF; in ms^2) band, the Low Frequency (LF; in ms^2) band and the ratio of LF to HF power (LF/HF). The LF band encompasses frequencies varying between 0.04 and 0.15Hz and reflects both sympathetic and parasympathetic activation, as well as baroreflex mechanisms (Shaffer et al., 2014). The HF band encompasses the signal power in the range from 0.15 to 0.4 Hz, and has been suggested to mainly reflect parasympathetic activity or vagal cardiac control (Shaffer et al., 2014; Shaffer & Ginsberg, 2017). Low HF power has been, therefore, associated with situations of anxiety and stress (Miu et al., 2009). This band also reflects variations in the cardiac signal related to the respiratory cycle (Shaffer et al., 2014). Therefore, alterations in the respiration rhythm are able to significantly modify HF power, and can also influence LF values under certain circumstances (e.g., during slow respiration rates; Shaffer & Ginsberg, 2017). For this reason, in this study,

the chosen filters to apply on the cardiac signal were designed in order to attenuate this physiological interference. The LF/HF ratio has been suggested to reflect the autonomic balance between sympathetic and parasympathetic activity, at least under certain conditions (Shaffer & Ginsberg, 2017). Higher values can indicate greater sympathetic activation in comparison with parasympathetic activity depending on the context; for instance, in challenging situations requiring increased SNS activation (Shaffer et al., 2014).

For details about the cardiac signal processing and the HRV indexes' calculation, please refer to S1 Fig in Supporting Information (SI). Separated repeated measures analysis of variance (ANOVA) were performed considering the psychophysiological and the self-report measures as dependent variables (LF/HF/Ratio/Happiness/Fear/Arousal), with Condition (Fear/Happy/Neutral emotional induction) and Moment (baseline/emotion) as within-subjects measures, and Group (high/low anxiety) as between-subjects measure. All the analyses were performed for groups divided considering trait-cognitive or trait-somatic anxiety. Whenever sphericity was not assumed, the values for main effects were reported using the Greenhouse-Geisser correction. Simple-effect analyses were performed for all the significant interactions, to clarify the direction of the effect. The significance levels of the comparisons were corrected using the Bonferroni correction (Howell, 2006). These procedures were conducted using IBM SPSS Statistics (version 23).

Results

Descriptive and preliminary analyses

Participants were divided in two groups considering the median of the cognitive dimension of STICSA-Trait (Mdn=19): the group with low trait-cognitive anxiety (LowCG; N=41) and the group with high trait-cognitive anxiety (HighCG; N=33). Similarly, two groups of trait-somatic anxiety were created considering the median (Mdn =16): the group of low trait-somatic anxiety (LowSG; N=42) and the group of high trait-somatic anxiety (HighSG; N=32). Descriptive statistics regarding the psychophysiological and subjective measures for each condition and moment of evaluation considering the four groups are outlined in S2 Table and S3 Table (SI). Before proceeding to the main analyses, differences across baselines were also tested to

assure no significant differences in the dependent variables at the beginning of each condition. Results of repeated measures ANOVAs revealed no differences between baselines across measures, except regarding the evaluation of happiness, $F(1.790)=3.352$, $p=.043$, partial $\eta^2=.044$. Nevertheless, the post-hoc tests were all non-significant.

Furthermore, we tested if the groups of cognitive and somatic anxiety were equivalent in the pre-test, considering the three emotional conditions and for all measures. Results of repeated measures ANOVAs suggested no differences between groups across conditions and measures, except regarding LF/HF ratio (trait-somatic anxiety groups) and self-reported happiness (trait-cognitive anxiety groups). In this line, there was a main effect of Condition regarding self-reported happiness, only when considering the groups of trait-cognitive anxiety, $F(1.793)=3.536$, $p=.037$, partial $\eta^2=.074$. Nevertheless, the post-hoc tests were all non-significant. Also, there was a significant main effect of Group (groups of trait-somatic anxiety) regarding the LF/HF ratio, $F(1)=7.513$, $p=.008$, partial $\eta^2=.094$, suggesting that the HighSG had significantly higher LF/HF ratio than the LowSG in the baseline moments. Yet, the interaction Condition x Group was non-significant ($p=.674$), suggesting that this group difference was systematically observed across conditions and is most likely due to the group characteristics (i.e., higher levels of self-reported trait-somatic anxiety may be associated with higher LF/HF ratio at rest, in comparison with lower levels of trait-somatic anxiety). For the detailed results of these preliminary analyses, please refer to S4 Table and S5 Table (SI). Altogether, these results indicate that the equivalence of the baselines can be assumed.

Differences considering groups of trait-cognitive anxiety

For an overview of the general results of the ANOVAs regarding the trait-cognitive anxiety groups, please refer to S6 Table and S7 Table (SI). Considering the psychophysiological measures, there was not a significant main or interaction effect of Group. However, in the self-report measures, a significant third order interaction effect Condition x Moment x Group emerged, $F(2)=3.414$, $p=.036$, partial $\eta^2=.045$. The simple-effect analysis suggested that, in the neutral emotional condition, the LowCG reported higher levels of arousal in the baseline in comparison with the emotional

condition, p=.025. Yet, the HighCG did not present significant differences between the baseline and the emotional condition, in the self-reported arousal, p=.833. In the other conditions, the performance was similar in both groups. Additionally, when analyzing the graph illustrating group differences across emotional conditions (Fig 1a), it is possible to observe that, in the LowCG, the self-reported arousal seemed to vary more across conditions, in comparison with the HighCG. Similarly, the variation between the baseline and the emotional conditions seems to be more consistent and marked in the LowCG in comparison with the HighCG, especially for the happy and neutral conditions (Fig 1b and 1c).

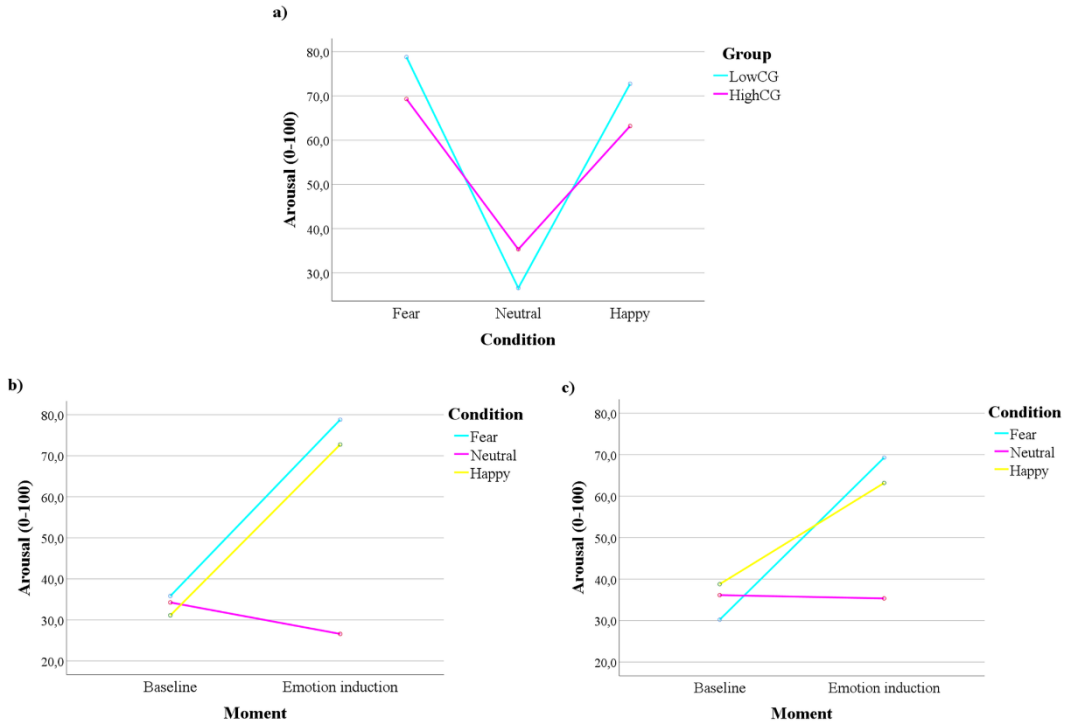

Figure 1. Cognitive groups' differences regarding the self-reported arousal.

Note. a) Group differences in the self-reported arousal after the emotional induction; b) LowCG self-reported arousal across Moment and Condition; c) HighCG self-reported arousal across Moment and Condition.

Differences considering groups of trait-somatic anxiety

For an overview of the general results of the ANOVAs considering the trait-somatic anxiety groups, please refer to S8 Table and S9 Table (SI). A significant

interaction Moment x Group emerged regarding the LF power, F(1)=5.132, p=.026, partial η^2=.067. The simple-effects inspection suggested that the LF power was always lower in the baselines in comparison with the emotional conditions, p<.001. Additionally, there were no significant differences between groups neither at the baselines nor at the emotional conditions. When inspecting the graph (Fig 2a), the HighSG seems to have slightly higher LF power in the baseline evaluation in comparison with the LowSG; however, the difference was small and not significant. Furthermore, a significant main effect of Group emerged regarding the LF/HF ratio, F(1)=6.390, p=.014, partial η^2=.082, suggesting that the HighSG presented higher values of LF/HF in comparison with the LowSG. Likewise, there was a significant interaction Moment x Group regarding the LF/HF ratio, F(1)=6.765, p=.011, partial η^2=.086, suggesting that the HighSG had a significantly higher LF/HF score in baseline and emotion evaluation, in comparison with the LowSG. Furthermore, the baselines and emotion evaluation were always significantly different in both groups. The analysis of the graph (Fig 2b) reinforced that the differences in the ratio score between the baseline and emotion evaluation were slightly less accentuated in the HighSG compared to the LowSG.

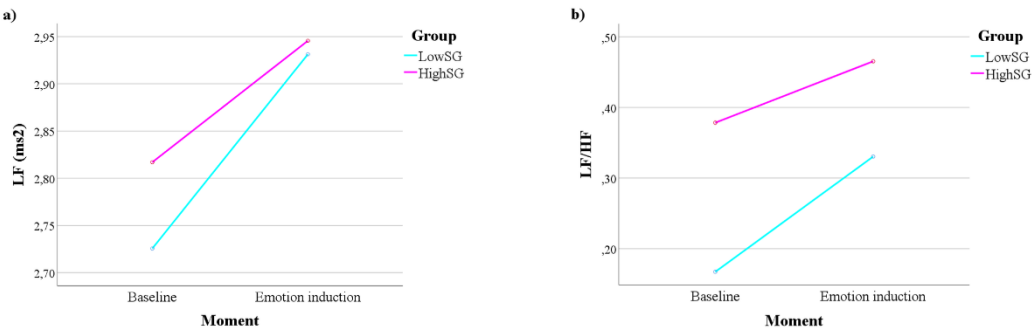

Figure 2. Somatic groups' differences considering the Moment of evaluation.

Note. a) LF power and b) LF/HF ratio.

Regarding the self-report measures, we observed a marginally significant interaction Condition x Group, F(2)=3.053, p=.05, partial η^2=.041, suggesting that, in both groups, the self-reported arousal was always higher in the fear condition compared

with the neutral condition, as well as in the happy condition compared with the neutral condition, $p<.01$. There were no significant differences between groups across the three emotional conditions. Importantly, a significant interaction Condition x Moment x Group emerged, $F(2)=3.102$, $p=.048$, partial $\eta^2=.048$. The post-hoc tests suggested that, while in fear and happiness conditions there were always significant differences between the self-reported arousal in the pre- and post-emotional induction in both groups, $p<.001$, in the neutral condition there were no significant differences between the self-reported arousal in the pre- and the post-emotional induction for the HighSG, $p=.805$. Moreover, after the neutral condition, the HighSG reported significantly more arousal than the LowSG, $p=.015$ (see Fig 3a). The analysis of the graphs allows to further observe that, in the neutral condition, while there is a slight decrease in the self-reported arousal in the post-induction in comparison with the pre-induction in the LowSG (Fig 3b), in the HighSG (Fig 3c) the values of self-reported arousal pre- and post-induction are quite similar. Also, the LowSG seems to have a higher difference between the self-reported arousal after the fear condition and the self-reported arousal after the happiness condition, while in the HighSG this difference is narrower. Lastly, the post-hoc tests suggest that, before the happiness induction, the HighSG also reported significantly more arousal than the LowSG, $p=.013$.

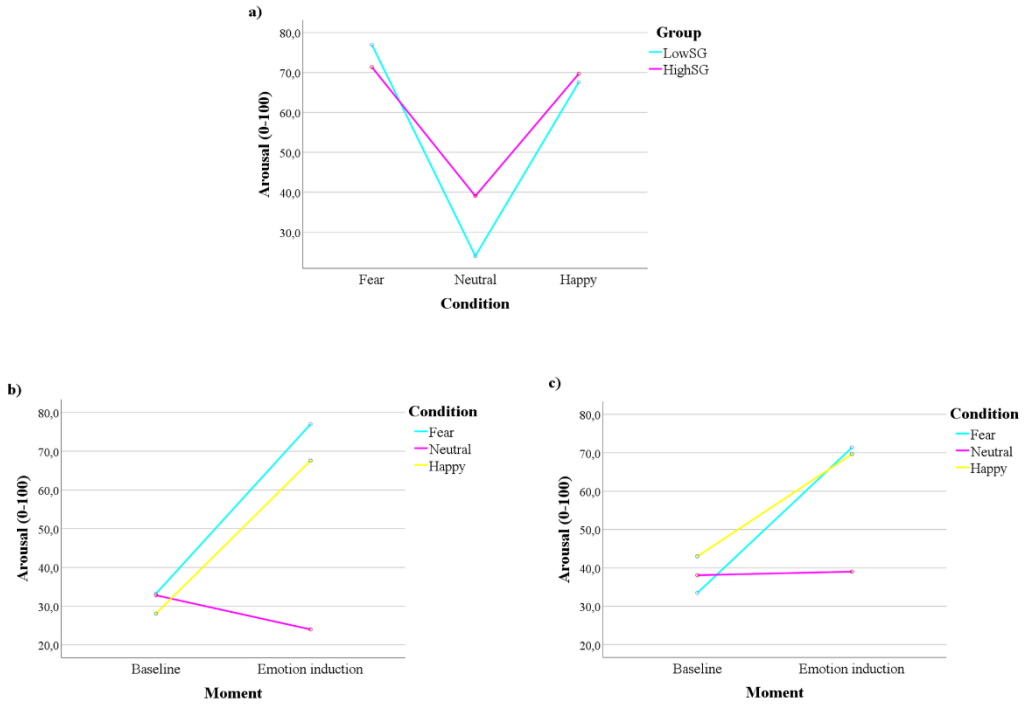

Figure 3. Somatic groups' differences regarding the self-reported arousal.

Note. a) Group differences in the self-reported arousal after the emotional induction; b) LowSG self-reported arousal across Moment and Condition; c) HighSG self-reported arousal across Moment and Condition.

Discussion

In a previous study, STICSA-Trait cognitive and somatic dimensions were found to predict state anxiety towards cognitive or somatic stressors, respectively (Ree et al., 2008). The present study sought to extend the knowledge about STICSA-Trait's predictive validity, by examining the performance of groups of high and low trait-somatic/cognitive anxiety considering the psychophysiological and subjective dimensions of the emotional response in three distinct laboratory-induced emotional situations. Results showed that the trait-cognitive anxiety dimension, as measured by STICSA, was related to differences between groups regarding self-reported arousal. Conversely, the trait-somatic anxiety dimension was observed to be associated with differences between groups regarding psychophysiological measures, specifically the LF and LF/HF indexes of HRV, as well as with differences regarding self-reported arousal. These results highlight the relevance of STICSA-Trait as a measure that

assesses different aspects of anxiety, distinctively associated with different components of the emotional response.

Considering the groups divided by trait-cognitive anxiety, our analysis suggested that the LowCG reported significantly more arousal in the baseline than in the emotionally neutral condition. This result suggests that the emotionally neutral condition contributed to a decrease in the experienced arousal in people with low trait-cognitive anxiety. However, people with high trait-cognitive anxiety reported constant levels of arousal, considering the transition from the baseline to the neutral condition. This may reflect the experience of increased physiological activation often reported by patients with anxiety disorders (Hoehn-Saric & McLeod, 2000). Visual inspection of the data further suggested that the self-reported arousal varied more across emotional conditions in the LowCG in comparison with the HighCG. Furthermore, variations between the baselines and each respective emotional condition seemed to be more consistent and noticeable in the LowCG in comparison with the HighCG. Together, these results suggest a relationship between lower emotional flexibility and high self-reported trait-cognitive anxiety, particularly considering the emotionally neutral or non-aversive conditions. This is consistent with the literature that observed lower variability regarding self-reported arousal in the context of anxiety disorders (e.g., Rosebrock et al., 2017). Nevertheless, these differences should be interpreted with caution, since they were non-significant; moreover, our sample is non-clinical, limiting direct comparisons.

Interestingly, the results considering the trait-cognitive anxiety groups suggested no differences regarding the psychophysiological measures. This suggests that the cognitive dimension of STICSA-Trait is more related to self-report, specifically the self-reported arousal, and is not related to differences in any of the indexes of HRV. This also highlights the frequently observed weak relationship between the self-report and physiology dimensions of emotion (Mauss et al., 2005), and particularly the frequently observed incoherence between heightened self-reported arousal and unchanged physiology in anxiety disorders (Lang & McTeague, 2009; Rosebrock et al., 2017). This particular relationship with the cognitive dimension of trait anxiety may reflect a set of cognitive factors that lead to an altered attention and perception of the self-arousal levels, inconsistent with the physiological changes (Fisher et al., 2010). Yet, although the pattern of self-reported arousal was different between the baseline and emotionally neutral condition within each group of low/high trait-cognitive anxiety, and

the self-reported arousal after the emotionally neutral condition was numerically higher in the HighCG, there were no significant differences between groups in the post evaluation; the significant results were only observed when considering the differences between the baseline and the emotionally neutral condition within each group.

Results regarding the groups divided by trait-somatic anxiety suggested that there were no differences within each moment of evaluation, neither differences between pre- and post-emotional induction within groups, considering the LF component of HRV. Even so, after visually inspecting data, it was possible to observe that the HighSG presented higher LF power in the baseline condition, in comparison with the LowSG (non-significant). The results regarding the LF/HF ratio add to the interpretation of this interaction; in fact, we also observed that the HighSG presented higher LF/HF values in comparison with the LowSG, both in the baseline and during the emotional conditions. By visually inspecting data, differences between the baseline and emotion conditions seemed to be lower in the HighSG in comparison with the LowSG, since the former started with a higher LF/HF score at the baseline, and thus had a less accentuated rise during the emotional conditions. Together, these results may suggest that self-reported trait-somatic anxiety is related to objective physiological alterations in healthy adults. Consistently with the previous literature, these alterations may be related to the often observed reduced physiological flexibility (Hoehn-Saric & McLeod, 2000) and abnormal ANS activity in anxiety (Friedman, 2007).

Considering that the ratio LF/HF has been suggested to reflect sympathovagal balance, with higher values associated with sympathetic dominance (Shaffer & Ginsberg, 2017), our findings may suggest that self-reported trait-somatic anxiety is associated with elevated sympathetic activation, particularly at rest and in non-threatening emotional situations. These results are not in accordance with the literature concerning healthy individuals with high levels of trait anxiety, which has been reporting reduced HF power in high trait anxiety (Bleil et al., 2008; Miu et al., 2009; D. Shepherd et al., 2015; L. L. Watkins et al., 1998). Nevertheless, some studies have observed greater sympathetic activity in patients with anxiety disorders in comparison with control groups at resting conditions, as suggested by higher LF power, higher LF/HF ratio or both (H. Cohen et al., 2000; Martinez et al., 2010). However, a meta-analysis conducted by Chalmers and colleagues (2014) suggested that anxiety disorders

do not have a significant impact in LF values, with the effects in HRV being more evident through differences in the HF band.

It is important to note that several aspects may contribute to differences in the HRV scores found across studies. These include variables associated with the recording, such as the methods employed to control for artifacts and respiration confounds, as well as variables associated with the subjects, including age and health status (D. S. Quintana & Heathers, 2014; Shaffer & Ginsberg, 2017). Importantly in the context of our results, the tasks used for the recording may add important differences that also preclude comparisons. In fact, previous studies have explored the relationship between HRV and trait anxiety solely at rest conditions (Bleil et al., 2008; D. Shepherd et al., 2015; L. L. Watkins et al., 1998) or during relaxation and mental stress conditions (Miu et al., 2009). Some of these studies have also analyzed only certain indexes of the ANS activity, such as vagally mediated components of HRV (Bleil et al., 2008; L. L. Watkins et al., 1998), which does not allow to draw conclusions about the other measures.

Similarly to the observed in trait-cognitive anxiety groups, our results also suggested that trait-somatic anxiety is associated with self-reported arousal – which would be expected, since self-reported arousal include the subjective experience of physiological activation. Specifically, group differences emerged after the neutral induction, where the HighSG evinced higher levels of self-reported arousal in comparison with the LowSG. By inspecting data visually, while the levels of self-reported arousal seemed to decrease from the pre-induction to the post-induction in the LowSG, in the HighSG the levels of self-reported arousal remained constant. Once more, these results point to increased self-reported physiological activation and lower variability regarding self-reported arousal in people with higher levels of anxiety (Hoehn-Saric & McLeod, 2000; Rosebrock et al., 2017).

Although our results contribute to unravel the association between trait-cognitive/somatic anxiety and different dimensions of the emotional response towards emotional situations, some factors limit the conclusions to draw. For instance, some authors have been claiming that the ratio LF/HF, which has been often addressed as an indicator of sympathovagal balance, should be interpreted with caution, especially in short-length recordings and considering the specific recording conditions (e.g., if it is recorded in a resting condition or not, if it includes paced or normal breathing; Shaffer et al., 2014; Shaffer & Ginsberg, 2017). In fact, the LF component of HRV is not a pure

index of sympathetic activity (Shaffer et al., 2014). Moreover, respiration can highly influence both the LF and HF parameters (Shaffer & Ginsberg, 2017), thereby, socioemotional tasks that are associated with changes in the respiratory pattern have an impact in these HRV parameters (for a review about this issue see D. S. Quintana & Heathers, 2014). In the present study, the respiratory pace was not explicitly controlled since it would affect the emotional response. Therefore, caution is needed when interpreting these HRV indexes, and future studies should further investigate the relationship between trait anxiety and variations in the different HRV parameters.

Another limitation of the present study concerns the relatively small sample size, which impacts statistical power. Furthermore, in lower sample sizes, small variations may conduct to group differences; this may explain why we observed trait-somatic anxiety group differences regarding self-reported arousal in the pre-induction moment of the happiness condition when analyzing the statistically significant Condition x Moment x Group interaction. In the preliminary analyses (see the "Descriptive and preliminary analyses" section of Results, where we performed Condition x Group repeated measures ANOVA), we did not find statistically significant group differences regarding self-reported arousal in the pre-induction moment across conditions. For this reason, we assumed that the groups have started from the same point across conditions. Yet, our results should be interpreted with caution and future studies should replicate these findings with larger samples. Finally, it is also important to note that participants' emotional response was elicited and evaluated following laboratory-induced emotions, which may have resulted in "artificial" emotional responses, differing in intensity and valence from the observed emotional responses in the daily life (McGinley & Friedman, 2017; Wilhelm & Grossman, 2010). Therefore, future studies should extend this research by assessing emotional response considering its subjective, psychophysiological, and behavioral components in more ecological contexts, using less invasive and limiting procedures, in order to closer understand the relationship between self-reported anxiety and emotional response across daily situations.

General discussion

The present study aimed to extend STICSA's validation studies to the Portuguese context, through several analyses of dimensionality, measurement

invariance, reliability and nomological and predictive validity. Results supported STICSA as a useful instrument measuring cognitive and somatic symptomatology dimensions within state and trait anxiety. The first study provided evidence for excellent psychometric properties and replicated previous findings by supporting the instrument's construct validity, with its four dimensions structure and with separate structures for trait and state anxiety. Furthermore, it supported the equivalence of measure across groups of sex when considering the factor structure and loadings (metric invariance). STICSA also evidenced good nomological validity, despite of being strongly correlated with depression, particularly its cognitive dimensions. Our results also suggested that STICSA is possibly a better instrument to differentiate anxiety from depression, which constitutes an important improvement in the context of anxiety assessment. The second study further highlighted the relationship between trait-somatic anxiety and differences in both the subjective and psychophysiological domains of the emotional response, as well as the association between the trait-cognitive anxiety and the subjective experience of arousal. The ability to provide a better differentiation between anxiety and depression, allied to excellent psychometric properties and a broader scope of evaluated symptoms, as well as to the knowledge about the distinct relationships between the cognitive and somatic dimensions and the subjective and psychophysiological components of the emotional response, makes STICSA a privileged source of information with the potential to critically shape a targeted assessment, monitoring and intervention.

Although the present study adds important evidence about the utility and validity of STICSA, some considerations about the conclusions to draw should be addressed. First, our results are restricted to the general population. It would be important to assess STICSA's utility, as well as the generalizability of the present results to other populations where anxiety may have distinct manifestations, such as in healthy older adults (Balsamo et al., 2015) and in clinical populations (Grös et al., 2007). Extending this research to clinical settings may be particularly critical to acknowledge the equivalence of measure between clinical and non-clinical populations and/or to evaluate the association with other psychopathological measures. Until date, only two studies investigated STICSA's properties in clinical samples (Grös et al., 2007; Van Dam et al., 2013); moreover, apart from the present research, there is only one more study assessing the predictive validity of STICSA-Trait, also in a non-clinical population (Ree et al.,

2008). Thus, future studies are needed to expand this knowledge and establish cut-off values for the four dimensions of STICSA, considering the Portuguese clinical population. Moreover, more than half of our sample was constituted by students (57.2%), which may limit the generalization of our results; future studies should extend STICSA validation studies by recruiting more diversified samples of the general population. Lastly, caution is needed when directly comparing our results with the results obtained by other psychometric studies of STICSA, since distinct studies have been using different response labels, specifically considering the STICSA-Trait form. In fact, Ree and colleagues (2008) described that the STICSA-State form should be scored from 1 (not at all) to 4 (very much), whereas the STICSA-Trait form should be scored from 1 (almost never) to 4 (almost always). This response scale, which differentiates the two STICSA forms in terms of both the response labels and instructions, was subsequently used by other researchers (Carlucci et al., 2018; Tindall et al., 2021). However, similarly to the indicated in the work of Grös and colleagues (2007), which was the first published psychometric study of STICSA, several other authors have been using the same response labels for both the STICSA-State and STICSA-Trait forms (i.e., both forms ranging from 1 [not at all] to 4 [very much]; Balsamo et al., 2015; Roberts et al., 2016; Styck et al., 2020). In the present study, we followed the response labels provided by Grös and colleagues (2007), which could have made it more difficult to distinguish between the state and the trait forms, since only the instructions differed. Yet, it is important to note that the studies, in general, have been supporting similar results in terms of STICSA dimensionality, reliability and nomological validity, which suggests that this difference in the response labels do not substantively affects the results.

Notwithstanding the limitations discussed throughout the article, important strengths are worth to note. First of all, an extensive psychometric evaluation was performed, including also a predictive validity analysis of STICSA-Trait, which is an analysis that is generally lacking in the literature concerning instruments' validation and can add very important information about the usefulness of an instrument in specific contexts. Moreover, we have assessed STICSA's predictive validity considering different emotional contexts (happy, fear and neutral). Most studies assess the relationship between anxiety and emotional response in neutral (at rest; e.g., Bleil et al., 2008) or under negative emotional inductions (such as stress-inducing situations; Miu et

al., 2009; Ree et al., 2008). Although these studies give valuable information about an individual's response, their results are limited to those contexts in particular. Thereby, it is important to extend this research and measure emotional response in other contexts that pose different demands and are associated with distinct responses (e.g., such as situations that induce positive emotions; Kreibig, 2010; Mauss & Robinson, 2009), in order to have a broader comprehension of how self-reported anxiety predicts an individual's behavior. Finally, we assessed STICSA's predictive validity by adopting a multidimensional approach of the emotional response, which gives important hints about the relationship between the self-reported anxiety (considering distinct dimensions) and its association with different aspects of the emotional response. This approach further allowed the analysis of the coherence among response systems (which is often low; Mauss et al., 2005; Rosebrock et al., 2017), as well as of the relationship between STICSA and HRV, a biomarker of autonomic dysfunction and psychopathology (Shaffer et al., 2014).

Conclusion

The present research supported STICSA as an adequate instrument to measure anxiety symptoms in the Portuguese population, by extending its psychometric studies and reinforcing it as a multidimensional and robust measure of anxiety in its four dimensions: state-cognitive, state-somatic, trait-cognitive and trait-somatic. By embracing a greater scope of symptoms, STICSA has the potential to meet the demands posed by the increasing prevalence of anxiety disorders and the multiplicity of symptom profiles manifested by different individuals in different contexts. Furthermore, considering the association between STICSA-Trait and the psychophysiological and subjective dimensions of the emotional response, this instrument may provide additional valuable information about how individuals would feel, think and respond in specific situations (Ree et al., 2008), improving the knowledge about their functioning, and leading to the development of a more appropriated evaluation, monitoring and intervention plans.

Supporting Information

Please refer to Supporting Information – Study 1, in Chapter 6 – Appendix; all files are also available in https://doi.org/10.1371/journal.pone.0262960.

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Study 2

Autism traits dimensionality and multivariate relationship with alexithymia and anxiety in the general population

This study was published online by Elsevier Ltd. in Research in Developmental Disabilities, on 12 October 2022. The reference list of the manuscript has been integrated in the bibliography of the thesis – please see Chapter 5. The necessary structural adaptations were performed to incorporate the content of the article in the present thesis.

Reference:

Barros, F., Figueiredo, C., & Soares, S. C. (2022). Autism traits dimensionality and multivariate relationship with alexithymia and anxiety in the general population. *Research in Developmental Disabilities, 131*, 104361. https://doi.org/10.1016/j.ridd.2022.104361

Abstract

Background: Autism is characterized by social and non-social alterations observed beyond the clinical diagnosis. Research analyzing the expression of autism traits in the general population helps to unravel the relationship between autism dimensions and other associated variables, such as alexithymia and anxiety. The Autism-Spectrum Quotient (AQ) was developed to assess autism traits in the general population; however, inconsistent results regarding its dimensionality have emerged.

Aims: This study aimed to extend evidence about the AQ measurement model, and explore the multivariate relationship between autism traits, alexithymia, and trait anxiety.

Methods: 292 adults of the general population were recruited. An Exploratory Factor Analysis and Confirmatory Factor Analysis were performed to assess the factorial structure of AQ. A path analysis was carried out to explore the relationship between autism traits, alexithymia, and trait anxiety.

Results: The results supported a three-factor model of AQ. The path analysis model showed evidence of a significant role of alexithymia as a mediator of the relationship between autism traits and anxiety.

Conclusions and implications: The present study provides empirical support for a three-factor model of AQ in the general population. The association between autism traits, alexithymia, and anxiety dimensions highlights the multidimensional nature of these variables and the need to account for their distinct impact on autism-related variables.

Keywords

Autism; Alexithymia; Anxiety; Autism Spectrum Quotient; Structural Equation Modeling

Highlights

- A three-factor model of the Autism Spectrum Quotient was supported.
- Alexithymia mediates the relationship between autism traits and anxiety.
- Autism traits, alexithymia, and trait anxiety dimensions relate differently.

What this paper adds?

This study extends the current knowledge about the dimensionality and reliability of the Autism-Spectrum Quotient, an instrument broadly used to measure autism traits in clinical and non-clinical populations. Providing support for adequate measurement of autism traits allows for the further assessment of the expression of these traits in the general population and their relationship with other symptoms, personality traits and comorbidities related to the autism spectrum. Furthermore, this study also contributes to a better understanding of the multivariate relationship between autism dimensions, alexithymia, and trait anxiety; these variables are often associated with the autism spectrum and impact socioemotional functioning, consequently influencing life outcomes. Thus, further knowledge about these relationships may contribute to improve assessment and, as a result, intervention protocols used in the spectrum.

Introduction

Autism Spectrum Disorder (henceforth autism) is a lifelong neurodevelopmental condition whose core features include atypical social interaction and communication, as well as restricted and repetitive patterns of behavior and interests (American Psychiatric Association, 2013). The considerable genetic variability, as well as the heterogeneity in the symptomatology and comorbidities presented by individuals with a diagnosis, make autism a complex condition, and also poses hindrances to the assessment, diagnosis, and intervention with this population (for a review see Masi et al., 2017). Importantly, autism heterogeneity has contributed to the evolution of the conceptualization of the condition, including its transition from a multi-categorical towards a more dimensional approach (Rosen et al., 2021). In a similar vein, research conducted in recent decades has been suggesting that autism characteristics are expressed in a continuum varying from clinically relevant symptoms observed in people with the diagnosis, to milder, subclinical characteristics observed in the general population (Baron-Cohen et al., 2001; Ingersoll & Wainer, 2014).

The subclinical manifestation of autism characteristics has been often addressed as the Broader Autism Phenotype (Piven et al., 1997). These autism characteristics, or autism traits, seem to be heritable (e.g., Hoekstra et al., 2007) and continuously distributed in the general population (e.g., Baron-Cohen et al., 2001; Hurst et al., 2007). Therefore, autism can be conceptualized as a spectrum which extends beyond a diagnosis, encompassing the subclinical expression of several spectrum-related characteristics in the general population. Furthermore, in line with the variability reported in the clinical diagnosis, the literature suggests the existence of "subtypes" defined as the presentation of different profiles of autism traits across individuals (e.g., Palmer et al., 2015). These traits are grouped in different dimensions, with evidence suggesting that not all dimensions of autism traits significantly correlate with each other (e.g., English et al., 2020), and that distinct dimensions may be associated with different outcomes. For instance, Davis et al. (2017) found that social and non-social dimensions of autism traits differently predicted social cognitive processes in a college sample; moreover, the distinct dimensions of autism traits seem to be differently associated with sensory processing in typically developing adults (e.g., Barros et al., 2021; Yaguchi & Hidaka, 2020). Therefore, investigating the expression of autism traits in the general

population may be especially useful to understand the contribution of each autism dimension to the cognitive, emotional and behavioral characteristics of the spectrum (Landry & Chouinard, 2016).

The development of instruments capable of reliably assessing and representing the range of autism characteristics in the general population is a critical requisite to further understand the nature of autism traits. In this regard, the Autism-Spectrum Quotient (AQ) was the first self-report instrument designed to multidimensionally measure autism characteristics in the general population (Baron-Cohen et al., 2001). This instrument originally encompassed five dimensions covering the typical social and non-social features observed in the spectrum. Although its original structure is theoretically sound, most of the subsequent studies examining dimensionality and general psychometric properties of AQ found inconsistent and unstable factorial results, with only a small set of items consistently loading in the same factors across studies (English et al., 2020). Also, with regard to the original dimensions, a number of them sometimes present inadequate internal consistency (J. L. Stevenson & Hart, 2017). Nevertheless, AQ yields potential as a multidimensional, reasonably brief, cost-effective and accessible instrument and has been extensively used to measure autism traits in adult clinical and non-clinical samples (e.g., English et al., 2020). However, more research is needed to understand the factor structure of this instrument and its ability to reliably reflect the distinct dimensions of autism across cultures and contexts.

Understanding the structure of autism traits and providing support for their adequate measurement allows for the further assessment of the relationship between these traits and other variables of interest in the spectrum, as the autism spectrum is often tightly associated with other features beyond its core characteristics, two of which are alexithymia (Kinnaird et al., 2019; Poquérusse et al., 2018) and anxiety (Hollocks et al., 2019). Alexithymia is a personality construct characterized by altered emotional experience and expression. This construct is multidimensional and involves difficulties in identifying feelings and emotions, difficulties in describing one's feelings and emotions to others, reduced imaginative processes and fantasizing, and an externally oriented cognitive style (Bagby et al., 2020; Sifneos, 1973; G. J. Taylor, 1984; G. J. Taylor et al., 1991). Although alexithymia was firstly introduced and studied in the context of psychosomatic disease (see G. J. Taylor, 2018), it is nowadays recognized as a transdiagnostic concept that is often associated with cognitive and emotional

processing difficulties in the general population (Grynberg et al., 2012; Luminet et al., 2021). Furthermore, it has been suggested that alexithymia influences physical and mental health, being associated with, for instance, hypertension (e.g., Grabe et al., 2010), as well as symptoms of anxiety (e.g., Fietz et al., 2018; Karukivi et al., 2010). Alexithymia also seems to be more prevalent in autism than in the typically developing population (Kinnaird et al., 2019). Importantly, a growing body of research has been suggesting that this personality trait may be a key-feature contributing to the socioemotional difficulties often observed in the spectrum (known as the "alexithymia hypothesis"; Bird & Cook, 2013; Oakley et al., 2020; Trevisan et al., 2016).

Apart from high levels of alexithymia, high levels of anxiety (Mertens et al., 2017; Zukerman et al., 2019) and anxiety disorders (Hollocks et al., 2019; van Steensel et al., 2011) are also often reported in youth and adults with autism, influencing their life outcomes (e.g., regarding health-related quality of life; van Steensel et al., 2012). Several studies have suggested that alexithymia influences the relationship between autism and anxiety (e.g., Maisel et al., 2016; Morie et al., 2019). Specifically, alexithymia was shown to mediate the relationship between autism traits and anxiety in adults with (Maisel et al., 2016; Morie et al., 2019) and without an autism diagnosis (Maisel et al., 2016). Yet, although this relationship has already been explored in the spectrum, previous studies did not completely uncover how the distinct dimensions of the constructs were associated with each other. In fact, in these studies, a total score was often used to represent a global measure of symptoms – as is the case in total AQ score. However, a total score is not informative about how each autism dimension relates with the dimensions of other constructs, and how they are linked to the specificities observed in the spectrum. This limits the interpretability of and the understanding about the diversity and heterogeneity of symptom profiles observed in the general population. Related to this, previous evidence suggests that the distinct dimensions of autism, alexithymia and anxiety are, indeed, differently related with each other. For instance, Bothe et al. (2019) observed that the distinct dimensions of autism traits, as measured by AQ, were differently associated with alexithymia in the general population. On the other hand, anxiety symptomatology seem to not correlate significantly with all dimensions of AQ, in the general population (Liss et al., 2008), and was shown to be distinctly associated with TAS-20 dimensions in a sample composed of adolescents and adults with and without autism (e.g., Oakley et al., 2020). Nevertheless, a model

exploring the multivariate relationship between autism traits, alexithymia and anxiety is still missing.

Considering this framework, this study has a twofold aim: 1) to extend AQ's psychometric studies by exploring its factor structure in relation to a sample of young Portuguese adults; and 2) to analyze the role of alexithymia as a mediator of the relationship between autism traits and trait anxiety, considering these constructs in a dimensional perspective. Although the previous literature is still scarce and involves extensive heterogeneity regarding analyzed samples, statistical procedures and the instruments employed to measure these constructs, we expect to observe a significant relationship between autism traits and alexithymia (e.g., Liss et al., 2008; Maisel et al., 2016; Oakley et al., 2020), especially between the social dimensions of AQ and both the identification and description of feelings dimensions of the TAS-20 (Liss et al., 2008; Oakley et al., 2020). Moreover, we hypothesize a significant relationship between alexithymia and trait anxiety, as observed by previous studies (Fietz et al., 2018; Oakley et al., 2020). Finally, a significant relationship between autism traits and trait anxiety, especially concerning the social dimension of AQ (Bothe et al., 2019; Liss et al., 2008) is expected, as well as a statistically significant indirect effect of autism traits in trait anxiety through alexithymia (Maisel et al., 2016). With the present research, we aim to add to the understanding of the nature of autism traits, as well as to contribute to an improved assessment of the construct. Furthermore, considering the heterogeneity in the profile of autism traits (e.g., Palmer et al., 2015), and since alexithymia and anxiety are both highly prevalent in the autism spectrum, this knowledge can be crucial in fostering adequate prevention of psychopathology and to support a shaped intervention (Kinnaird et al., 2019; McVey, 2019).

Material and methods

Participants

A convenience sample of 295 adults (180 women; 61%), with ages between 18 and 42 years old, was recruited. Inclusion criteria included having Portuguese nationality and being at least 18 years old. Exclusion criteria encompassed not having Portuguese as a native language, presenting a formal autism diagnosis, and having a first degree relative diagnosed with autism. The two last exclusion criteria were

considered since we were interested in studying the expression of autism traits in the general population. Considering these criteria, three participants were excluded; therefore, the final sample was composed of 292 adults (178 women; 61%), with ages between 18 and 42 years old (M=24.22; SD=4.45). Most of the participants were full-time students (n=194; 66.4%). Thirty-seven participants (12.7%) reported having a psychological or psychiatric disorder. Of these, 10 participants (27%) reported anxiety, five (13.5%) reported both anxiety and depression, and two (5.4%) reported other mental health disorders. Fifteen of these 37 participants (40.5%) were also taking medication such as antidepressants and benzodiazepines. Please refer to Table S1 (Supplementary Material – Study 2; see Chapter 6 - Appendix) for information about the sample's characteristics.

For the path analysis, only participants who answered all the items in the instruments were considered. Consequently, 14 participants (5%) were excluded due to incomplete data. Nevertheless, these 14 participants were kept in the factorial analyses since we observed that they did not have a systematic pattern of response (e.g., they did not choose one point of the response scale for all the items) and showed coherence in their responses to similar items (i.e., items similar in meaning or items that were originally associated with the same sub-construct or dimension). Furthermore, 34 participants (12.2%) reported psychological or psychiatric disorders, mostly associated with anxiety. Since anxiety symptomatology is a central variable in this analysis, these participants were excluded. The sample considered for the path analysis was, ultimately, composed of 244 participants (141 women; 57.8%), with ages between 18 and 36 years old (M=24.18; SD=4.31). Most of these participants were students (n=166; 68%).

All participants provided informed consent before the study. The study was approved by the institutional Ethics and Deontology Committee and was performed according to the guidelines of the Declaration of Helsinki and the American Psychological Association.

Materials

Autism-Spectrum Quotient. The Autism-Spectrum Quotient (AQ) is a self-report instrument developed by Baron-Cohen et al. (2001) to measure autism traits in adults of the general population with normal intelligence. A five-domain theoretical

structure was the basis of the development of this instrument, covering key-areas such as social skills, attention switching, attention to detail, communication and imagination. This instrument is comprised of 50 items, distributed across these dimensions. For each one of the AQ's items, individuals may select one of four possible answers: "definitely agree", "slightly agree", "slightly disagree" and "definitely disagree". In the original work, each item was scored considering a binary scoring method. Alternatively, several studies have been scoring AQ's items considering a 4-point Likert-type scale, in order to account for all the response variability and, thus, better differentiate between individuals (Austin, 2005; English et al., 2020; Hoekstra et al., 2008). In the present work, the items were analyzed considering the 4-point scale and only those AQ dimension scores that showed factorial validity were considered.

In the present study, reliability was calculated using Cronbach alpha, considering a total score (50 items), which turned out to be satisfactory, α= .76. Yet, 36 of the 50 items showed item total-correlations inferior to .30, considered to be an acceptable cut-off to assume the item to be a good marker of the measure (J. Cohen et al., 2002). The internal consistency regarding the five original AQ's subscales was also computed. The Cronbach alfas were α= .75 (social skills), α= .56 (attention switching), α= .71 (attention to detail), α= .58 (communication), and α= .55 (imagination). Overall, all subscales presented at least one item with item total-correlation inferior to .30, with the attention switching, communication and imagination dimensions showing between 6 and 7 items in this condition.

Translation and adaptation for the Portuguese context. In order to explore the dimensionality of the AQ and the internal consistency of its subscales, the 50 items were firstly translated by the research team and further discussed with experts in psychological instrument development. Next, a retroversion was performed by an English native speaker, followed by an analysis and discussion of the two versions by the research team, resulting in a preliminary Portuguese version of the AQ. This version was then tested in a small group of adult individuals (> 18 years old) of both sexes to evaluate if the items and instructions were clear and correctly interpreted, as well as to measure the average time needed to answer the AQ. The results of this pre-test suggested that no further correction was needed.

State-Trait Inventory for Cognitive and Somatic Anxiety – trait version. The State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA; Ree et al., 2008) is a self-report questionnaire which aims to assess anxiety as a multidimensional emotional response. It is divided into two forms, one comprising a more transitory manifestation of anxiety, and the other addressing a stable trait, reflecting the predisposition of an individual to experience anxiety (Cattell & Scheier, 1958; Spielberger et al., 1983). Moreover, each form encompasses items assessing the cognitive aspects of anxiety (such as intrusive thoughts), as well as items reflecting somatic symptoms (such as muscular tension). A 4-point scale, varying between 1 (not at all) and 4 (very much so), is used to measure the frequency of anxiety symptoms across the items in the scale.

Taking into account our rational, in the present research only the trait dimension of anxiety was assessed, including its cognitive and somatic dimensions (henceforward STICSA-Cognitive and STICSA-Somatic, respectively). For this purpose, a Portuguese version of STICSA was used (Barros, Figueiredo, Brás, et al., 2022). In the present research (N=244), Cronbach alfas were .86 for cognitive and .83 for somatic trait anxiety, supporting excellent reliability.

Toronto Alexithymia Scale. The Toronto Alexithymia Scale of 20 items (TAS-20) is one of the most used self-report questionnaires measuring alexithymia considering its distinct dimensions: difficulty in identifying feelings (DIF), difficulty in describing feelings (DDF) and externally oriented thinking, i.e., a "concrete and reality-based" cognitive style (EOT; Bagby, Parker, et al., 1994; Kooiman et al., 2002; Sekely et al., 2018; G. J. Taylor et al., 1985). Across its 20 items, responses are given in a 5-point Likert scale, ranging from "strongly disagree" to "strongly agree".

A Portuguese adaptation of TAS-20 (Prazeres et al., 2000) was used in the present study. A reliability analysis of the TAS-20 subscales was performed (N=244), suggesting very good internal consistency for the DIF dimension, $\alpha = .85$, as well as for DDF, $\alpha = .81$. However, in line with previous research (Bagby et al., 2020; Kooiman et al., 2002), the EOT dimension had poor reliability, $\alpha = .58$. Nevertheless, to preserve the alexithymia construct and better understand the influence of all the inherent dimensions, we kept the EOT in the analysis and discussed the limitations to the interpretability of this dimension in the Discussion section.

Procedure

This study was part of a broader research project which sought to study, among others, the relationship between autism traits, alexithymia, and anxiety. The assessment protocol was comprised of a sociodemographic questionnaire with relevant information for the sample's characterization, followed by AQ (50 items), STICSA-Trait (21 items) and TAS-20 (20 items). Participants were recruited and informed about the study mainly through e-mail, class visits, distribution of flyers, and social networks. Data collection was performed through a digital platform of the institution. Before answering the protocol, all participants were made aware of the voluntary nature of their participation and confidentiality of the collected data, and provided their informed consent. There was no compensation for their participation in the study. The protocol took approximately 15 minutes to complete.

Analytical procedures

The statistical procedures were conducted using IBM SPSS Statistics (version 26) when considering descriptive, Exploratory Factorial Analysis (EFA) and reliability analyses. The EFA were performed with the Principal Axis Factoring (PAF) method of parameter estimation to explore the observed interdependencies of the variables that underly theoretical constructs represented by factors. This estimator is considered quite robust when dealing with small to medium deviations from normal distribution. To better interpret the final solution, an oblique rotation, namely a direct oblimin, was conducted assuming the dimensions were correlated. This option was used for comparability purposes as it was the choice of most of the authors from previous dimensionality analyses of the AQ scale. The cut-off point for item retention loading was set to at least .40 and the final results were reported from the pattern matrix (Howard, 2016; Tabachnick & Fidell, 2007).

The Mplus version 8 (Muthén & Muthén, 2017) was used for the Confirmatory Factorial Analysis (CFA). The CFA were carried out using the Weighted Least Square Estimator (WLSMV; Muthén & Muthén, 2017), a categorical robust estimator which considers observed variables as ordinal and latent variables as continuous, being also

the most suitable option for small to medium samples (Flora & Curran, 2004). The assessment of model fit was supported on a robust chi-square statistic, but mostly on the following fit indexes and cut-offs: Comparative Fit Index (CFI) and Tucker and Lewis Index (TLI), both of which should present values higher than .95; Root Mean Square Error of Approximation (RMSEA), and Standardized Root Mean Residual (SRMR), which should be equal or less than .07 (Brown, 2006; Schermelleh-Engel et al., 2003). The reliability analysis was assessed using Cronbach alpha, with values higher than .60 being considered acceptable for internal consistency (DeVellis, 2012). The categorical Omega was computed assuming the factor loadings extracted in the performed factorial analysis (Viladrich et al., 2017).

The path analysis model was performed using IBM AMOS (version 22). This analysis was carried out to consider the relationship between observed variables, using the Maximum Likelihood (ML) estimator. The model fit was evaluated using the chi-square statistics and the same four fit indexes considered for the CFA models (R. Kline, 2005).

Pearson correlations were computed to consider the association between numeric variables, and the effect sizes were assessed using Cohen et al. (2002) rule of thumb: low – between .10 till .30; medium – between .30 till .50 medium; high – from .50. The number of recruited participants allows the sample significance of the analyses. With regard to the EFA, nearly 300 participants were recruited (n=292), as recommended by Tabachnick and Fidell (2007). Additionally, there is a ratio of 5.82 participants per item for the analysis with all the items, and a ratio of 12.70 participants per item for the final solution, which is above the recommended cut-point of five participants per item (Hair et al., 2014). Furthermore, in the structural equation modelling analyses we had a ratio of 11.68 participants per estimated parameter for the CFA, and a ratio of 18.53 participants per parameter for the path analysis, which is above the recommended cut-point of ten participants per parameter (R. Kline, 2005). All measures and exclusions are reported in the results section.

Results

AQ dimensionality

The set of 50 items corresponding to the original five-factor structure of AQ was tested through a CFA. The model revealed very poor fit and, by observing the standardized factor loadings and the modification indexes, it was possible to conclude that the imposed hypothetical structure was problematic. Therefore, to better understand the underlying structure of the AQ scale, the factor structure was explored using an EFA with a PAF estimation. This procedure was conducted with the 50 items and the initial solution was analyzed in order to provide the indication about the number of factors to retain. The Cattell's scree plot showed a clear marked declination after the third point, indicating the optimal number of factors to retain in the structure. In a subsequent step, the five-factor original structure was explored, but it was uninterpretable and very distinct from the proposal by Baron-Cohen et al. (2001). The next option was to retain the three factors indicated by the Cattell's scree test, even though it required the exclusion of 26 items, that either had factor loadings lower than .40 or loaded in more than one factor (cross-loadings). The final solution was a comprehensible factor structure with the items marking three autism dimensions, namely Social Skills, Communication, and Restricted Interests and Detail Orientation (RIDO). One last item (32: "I find it easy to do more than one thing at once") was excluded due to a theoretical criterium, since it loaded in a factor where a conceptual fit was not made.

The final solution was, then, obtained conducting an additional EFA with a PAF procedure for the remaining 23 items, after ensuring suitable sample and matrix values for this statistical procedure to be conducted (Kaiser-Meyer-Olkin=.78; Bartlett's Test of Sphericity, $\chi^2(253)=1751.50$, $p<.001$). The structure retained three factors after a direct oblimin rotation: The first factor, Social Skills, consisting of 10 items, had loadings between .73 and .40, and presented an eigenvalue of 3.60, explaining 16.86% of variance. The second factor, Communication, explained 8.05% of variance (eigenvalue =2.51) with 7 items loading between .67 and .44. The third factor, RIDO, had 6 items with loadings between .62 and .45, and presented an eigenvalue of 1.81, explaining 7.80% of variance.

Of the 23 items, 12 loaded in a factor similar to that originally proposed by Baron-Cohen et al. (2001). Although the remaining 11 items loaded in distinct factors in

comparison with the original structure, they are consistent with the results observed by previous studies exploring the dimensionality of AQ (e.g., English et al., 2020; Palmer et al., 2015); nevertheless, item 3 ("If I try to imagine something, I find it very easy to create a picture in my mind") and item 8 ("When I'm reading a story, I can easily imagine what the characters might look like") are exceptions, since they loaded in different factors when comparing with previous studies.

The model extracted in the preceding analysis was tested using a CFA procedure to corroborate the robustness of the extracted factor structure. In a first step, one model was tested taking into account that the 23 items loaded on the same factor, revealing, as expected, very poor fit values (Table 1). In a next step, a model assuming the final structure extracted in the EFA was tested, and the chosen fit indexes showed results not far from the cut-off points to be accepted as a suitable model. By exploring the modification indexes, it was possible to verify a high level of association between the error terms of two items of the first factor, namely item 17 ("I enjoy social chit-chat") and item 38 ("I am good at social chit-chat"). This high association was expected since these items relate to social conversation and have the exact same wording ("chit-chat"). This observation allowed the decision to be made of running a nested model with one modification (assuming the covariance of the errors of items 17 and 38). This new model revealed a significant decrease of chi square units, $\chi^2(1)=69.30$; $p<.001$. The observation of the goodness of fit, and the modification indexes revealed another two items, namely item 3 ("If I try to imagine something, I find it very easy to create a picture in my mind") and item 8 ("When I'm reading a story, I can easily imagine what the characters might look like") in the factor 2, which were equally highly associated. Both items 3 and 8 relate to the ability to create a mental representation of something; yet, although highly associated, both still measure different aspects, since item 3 is more general (and not necessarily related to social stimuli) and item 8 is more specific, alluding to characters' features. Therefore, another nested model assuming the two modifications was then tested, presenting an acceptable fit (CFI=.91, TLI=.90, RMSEA=.06, SRMR=.07). The chi square difference between the models was also significant, $\chi^2(1)=37.03$; $p<.001$ (Table 1).

Table 1. CFA results – Goodness of fit of the tested models (N=292).

Models	χ^2	$\Delta\chi^2$(df)	df	CFI	TLI	SRMR	RMSEA (CI90%)
Unidimensional model	1280.54*		230	.58	.54	.12	.13 (.12 -.13)
Three factor model	550.65*		227	.87	.86	.08	.07 (.06 -.08)
		69.30*(1)					
Three factor model with one modification	481.35*		226	.90	.89	.08	.06 (.06 -.07)
		37.03*(1)					
Three factor model with two modifications	444.32*		225	.91	.90	.07	.06 (.05 -.07)

*p < .001

The final model supported the factor structure of the three factors with all the items presenting significant factor loadings. The Social Skills factor was composed of 10 items with loadings ranging between .44 and .78, and two error terms allowed to covariate (r=.61). The Communication factor encompassed 7 items with loadings ranging from .43 and .74, and two error terms of items correlated (r=.47). The RIDO factor was composed of 6 items with factor loadings between .48 and .66 (Table 2). Results further revealed non-significant correlations between RIDO and Social Skills factors, r=-.05, as well as between RIDO and the Communication factor, r=-.012, p>.05. The Social Skills and the Communication factors were moderately correlated, r=.36, p<.001.

The ordinal Omega showed adequate levels of reliability for the three factors (social skills: .86; Communication: .78; RIDO: .74). Also, and for comparability purposes, the Cronbach's alpha was computed, and it supported adequate to very good levels of internal consistency for the three factors, with values of .68 (RIDO), .74 (Communication) and .83 (Social Skills; DeVellis, 2012). The item-total correlations were all above .36, meaning that all the retained scale items contributed positively to the construction of the final measure in the three factors of organizational cooperation (DeVellis, 2012).

Relationship between autism traits, alexithymia, and anxiety: A path analysis

In order to explore the relationship between autism traits, alexithymia and trait anxiety, taking into account the distinct dimensions within each construct, a path analysis was performed. This analysis considered the computed score factors as observed variables, namely the three dimensions of AQ as predictors, the two dimensions of TAS-20 as mediator variables, and the two dimensions of STICSA as dependent variables. The means and standard deviations of the autism traits, alexithymia, and trait anxiety dimensions, as well as the Pearson correlations between these variables, are outlined in Table S2 (Supplementary Material – Study 2; see Chapter 6 – Appendix). Considering the fact that the literature often reports a significant (and moderate) correlation between the Social Skills and Communication dimensions of the AQ (e.g., Bothe et al., 2019; English et al., 2020), this correlation was previously assumed by the model.

The tested model presented a non-significant chi-square, $\chi 2(2)=1.52$; $p=.47$, meaning that the empirical data does not significantly differ from the imposed structure. In the model specification, the errors of the mediators (DIF and DDF, r=.51; DIF and EOT, r=.15; DDF and EOT, r=.24), as well as the errors of the dependent variables (STICSA-Cognitive and STICSA-Somatic, r=.52) were allowed to correlate.

The model representing the relationship between autism traits, alexithymia and trait anxiety is outlined in Figure 1. Results showed that the imposed model explained 37.9% of variance of the STICSA-Cognitive and 26.6% of the STICSA-Somatic. The predictors were found to explain 16.2% of DIF, 18.2% of DDF, and 9.3% of EOT. The Social Skills factor had a significant impact on both DIF, $\beta=.31$; $p<.001$, and DDF, $\beta=.21$; $p<.001$. The Communication factor showed a significant impact on the DDF, $\beta=.40$; $p<.001$, and on EOT, $\beta=.41$; $p<.001$. The RIDO factor explained a significant variance of DIF, $\beta=.47$; $p<.001$, as well as of DDF, $\beta=.23$; $p=.005$. The DIF dimension showed a large and significant impact on both anxiety dimensions (STICSA-Cognitive, $\beta=.58$; $p<.001$, and STICSA-Somatic, $\beta=.46$; $p=.01$). The DDF and the EOT dimensions did not present a significant impact in both dependent variables. None of the AQ dimensions had a significant direct effect in anxiety dimensions.

Table 2. CFA results - Item loadings by factor.

Item	Item content	Factor loadings		
		SOC	COM	RIDO
AQ11	I find social situations easy.	.77		
AQ13	I would rather go to a library than a party.	.45		
AQ15	I find myself drawn more strongly to people than to things.	.45		
AQ17	I enjoy social chit-chat.	.44		
AQ22	I find it hard to make new friends.	.67		
AQ26	I frequently find that I don't know how to keep a conversation going.	.65		
AQ34	I enjoy doing things spontaneously.	.56		
AQ38	I am good at social chit-chat.	.65		
AQ44	I enjoy social occasions.	.76		
AQ47	I enjoy meeting new people.	.78		
AQ3	If I try to imagine something, I find it very easy to create a picture in my mind.		.43	
AQ8	When I'm reading a story, I can easily imagine what the characters might look like.		.45	
AQ20	When I'm reading a story, I find it difficult to work out the characters' intentions.		.45	
AQ27	I find it easy to "read between the lines" when someone is talking to me.		.74	
AQ31	I know how to tell if someone listening to me is getting bored.		.55	
AQ36	I find it easy to work out what someone is thinking or feeling just by looking at their face.		.73	
AQ45	I find it difficult to work out people's intentions.		.69	
AQ6	I usually notice car number plates or similar strings of information.			.57
AQ9	I am fascinated by dates.			.57
AQ16	I tend to have very strong interests, which I get upset about if I can't pursue.			.48
AQ19	I am fascinated by numbers.			.60
AQ23	I notice patterns in things all the time.			.66
AQ41	I like to collect information about categories of things (e.g. types of car, types of bird, types of train, types of plant, etc.).			.50

Note. All items are significant at $p < .01$.

Discussion

Study of the relationship between the expression of autism traits in the general population and other autism-related features, including alexithymia and anxiety, contributes to autism conceptualization, as well as to a better understanding of the specific versus shared mechanisms between the clinical and non-clinical manifestations. Furthermore, exploring these relationships in a multidimensional perspective can be highly informative about the specific associations between the distinct dimensions, which relates to the heterogeneity observed in the spectrum. To this end, the development of reliable instruments, capable of quantifying and reflecting the core characteristics of autism and its heterogeneity, is also vital. The present research aimed to tackle some of these aspects by: 1) contributing to the clarification of the AQ measurement model; and 2) further exploring the relationship between autism traits, alexithymia and anxiety in a multidimensional perspective. Our results suggested that the original five-factor model of the AQ yielded poor statistical fit, providing empirical support for a three-factor model. In addition, results of a path analysis supported the role of alexithymia, particularly of the DIF dimension, as a mediator of the relationship between autism traits and self-reported trait anxiety in the general population, especially in relation to cognitive anxiety.

AQ's measurement model

In order to extend previous psychometric studies of AQ, we firstly tested the adequacy of the originally proposed five-factor model (Baron-Cohen et al., 2001) through a CFA. Consistent with previous studies (e.g., do Egito et al., 2017; English et al., 2020), results indicated that this model did not present adequate statistical fit. Subsequent EFA and CFA analyses supported a three-factor model composed of 23 items, with goodness-of-fit indices evidencing acceptable fit.

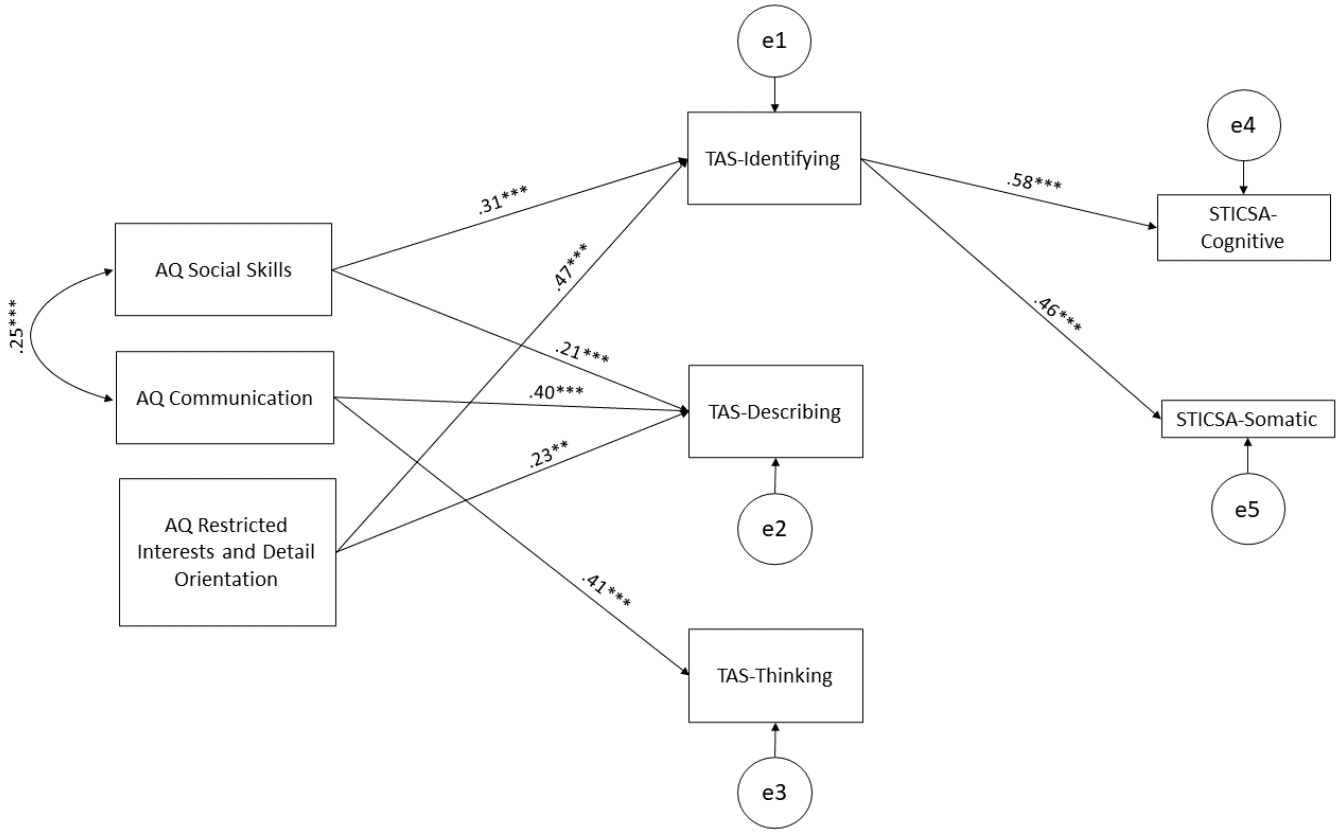

Figure 1. Path analysis model representing the moderator role of alexithymia in the relationship between autism traits and trait anxiety.

Note. All the presented coefficients are standardized. Only the significant relationships are depicted. The levels of significance considered were *p<.05, **p<.01, ***p<.001.

After an examination of the corresponding items, the proposed dimensions were Social Skills, Communication and Restricted Interests and Detail Orientation, reflecting, respectively: 1) social interest, deficits in developing and maintaining relationships and other social abilities; 2) difficulties related to the use of pragmatic language and to the inference of thoughts, feelings, and intentions of others; 3) a pattern of restricted interests and behaviors, and a tendency to focus on details.

The Social Skills and Communication dimensions were found to correlate moderately (r=.36), whereas none of these dimensions were found to significantly correlate with the RIDO dimension. As previously argued by Palmer et al. (2015), the observed three-factor model and the fact that the social dimensions were moderately associated with each other (but not with the RIDO dimensions) seems to be in line with the current proposed diagnostic criteria for autism, when considering DSM-5 (American Psychiatric Association, 2013). The lack of association between RIDO and the social dimensions, as similarly observed by several other studies (Austin, 2005; Bothe et al., 2019; English et al., 2020; Palmer et al., 2015; Russell-Smith et al., 2011) further supports the notion that autism is comprised of distinct social and non-social dimensions which, together, contribute to the weaknesses and strengths of the spectrum (e.g., Happé & Ronald, 2008; Palmer et al., 2015; Ronald et al., 2006).

The Social Skills factor retained 10 items in total, six of which were part of the ten originally proposed items for this dimension (Baron-Cohen et al., 2001). This subscale also encompassed items originally from other dimensions, namely from communication – item 17, 26 and 38 – as well as from attention switching dimension – item 34. This is in line with previous studies that observed a moderate association between social and communication dimensions of autism, which explains why some items originally thought to be more associated with communication abilities are, in fact, also associated with social abilities (English et al., 2020; Palmer et al., 2015). Item 34, originally from attention switching dimension, is also highly related to social skills, reflecting attentional processes relating to a social context (do Egito et al., 2017; Palmer et al., 2015).

The Communication factor retained seven items, with only two of the ten items originally proposed for this dimension (Baron-Cohen et al., 2001). The remaining items were originally from the Social Skills (items 36 and 45) and Imagination dimensions (items 3, 8 and 20). Items 8 and 20 were previously found to be associated with a

communication dimension by other studies (e.g., Lau, Kelly, et al., 2013; Palmer et al., 2015). However, this is the first time that item 3 is associated with a factor other than Imagination; it is possible that these items also reflect difficulties in representing mental states, intentions, emotions and general attributes of others, which are important for social communication (U. Frith, 1994) and may be also closely related with imagination abilities (Fonagy & Target, 2006; U. Frith, 1994). In line with this, difficulties in sharing imaginative play are included in the social communication and social interaction criterion of DSM-5 (American Psychiatric Association, 2013).

The RIDO factor retained four of the original items proposed by Baron-Cohen et al. (2001) for the correspondent dimension. The remaining two items were originally from the attention switching (item 16) and imagination subscales (item 41). A close inspection of these items suggests that they seem to be highly associated with narrow interests and behaviors, which may explain why they loaded in this dimension, similarly to those observed in other studies (e.g., Russell-Smith et al., 2011).

The final solution was theoretically sound, even though it only retained 46% of the proposed original items, which may limit the range of autism characteristics assessed by AQ; nevertheless, the excluded items were, in general, previously shown to be problematic, when taking into consideration their association with different factors or their exclusion by the final solutions across studies. Although our factor structure differs from some of the other models reported in the literature, this latter aspect is especially true for the Communication and RIDO dimensions. The Social Skills dimension is very close to the correspondent dimension in other studies, retaining most of the originally proposed items. This dimension is, in fact, always present across studies, and explains a significant portion of variance, indicating the importance of social abilities for the structure and understanding of autism traits. Conversely, the other dimensions are less stable in terms of the retained items, which may suggest that they are more culture-dependent (e.g., Hurst et al., 2007), or their items may be inadequate to measure the respective component, which leads to different interpretations from the respondents (English et al., 2020; Palmer et al., 2015).

The mediation role of alexithymia in the relationship between autism traits and trait anxiety

Results of the path analysis performed in the present study, as hypothesized, supported an indirect effect of autism traits in trait anxiety through alexithymia, with the model yielding acceptable fit and explaining 37.9% of the variance in STICSA-Cognitive and 26.6% of the variance in STICSA-Somatic. This is in line with the findings reported by Maisel et al. (2016) who found that alexithymia, together with emotional acceptance, explained a substantial amount of the effect between autism traits and trait anxiety in a sample of adults with and without autism. Moreover, in a sample of adults with autism, Morie et al. (2019) also observed that the relationship between alexithymia and emotion regulation mediated the association between autism traits and anxiety. However, these studies considered autism traits as reflected by a total score, used distinct questionnaires to measure autism traits and anxiety, had distinct samples (only clinical or mixed) and introduced other constructs in their models, which precludes direct comparison between studies.

In our model, Social Skills and RIDO dimensions had a moderate impact in DIF, which, in turn, had a moderate impact in both STICSA-Cognitive and STICSA-Somatic anxiety. On the other hand, although the three dimensions of AQ had an impact in DDF, and the Communication dimension had a moderate impact in EOT, these two alexithymia dimensions did not significantly predict trait anxiety. These results suggest that difficulties in social interaction, as well as the presence of restricted interests and tendency for detail orientation, are associated with deficits in identifying feelings and emotions, which together contribute to anxiety symptomatology (cognitive and somatic). In line with these results, it has been proposed that difficulties in emotional processing are associated with impairments in social interaction and communication (Poquérusse et al., 2018; Scheerer, Boucher, et al., 2021). Therefore, alexithymia may contribute to the difficulties in social interaction and communication often observed in the spectrum, probably due to poor emotional awareness, difficulties in expressing emotions, and impaired emotion regulation (Bird & Cook, 2013; Maisel et al., 2016; Morie et al., 2019). In addition, we also found detail orientation and restricted interests to be associated with alexithymia, especially concerning difficulties in identifying feelings. It has been suggested that alexithymia is characterized by attentional deficits,

particularly concerning affective stimuli (Luminet et al., 2021), as well as heightened attention to somatic changes (G. J. Taylor et al., 1997). These attentional alterations, allied with difficulties in the cognitive processing of emotional stimuli, may be associated with greater difficulty in identifying one's own feelings and emotions and, to a lesser extent, in communicating them to others.

Similarly to our results, Liss et al. (2008) found that the Social Skills dimension was related to DIF and DDF dimensions of alexithymia, and that the Communication dimension was related to the EOT dimension of alexithymia. However, these authors did not observe a relationship between the dimension which corresponds to RIDO and alexithymia. On the other hand, Bothe et al. (2019) found a marginally significant ($p<.07$), negative and weak association between the attention to detail dimension and alexithymia. One explanation for the discrepancy between these results may be the differences observed in the structure of RIDO subscales across these studies and ours. Importantly, the analyses performed to explore the relationships of these variables were also different; in our study, a path analysis was conducted, while in Liss et al. (2008), only bivariate correlations were computed, which do not account for the multivariate nature of the phenomena. Bothe et al. (2019) also did not analyze the distinct dimensions of alexithymia, and this precludes direct comparisons with our results.

Alexithymia, specifically DIF dimension, was found to significantly and moderately predict both dimensions of trait anxiety. This is partially in line with the previous literature, which found significant and small-to-moderate relationships between alexithymia and anxiety in the general population (Bothe et al., 2019; Fietz et al., 2018; Liss et al., 2008). Interestingly, although the three dimensions of AQ had a small impact on DDF, especially the Communication dimension, this alexithymia dimension did not evince a significant impact in either dimension of anxiety in our model. This suggests that the difficulty in identifying one's own emotions is more closely associated with anxiety symptomatology. Oakley et al. (2020) also observed that DIF was more associated with anxiety and this predicted later anxiety symptoms in a sample of adolescents and adults (with and without autism), although they evaluated current anxiety symptomatology rather than trait anxiety.

Our model explained more variance in cognitive trait anxiety than in somatic trait anxiety, which suggests that autism traits and alexithymia play a major role specifically in the former dimension of anxiety. It is possible that higher difficulties in

identifying feelings, allied with poor social abilities and detail orientation, are associated with a more negative appraisal about future social situations, with their being perceived as demanding and unpredictable, which is related to more cognitive anxiety symptoms, such as higher anticipation of negative outcomes and difficulties concentrating (see, for instance, Sharma et al., 2014).

In line with the results observed by similar studies (Fietz et al., 2018; Maisel et al., 2016), after accounting for the impact of alexithymia on trait anxiety, none of the dimensions of autism traits directly influenced anxiety. Yet, direct comparisons with past studies are limited, since they used a total AQ score instead of its dimensions, had distinct samples and carried out other statistical analyses. It has been previously suggested that poor social abilities can be associated with social rejection and can make social situations unpredictable, uncomfortable and highly demanding, which may contribute to the anxiety symptomatology experienced by people with autism (White et al., 2014; Wood & Gadow, 2010). And, in fact, some authors previously observed a relationship between the Social Skills dimension of AQ and anxiety (Bothe et al., 2019; Liss et al., 2008). Although we also observed a significant positive correlation between the Social Skills dimension of the AQ and cognitive anxiety, when accounting for alexithymia, the impact is no longer significant. Our results, then, suggest that the self-reported trait anxiety symptomatology in the general population is not significantly affected by autism traits per se, but rather by the combined effect of specific dimensions of autism traits and alexithymia.

Strengths, limitations, and future directions

Grounded in the importance of further understanding the complexity of autism and its dimensional nature, as well as the relationship between its distinct core features and other variables often associated with the spectrum, the present research sought to extend previous studies about the assessment of autism traits and their association with alexithymia and anxiety in the general population. Taking into consideration the often-observed high variability in the profiles of symptoms and their severity across the spectrum, and the high association between autism traits with both alexithymia and anxiety symptoms, this knowledge may be crucial to further shape assessment, mental health prevention and intervention. As suggested by Landry and Chouinard (2016),

studying these relationships in the general population may be helpful to understanding autism in a dimensional perspective, since it is easier to isolate the effect of one variable on another, and it is also possible to account for a wider range of variability in the different traits. Although the results obtained for the general population cannot be generalized to autism, considering the differences between the clinical and nonclinical manifestations, they offer an important insight about the potential shared or unique mechanisms underlying the symptoms and features associated with both phenotypes (Landry & Chouinard, 2016). To study these mechanisms, it is crucial to ascertain the adequacy and reliability of the assessment instruments, as well as their ability to capture and represent the essential features of autism. This issue was addressed by the present study and provided further support for AQ's measurement model, with the three proposed dimensions evincing acceptable-to-good factorial validity and reliability.

Our results also provide further evidence of the heterogeneous nature of autism traits by showing, in line with previous research, that the social and non-social dimensions do not significantly correlate (e.g., Bothe et al., 2019; English et al., 2020; Russell-Smith et al., 2011). Previous research also suggested that social and non-social autism traits are genetically dissociable (Warrier et al., 2019), and are also associated with distinct outcomes, for instance, regarding socioemotional processing (e.g., Bothe et al., 2019) and social cognitive processes (e.g., J. Davis et al., 2017). In this study, distinct relationships were observed between the autism traits, alexithymia, and anxiety dimensions. In fact, the conceptualization of autism has been evolving towards the recognition that, instead of being a unitary construct and having a single explanation about its basis, autism comprises distinct features (e.g., social skills, and repetitive behaviors and interests) which seem to be independent at the cognitive, behavioral, genetic and neural level (Happé & Frith, 2020; Happé & Ronald, 2008). Therefore, distinct patterns of autism traits along the spectrum may be associated with distinct strengths and difficulties, which reinforces the need for implementing a comprehensive, multidimensional, and multidisciplinary assessment to properly support people with an autism diagnosis and/or with a higher manifestation of autism traits.

The present research also contributes to a better understanding of the mechanisms underlying trait anxiety in the general population, including the role of alexithymia, especially the DIF dimension, in the relationship between autism traits, particularly regarding the Social Skills and RIDO dimensions, and trait anxiety. This

knowledge is relevant considering that anxiety is highly prevalent in autism (e.g., Hollocks et al., 2019) and greatly impacts the quality of life people with autism (e.g., I. C. Smith et al., 2019). By using STICSA, we were also able to understand the mechanisms underlying cognitive and somatic trait anxiety, which can be useful since different people can present distinct profiles of anxiety symptomatology (Schwartz et al., 1978). Furthermore, considering the specificity of anxiety manifestation in autism, which often includes traditional anxiety symptoms coupled with atypical, autism-related anxiety symptoms (Kerns et al., 2014), it is important to further explore and characterize these distinct profiles and the underlying mechanisms to be able to develop adequate intervention plans.

Autism and high levels of alexithymia frequently co-occur (Kinnaird et al., 2019; Poquérusse et al., 2018), with the latter being linked to many of the socioemotional impairments observed in the spectrum (Bird & Cook, 2013). As alexithymia was shown to mediate the relationship between autism traits and trait anxiety, assessment and intervention targeting this personality trait may be critical to decrease anxiety symptoms and increase the success of the intervention with people on the spectrum (e.g., Albantakis et al., 2020; Kinnaird et al., 2019; Milosavljevic et al., 2016). Specifically, the results support the central role of difficulties in identifying one own's emotions and feelings in the impact that social difficulties and repetitive/restricted behaviors and interests have on trait anxiety, especially cognitive trait anxiety. These results also suggest that interventions whose aim is to reduce the general predisposition of experiencing cognitive anxiety (and, to a lesser extent, somatic anxiety) should place a major focus on the DIF dimension of alexithymia. For this purpose, and as observed by Cameron et al. (2014), interventions encompassing psychoeducation with skills training, and/or focused on the identification, expression, and understanding of emotions and body changes, may be effective at reducing alexithymia. Research exploring the efficacy of interventions targeting alexithymia in anxiety symptomatology, well-being, and general functioning (including interpersonal functioning; Cameron et al., 2014) is lacking, including in people with autism and/or with both a high expression of autism traits and alexithymia. Nevertheless, as previously stated, this study aimed to explore the relationship between autism traits, alexithymia, and anxiety in the general population and, thus, our results cannot be generalized to the clinical spectrum or be used to inform assessment and intervention in

this group. Exploring these mechanisms in both clinical and nonclinical samples could further contribute to the understanding of the variables associated with anxiety etiology across the spectrum, which would, in turn, serve assessment, diagnosis and intervention in individuals with and without clinically significant autism symptomatology.

As supported by the results, individuals without a formal diagnosis, but with more autism traits and higher alexithymia may have a higher tendency to experience anxiety symptoms. Previous evidence suggests that anxiety disorders are often associated with other mental health disorders (e.g., Lamers et al., 2011), physical problems (e.g., Celano et al., 2016) and poor quality of life (e.g., Olatunji et al., 2007); similarly, high levels of anxiety were also related to depressive symptoms (e.g., Zukerman et al., 2019), sleep problems (e.g., Mazurek & Petroski, 2015), and lower quality of life (e.g., I. C. Smith et al., 2019) in individuals with an autism diagnosis. Even though individuals with high levels of autism traits, alexithymia and trait anxiety do not formally meet the criteria for an autism and/or an anxiety diagnosis, many of them may be suffering or may present difficulties across contexts. This stresses the need to view and evaluate these traits by implementing a multidimensional approach, in order to support a personalized intervention plan that can benefit the specific needs of these individuals. For this purpose, using a dimensional approach can, indeed, be crucial to get information and characterize the distinct profiles of the manifestation of symptoms along the spectrum and their developmental trajectories, as well as to improve intervention planning and monitoring (see Happé & Frith, 2020; Rosen et al., 2021). Moreover, not only is it important to intervene when there is a formal diagnosis, but it is also crucial to prevent further psychopathological conditions and promote the well-being and quality of life of these individuals.

The contributions of this study notwithstanding, it is important to discuss some limitations to our conclusions. First, the sample size reported for the dimensionality study of AQ is relatively small, which may limit the generalization of our results. Although we do not lack statistical power for conducting the analysis, it is possible that the small sample does not represent all the variability in autism traits, taking into consideration all the different dimensions, which can have impacted the results underlying the factorial structure of AQ. Furthermore, as discussed above, our final model is slightly distinct from the original model, and from others previously found (although it is similar, for instance, to the model of Palmer et al., 2015), which may

limit direct comparisons. Also, the two factorial analytic procedures were implemented in the same sample, which hinders the generalization of the structure tested. Further studies should test the three-factor structure that emerged in the present study in an independent sample. Finally, the EOT dimension of TAS-20, used in the path analyses, yielded poor reliability, limiting the conclusions which were possible regarding this specific facet of alexithymia.

As previously argued, future studies should extend our findings to the clinical spectrum. Firstly, and in relation to the first study, it is essential to assess measurement invariance between clinical and non-clinical groups (Murray et al., 2014). This would allow the use of AQ in future studies with clinical and non-clinical samples, and reliably compare the two groups in terms of the expression of autism traits and its relationship with other variables. With regard to the second study, the relationships between the distinct dimensions of autism traits, alexithymia and trait anxiety may differ between the clinical and nonclinical sides of the spectrum (e.g., Albantakis et al., 2020), possibly due to the combined presentation of symptoms in autism, and to other features that are not markedly present in the general population. Furthermore, other variables should be further explored in future studies, taking account of their previously shown association with the autism spectrum and anxiety. For instance, anxiety symptoms in the autism spectrum may be linked to altered sensory processing (Amos et al., 2019; Liss et al., 2008; MacLennan et al., 2020), as well as with cognitive variables, such as emotional acceptance and intolerance of uncertainty (Maisel et al., 2016), cognitive inflexibility (Ozsivadjian et al., 2020), and other variables such as emotional regulation (Morie et al., 2019). By recruiting larger samples and comparing groups of individuals with and without an autism diagnosis, it would be possible to extend our knowledge about how the different aspects of autism (clinically relevant or not) are related to these other variables and which outcomes emerge from those associations, and also allowing for the use of a latent variable approach for model testing.

Conclusions

The present study provided empirical support for a three-factor model of AQ, with two correlated social dimensions and one distinct non-social dimension, reflecting the core characteristics of autism, as well as the heterogeneity associated with this condition.

Therefore, notwithstanding its limitations, AQ may constitute a useful instrument for the measurement of autism characteristics in the general population, within the three proposed distinct dimensions that characterize the autism spectrum. Furthermore, empirical support was also provided for the differential relationships between the distinct components underlying autism traits, alexithymia, and trait anxiety, which corroborates the multidimensional nature of these variables and the need to account for their distinct dimensions in the study of autism. Study of the nature of autism traits and their relationship with the strengths and difficulties evinced in the spectrum is crucial to the understanding of autism, and it also has the potential to foster important developments in the conceptualization, assessment and intervention in the clinical phenotype (Landry & Chouinard, 2016).

Supplementary Material

Please refer to Supplementary Material – Study 2, in Chapter 6 - Appendix. This material is also available in https://doi.org/10.1016/j.ridd.2022.104361.

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Study 3

Sensory processing in the Autism Spectrum: The role of attention to detail and somatic trait anxiety in the olfactory perception of the general population

This study was published online by Springer Nature in Journal of Autism and Developmental Disorders, on 22 September 2020. The reference list of the manuscript has been integrated in the bibliography of the thesis – please see Chapter 5. The necessary structural adaptations were performed to incorporate the content of the article in the present thesis.

Reference:

Barros, F., Figueiredo, C., Costa, A., & Soares, S. C. (2021). Sensory Processing in the Autism Spectrum: The Role of Attention to Detail and Somatic Trait Anxiety in the Olfactory Perception of the General Population. Journal of Autism and Developmental Disorders, 51, 2338-2353. https://doi.org/10.1007/s10803-020-04711-0. Reproduced with permission from Springer Nature.

Abstract

Autism Spectrum Disorders, as well as autism traits (AT), have been associated with altered sensory processing. However, the role of AT in olfactory processing is still unclear. We analyzed the impact of AT and trait anxiety (TANX), relevant in the context of autism and olfactory perception, in the olfactory abilities of a nonclinical adult sample. Participants (N=116) completed the Autism-Spectrum Quotient (AQ), the State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA) and the *Sniffin' Sticks* Extended Test to measure AT, TANX and olfactory abilities, respectively. A hierarchical multiple regression analysis suggested that women and higher scores on the Attention to Detail subscale of AQ were associated with better odor discrimination, and higher somatic TANX was related to poorer odor discrimination.

Keywords

Autism Spectrum Disorders; Autism Quotient; Olfaction; Sensory processing; *Sniffin' Sticks*; Trait Anxiety.

Introduction

Autism Spectrum Disorders (ASD) are characterized by atypical social communication skills, as well as by patterns of repetitive and restrictive behaviors and interests (American Psychiatric Association, 2013). Altered sensory processing has been also established as a core characteristic of this population, frequently involving multiple sensory modalities and extending over the course of development (American Psychiatric Association, 2013; Baranek et al., 2014; Leekam et al., 2007). Although atypical sensory processing has been observed across modalities in ASD, some senses, such as olfaction, seem to have been less explored (Galle et al., 2013). Olfaction is a complex sensory system that plays a pivotal role in survival, since it allows the identification and management of resources, opportunities and threats, and is also critical in human social interaction and communication (R. J. Stevenson, 2010). Therefore, alterations in olfactory processing may underlie important consequences, influencing not only behaviors associated with food intake and safety, but also social communication and relationships with others (R. J. Stevenson, 2010). In this line, several medical conditions have been associated with altered olfactory perception (e.g., Greenberg et al., 2013), including ASD (Larsson et al., 2017).

Available evidence about olfactory perception in ASD mostly targets three olfactory abilities: 1) olfactory detection thresholds, which correspond to the lowest concentration that an odor must possess to be detected by a certain individual; 2) olfactory discrimination, which requires the distinction between odors with different qualities; and 3) olfactory identification, the ability to correctly identify odors (Doty, 2017). Regarding detection thresholds, some studies found no differences between people with ASD and typically developing (TD) individuals (e.g., Galle et al., 2013; Tavassoli & Baron-Cohen, 2012), and others observed either lower olfactory sensitivity (e.g., Dudova et al., 2011; Muratori et al., 2017), or higher olfactory sensitivity (Ashwin et al., 2014). Olfactory discrimination seems to be less studied in comparison with detection and identification abilities, with evidence in favor of intact odor discrimination in ASD (Galle et al., 2013; Muratori et al., 2017). Lastly, most of the studies analyzing olfactory identification show impairments in this domain (e.g., Galle et al., 2013; Muratori et al., 2017; Wicker et al., 2016). These contradictory results across studies may be related to sample and methodological differences (Larsson et al.,

2017; Tonacci et al., 2015), such as variations in participants' age, sample sizes, in the stimuli and tests employed to measure olfactory abilities, in the inclusion and exclusion criteria for participants' selection, as well as in the evaluation and controlling of important variables (such as atypical language development or autism symptoms severity; Ashwin et al., 2014; Galle et al., 2013). Therefore, research about olfactory perception in ASD still raises questions about the nature and impact of possible alterations in the social and general everyday experience of this population.

Since ASD constitute a heterogeneous spectrum with different clinical symptomatology and comorbidities across individuals (Lecavalier, 2014), efforts should be made to disentangle the effect of these variables in olfactory perception. In fact, human olfaction is also shaped by marked interindividual differences, including variables such as genetics, sex, age, smoking habits and environmental characteristics (for a review, see Greenberg et al., 2013). Given that olfaction shares common neural substrates with the limbic system (e.g., the amygdala; Gottfried, 2006), olfactory cues are also able to greatly influence emotion and vice-versa (D. Chen & Dalton, 2005; de Groot et al., 2012). In fact, some studies have been showing the influence of emotion in olfactory perception, including the role of symptomatology often associated with emotional disorders, such as depressive (e.g., Pollatos, Albrecht, et al., 2007) and anxiety symptoms (e.g., La Buissonniere-Ariza et al., 2013; Takahashi et al., 2015). Indeed, the role of anxiety in olfactory perception, especially considering related personality traits, such as trait anxiety (TANX; Schienle et al., 2018; Takahashi et al., 2015) and neuroticism (Havlíček et al., 2012), has been receiving increasing attention.

Anxiety is a multidimensional emotional response triggered by the anticipation of perceived or real threat (American Psychiatric Association, 2013). It yields adaptive meaning, by allowing a fast and efficient allocation of resources when a potential threat is detected or anticipated; however, it is often followed by significant distress and functional impairment when persistent and/or intense (American Psychiatric Association, 2013; Calvo & Miguel-Tobal, 1998). Anxiety symptoms are often long lasting in comparison with other emotions, and persevere even without well-defined triggers (e.g., Lang et al., 2000). Furthermore, the profile of anxiety symptoms may be heterogeneous across individuals (e.g., Schwartz et al., 1978) and, importantly, may play a notable role in task performance, being able to benefit certain tasks and impair

performance in others, depending on their nature and cognitive demands (Eysenck et al. 2007; Eysenck and Calvo 1992).

Several studies have suggested that higher levels of anxiety and/or neuroticism, a personality trait with overlapping components with anxiety (S. Bishop & Forster, 2013), may enhance odor detection (D. Chen & Dalton, 2005; La Buissonniere-Ariza et al., 2013), olfactory sensitivity (Havlíček et al., 2012; Pause et al., 1998), odor discrimination (Havlíček et al., 2012), and odor identification (Larsson et al., 2000). However, many other studies observed impaired odor perception following higher levels of anxiety (Rovee et al., 1973; Takahashi et al., 2015), or, instead, failed to find a relationship between anxiety and/or neuroticism and certain dimensions of olfactory perception (e.g., Croy et al., 2011; Havlíček et al., 2012; Larsson et al., 2000). Once again, these contrasting results may be due to differences across studies, including the instruments used to measure both anxiety symptoms and odor perception, and greatly limit conclusions about the nature of the relationship between anxiety and olfactory perception.

Although anxiety has been shown to play a role in olfactory perception, this relationship has been sometimes neglected, even when analyzing populations where anxiety is highly incident, as it is the case of ASD (Hollocks et al., 2019). Anxiety is, in fact, a frequently co-occurring symptom of ASD (Kerns & Kendall, 2012), and has been associated with distinct patterns of reactivity to sensory stimuli in ASD (e.g., MacLennan et al., 2020). Parma and colleagues (2019) further observed that anxiety and an ASD diagnosis were associated with distinct patterns of resting autonomic activity. Altogether, this suggests that measuring the independent effect of anxiety, especially as a stable personality trait, may be important when evaluating the response to sensory stimulation in ASD. In fact, previous studies evaluating olfactory perception in ASD have not considered the role of anxiety in this process. Given the often-observed association between anxiety and olfactory perception, it is possible that this variable explains part of the observed variability in results.

Several researchers have suggested that the group of features that characterize ASD goes beyond the diagnosis, expressing as autism traits (AT) in the general population (e.g., Baron-Cohen et al., 2001; Constantino & Todd, 2003). These traits are heritable, continuously distributed (Baron-Cohen et al., 2001; Constantino & Todd,

2003; Ronald & Hoekstra, 2011) and their manifestation reflects a dimensional overview of ASD, constituting the Broader Autism Phenotype (BAP; Piven et al., 1997). The expression of AT in the general population has been associated with sensory processing differences very similar to those observed in ASD, suggesting shared mechanisms (e.g., Horder et al., 2014; Mayer, 2017; A. E. Robertson & Simmons, 2013). Nevertheless, knowledge about the relationship between olfactory perception and the expression of AT is limited. Robertson and colleagues (2012) evaluated the relationship between olfactory abilities and AT in a sample of healthy adults. Results suggested no relationship between these variables. However, these authors used a total score provided by the Autism-Spectrum Quotient (AQ; Baron-Cohen et al., 2001), an instrument broadly used to measure AT. Although it is frequently reported in studies measuring AT, this total score has been suggested to yield low interpretability (English et al., 2020) and does not allow the assessment of how the different characteristics of autism are distinctively related to the several domains of olfactory perception. Furthermore, some individual differences, relevant both in the context of autism and in the context of olfactory perception, such as TANX (Hollocks et al., 2019; Takahashi et al., 2015), were not evaluated in this study. In fact, as observed in the clinical phenotype, TANX was found to be associated with both sensory processing and AT in the TD population (Horder et al., 2014; Liss et al., 2008). Therefore, studying the association between AT, as well as TANX, and olfactory perception in the general population, may be useful to characterize the subclinical phenotype, as well as to improve the knowledge about the dimensional manifestation of autism (Ingersoll & Wainer, 2014; Ronald & Hoekstra, 2011).

The present study sought to evaluate olfactory perception in the subclinical extreme of the autism spectrum, by considering both the impact of AT and TANX. By analyzing the independent effects of these variables, we intend to disentangle possible confounds observed in the literature, as well as to provide new hints about olfactory perception in the autism spectrum. We hypothesize a significant association between a higher expression of AT and olfactory perception, since olfactory alterations have been linked with ASD, although in an inconsistent way (Larsson et al., 2017; Tonacci et al., 2015). We also expect to find a significant relationship between olfactory performance, especially olfactory detection thresholds, and TANX, given its association with

olfactory sensitivity in adults (Takahashi et al., 2015). Lastly, since ASD and a higher expression of AT seem to be more observed in males (Baron-Cohen et al., 2001; Loomes et al., 2017) and women have been reported to be better in olfactory tasks (Sorokowski et al., 2019), the association between sex and olfactory performance will also be analyzed.

Methods

Participants

A sample of 118 healthy university students and researchers (69 females; 58.5%) was recruited at the University of Aveiro, Portugal. The inclusion criteria encompassed the following: 1) Age between 18 and 35 years old; 2) Being Caucasian; 3) Having Portuguese nationality. As exclusion criteria, the following factors were considered: 1) Having any reported psychiatric, neurological, endocrine, respiratory or immunological disorder, or any other condition with significant impact in the olfactory functioning; 2) Taking medication with significant impact in the olfactory functioning, such as hormonal drugs (except oral contraceptives); 3) Having an ASD diagnosis or a 1st degree relative with this diagnosis, since our aim encompassed the study of the subclinical phenotype in the general population. Considering these criteria, one participant was excluded for breastfeeding in the last months, since pregnancy and hormonal variations influence odor perception (Doty & Cameron, 2009). Another participant was later excluded from the analysis for being an extreme case in the predictive models of the olfactory performance variables. Therefore, the final sample was constituted by 116 participants (68 females; 58.6%), with a mean age of 24.30 years old (SD=4.24).

All participants gave their written informed consent after a complete description of the study was provided. This study was performed considering the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the University of Aveiro, Portugal (Ref.: 04-CED/2019).

Materials

Evaluation of the olfactory performance. The *Sniffin' Sticks* Extended Test (Hummel et al., 1997) is a broadly used olfactory test that evaluates olfactory abilities in three dimensions: detection, discrimination and identification. This test considers the individual's self-report towards the presentation of odor stimuli imbued in felt tip pens, and was adapted and validated for the Portuguese population by Ribeiro and colleagues (2016), showing adequate measurement properties. Considering individuals with ages between 18 and 35 years old, the means obtained in the validation study for the three tests were 10.83 (SD=2.31) for odor threshold, 12.05 (SD=2.16) for odor discrimination and 14.10 (SD=1.21) for odor identification.

Odor threshold test. The odor threshold test intends to evaluate the minimal concentration that an odor must possess to be detected by a certain individual (Hummel et al., 1997). This test contains 16 triplets of pens, in which only one pen of each triplet has the target-odor in a certain concentration; the remaining two pens of the triplet contain only the solvent used to dilute the target-odor. The target-odor can be n-butanol or phenylethanol (PEA), since the thresholds provided by each one are comparable (Croy et al., 2009). In this study, PEA was used as target odor.

The triplets of pens were presented using a staircase procedure, starting by the triplet with the smallest concentration of the target odor. Each trial corresponded to the presentation of a triplet, which in turn involved the presentation of each one of the three pens by 3 seconds. Between the presentation of each pen, there was a 5-second gap and between triplets' presentation there was an interval of 30 seconds. Following a forced-choice paradigm, the participant's task was to identify the pen that contained the target odor. The participant's answers per trial were registered in a table. When the participant correctly identified the pen with the target odor twice in a row, a staircase reversion occurred and the following triplet with lower concentration was presented. Similarly, when the participant gave an incorrect answer, a new reversion took place and the following triplet with higher concentration was presented. The odor threshold score corresponded to the average of the last four reversals registered in the table and could vary between 1 and 16 (higher scores associated with lower thresholds and, therefore, with better odor detection performance).

Odor discrimination test. The odor discrimination test encompassed the presentation of 16 triplets of pens with common odors. Each trial corresponded to a triplet presentation, which in turn implied the presentation of each pen separately for 3 seconds. Between pens, there was a 5-second interval, and between triplets there was a 30-second interval. The participants task consisted in the identification of the pen that contained an odor different from the remaining two pens. The final score corresponded to the sum of the correct answers, varying between 0 and 16 (higher scores correspond to better odor discrimination ability).

Odor identification test. The odor identification test encompassed 16 pens with different everyday odors, such as orange and rose. Each pen was presented separately by 3 seconds, with an interval of at least 30 seconds between pens. Before the presentation of each pen, a card with four options was placed in front of the participant, who should choose the word that corresponded to the odor. The score corresponded to the sum of correct answers and could vary between 0 and 16 (higher scores relate to better odor identification ability).

Autism-Spectrum Quotient. The AQ (Baron-Cohen et al., 2001) is a self-report psychological instrument that measures characteristics associated with the autism spectrum. It encompasses 50 items divided into 5 subscales: social skills (e.g., “I find it hard to make new friends”), attention switching (e.g., “I prefer to do things the same way over and over again”), attention to detail (“I often notice small sounds when others do not”), communication (e.g., “Other people frequently tell me that what I've said is impolite, even though I think it is polite”) and imagination (e.g., “When I'm reading a story, I find it difficult to work out the characters' intentions”). The participant’s answers were given in a 4-point Likert-type scale: 0 “definitely disagree”, 1 “slightly disagree”, 2 “slightly agree” and 3 “definitely agree”. Some items were scored reversely (e.g., “I prefer to do things with others rather than on my own”), according to the guidelines provided by the authors (Baron-Cohen et al., 2001). Although the scoring is usually performed binarily (0 and 1), in the present study the scoring accounted for all the response range to allow more variability and a better ability to differentiate between

individuals (Austin, 2005; DeVellis, 2012). Therefore, the AQ total score could vary between 0 and 150 and each one of its subscales could vary between 0 and 30 (higher scores correspond to higher manifestation of AT).

In this study, a Portuguese adaptation of the instrument was used (Barros et al., unpublished work). To evaluate the reliability of the instrument's subscales, Cronbach Alfa and item-total correlation were computed. Only two of the subscales presented adequate levels of reliability with item-total correlation higher than .30 and Cronbach Alpha of at least .70: social skills (α=.72) and attention to detail (α=.73) (DeVellis, 2012; Nunnally, 1978). Therefore, only these subscales were considered in further analysis. The Cronbach Alpha of the remaining subscales corresponded to .52 (attention switching), .45 (communication), and .57 (imagination).

State-Trait Inventory for Cognitive and Somatic Anxiety – Trait Version. The State-Trait Inventory for Cognitive and Somatic Anxiety (STICSA; Ree et al., 2008) is a self-report instrument that evaluates anxiety in four dimensions: state-cognitive, state-somatic, trait-cognitive and trait-somatic. It encompasses a state form, which evaluates the symptoms of anxiety felt at the moment (anxiety as a transient phenomenon), and a trait form, that evaluates the symptoms of anxiety generally experienced (anxiety as a stable trait; Spielberger et al., 1983). Additionally, each form encompasses items measuring cognitive symptoms (such as intrusive and catastrophizing thoughts) and items measuring somatic symptoms (such as palpitations and muscular tension). Each item was measured in a 4-point scale, varying between 1 (not at all) and 4 (very much so; Ree et al., 2008). In this study, only the trait dimension was assessed (cognitive and somatic TANX), using a Portuguese adaptation of the instrument (Figueiredo et al., 2019). Regarding the present study, the reliability analysis of the subscales showed good internal consistency of the cognitive TANX subscale (α=.87) and somatic TANX subscale (α=.83).

Procedure

Initially, all participants filled a questionnaire with relevant sociodemographic items (such as sex and age) and questions regarding physical and psychological

symptoms and disorders, as well as STICSA-Trait and AQ. After verifying inclusion and exclusion criteria, eligible participants were contacted to schedule the olfactory evaluation. Periods of exams and other emotionally intense situations that could significantly impact participants emotional state, attention, and olfactory performance were avoided. Participants were instructed to not ingest alcoholic drinks, chew gums, or eat sweets/odorous food (as garlic and onions) in the day of the olfactory evaluation. Moreover, they should not use parfum or scented creams, and should not be sick or with their nose congested. Additionally, they should not consume coffee and tobacco in the hour before the evaluation. Finally, in the 15 minutes before the evaluation they should eat or drink nothing at all, except for water.

Immediately before the olfactory evaluation, the experimenter confirmed if the previously given instructions were followed. Then, the experimenter followed the guidelines of *Sniffin' Sticks* Extended Test, explaining and presenting the three tests in the default order: Threshold, Discrimination, and Identification. Between tests an interval of at least 3 minutes occurred, during which the experimenter explained the following test. In the end of the three tests, the researcher informed the participant about the results of the test and explained their meaning considering the standardized values for the Portuguese population. The olfactory evaluation session lasted approximately 45-60 minutes and was performed in a clean, quiet and well-ventilated room.

Statistical analysis

Data analysis was conducted using IBM SPSS, version 25, considering a significance value of at least .05 for inferential analysis (Howell, 2006). Independent samples t-tests were computed to test mean differences between two groups (e.g., between groups with respiratory problems vs. without respiratory problems; between groups of women taking oral contraceptives (OC) vs. women free of OC), and non-parametric Mann-Whitney U test was conducted to assess distribution differences in the two groups of women free of OC (in follicular vs. luteal menstrual phase), which had less than 30 cases. Additionally, one-way ANOVA was computed to test mean differences in olfactory abilities between the three groups of women (women free of OC, in the follicular phase; women free of OC, in the luteal phase; and women taking

OC; Field, 2009; Howell, 2006). Pearson correlation coefficient was performed to analyze the level of association between two continuous variables (e.g., between the different olfactory abilities; Howell, 2006).

To test the multivariate impact of a set of predictors in olfactory function dimensions, a hierarchical multiple linear regression analysis was performed. In step 1, sex was added as a control variable; in step 2 attention to detail and social skills dimensions of AQ, as well as cognitive and somatic TANX, were added as predictors. Predictors in the equation were assessed for multicollinearity considering the correlation between them (less than .70) and values of tolerance up to .20. Influent outliers were assessed regarding standardized residual and the specific impact in Cook and Mahalanobis distance. Goodness of fit and the magnitude of improvement of the model and individual steps were assessed through R^2 and F-ratio. Standardized beta coefficients were further interpreted (Howell, 2006; Tabachnick & Fidell, 2007).

Results

Preliminary analysis

Before proceeding with the main analyses considering the proposed objectives, we carried some preliminary analyses regarding our sample's characteristics. The literature reports that some respiratory conditions may influence olfactory performance, such as allergic rhinitis, asthma or sinusitis (e.g., Stuck & Hummel, 2015). Thirty-four participants reported having such problems, despite being seasonal or medically controlled (i.e., participants were asymptomatic). Four of these participants were also taking medication for these pathologies (such as antihistamines). Therefore, in order to analyze potential differences in the olfactory performance of our sample due to respiratory problems, we compared the means of the olfactory performance of these participants with the remaining, healthy participants. Results showed no significant differences between groups for the odor detection $t(114)=0.424$; $p=.672$, odor discrimination $t(114)=1.184$; $p=.239$, and odor identification ability $t(114)=0.274$; $p=.785$. Therefore, all these participants were considered in posterior analysis.

Additionally, 39 of the 68 female participants (57.35%) reported to be taking OC. Since it has been reported that OC can also impact the olfactory function (Derntl et al., 2013; Kollndorfer et al., 2016), we conducted an analysis to evaluate differences

between the performance of these participants and the remaining female participants. Results showed no differences between women taking OC and women free of OC for odor detection t(66)=0.012; p=.991, odor discrimination t(66)=0.555; p=.581, and odor identification t(66)=0.625; p=.534. Furthermore, the literature also suggests that women's olfactory perception, particularly olfactory sensitivity and odor identification, may vary depending on the phase of the menstrual cycle (e.g., Nováková et al., 2014). To evaluate menstrual cycle's influence in olfactory perception, we calculated the phase of the menstrual cycle for each woman free of OC (n=29). We followed the same standardization procedure employed by Parma and colleagues (2012). This procedure consists in keeping the luteal phase constant and adjusting the follicular phase of each woman to a cycle length of 28 days, considering women's menstrual cycle variability. When women were on the first 14 days of their cycle, the standardized day of the menstrual cycle was calculated with the following formula: [Day/(Cycle-14)]*14[1]. For instance, if a woman had a cycle of 30 days and was on the 8th day of her cycle, her standardized day of the cycle would be: [8/(30-14)]*14=7. Additionally, when women were on the last days of their cycle, the following formula was applied: 28 – Cycle + Day[1]. For further details about this procedure please refer to Parma and colleagues (2012). Only twenty-two of the initial 29 women free of OC were included in these analyses, after reporting their menstrual cycle, history of OC intake and date of last period; three women were excluded because they were not free from taking OC for at least six months before the evaluation, and other three participants were excluded due to an irregular menstrual cycle. The remaining women were then divided in two groups considering their phase of the menstrual cycle at the time of the evaluation: women in follicular phase (first 14 days of the menstrual cycle; n=11) and women in luteal phase (last 14 days of the menstrual cycle; n=11; Derntl et al., 2013; Parma et al., 2012). Three Mann-Whitney tests were performed to analyze differences between these two groups regarding odor perception. The results suggested no differences between groups regarding odor detection, U=41.000, z=1.284, p=.199; odor discrimination, U=50.000, z=0.730, p=.465; and odor identification, U=54.000, z=0.440, p=.660. These two groups were then compared with the group of women taking OC, which reported their pill type, brand, intake duration and length of menstrual cycle. Of the initial 39 women taking

[1] [Cycle] corresponds to the number of days of the menstrual cycle, and [Day] to the current day of the cycle.

OC, seven were excluded due to the following reasons: one reported OC intake for less than six months, three did not report for how they were taking OC, one did not report her menstrual cycle length and two were not taking low-dose (ethinyl estradiol ≤35 μg) and monophasic (all pills delivering the same amount of hormones) OC. This group (n=32) was then constituted only by women taking combined, low-dose and monophasic OC, based on estrogen and progesterone derivates. A one-way ANOVA was conducted to analyze differences regarding olfactory perception between (1) the group of women not taking OC, in follicular phase (n=11); (2) the group not taking OC, in luteal phase (n=11); and (3) the group taking OC (n=32). Results suggested no differences between groups regarding odor detection, $F(2, 51)= 0.914$; $p=.407$, odor discrimination, $F(2, 51)=1.135$; $p=.329$, and odor identification, $F(2, 51)=1.177$; $p=.316$. These results suggested that the menstrual cycle did not have a significant impact in women's olfactory perception in our sample, therefore a homogeneous, single group constituted by all women was considered in additional analysis.

The impact of AT and TANX in olfactory perception

Means and standard deviations for predictors and the three outcome variables are presented in Table 1. Since sex has been differently associated with olfactory ability, AT and TANX, the results were divided considering this attribute. T-tests were computed in order to acknowledge if there were sex differences in predictors and outcome variables. The results suggest that females had significant higher means only regarding olfactory discrimination, $t(114)=2.342$, $p=.021$. Gender differences in the predictor variables were also tested and non-significant results were observed. The relationship between olfactory functions was further explored considering the correlation between the three dimensions. Results showed a small effect association (between .10 till .30; J. Cohen et al., 2002) ranging between a non-significative .129 (threshold and discrimination) and a significative .222 (identification and discrimination), pointing to the relative independence of these dimensions.

Table 1. Means (M), standard deviations (SD), independent-sample t test and Cronbach alphas (α) for olfactory performance dimensions, autism traits and anxiety dimensions.

Variables	Items	α	Male (n=48)		Female (n=68)		t (114)	p
			M	SD	M	SD		
Olfactory performance								
Odor Threshold			8.35	2.96	8.95	2.78	1.111	0.269
Odor Discrimination			12.85	1.82	13.57	1.48	2.342	0.021*
Odor Identification			13.19	1.43	13.57	1.42	1.436	0.154
Predictors								
AQ SS	10	0.72	11.52	4.06	11.16	4.50	0.441	0.660
AQ AD	10	0.73	13.63	4.44	13.37	5.53	0.267	0.790
STICSA Cog Anx	9	0.87	18.69	6.22	18.75	5.42	0.035	0.972
STICSA Som Anx	11	0.83	17.56	4.94	17.88	5.17	0.334	0.739

*p<.05

Note: AQ SS – Social Skills; AQ AD – Attention to detail; Cog Anx – Cognitive Anxiety; Som Anx – Somatic Anxiety

Three hierarchical multiple regression models were conducted to test the impact of AT (social skills and attention to detail) and TANX (cognitive and somatic) on the three dimensions of olfactory function (threshold, discrimination and identification), controlling for sex in the first step (Table 2). The three models were computed assuming the same sample, predictors and steps, in order to be interpreted as equivalent. Sex had a significant impact only in odor discrimination, accounting for 4.6% of total variance. Also, autism and anxiety traits had significant impact only in discrimination, accounting for 8.9% of variance. The final model was significant and accounted for 13.5% of variance, $F(5, 110)=3.426$, $p=.006$. The regression coefficients showed that attention to detail, $\beta=.257$, $t(115)=2.812$, $p=.006$, somatic TANX, $\beta=-.245$, $t(115)=2.054$, $p=.042$, and sex, $\beta=.230$, $t(115)=2.585$, $p=.011$, were significant predictors. Therefore, on explaining odor discrimination, a significant positive impact of attention to detail was observed, as well as a negative impact of somatic anxiety and a higher performance in females.

Table 2. Multiple regression models of the three dimensions of olfactory function (n=116).

Predictors	Odor threshold				Odor discrimination				Odor identification			
	R2	F	β	p	R2	F	β	p	R2	F	β	p
Step 1	0.011	1.234		0.269	0.046	5.485		0.021*	0.018	2.061		0.154
Sex			0.103	0.269			0.214	0.021*			0.133	0.154
Step 2	0.021	0.472		0.796	0.135	3.426		0.006*	0.054	1.252		0.290
Sex			0.104	0.273			0.230	0.011*			0.145	0.123
AQ SS			0.013	0.895			0.042	0.661			0.156	0.118
AQ AD			-0.074	0.446			0.257	0.006*			0.058	0.543
Cog Anx			0.063	0.629			0.098	0.425			0.089	0.489
Som Anx			-0.067	0.598			-0.245	0.042*			-0.119	0.341

*p<.05
Note: AQ SS – Social Skills; AQ AD – Attention to detail; Cog Anx – Cognitive Anxiety; Som Anx – Somatic Anxiety

Discussion

The present study investigated the relationship between the expression of AT and the olfactory abilities of the general population. Concomitantly, we investigated if sex and TANX account for the differences observed in odor detection, discrimination, and identification. The multiple linear regressions models performed for each olfactory domain suggested that variables such as sex, AT and TANX have indeed a significant impact in the olfactory function, particularly in the odor discrimination ability. This model further showed that, in the overall explanation of the odor discrimination, women outperformed men, and subjects with higher scores in the attention to detail subscale of AQ and lower levels of somatic TANX have better odor discrimination abilities.

A significant relationship between the attention to detail subscale of AQ and odor discrimination was observed. To the best of our knowledge, no studies to date have reported a significant relationship between AT and olfactory performance. For instance, Robertson (2012) has indeed investigated the association between AT, as reflected by the total score of AQ, and olfactory performance, as measured by the *Sniffin' Sticks* Extended Test, but observed no relationship between these variables. Similarly, Stafford and colleagues (2017) explored the relationship between olfactory sensitivity and AT in a sample of TD adults and also found no association between variables. Yet, it is important to note that these authors used a total score, which is commonly used in studies that evaluate AT. English and colleagues (2020), who conducted a series of

confirmatory analysis of AQ factor structure using large samples of healthy individuals, do not recommend the use of a total score, since the underlying factors often do not correlate positively, which limits the interpretability of this score. Considering this, AT were analyzed separately by subscales in the present study. In fact, each one of the subscales of AQ encompass distinct characteristics of the spectrum that are associated with different cognitive and socioemotional processes, abnormal in ASD, including attentional processes, communication and social skills (American Psychiatric Association, 2013; Baron-Cohen et al., 2001). Therefore, these processes may be differently associated with distinct olfactory abilities, since the latter also encompass different cognitive and neural correlates (e.g., Frasnelli et al., 2010; Hedner et al., 2010). The observed relationship between the attention to detail subscale and the odor discrimination ability may reflect the atypical attentional and perceptual processing often observed in ASD (e.g., Ames & Fletcher-Watson, 2010; Mottron et al., 2006). Attentional processes seem to influence sensory processing, through the selection and orienting to relevant sensory stimuli, the scanning and discrimination of their features and the switching between sensory information (Baranek et al., 2014; Pessoa et al., 2003). Hence, it is possible that atypical attentional processes are related to abnormal sensory processing in ASD. For instance, some studies observed an association between over-focused attention and sensory hypersensitivity (e.g., Baron-Cohen et al., 2009; Liss et al., 2006). Additionally, abnormalities in attentional disengagement (Sabatos-DeVito et al., 2016) and symptoms of Attention Deficit/Hyperactivity Disorder (Sanz-Cervera et al., 2015) seem to be also associated with distinct sensory response patterns. Importantly, heightened attention to detail and an atypical bias for local information is often reported in ASD (Happé et al., 2006; Mottron et al., 2006). This cognitive style may comprise an advantage in certain tasks that demand a deeply and efficient scanning and discrimination of similar sensory features, such as visual search tasks (e.g., O'Riordan et al., 2001), and odor discrimination by quality, since this task also involves the processing of the features of a stimulus in order to differentiate it from other available sensory information (Hedner et al., 2010). Nevertheless, literature about odor discrimination in ASD is yet inconclusive, preventing a strong support of this hypothesis in the clinical extreme of the spectrum.

In fact, the scarce available evidence suggests normative odor discrimination in ASD (Galle et al., 2013; Muratori et al., 2017). However, there are important differences between the present research and these studies. For instance, Galle and colleagues (2013) recruited a small sample of adults (5 ASD and 5 TD individuals) and used a different olfactory test encompassing "same/different" judgments of pairs of stimuli, which can have posed less cognitive demands in comparison with our task (where we used triplets of stimuli). On the other hand, the study of Muratori and colleagues (2017) analyzed a sample of male children and adolescents. Age is known to be an important variable to consider when analyzing olfactory abilities in ASD (Larsson et al., 2017; May et al., 2011; Tonacci et al., 2015), although, to the best of our knowledge, there is no literature addressing the role of age in olfactory discrimination. Therefore, caution is needed when comparing studies encompassing samples of different age groups. These differences in samples and methodology add, therefore, important confounds that may influence test results and limit comparisons across clinical studies and, consequently, across both the extremes of the spectrum (Doty, 2017).

For people with higher expression of AT, especially regarding attention to detail, having an increased ability to determine differences between odors may result in advantages in certain tasks (Mottron et al., 2006). For instance, it may benefit the distinction between a broad range of odors, associated with certain benefits or hazards (e.g., distinguish between food which is suitable for consumption vs. spoiled food), which may be crucial for quick and adaptive responses to posed challenges. On the other hand, having a cognitive style characterized by preferential local processing and enhanced attention to detail may be disadvantageous in situations that require a more global processing, such as in social situations (Happé & Frith, 2006; Hill et al., 2014). In fact, odors also play an important role in social domain, where information about our conspecifics, resulting from multiple sensory sources, need to be effectively integrated to provide an adequate response (Pause, 2012; R. J. Stevenson, 2010). A default detail-focused style may be, therefore, associated with difficulties in prioritizing and integrating relevant information, which can, in turn, be related to the often reported "sensory overload" in this population (R. S. P. Jones et al., 2003). Furthermore, atypical sensory processing, including increased sensory sensitivity, has been also associated

with negative outcomes in ASD, such as anxiety symptoms (for a review see South & Rodgers, 2017). Nevertheless, whether increased odor discrimination benefits or impairs functioning in the autism spectrum, including safety, eating and social behaviors, needs to be further investigated in future research.

A significant relationship between perceived somatic anxiety and odor discrimination was also found in our study. Particularly, higher somatic anxiety was associated with lower discrimination ability. This is consistent with the literature that links anxiety, as well as anxiety disorders, with odor perception (e.g., Burón & Bulbena, 2013; Havlíček et al., 2012). However, the two studies addressing the relationship between anxiety and olfactory discrimination in the general population suggest that higher levels of anxiety are associated with better odor discrimination (Havlíček et al., 2012; Krusemark & Li, 2012). Although our results seem to disagree with the literature in a first instance, some considerations should be noted. First of all, the study of Havliček and colleagues (2012) used a different instrument (NEO-Five Factor Inventory; Costa & McCrae, 1992), which does not measure TANX, but rather several dimensions of personality, including neuroticism. Also, in the study of Krusemark and Li (2012), state anxiety was evaluated instead of TANX, and it was associated with improved odor discrimination of negative odors specifically. Therefore, direct comparisons with these studies are highly limited due to important differences in measured constructs and methodology.

Anxiety may play a significant role in olfactory perception, due to a close relationship between olfaction, emotion and the limbic system (Gottfried, 2006). Through a tight relationship with emotional processing, attentional processes may direct and facilitate the selection of relevant sensory information that conveys a better adapting to the environment demands (Carretié, 2014; Vuilleumier, 2005). Corroborating this assumption, people with higher anxiety often presents heightened sensitivity to potential environmental threats across sensory domains, being better at the detection of negative stimuli and more prone to be distracted by them (e.g., Fox et al., 2001; Soares et al., 2015). In line with this, several studies observed that higher levels of anxiety and/or neuroticism are associated with improved odor detection (D. Chen & Dalton, 2005; La Buissonniere-Ariza et al., 2013), higher odor sensitivity (Havlíček et al., 2012; Pause et al., 1998), better odor discrimination (Havlíček et al., 2012),

particularly of negative odors (Krusemark & Li, 2012), and better odor identification (Larsson et al., 2000). Nevertheless, some other studies have found contrasting results. For instance, some studies found no relationship between levels of anxiety/neuroticism and both odor thresholds (e.g., Croy et al., 2011; Havlíček et al., 2012; Larsson et al., 2000) and odor identification (Havlíček et al., 2012). Furthermore and similarly to our results, some other studies found a negative relationship between anxiety and odor perception (Rovee et al., 1973; Takahashi et al., 2015). When analyzing studies encompassing patients with anxiety disorders, evidence mainly suggests impairments in odor identification abilities (for a review see Burón & Bulbena, 2013); however, there is also evidence in favor of increased odor sensitivity (e.g., Burón et al., 2015), as well as evidence of impairments affecting all the three olfactory abilities, including odor discrimination (e.g., Clepce et al., 2012).

In fact, the presence of high levels of anxiety may either enhance or impair performance, depending, for instance, on the nature of the task in course (Eysenck et al., 2007; Eysenck & Calvo, 1992). Thereby, more cognitive loaded tasks, that require substantial working memory, may be more disrupted by higher levels of anxiety (Eysenck et al., 2007; Eysenck & Calvo, 1992). Odor discrimination involves the ability to determine if an odor is different from another (Wilson & Stevenson, 2003), requiring the representation of the target odor features, which will be subsequently compared with the remaining odors in order to allow the selection of the target (Hedner et al., 2010). Therefore, semantic skills, learning, executive functioning, memory and decision making may be necessary abilities to successfully deal with the task demands (Hedner et al., 2010; Hummel et al., 1997; La Buissonniere-Ariza et al., 2013; Wilson & Stevenson, 2003). It may be the case that the discrimination task may have required substantial attentional and working memory components that were impaired by perceived somatic anxiety specifically. Additionally, it has been hypothesized that over-arousal may be associated with sensory abnormalities in ASD (e.g., Green & Ben-Sasson, 2010), which may include olfactory perception. Specifically in the study of Clepce and colleagues (2012), where impaired odor discrimination was found in a sample of patients with anxiety disorders, most of the patients had a panic disorder diagnosis. This particular anxiety disorder is characterized by severe somatic symptoms, great arousal and hypervigilance (Geiger et al., 2014; Hoehn-Saric et al., 1991).

Therefore, it may be that high levels of anxiety, and somatic anxiety specifically, have a disrupting impact in odor discrimination, a substantially cognitive loaded task. Even so, these assumptions are very preliminary. For instance, it was reported that individuals with diminished olfactory functions have significantly less reported somatic symptoms than individuals with normal olfactory functioning (Schienle et al., 2018). Nevertheless, relationships with specific olfactory tests were not inspected in this study, and it is possible that somatic anxiety has a major impact in specific abilities, such as odor discrimination, by means of impaired attentional processes. Previous literature with both healthy individuals and patients diagnosed with anxiety disorders does not allow to draw further conclusions, since in most cases they are not comparable in terms of sample (e.g., different anxiety disorders), specific olfactory functions addressed (e.g., few addressed olfactory discrimination) and olfactory tests used.

Our findings are also consistent with previous studies reporting that women have general better olfactory abilities in comparison with men (e.g., Hummel et al., 2007; Oleszkiewicz et al., 2019; Sorokowski et al., 2019), although this is not always a clear picture (Majid et al., 2017). This advantage seems to emerge more consistently when higher-order processing olfactory abilities, such as identification abilities, are addressed (e.g., Doty et al., 1985; Larsson et al., 2004). Congruently with our results, a recent meta-analysis indeed suggests that women tend to have better odor discrimination performance in comparison with men (Sorokowski et al., 2019). These sex differences may be driven by a panoply of factors, including hormonal influences (Doty & Cameron, 2009), expertise and odor awareness (Havlicek et al., 2008; Smeets et al., 2008), as well as social and cognitive factors (Sorokowski et al., 2019). For instance, women seem to rely and value more odor cues and have general higher odor awareness in comparison with men (Demattè et al., 2011; Havlicek et al., 2008). This may reflect an advantage in odor discrimination, since they probably pay more attention to distinct odors and their features (Smeets et al., 2008), facilitating also important processes for this task, such as odor memory and learning (Arshamian et al., 2011).

Nevertheless, it is somewhat interesting that we only found a significant impact of sex, AT and anxiety in odor discrimination and not on odor identification ability. For instance, despite some literature observing the absence of odor identification impairments in ASD (e.g., Brewer et al., 2008; Dudova et al., 2011), most of the studies

point out impairments in this domain, in both children and adolescents (e.g., Muratori et al., 2017), as well as in adults with ASD (Galle et al., 2013; Wicker et al., 2016). Some studies further suggested that olfactory functions, including odor identification, may be associated with a distinct developmental trajectory in ASD, since olfactory deficits are more evident in samples constituted by older individuals, contrary to what is observed in TD (Brewer et al., 2008; May et al., 2011). Also, the meta-analysis of Larsson and colleagues (2017) highlighted the role of IQ as an additional important moderator in olfactory identification, with higher IQ being associated with lower identification ability. It is, indeed, intriguing, because our sample was constituted by young adults, all with higher education levels, and we still found no differences in this domain.

In this regard, it is important to consider the following aspects: First, our sample was constituted by healthy adults, while the previously mentioned literature was referring to individuals with a diagnosis of ASD. It is possible that other features of the disorder, which surpass the scope of the evaluated dimensions in this study (social skills and attention to detail) are more directly related to impairments in odor identification. For instance, it has been suggested that the performance in tasks that rely on verbal labels, as it is the case of odor identification (Hedner et al., 2010) may be impaired by atypical language development (Galle et al., 2013). Our sample, in fact, did not present language issues; however, language impairment is a common feature of ASD (American Psychiatric Association, 2013), and can be, therefore, linked to altered odor identification in this population (Galle et al., 2013). Second, our results are probably associated with the nature of the identification task used in the present study, which includes very familiar odors and may not be very cognitive loaded, since it is based in a cued, forced-choice paradigm (Havlíček et al., 2012; Hedner et al., 2010). Since verbal cues indeed influence olfactory identification (Yeshurun & Sobel, 2010), it is highly likely that a ceiling effect have guided our results, as in other studies with similar results (Havlíček et al., 2012; Hedner et al., 2010).

In the current study, we also failed to find a significant regression model for olfactory detection threshold, despite the existence of some studies suggesting a relationship between olfactory sensitivity and sex (Sorokowski et al., 2019), as well as its relationship with anxiety (Havlíček et al., 2012; La Buissonniere-Ariza et al., 2013; Takahashi et al., 2015) and even autism (Ashwin et al., 2014; Dudova et al., 2011).

Nevertheless, the literature regarding these relationships is not very clear. For instance, although many studies support that women have a better olfactory performance in comparison with men, the reported effect sizes seem to be very small, and other studies observed no sex effect at all (e.g., Sorokowski et al., 2019). In the case of ASD, results are also mixed, with studies suggesting no differences between ASD and TD individuals (Galle et al., 2013; Tavassoli & Baron-Cohen, 2012), as well as higher (Ashwin et al., 2014) or decreased sensitivity in ASD (Dudova et al., 2011; Muratori et al., 2017). It is important to note that the studies observing decreased odor sensitivity encompass samples of children and adolescents, whereas the studies observing no differences between groups encompass adult samples (similarly to our study). As previously said, age may be a critical factor for olfactory performance in ASD (Dudova et al., 2011; Tonacci et al., 2015), thereby caution is needed when directly comparing different age groups. The only study denoting higher odor sensitivity in ASD encompassed a sample constituted by male adults, but used a different olfactory stimulus (isopropyl alcohol), as well as a different detection task, consisting in varying the distance between the olfactory stimulus and the individual. Therefore, these differences in detection methods, stimuli and sample's characteristics may explain the discrepant results observed.

Regarding anxiety, the literature is also divided, with studies suggesting either a positive (D. Chen & Dalton, 2005; Havlíček et al., 2012; La Buissonniere-Ariza et al., 2013; Pause et al., 1998), negative (Rovee et al., 1973; Takahashi et al., 2015) or no relationship (e.g., Croy et al., 2011; Larsson et al., 2000) between anxiety/neuroticism and olfactory sensitivity. These discrepancies across studies may be, once more, due to variations in the methodology, since studies often recruit individuals with different characteristics and use different tests and stimuli to evaluate olfactory processing. Finally, it is important to be aware that we obtained lower means for olfactory detection threshold in comparison with the means reported by the Portuguese validation of *Sniffin' Sticks* Extended Test for the same age group, despite using the same target odor (PEA), the same procedure and a sample of the same population. Our mean (M=8.70; SD=2.86) and median (Mdn=9.5) correspond to the percentile 25 of the results found in this study, which may be interpreted as a diminished detection ability of our sample. Nevertheless, we believe it is not the case, since the mean reported by Ribeiro and colleagues (2016) is higher than the means often reported by most validation studies

(Hummel et al., 2007; Kobal et al., 2000; Oleszkiewicz et al., 2019) and our results are closer to other validations and studies analyzing olfactory performance with *Sniffin' Sticks* (e.g., Kobal et al., 2000; C. Neumann et al., 2012; A. E. Robertson, 2012).

Although we observed a statistically significant model for discrimination, the percentage of explained variance is still very low (13.5%). Nevertheless, it is still comparable to the explained variance of other models presented for olfactory performance, varying, for instance, between 9.1% and 23.4% (Hedner et al., 2010; Mahmut & Stevenson, 2012). This may suggest that olfactory performance is indeed a complex variable that involves a multiplicity of factors that have not been completely uncovered. Furthermore, our results should be interpreted with caution, considering that we used a Portuguese adaptation of the AQ, which is not yet supported in further validation studies. Moreover, our reliability analyses suggested that dimensions regarding communication, attention switching, and imagination did not yield consistent results, which limited our analyses and conclusions to specific characteristics of the autism spectrum, namely the attention to detail and social skills dimensions. The fact that few participants scored in the extremes of the AQ, which is expected in a sample of the general population, may have also limited our conclusions about the relationship between the expression of AT and olfactory function. Therefore, future research should extend the knowledge about the relationship between AT, TANX and olfactory perception, by recruiting more diversified samples, with a broader manifestation of AT, especially with more individuals with higher levels of AT. Moreover, future research should also investigate the impact of other relevant variables in the context of autism and olfactory variability, such as odor awareness and cognitive functioning. For instance, higher odor awareness seems to be associated with better odor discrimination and identification (Arshamian et al., 2011). On the other hand, since distinct olfactory variables rely differently in cognitive abilities, such as executive functioning and semantic memory (Hedner et al., 2010), measuring these abilities could allow a better differentiation between individuals and, thus, a better explanation of olfactory variability.

Even though we have asked participants to avoid emotionally intense situations, which could impact olfactory perception, we did not measure the emotional state of participants at the moment of the olfactory evaluation. Given the emotional distress and

emotion regulation difficulties often experienced by individuals with ASD (e.g., Mazefsky, 2015), and since some studies suggest that emotional state may be associated with differences in olfactory perception (e.g., D. Chen & Dalton, 2005; Pollatos, Kopietz, et al., 2007), future studies should also evaluate these variables to further understand their role in olfactory outcomes. Finally, the emotional appraisal of odors may also play a role in olfactory abilities, especially in the context of autism. For instance, Dudova and colleagues (2011) found that children with ASD were significantly better than their TD peers at identifying the odor of orange, and were also significantly worse at identifying the odor of cloves. Interestingly, in the study of Hrdlicka and colleagues (2011), the odors of orange and cloves were rated as the most pleasant and unpleasant odors for children with ASD, respectively. In fact, some studies reported altered perceived valence (Hrdlicka et al., 2011; Legisa et al., 2013; Wicker et al., 2016), as well as intensity (Wicker et al., 2016) of common odors in ASD. However, conclusions are limited due to few studies and differences in samples' characteristics and evaluated odors. Furthermore, to the best of our knowledge, there are no studies exploring the relationship between odor ratings (e.g., regarding odor valence and intensity) and AT. Therefore, differences in subjective odor ratings, as well as their relationship with olfactory abilities, and the influence of other variables in this process, such as odor familiarity (e.g., Luisier et al., 2015), remain far from being understood in the autism spectrum. The analysis of these variables could greatly improve the current knowledge about olfactory perception in this population, as well as about the relationship between potential alterations and the sensory-related hardships often experienced in the clinical extreme of the spectrum, including, for instance, feeding problems (Luisier et al., 2015).

In the present study, we observed a statistically significant model for the explanation of the variability in odor discrimination, with AT, TANX and sex as significant predictors. This research provides an important contribution for the current knowledge about olfactory functioning and its relationship with autism, considered in a dimensional perspective, as well as with other relevant individual differences, such as cognitive and somatic TANX. Since atypical sensory processing is a well-established feature of ASD, and considering that these sensory symptoms are present since early and extend over the course of development, may involve multiple sensory systems and

may be associated with differentiated response patterns across and within individuals, studying these processes is crucial to understand their influence in everyday experience and in the development of the disorder (Woodard et al., 2012). Furthermore, understanding and characterizing these sensory symptoms and their relationship with other variables may be helpful for an early and accurate diagnosis and, importantly, for an adequate intervention. Olfaction may be especially relevant in ASD in this context, considering its close relationship with social and emotional structures of the brain and, therefore, its potential to act as a privileged and effortless channel of socioemotional information in this population (Barros & Soares, 2020; Parma et al., 2013). By investigating sensory profiles considering a broader spectrum that surpasses the boundaries of a diagnosis, it is also possible to reduce a set of challenges posed by the clinical extreme of a complex diagnosis such as ASD, including multiple comorbidities and behavioral and compliance problems. But, importantly, it may also allow the understanding of the non-clinical phenotype, of the spectrum's functioning in general and of the relationship between the clinical and non-clinical manifestation. Research in the field is still scarce and faces multiple challenges, especially considering olfactory functioning both in ASD and in the BAP, whereby further studies are needed to improve both knowledge and practice with this population.

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Study 4

Giving meaning to the social world in Autism Spectrum Disorders: Olfaction as a missing piece of the puzzle?

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Reference:

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Abstract

Altered social cognition is a core feature of Autism Spectrum Disorders (ASD). These impairments have been explained as the consequence of compromised social motivational mechanisms that limit social interest and activate a cascade of social deficits. Following this rational, we argue that approaches capable of surpassing ASD usual restraints (e.g., deficits in verbal abilities), and able to assign social meaning, could be more effective at responding to these difficulties. In this framework, we propose that olfaction, as well as cross-modal integration strategies involving both visual and olfactory domains, may have such potential. In fact, most of socioemotional processing deficits in ASD have been shown in a unimodal perspective, mainly with visual stimuli. However, the social environment involves other modalities and is typically multisensorial. Given the potential of olfaction as a gateway for socioemotional information in ASD, we argue in favor of studying olfactory perception, as well as visuo-olfactory integration, given the potential of these approaches to drive effective interventions and give the access to a meaningful social world in ASD.

Keywords

Autism; social cognition; emotion; face processing; olfaction.

Introduction

Autism Spectrum Disorders (ASD) are characterized by early core impairments in the social domain, accompanied by restrictive and repetitive behaviors and interests (American Psychiatric Association, 2013). Dysregulations in social cognition are paramount in ASD and play a vital role in patients' inadequate social functioning. Social cognition refers to the processes through which people perceive and give meaning to the social world by understanding and managing self and other's emotions, beliefs, intentions and behaviors, which is crucial to respond successfully to the complexity of social situations (Beer & Ochsner, 2006; C. D. Frith, 2008; C. D. Frith & Frith, 2008). These abilities have been shown to be compromised in ASD, as reflected in impaired recognition of emotional expressions (Harms et al., 2010; Uljarevic & Hamilton, 2013), abnormal emotion regulation (Mazefsky et al., 2013), difficulties in sharing feelings and experiences (e.g., Kasari et al., 1990) and difficulties in working out people's intentions (Baron-Cohen, 2000). Concomitantly, individuals with ASD often present difficulties in imitation behavior (J. H. G. Williams et al., 2004), as well as abnormal non-verbal communication, including poor eye-contact (Dawson et al., 2004; Senju & Johnson, 2009), and overall difficulties in engaging in relationships with others (Orsmond et al., 2004). These impairments involve significant psychosocial, occupational and economic burdens (Cappadocia & Weiss, 2011; Leigh & Du, 2015), as they seem to be present across the spectrum and do not improve spontaneously with time (A. J. Smith et al., 2010; White et al., 2007).

Since vision may be the most significant way to effectively interact with the world (Pazzaglia, 2015), especially in the context of complex social interaction, most research focused on social cognition in ASD have only relied on this sensory modality. In the present article we argue, based on a large bulk of studies published in the last decade (Semin & Groot, 2013; R. J. Stevenson, 2010), that olfaction may be an important mean of conveying social information in ASD, one that may surpass the barriers of language and intellectual impairments. As such, we propose that olfaction should be considered in the context of social cognition in ASD, either by using a cross-modal visuo-olfactory approach in which olfactory cues may act as contextual cues that give meaning to visual social information, or as an exclusive sensory cue that provides

social information, which is of high relevance to ASD individuals adequately adjust to the social world.

Social cognition in ASD: From vision to olfaction

Social cognition impairments in the visual domain

Research in social cognition in ASD seems to be consistent in showing that ASD individuals have an apparent lack of spontaneous interest in people and social interaction (Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Senju, 2013), with decreased visual attention to social stimuli across development, when compared to typical developing (TD) population (Chita-Tegmark, 2016; Guillon et al., 2014). This atypical attentional performance seem to impact face processing (Dawson et al., 2005), as well as the development of a larger spectrum of social skills, such as joint attention, i.e., the ability to share and coordinate orientation of attention to an object with another person (Dawson et al., 2004; Mundy & Newell, 2007), and theory of mind, which reflects the ability to attribute and represent mental states of other people (Baron-Cohen, 1991).

Most of the research in social cognition in ASD use human faces as social stimuli, as they convey important cues about the social environment and facilitate communication with others (Haxby et al., 2000). Being able to successfully extract meaningful information from others' faces, such as the individual's identity, intentions or emotional state, can help to predict their behavior and adjust self-behavior accordingly, hence providing valuable social advantages (Leopold & Rhodes, 2010). Although social information carried by faces seems to be crucial and salient enough to capture TD individual's attention (Palermo & Rhodes, 2007; Theeuwes & Van der Stigchel, 2006), that does not seem to be the case in ASD. Studies examining social attention in ASD observed a general lack of spontaneously directing eye-gaze for faces in children and adolescents (Riby et al., 2013; Riby & Hancock, 2009, 2008), as well as increased eye-gaze to the mouth and decreased eye-gaze to the eye region of the face in adults (D. Neumann et al., 2006; Pelphrey et al., 2002), reduced orienting to eye-gaze cues in adolescents and adults (Ristic et al., 2005) and overall reduced eye-contact (Senju & Johnson, 2009). Interestingly, children with ASD seem to be faster than TD

(e.g., Kikuchi et al., 2011) and developmentally delayed peers (e.g., Chawarska et al., 2010) at disengaging their attention from a social stimulus. Despite being scarce, studies with adults report a similar pattern (e.g., Vlamings et al., 2005), suggesting that this difficulty may be stable across development. In visual exploration tasks, children with ASD also seem to look more and/or longer to objects of high interest rather than to other objects (Sasson et al., 2011) or social stimuli (Sasson et al., 2008, 2011). Furthermore, the lack of spontaneous attention to faces seems to be even more evident when there are non-social stimuli competing for attentional resources, independently of whether the faces are relevant for the task or not (Klin et al., 2002; Remington et al., 2012; Riby et al., 2012). Together, this literature suggests a weaker engagement by social features (Kikuchi et al., 2011; Sacrey et al., 2014). Yet, there is also literature suggesting normative social orienting and disengagement in children (Fischer et al., 2014, 2016; Pruett et al., 2011) and adults with ASD (Kuhn et al., 2010; Skripkauskait, 2018). These discordant results may be driven essentially by sample and methodological differences across studies (Sacrey et al., 2014).

Importantly, it is not the case that people with ASD are unable to attend to social cues. Instead, they seem not to attend to such cues in a spontaneous fashion, as observed in TD people (Senju, 2013). This is in line with the assumption that diminished social motivation, present since very early in development, may be subserving socioemotional deficits in ASD (Chevallier et al., 2012; Dawson et al., 2005). According to this approach, since social information, such as human faces, do not seem to carry the same rewarding nature for people with ASD as it does for TD people, they are hence less attended and, thus, the experience with these stimuli remains limited. This, in turn, impacts both the development of specific social skills and the normative cortical specialization for face processing (Dawson et al., 2005).

Indeed, a significant number of studies in the area support altered face processing, especially concerning face recognition in children and adolescents with ASD (Boucher & Lewis, 1992; de Gelder et al., 1991; McPartland et al., 2011; Wolf et al., 2008), as well as facial emotional processing in children (Farran et al., 2011; Gross, 2008) and adults with ASD (Ashwin et al., 2006; Baron-Cohen et al., 1997; Pelphrey et al., 2002). Yet, studies have been showing that ASD individuals process face identity in a similar fashion as TD people but have an overall weaker performance (Weigelt et al.,

2012), especially in face memory tasks (Boucher & Lewis, 1992; McPartland et al., 2011) and face perception tasks requiring the discrimination of the eye region (Wolf et al., 2008).

In addition, the ability to rapidly and correctly detect and recognize emotions in other's faces, which is crucial to coordinate social interaction by helping to predict other's intentions and behavior and to adjust oneself accordingly (Keltner & Kring, 1998; Niedenthal & Brauer, 2012), is also dampened. Difficulties in this domain are thought to be linked to other crucial socioemotional processes, such as empathy and mimicry behavior (Atkinson, 2007), abilities also known to be impaired in ASD (T. F. Clark et al., 2008; McIntosh et al., 2006; Schulte-Rüther et al., 2011; J. H. G. Williams et al., 2004). Regarding emotional processing in particular, some studies suggest that dysregulations may be confined to certain social and complex emotions and mental states, with intact recognition of basic emotions both in children (e.g., Baron-Cohen et al., 1993) and adults with ASD (e.g., Adolphs et al., 2001; Baron-Cohen et al., 1997). However, other studies suggest impairments in processing basic emotions, particularly negative emotions such as fear, both in children (e.g., Farran et al., 2011) and adults with ASD (e.g., Ashwin et al., 2006). In comparison with the facial expression of fear, the emotional processing of happy faces seems to be only slightly impaired in ASD (Uljarevic & Hamilton, 2013). Difficulties with the processing of fear can be hypothesized as the consequence of a lack of attention to the eye region, crucial for fear processing (Pelphrey et al., 2002), as well as the consequence of abnormalities in amygdala functioning (Ashwin et al., 2006). Some studies further corroborate this idea, by observing evident difficulties posed by children and adults with ASD when processing emotional information of the eye-region of the face (Baron-Cohen et al., 1997; Gross, 2008).

Importantly, face processing in ASD seems to depend on the nature of the employed stimuli (e.g., human faces vs. cartoon, animal or robot faces). For instance, children (Grelotti et al., 2005; Rosset et al., 2008; Sedeyn, 2017), and adolescents with ASD (Brosnan et al., 2015) seem to respond similarly to their TD peers when processing cartoon faces, possibly due to greater interest in this type of stimuli (Rosset et al., 2008). This interest in certain types of stimuli may increase expertise and specialization, facilitating their processing (Grelotti et al., 2002). Similarly, a study

analyzing motivational approach and avoidance responses in adolescents with ASD also found that, for positive stimuli, these individuals showed faster avoidance from photographs of people but increased approach to cartoons (C. Silva et al., 2015). The authors interpreted these results as difficulties in assigning reward to socioemotional stimuli in ASD. Some studies further suggest that animal faces may be also processed differently from human faces in ASD (Cross et al., 2019; Davidson et al., 2019; Whyte et al., 2016). For instance, children and adolescents with ASD seem to be better at recognizing emotions in animal faces rather than in human faces (Cross et al., 2019; Davidson et al., 2019). Adolescents with ASD have been also shown to have decreased neural activation for human but not for animal faces (Whyte et al., 2016). Additionally, individuals with ASD seem also to process robot faces similarly as their TD peers (Jung et al., 2016). Lastly, it is also suggested that adults with ASD respond differently to unfamiliar and familiar faces, being the latter associated with a stronger neural activation, specifically of the fusiform face area (e.g., Pierce et al., 2004). The nature of the stimuli seems to also play an important role in social attention. For instance, some studies suggest that differences between children with ASD and their TD peers emerge when using more ecologically valid stimuli (Chevallier et al., 2015; Saitovitch et al., 2013). Furthermore, a review and meta-analysis of eye-tracking studies inspecting social attention in ASD suggested that the latter is, in fact, diminished in ASD, and this seems to not depend on the complexity of the used stimuli but rather on the quantity of social content. This suggests that individuals with ASD have more difficulties when more people is included in the presented stimulus (e.g., in a social situation scene; (e.g., in a social situation scene; Chita-Tegmark, 2016). Therefore, it is important to consider the nature of the stimuli when studying face processing and social attention in ASD, and also when designing interventions, given the potential of more motivating stimuli to enhance face processing and social skills in ASD (Cross et al., 2019; Jung et al., 2016).

Yet, independently of the nature and extension of the impairments, general difficulties in human face processing have been observed and they may have significant consequences for the social functioning of people with ASD (Dawson et al., 2005; Uljarevic & Hamilton, 2013; Weigelt et al., 2012). Herein, we argue that, given the diminished social motivation in ASD, possibly associated with other cognitive and neural impairments that disrupt general socioemotional processing, ideal interventions

should include strategies that carry socioemotional meaning on their own and/or that are also able to orient attention and assign meaning to certain social stimuli, such as faces, that alone may not have an inherent rewarding nature for this population. Given the recent evidence in favor of the striking role of olfaction as an important channel of socioemotional communication (de Groot et al., 2017; R. J. Stevenson, 2010), we regard olfaction as a privileged candidate to facilitate socioemotional processing in ASD and to reduce the social deficits observed in this population.

What makes olfaction special in this context?

The human olfactory system and its relevance in daily life has been receiving exponential attention in last decades (Heymann, 2006). This system is involved in the detection, identification, recognition and memory of a wide range of odors around us (Boudjarane et al., 2017), which can be non-social, coming from a variety of sources such as food, flowers or perfumes, or social, i.e., originating from human body fluids. These chemical signals constitute a valuable source of information about the world, providing important cues about available resources, opportunities and threats, influencing emotion, cognition and behavior (de Groot et al., 2012; H. S. Seo et al., 2010; Smeets & Dijksterhuis, 2014; Spehr, 2017b) and, more importantly in this context, communicating crucial social information that mediates social interaction (Pause, 2012; Spehr, 2017b). Thus, relying in olfactory information may be crucial, especially in ambiguous situations wherein the other senses may fail (Pause, 2012; Pazzaglia, 2015).

Olfaction encompasses a set of particularities that make it somewhat special in comparison with other modalities (Gottfried, 2006). First, the sense of smell develops very early in the development, being already present before birth (Schaal et al., 1998). Also, chemical signals are able to surpass physical barriers, having the potential of travelling for long distances (Pause, 2012). Furthermore, olfactory information is able to influence cognition, emotion and behavior subliminally or without the awareness of the receiver (Keller, 2011; Merrick et al., 2014; Sela & Sobel, 2010), directly accessing structures of the olfactory cortex without a thalamic relay (Gottfried, 2006). Also, structures involved in olfactory processing often overlap with others involved in

emotional processing (e.g., amygdala), creating a strong interplay between olfaction and emotion. Indeed, odors are able to induce emotions, as well as emotions are also able to influence our olfactory perception (D. Chen & Dalton, 2005; de Groot et al., 2012).

Importantly, social communication is pointed as one of the main purposes of the human olfactory system (R. J. Stevenson, 2009). Each human carries a unique body odor (BO), receiving both genetic (Penn et al., 2007) and environmental influences, including reproductive status, diet, and general health (Havlicek & Lenochova, 2008). Many studies support the remarkable role of BOs as effective communicative agents in the social domain (de Groot et al., 2017). Human social chemosignals carry unique information about its owner's, such as age (Mitro et al., 2012), gender (Penn et al., 2007), genetic relatedness and compatibility (Porter et al., 1985; Ruff et al., 2012), hormonal variations (S. L. Miller & Maner, 2011), disease (Olsson et al., 2014), personality traits (Sorokowska et al., 2012), and emotional state (D. Chen & Haviland-Jones, 2000). This information is thought to modulate the dynamics of social interaction by, for instance, recruiting empathy-related neuronal areas in the receiver (Prehn-Kristensen et al., 2009), influencing interpersonal trust (P. Quintana et al., 2019) and social judgement (Dalton et al., 2013), inducing pro-social behavior (Camps et al., 2014), cooperative behavior (Huoviala & Rantala, 2013), risk behavior (Haegler et al., 2010), generosity (Perrotta et al., 2016), and by influencing pivotal social processes, such as the mother-child bonding (Lübke & Pause, 2015; Schaal, 1988, 2015), and sexual functioning and behavior (Alves-Oliveira et al., 2018; Havlicek et al., 2008; Herz & Inzlicht, 2002). Furthermore, there is evidence that social and biologically relevant stimuli, such as human BOs, have a differential and preferential processing, by recruiting brain structures related to emotional processing (e.g., the amygdala), attentional regulation (e.g. anterior cingulate cortex), visual processing (e.g., occipital cortex), processing of social stimuli (e.g., fusiform gyrus) and creation of a basic perception of a human body (e.g., the angular gyrus), contrasting with the more traditional olfactory areas recruited by common odors (COs), such as the medial orbitofrontal cortex and the piriform cortex (Lundström et al., 2008; Prehn-Kristensen et al., 2009). Nevertheless, even if human chemosignals seem to be a critical mean of receiving information about the social environment, COs also play an important role in the way we interact with the world, by influencing, for instance, pro-social behavior,

interpersonal trust and social relationships (e.g., Baron, 1997; Guéguen, 2012a, 2012b; Sellaro et al., 2014).

Independently of the social or non-social nature of an odor, odors have certain properties that determine how they will be further processed and attended, such as familiarity and valence (Seubert et al., 2017). For instance, evidence suggests that the BO of a kin elicit differentiated responses, compared with the BO from strangers (Lundström et al., 2008) and, accordingly, familiarity also seems to facilitate the detection of chemosensory emotional cues (Zhou & Chen, 2011). On the other hand, odor valence is similarly important to signal potential threats or positive and safe chemical signals, eliciting congruent emotions and behaviors (Seubert et al., 2017). Therefore, emotional-congruent responses are elicited in the receivers following different COs presence (Alaoui-Ismaili et al., 1997; Bensafi, 2002). Crucially, BOs collected in specific socioemotional situations, such as fear (de Groot et al., 2012), disgust (de Groot et al., 2012), happiness (de Groot et al., 2015), anxiety (Prehn et al., 2006), competition (Adolph et al., 2010) and aggression (Mutic et al., 2016), have also demonstrated the ability to induce congruent affective responses in the receivers, including changes in the activity of certain facial emotional muscles (de Groot et al., 2012, 2015), inhalation magnitude (de Groot et al., 2012), eye-scanning behavior (de Groot et al., 2012), as well as motor (Prehn et al., 2006) and psychophysiological responses (Adolph et al., 2010; Ferreira et al., 2018). These results indicate that odors, especially BOs, indeed carry important social information that is effectively processed and produces compatible emotional and behavioral changes in the receiver, possibly to increase the chances to deal adaptively with the social environment.

Olfactory (dis)abilities in ASD

Alterations in olfactory abilities and their impact in social and occupational functioning and health have been studied and documented across populations (Boesveldt, Postma, et al., 2017; Croy, Nordin, et al., 2014; Hummel et al., 2016). Impairments in these abilities may affect not only health, safety and work ability, but also important dimensions of social functioning (Croy et al., 2012; Croy, Nordin, et al., 2014; Hummel et al., 2017). Olfactory dysfunction also often occurs in

neurodegenerative disorders, such as Parkinson disease (Haehner et al., 2009) and psychiatric disorders, such as schizophrenia (Moberg et al., 2014), and some studies have been suggesting that olfactory alterations are present in children and adolescents (Dudova et al., 2011; Hrdlicka et al., 2011; Rozenkrantz et al., 2015), as well as in adults with ASD (Ashwin et al., 2014; Galle et al., 2013; Suzuki et al., 2003; Wicker et al., 2016).

Atypical sensory processing is a well-established feature of ASD (American Psychiatric Association, 2013). It seems to be pervasive across development (e.g., Leekam et al., 2007) and involves distinct and frequently co-occuring response patterns that may include hyperresponsiveness (heightened sensitivity or response to a stimulus), hyporesponsiveness (diminished response to a stimulus) and sensory seeking (persistent search for stimulation; Baranek et al., 2006; L. J. Miller et al., 2007). Although evidence suggests that these sensory abnormalities extend to multiple modalities, most of the studies target visual or auditory perceptual processing, with very few literature addressing how individuals with ASD process olfactory cues (Baranek et al., 2014; Baum et al., 2015; Marco et al., 2011). Furthermore, although informative, studies about olfactory perception in ASD are still inconclusive, given the great heterogeneity in crucial aspects, such as the sample's characteristics (e.g., age of participants) and the type of tasks and olfactory stimuli used to measure olfactory function (Larsson et al., 2017; Martin & Daniel, 2014; Tonacci et al., 2015). For instance, the literature suggests lower ability to detect odors in children with ASD (Dudova et al., 2011; Muratori et al., 2017), but also points to normal (Galle et al., 2013; Suzuki et al., 2003; Tavassoli & Baron-Cohen, 2012) or enhanced (Ashwin et al., 2014) olfactory detection abilities in adults. Olfactory discrimination, the ability to distinguish odors considering their distinct qualities (Doty, 2017), seems to be unimpaired either in children (Muratori et al., 2017) and adults with ASD (Galle et al., 2013). Lastly, the ability to identify odors seems to be impaired both in children (Bennetto et al., 2007; Legisa et al., 2013; Muratori et al., 2017) and adults with ASD (Galle et al., 2013; Suzuki et al., 2003).

Importantly, in the socioemotional domain there is also some evidence of altered perception and emotional responses to odors, especially regarding subjective ratings of COs valence in children and adolescents with ASD (Hrdlicka et al., 2011; Legisa et al., 2013), as well as regarding perception and physiological response towards "fear BOs"

in adults with ASD (Endevelt-Shapira et al., 2018). For instance, children and adolescents with ASD seem to perceive the pleasantness of some COs, such as cinnamon and sweat, in a significantly more "neutral" way than their TD peers (as less pleasant and less unpleasant, respectively; Hrdlicka et al., 2011; Legisa et al., 2013). On the other hand, Endevelt-Shapira and colleagues (2018), who performed the only study, to our knowledge, that evaluated the processing of BOs in adults with ASD, observed that emotional BOs, specifically BOs collected during emotional induction of fear, significantly increased electrodermal response in TD people but not in ASD. Additionally, these "fear BOs" reduced measures of trustworthiness in TD people but displayed the opposite effect in ASD (Endevelt-Shapira et al., 2018). The authors argued that ASD people may process social odors in a distorted fashion (which they called "social dysosmia"), similarly to what happens in other sensory modalities, such as vision. This social distortion is further argued as a potential mechanism behind the emotional processing deficits observed across modalities in ASD. Nevertheless, despite these dysregulations, adults with ASD seem to be as able as TD individuals to spontaneously sample BOs, adjusting their sniffing pattern and being able to detect and discriminate them (Endevelt-Shapira et al., 2018).

On the other hand, and interestingly in the context of olfactory abilities, Rozenkrantz and colleagues (2015) observed impairments in the adjustment of the sniffing pattern according to CO valence, in children with ASD. The adjustment of the sniffing response is important because it may reflect the adaptive functioning of sensory acquisition and rejection mechanisms that allows and regulates the amount of information that is received when a potential contaminant, threat or positive chemical signal is encountered (de Groot et al., 2012). One could argue that having a compromised functioning of these mechanisms can be a serious limitation and, in ASD particularly, may be favoring the observed social deficits (Rozenkrantz et al., 2015). On the other hand, we can also argue that an undifferentiated sniffing pattern can actually be an opportunity rather than a deficit, because it allows people with ASD to obtain the maximum of socioemotional information as possible from an odor, even if negative. This is especially meaningful in the context of impaired visual emotional processing in ASD (Uljarevic & Hamilton, 2013) because, when vision fails, olfaction may be an important, if not the only one, way to access the available socioemotional information in

the environment (Pazzaglia, 2015). The research of Parma and colleagues (2013) may provide an additional and important reinforcement of this potential. These authors conducted a study to examine if an olfactory social cue would be able to facilitate social behavior in ASD, particularly imitation behavior. The results showed that children with ASD were able to imitate the action of a model only when a socially meaningful BO, namely the odor of their own mother, was present in the scene, reinforcing the role of socioemotional meaningful olfactory cues as facilitators in social context.

The role of olfaction in visual processing

We live in a multisensory world, which constantly provides us an unimaginable amount of diversified information. Hence, it is not surprising that the information coming from the different senses interact to provide a faster and clearer experience of the environment, directing also our focus of attention (Driver & Noesselt, 2008; Driver & Spence, 1998; Pazzaglia, 2015). Cross-modal interactions occur when a stimulus from one modality influences the processing of another stimulus from other sensory modality (Spence, 2018). When stimuli from different modalities are presented approximately in the same spatial location and time window, multisensory integration processes may occur, resulting in a distinct percept that may facilitate performance (Holmes & Spence, 2005; Spence, 2010; Stein & Rowland, 2020). These processes may be especially relevant when the information coming from the individual sensory channels is ambiguous or little informative (De Gelder & Vroomen, 2000; Zhou & Chen, 2009). Indeed, according to the principle of inverse effectiveness (Holmes & Spence, 2005; Meredith & Stein, 1986), the less effective the individual stimuli are, the most pronounced and beneficial multisensory integration is.

Much research has, therefore, been interested in studying cross-modal interactions, especially between vision, audition and touch (Driver & Noesselt, 2008). More recently, the interest in studying the role of olfactory cues in cross-modal interactions has also been growing. For instance, in the non-social domain, olfactory cues have been showed to enhance visual perception (Frassinetti et al., 2002; Ohla et al., 2018), to orient attentional resources to congruent objects or images (K. Chen et al., 2013; Seigneuric et al., 2010; H. S. Seo et al., 2010), to influence visual distraction and

visual attentional capture (Michael et al., 2003, 2005), as well as the salience of congruent images during attentional blink (A. K. Robinson et al., 2013). Moreover, studies have shown that olfactory cues seem to influence reaction time in visual and auditory tasks (Millot et al., 2002) and subjective ratings of valence regarding pictures (Banks et al., 2012). They also seem to facilitate the localization of sounds (La Buissonnière-Ariza et al., 2012), the processing of auditive and visuo-auditive stimuli (Ohla et al., 2018), and have the ability to change the pleasantness of touch (Croy, D'Angelo, et al., 2014). Hence, in the social domain, olfactory cues do represent a promising socioemotional context when interacting with the visual domain.

In fact, social communication and social interaction are multisensory-based. To effectively disentangle the cues imbued in a social situation, we may have to rely on vision, but it is unlikely that this is the only information we receive and rely on (de Groot et al., 2017; Pause, 2012). For instance, speech cues, touch and chemical signals interact and possibly integrate to provide us a better understanding and, hence, an adaptive responding to social interaction demands. Thereupon, and corroborating this assumption, anxiety chemical signals have been shown to boost attentional allocation, to induce stronger startle responses following the presentation of fear facial expressions in high social anxiety (Adolph et al., 2013), to enhance the perception of fearful faces (Wudarczyk et al., 2016), to decrease the priming effect of positive facial emotional expressions (Pause et al., 2004), to facilitate dynamic facial emotional recognition (Rocha et al., 2018), and to decrease subjective ratings of pleasantness of ambiguous faces (Zernecke et al., 2011). Similarly, fear chemical signals seem to modulate the appraisal of ambiguous faces (Zhou & Chen, 2009) and to facilitate the detection of congruent fearful facial expressions but not other negative expressions (Kamiloglu et al., 2018).

Furthermore, a set of studies have crossed social and non-social domains of olfactory and visual stimuli, by demonstrating that COs also affect subjective ratings of human faces (S. Cook et al., 2015, 2017), modulates neural responses towards faces (S. Cook et al., 2015, 2017; Leleu, Godard, et al., 2015), facilitates the perception of emotional faces, either emotionally congruent (Leppänen & Hietanen, 2003) or not (Seubert et al., 2010), guides affective decision-making towards faces in children (Cavazzana et al., 2016) and facilitates the perception of ambiguous facial expressions (Leleu, Demily, et al., 2015; Novak et al., 2015). Together, this evidence supports the

notion that olfactory cues, both social and non-social, are able to act as special primes in visual processing, orienting attention for congruent visual stimuli and enhancing facial perception, especially when a stimulus is ambiguous or congruent with the emotional tone of the odor.

Olfaction as a unique contextual socioemotional cue for ASD?

The arguments

By merging pieces of evidence together, and although the nature of olfactory abilities in ASD remains far from uncovered, we argue that the promising results obtained in the last decades unravel new opportunities for developing comprehensive and integrative research about the relevance of social information imbued in olfactory cues for the social behavior in ASD. We aim to further support this idea, by arguing that olfaction may be a privileged channel of socioemotional communication in ASD, and also a strong candidate to surpass the difficulties often observed in visual domain by means of cross-modal visuo-olfactory integration of information. Our arguments are the following:

1. Olfaction is unique in its privileged relationship with emotional brain structures (Gottfried, 2006), and emotionally relevant cues exert great influence in general attentional and perceptual processes (Pourtois et al., 2013). Also, some of these structures have been showed to be abnormally functioning in ASD (e.g., the amygdala and fusiform gyrus) in facial processing tasks (Schultz, 2005). Hence, we believe that olfaction may be able to easily and rapidly recruit these areas, enhancing emotional communication in ASD. Furthermore, since visual stimuli may fail to properly recruit these areas in ASD, possibly due to lack of socioemotional salience (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998), olfaction may be able to directly influence the structures involved in socioemotional processing (Gottfried, 2006; Schultz, 2005) and, thus, provide the salience that visual stimuli is lacking (Michael et al., 2003).

2. Olfaction can be used as an effortless and subliminal aid in ASD (Merrick et al., 2014; Parma et al., 2013). This is absolutely critical for these population, since it helps to deal with the verbal language, severity, sensory hypo and hyper-sensibility, intellectual functioning and compliance problems often described by researchers and

clinicians (Kasari & Patterson, 2012; Larsson et al., 2017; Martin & Daniel, 2014; L. Watkins et al., 2015).

3. Olfaction may provide social meaning and disambiguation of other modalities, including the visual system, by being able to recruit areas related to both visual and social perception (Lundström et al., 2008), since it has been suggested that integrating redundant information by two or more modalities may reduce ambiguity (Ohla et al., 2018). This assumption is corroborated by the research in cross-modal visuo-olfactory integration conducted with TD population, that highlights the role of olfactory cues as a facilitator in the perception of ambiguous faces (Leleu, Demily, et al., 2015; Zernecke et al., 2011) and in visual attention to congruent stimuli (H. S. Seo et al., 2010).

4. Adding information about two or more modalities not only facilitates perception (Ohla et al., 2018), but is closer to how reality works. Notwithstanding the robust difficulties observed in ASD regarding spontaneous allocation of attention to visual social cues and in face processing (Dawson et al., 1998, 2005; Klin et al., 2002), human faces are rarely (if not never) encountered alone in a social interaction, but instead show up together with more information coming from the other senses. As human BOs are highly informative about its sender, communicating striking information in the context of the social encounter and modulating receiver's emotions and behaviors (de Groot et al., 2017; Semin & Groot, 2013), they possibly constitute a key element for understanding social processes in ASD. To close the argument, authors have been claiming that the context matters when studying social attention in ASD (Chawarska et al., 2013), highlighting the need to employ more ecological social stimuli when addressing social processing in ASD. Indeed, when more ecologically valid visual stimuli are used (e.g., complex scenes involving more than one person), the differences between ASD people and TD people seem to be more salient (Chevallier et al., 2015; Chita-Tegmark, 2016; Risko et al., 2012). Moreover, people with ASD are able to look at faces when told to, but instead do not seem to do that spontaneously (Senju, 2013), and are also able to learn emotion recognition strategies and other social skills, but often do not know how to apply them to multiple settings (Kasari & Patterson, 2012). These results seem to suggest that, in fact, ASD people present social difficulties in real-life, complex, social interactions, and, still, we may have been failing to assign and adjust

this social and ecological meaning with the adopted approaches. Adding olfactory cues to improved paradigms would mimic more closely the complexity of real-life.

5. Finally, olfaction does not depend on a thalamic relay (Gottfried, 2006). Yet, the existence of a direct route connecting olfactory receptors with the cortex does not necessarily imply that the thalamus is not involved in olfactory processing at all (Courtiol & Wilson, 2015). In fact, the role of thalamus in olfaction is still underexplored, but some studies suggest that it may be involved in olfactory attentional processing, as well as in cortico-cortical communication, hedonic processing, learning and memory (for reviews see Courtiol & Wilson, 2015; Gottfried, 2010; Tham et al., 2009). Furthermore, this structure seems to be compromised in ASD (e.g., Nair et al., 2013; Tamura et al., 2010), although the impact of these abnormalities in olfactory processing is still unknown. Nevertheless, the fact that at least not all olfactory information relies in a thalamic stay, thus being able to directly access the olfactory cortex, confers a more direct and rapid processing (Gottfried, 2006).

By putting these arguments together, we hypothesize that olfactory stimuli, used as an emotional prime or context, may facilitate socioemotional processing in ASD, especially concerning the orientation of attentional resources to social cues and socioemotional perception, which is critical in this population.

Implications of the present work

We would like to make some considerations, starting by the point of this framework, which does not aim to propose ungrounding new research or to directly propose new interventions in ASD without evidence, but rather: 1) to review the actual knowledge about social cognition in ASD, with particular focus in visual social attention and face processing, which have been showing impairments across the spectrum and may be linked to broader social deficits; 2) to review the knowledge about olfactory processing in ASD, which remains a very under-explored area for the potential we show that effectively exists for olfaction and socioemotional communication; and 3) to present evidence-based arguments to support why olfactory processing, as well as cross-modal and visuo-olfactory integration approaches could be used to study social

perception and behavior in ASD. Thus, we intend to provide new opportunities to further investigate the role of olfaction in socioemotional processing in ASD, as well as its role in cross-modal and multisensory integration processes in ASD, which have the potential to critically drive effective practices.

More than providing answers, we sought to open a new avenue of questions. For instance, until date, no research had addressed the use of COs in comparison to BOs in visual social processing, and the research using them in separate have demonstrated very similar abilities to influence facial perception (Leleu, Demily, et al., 2015; Zernecke et al., 2011). It would be interesting to investigate if the presence of an olfactory cue is sufficient to facilitate visual social processing or if the socioemotional meaning of the odor is important in this context. Also, what are the remaining odor properties that guide the effect? Does familiarity, intensity and valence play equally important roles in facilitation effects? Parma and colleagues (2013), for instance, observed that only familiar odors (maternal BOs) facilitated imitation in ASD children. This suggests that familiarity may be an important property, especially in this population, but we keep unaware of what would have happened if the BOs had a strong emotional content (e.g., being collected in different emotional induction situations). May the effect of these properties be modulated by the characteristics of the spectrum as, for instance, the existence of anxiety symptoms? For instance, Parma and colleagues (2019) found that ASD and anxiety are associated with differentiated patterns of psychophysiological response. These findings support that measuring anxiety is relevant when addressing, for instance, responses to emotional stimuli, and that this variable may be modulating some of the mixed results observed in uni-sensorial emotional research in ASD.

To test the role of olfaction as a facilitator in social cognition processes in ASD, it is possible to adapt multiple experimental paradigms, some of them typically used in visual perception research in ASD. These include eye-gaze cueing, spatial attention and scene viewing paradigms (for a review see Ames & Fletcher-Watson, 2010). Tasks already employed in cross-modal visuo-olfactory interaction studies in TD population, such as facial emotion recognition/categorization tasks (e.g., Leppänen & Hietanen, 2003; Rocha et al., 2018; Wudarczyk et al., 2016), can also be explored in ASD. For instance, analyzing eye movements in scene-viewing paradigms (Klin et al., 2002) in

which visual social stimuli are presented after and/or during an olfactory cue, would allow to evaluate differences in the scanning behavior of visual social stimuli between both sensory conditions. Based in previous research in visual domain, we would expect less spontaneous scanning of social features in the absence of an olfactory cue, in people with ASD (e.g., Klin et al., 2002). Additionally, we hypothesize that the presentation of an olfactory stimulus could approximate the performance of people with ASD and TD individuals by orienting their attention to congruent visual cues (Kamiloglu et al., 2018; H. S. Seo et al., 2010). We further expect these effects to be more robust in the presence of BOs, since they would be socially relevant and congruent with the visual stimuli (H. S. Seo et al., 2010), and that affective congruency between visual and olfactory stimuli would guide or augment the effects (Kamiloglu et al., 2018). Considering research in more applied settings, it would be also interesting to study specific components of social behavior/interaction in ASD by using odors as a context in semi-realistic and real-life settings, similarly to the approach employed by Parma and colleagues (2013). It would be also interesting to understand if, assuming that olfaction does have a facilitation effect in visual domain and social behavior in ASD, a visuo-olfactory training would have benefits regarding facial processing, social attention and social functioning in general.

Apart from the potential to facilitate visual processing, olfactory cues may also play a fundamental role in other dysfunctional processes and behaviors in ASD, such as eating behavior and food selection (Boesveldt, 2017). It has been reported that people with ASD presents more feeding problems than their TD peers, resulting in, for instance, nutritional intake deficits (Sharp et al., 2013). These feeding problems may be associated with variables including alterations in sensory processing (e.g., Cermak et al., 2010; Suarez et al., 2014), the emotional valence attributed to visual and olfactory food stimuli (Luisier et al., 2015; Luisier, Petitpierre, Bérod, et al., 2019) and the child's family reported eating preferences (Schreck & Williams, 2006). For instance, Luisier and colleagues (2015) found an association between the valence attributed to olfactory food stimuli and food neophobia, the tendency to reject new food, in children with ASD. Furthermore, other study enrolled TD children and children with ASD in several sessions to allow an increase in familiarization with olfactory food stimuli (Luisier, Petitpierre, Clerc Bérod, et al., 2019). The authors observed not only a more positive

appraisal of the “familiarized odors”, but also that children tended to choose food associated with the “familiarized odor”. These findings suggest that odors, especially familiar and positive odors, may facilitate food education and the expansion of food repertoire of children with ASD. Future research should further investigate if, for instance, familiarization with food odors brings long-term enhancement of eating behavior in ASD.

To conclude suggestions of future research, it would be also interesting to investigate sensory processing and cross-modal integration considering ASD in a dimensional perspective, including the manifestation of autism symptoms in the general population (Baron-Cohen et al., 2001; Constantino & Todd, 2003). These symptoms, often addressed as autism traits, seem to have a hereditary component (Hoekstra et al., 2007) and to be continuously distributed in the general population (R. M. Hurst, Mitchell, et al., 2007). Importantly, differences in the manifestation of these traits have been associated with altered attention mechanisms (e.g., Dunn et al., 2016; Freeth, Foulsham, et al., 2013; Muller Spaniol et al., 2018), sensory processing (e.g., Bayliss & Tipper, 2005; Cribb et al., 2016; Mayer, 2017; A. E. Robertson & Simmons, 2013) and multisensory integration (e.g., Donohue et al., 2012; Kawakami et al., 2018; van Laarhoven et al., 2019), in a similar fashion as observed in the clinical extreme of the spectrum. Nevertheless, there is limited knowledge about olfactory perception and cross-modal visuo-olfactory integration in the sub-clinical part of the spectrum. By studying these processes considering a dimensional perspective, we would have the opportunity to not only access their manifestation and variability in the general population (Donohue et al., 2012), but also to expand the knowledge about this subclinical phenotype of autism and its relationship with the clinical manifestation (Ingersoll & Wainer, 2014; Ronald & Hoekstra, 2011).

Extending research about olfactory and visuo-olfactory perception in ASD would, thus, allow to improve knowledge about if and how olfaction influence social cognition in this population and also how visual and olfactory stimuli combine to modulate attention, emotion and behavior in ASD. In addition to the theoretical and research value, we believe that investigating these processes may have a great impact for the development of new cost-effective and non-invasive intervention programs, with

potential to generalize their effects to multiple contexts and to reach positive effects in a cascade of social skills.

Challenges and opportunities

Throughout the document we have been discussing why studying olfactory perception, as well as cross-modal and multisensory integration involving olfactory cues, may be relevant in the context of sensory and social processing difficulties in ASD. Yet, we disclose some challenges and opportunities regarding these topics that should be carefully considered in future research. First of all, as previously reviewed in this paper, people with ASD often presents altered visual perception (e.g., Behrmann et al., 2006) and may also exhibit either normal (e.g., A. E. Robertson, 2012), increased (Ashwin et al., 2006), decreased (e.g., Suzuki et al., 2003) or even distorted olfactory perception (e.g., Endevelt-Shapira et al., 2018). This could imply that, if both visual and olfactory cues are processed in an atypical fashion when isolated, the integration of both signals could also lead to a different, most likely maladaptive percept of the world. Still, the methodological and sample differences across studies strongly difficult direct comparisons, as well as the understanding of how individual differences in this spectrum, such as developmental stage, comorbid conditions (e.g., anxiety disorders) or symptoms' severity, could be associated with sensory processing. Although ASD comprise a complex condition, with great variability regarding symptoms and levels of functioning, efforts should be made to isolate as much as possible the effects of these variables to better understand sensory and social processing in this population.

Furthermore, some studies have been suggesting that the ability to combine information from multiple sensory channels is often compromised in ASD (for reviews see Baum et al., 2015; Beker et al., 2018; Feldman et al., 2018; Wallace et al., 2020). Nevertheless, these studies rely mostly in the integration of visual, auditory and tactile sensory information. Regarding the integration of information involving olfactory cues, little is known. To the best of our knowledge, only one study has, so far, addressed the behavioral effects of cross-modal integration of visuo-olfactory stimuli in ASD (Endevelt-Shapira et al., 2018). The authors examined olfactory perception of "fear BOs" in adults with ASD and TD people, including the influence of these olfactory cues

in a face perception task and in a social judgement task. The authors did not find behavioral effects of exposure to BO in either group in the face perception task, but observed altered estimation of trustworthiness following the perception of fear social chemosignals in ASD. These results open a new avenue of questions about the processing of emotional odors, especially BOs, and their relevance in visual and social perception across contexts, in ASD. Still, conclusions are limited due to scarcity of literature, as well as by the fact that this study only tested adults, mainly male and only used BO, collected in specific emotional situations.

Regarding the neural correlates of multisensory visuo-olfactory integration, the scarcity of studies even in typical development greatly limits the knowledge about how the brain processes this information (e.g., Ripp et al., 2018; Sijben et al., 2018). In ASD, there is only one study analyzing brain activation following visuo-olfactory stimulation, suggesting that visuo-olfactory integration of congruent images and common odors (e.g., rose), is associated with a pattern very similar to that observed in audio-visual integration (Stickel et al., 2019). Furthermore, results evidenced similar brain activation for TD and adults with ASD, suggesting intact visuo-olfactory integration in adults with ASD (Stickel et al., 2019). Therefore, despite the evidence in favor of altered multisensory integration regarding other sensory modalities (which is not true for all the people with ASD and for all the types of sensory stimuli; see, for instance, Beker et al., 2018), it is very preliminary to assume that visuo-olfactory integration is also atypical in ASD. As argued throughout the paper, future studies should further investigate olfactory perception and visuo-olfactory integration in ASD (and also in typical development), to provide a better understanding of how the brain processes this information separately and together, and how this perception influences cognition, emotion and behavior in this population.

Despite inconsistent results regarding olfactory perception in ASD, olfactory cues have already been shown to have the potential to foster previously impaired adaptive behaviors in this population. For instance, the research of Luisier and colleagues (2019) suggested that it is possible to increase familiarization with odors from food stimuli, allowing a more positive evaluation of these stimuli and a preference for the associated food. This may have important implications for future research regarding eating behavior and food refusal in ASD, but also hints that it may be

possible, through the process of familiarization with odors, to use familiar olfactory stimuli to improve behavior in other contexts. Similarly, the research of Parma and colleagues (2013) also suggests that familiar and socioemotionally significant BO, such as the mother's BO, is able to promote social behaviors. Familiarity seems to be a central property to unlock previously impaired behaviors, yet further research is necessary to extend these results and explore other possibilities.

An additional important challenge is to understand how to transpose this laboratory research to daily life context, where people deal with the typical complexity of a real social interaction. In a real social interaction, we are overwhelmed with sensory information that we must be able to successfully filter, select, integrate and segregate to identify rapidly and correctly the presence of a kin, rivals and potential partners, as well as their emotions, thoughts, intentions and behaviors (Carretié, 2014; Keltner & Kring, 1998; Stein & Rowland, 2020; Vuilleumier, 2005). In addition to relevant social information, we also receive cues about nonsocial objects present around us, that may possess different properties and, therefore, signal events of distinct relevance in the environment. Even social information can be masked or modified by nonsocial cues, such as parfum or hygiene products, that mix with the BOs of people around us (Allen et al., 2019). It is up to our perceptual system to prioritize socially and evolutionarily relevant information, allowing an appropriated response to the challenges and opportunities posed by the environment (Carretié, 2014; Desimone & Duncan, 1995; Egeth & Yantis, 1997). This may be a challenge for people with ASD, that frequently report to be overloaded with sensory information (R. S. P. Jones et al., 2003; O'Neill & Jones, 1997). Considering that individuals with ASD may process social information in a nonsocial fashion, either due to motivational deficits (e.g., Chevallier et al., 2012), or broader atypical perceptual functioning (e.g., Mottron et al., 2006), or both, then we could argue that they may have difficulties in prioritizing and using relevant information to act accordingly, especially in more complex scenarios. Difficulties in multisensory integration may also play a significant role here, as previously described, as well as difficulties in effectively value and learn from prior sensory experiences and use this knowledge to deal with current sensory information (Pellicano & Burr, 2012). All these accounts seem plausible and may not be mutually exclusive in their role to explain sensory and social difficulties in ASD. Together, they may hint why olfactory

cues, which are present in the environment together with other sensory information, do not help in face processing and social behaviors in ASD in everyday life. Therefore, it may be necessary to: 1) First, understand how cross-modal and multisensory integration processes work in ASD, in controlled and well-defined laboratory experiments, by isolating the stimuli of interest from other irrelevant sensory stimuli as much as possible; 2) Second, evaluate the feasibility and efficacy of sensory trainings that systematically combine relevant sensory information to improve, for instance, face processing and/or social attention. Starting with more motivating visual (e.g., cartoons and animal facial expressions; Rosset et al., 2008; Whyte et al., 2016) and olfactory (e.g., familiar and socioemotionally relevant odors; Luisier, Petitpierre, Clerc Bérod, et al., 2019; Parma et al., 2013) stimuli may help in the process; 3) And third, gradually transpose the experimental paradigms to more applied contexts, evaluating not only measures of social attention, but also how the sensory training improved social functioning in general. We hypothesize a long way to go with many challenges subserved by a complex and fascinating spectrum. Nevertheless, independently of the outcome and as argued before, we argue that investigating olfaction and cross-modal interplay in ASD will provide certainly several answers and, importantly, many other important questions.

Conclusion

In this article, we have reviewed evidence that supports that olfaction possesses a set of unique properties whose processing is worthy of studying in ASD, and that olfactory cues can be helpful to disentangle social meaningless and ambiguity, as well as to overcome the consequent social deficits observed in ASD. Despite the still scarce literature in olfactory processing, studies suggest that the interplay between olfaction and emotion is, indeed, significant, and that odors may be an effective way to correspond to the complexity of social interaction. We also believe that the knowledge about olfactory abilities in ASD and how odors interact with other sensory modalities may be helpful to better understand how these modalities work alone, how cross-modal and multisensory mechanisms work in general, how the sensory profile of this population works in particular, and this knowledge may be crucial to design cost-

effective, embracing, generalizable, inclusive and effortless intervention plans. We further suggest that this knowledge would be also capable of open new possibilities for other populations with deficits in social domain (e.g., schizophrenia). For now, this framework lights up new opportunities for research and, posteriorly, practice in social domain, by providing one more piece for the intriguing and complex puzzle of ASD functioning.

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CHAPTER 4 – GENERAL DISCUSSION

4.1. Main results

The present work sought to provide theoretical and empirical support in a specific area where investigation was lacking regarding olfactory processing in the autism spectrum, as well as about the assessment of variables that are critical to better understand autism and its associated features from a dimensional perspective. Although olfactory processing has gained momentum in autism, studies exploring olfactory abilities in this condition are mixed, and investigations exploring the role of autism-related variables in these inconsistent results are missing. Studying autism from a dimensional perspective could help to understand which specific variables are associated with the several components of altered olfactory processing – as well as with other outcomes observed in the autism spectrum. Furthermore, exploring the expression of autism traits in the general population may provide a better understanding of the heterogeneity observed in the spectrum regarding cognitive, social, and emotional variables; for instance, it may help to uncover which autism dimensions are related to the comorbidities and symptoms often observed in the spectrum. To this end, it is crucial to have validated and adequate instruments that reliably measure these variables, considering their multiple dimensions and the variability observed in the general population. In this regard, Studies 1 and 2 shed some light on the psychometric properties of the STICSA and the AQ, which are two widely used instruments to measure anxiety symptoms and autism characteristics in a multidimensional perspective in the general population, respectively. Study 2 also provided further evidence of the role of alexithymia, a trait highly comorbid with autism, as a mediator of the relationship between autism traits and trait anxiety. Study 3 benefited from these instruments to add to the understanding of the role of autism symptomatology and trait anxiety on the olfactory abilities of the general population. Finally, Study 4 provided an integrative and orienting perspective of the current results regarding olfactory processing in autism and how future studies can tackle the gaps in this research.

STICSA is an adequate measure of anxiety in the general population

High levels of anxiety are often observed in the general population, with anxiety disorders being one of the most prevalent psychiatric disorders (Castaldelli-Maia & Bhugra, 2022; Kessler et al., 2009; Steel et al., 2014). These disorders are also highly comorbid with other physical, mental, and neurodevelopmental conditions, including autism (e.g., Hollocks et al., 2019). Assuring the adequacy and validity of instruments measuring anxiety symptomatology is, therefore, critical to an accurate assessment and, consequently, a timely and effective intervention and monitoring. In this regard, the psychometric properties of the STICSA, an instrument measuring the state-cognitive, state-somatic, trait-cognitive, and trait-somatic dimensions of anxiety, were explored in Study 1. Although some studies have already analyzed the psychometric properties of the STICSA in non-clinical populations (e.g., Carlucci et al., 2018; Gros et al., 2010; Ree et al., 2008; Roberts et al., 2016; Styck et al., 2020; Tindall et al., 2021), mixed results about its dimensionality have emerged. In fact, a 4-factor model (Balsamo et al., 2015; Grös et al., 2007; Roberts et al., 2016), a hierarchical model with a global anxiety factor and four correlated first-order factors (Roberts et al., 2016), and a 2-factor model within both trait and state anxiety forms (Carlucci et al., 2018; Ree et al., 2008; Roberts et al., 2016; Styck et al., 2020; Tindall et al., 2021) were all supported in past studies. These models were tested in Study 1, together with the unidimensional model and two additional hierarchical models that were more complex and theoretically sound. In line with previous literature, results supported the construct validity of the STICSA and the distinction between the four dimensions of anxiety (state-cognitive, state-somatic, trait-cognitive, and trait-somatic), with the full factorial model, as well as the 2-factor model within state and trait forms presenting the best adjustment (Barros, Figueiredo, Brás, et al., 2022).

Results also supported very good reliability for all STICSA's dimensions, in line with previous studies (Balsamo et al., 2015; Carlucci et al., 2018; Grös et al., 2007; Ree et al., 2008; Roberts et al., 2016; Tindall et al., 2021). A measurement invariance analysis was also considered relevant in this context since women often present higher anxiety symptomatology in comparison with men (e.g., McLean et al., 2011). Results

supported STICSA's metric invariance, suggesting that its items contribute to the latent construct to a similar degree in women and men (Barros, Figueiredo, Brás, et al., 2022). Although metric invariance was also attained in previous studies, full measurement invariance (i.e., scalar invariance) was previously established for groups of sex (Carlucci et al., 2018; Tindall et al., 2021). While scalar invariance was not assumed in Study 1 as observed in past studies, these results were expected and are in line with the often-observed sex differences regarding anxiety symptomatology.

The ability of the STICSA to distinguish between anxiety and depression was also a concern raised by previous studies, even though STICSA performs better in comparison with other similar instruments (Balsamo et al., 2015; Grös et al., 2007; Tindall et al., 2021). In the present work, the nomological validity of the STICSA was explored, with results showing a high level of association between the dimensions of the STICSA and those of the STAI, thus supporting good convergent validity (Barros, Figueiredo, Brás, et al., 2022). The association was particularly strong regarding the cognitive dimensions of the STICSA, which suggests that the STAI measures mostly cognitive anxiety, while the STICSA covers broader anxiety symptomatology. However, in line with previous studies (Carlucci et al., 2018; Roberts et al., 2016), the cognitive dimensions of the STICSA were strongly correlated with a measure of depression (Barros, Figueiredo, Brás, et al., 2022). Although this may seem problematic, these results are expected considering that anxiety and depression share a common component of negative affect, according to the tripartite model of anxiety and depression (L. A. Clark & Watson, 1991). Furthermore, the inclusion of a measure of positive and negative affect (PANAS) was a critical piece to support the STICSA as a measure of anxiety. Results showed that the cognitive dimensions of the STICSA were more strongly associated with high negative affect than with low positive affect, which is a component specific to depression (L. A. Clark & Watson, 1991; Watson et al., 1995). Since the correlations with depression and low positive affect were lower for the STICSA than for the STAI, STICSA – and particularly its somatic subscales – may be more suitable to differentiate anxiety from depression (Barros, Figueiredo, Brás, et al., 2022; Roberts et al., 2016; Tindall et al., 2021). In terms of clinical assessment and intervention, this distinction between anxiety and depression may entail important implications since these two conditions are often comorbid with each other (Choi et al.,

2020; Cummings et al., 2014). In comorbid anxiety and depression, specific clinical features may be present (see Choi et al., 2020 for a review); for instance, previous evidence found an increased risk of suicidal ideation and lower self-reported quality of life (e.g., H.-J. Seo et al., 2011), as well as lower remission rates and poorer response to treatment (e.g., Fava et al., 2008) in patients with anxious depression in comparison with patients with non-anxious depression. Therefore, it is critical to ensure a proper assessment that can capture the unique characteristics of these constructs and that, consequently, allows the adoption of therapeutic approaches adjusted to the needs of the individuals (Choi et al., 2020).

Finally, the ability of the STICSA to predict the emotional response in distinct contexts was also explored in Study 1. Previous evidence only explored the predictive validity of the STICSA in regard to self-reported state anxiety towards cognitive and somatic stressors (Ree et al., 2008). The current work added another dimension of the emotional response, namely the psychophysiological component, evaluated in three distinct conditions (fear, happiness, and emotionally neutral condition). Increased levels of trait anxiety were previously shown to be associated with autonomic dysfunction, particularly with reduced HRV (Bleil et al., 2008; Miu et al., 2009; D. Shepherd et al., 2015), which negatively impacts health and well-being (see, for instance, Shaffer et al., 2014). For this reason, Study 1 also evaluated how self-reported trait-cognitive and trait-somatic anxiety, as measured by the STICSA, were related to HRV indexes in distinct emotional contexts. Results showed that the trait-cognitive dimension was associated with self-reported arousal, while trait-somatic anxiety was associated with both self-reported arousal and the psychophysiological response, particularly with the LF/HF component of HRV. As a first main conclusion, this suggests that the cognitive and somatic dimensions of trait anxiety, as measured by the STICSA, are associated with distinct aspects of the emotional response (Barros, Figueiredo, Brás, et al., 2022). Additionally, on the one hand, both trait-cognitive and trait-somatic anxiety dimensions were associated with differences in self-reported arousal. The groups of lower anxiety (both cognitive and somatic) demonstrated higher physiological flexibility than the respective groups of higher anxiety, in the neutral condition. This means that, while the groups of low anxiety exhibited a decrease in self-reported arousal during the neutral emotion induction in comparison with the baseline, the groups of higher anxiety kept

their self-reported arousal constant (Barros, Figueiredo, Brás, et al., 2022). When comparing the different emotional conditions, or the baseline with the emotional induction, there was a trend for higher variability in the groups with lower anxiety in comparison with the groups with higher anxiety. Although evidence about the relationship between trait anxiety and the physiological response across emotional situations is still lacking, our results seem to be in line with previous studies observing lower variability in self-reported arousal in people with increased levels of anxiety, namely in anxiety disorders (e.g., Rosebrock et al., 2017).

On the other hand, while an association between the cognitive component of trait anxiety and the HRV indexes was not observed, the somatic component, as expected, evidenced an association with the psychophysiological response. In fact, the group with higher trait-somatic anxiety (HighSG) presented significantly higher LF/HF values, both in the baseline and emotional conditions, in comparison to the group with lower trait-somatic anxiety (LowSG). As the LF/HF ratio may be an indicator of sympathovagal balance, a higher LF/HF ratio suggests a general higher sympathetic activation in individuals with higher levels of trait-somatic anxiety. These results are intriguing since previous studies observed a relationship between trait anxiety and the HF component of HRV specifically (Bleil et al., 2008; Miu et al., 2009; D. Shepherd et al., 2015). Finally, results also suggested that the differences between the baselines and emotional conditions were less accentuated in the HighSG in comparison with the LowSG (regarding the LF/HF component), although this was only a trend and not a statistically significant result. Nevertheless, these results may further suggest that high levels of anxiety, particularly regarding trait-somatic anxiety, may be associated with both lower physiological flexibility (e.g., Hoehn-Saric et al., 2004) and ANS dysfunction (e.g., Friedman, 2007). Yet, caution is needed when interpreting our results. First, the LF component is not a pure index of sympathetic activation, which limits the interpretation of the LF/HF ratio (see Shaffer et al., 2014; Shaffer & Ginsberg, 2017). Second, it is important to note that past studies did not explore distinct dimensions of trait anxiety, only explored certain indexes of HRV (e.g., only HF), and explored the relationship between anxiety and HRV in neutral situations only, which preclude direct comparisons (Barros, Figueiredo, Brás, et al., 2022).

It is interesting to observe that, in general, the differences between high and low anxiety seem to be more marked in the neutral or non-aversive conditions, although we could also see some trends in the other conditions by visually inspecting data. Also, in general, lower anxiety levels are associated with more variability in emotional response. Since reduced HRV, particularly considering the HF component, has been associated with lower physiological flexibility, a relationship between trait anxiety dimensions and the HF component of HRV was expected, although not corroborated by our data. However, although these results are indicative of distinct patterns of emotional response in function of anxiety levels and different relationships between the two dimensions of trait anxiety and HRV, they should be interpreted with caution since there are some important limitations in Study 1, apart from the ones that were already stated. For instance, our sample was relatively small, and the emotional situations were "artificially" induced in the laboratory (see Barros, Figueiredo, Brás, et al., 2022). Future studies should replicate this procedure with larger samples of the general population and should extend to specific anxiety disorders and/or to other conditions with unique presentations of anxiety symptomatology, to explore the pattern of response across contexts and to contribute to the characterization of the STICSA. Larger samples would also allow to explore distinct patterns of trait anxiety in addition to those that have been explored; for instance, it would allow to explore if an individual with low cognitive anxiety and high somatic anxiety has a distinct response in comparison to an individual with low levels of both cognitive and somatic anxiety. Notwithstanding these limitations, overall, Study 1 contributed to the characterization of the STICSA as an adequate and useful instrument to measure anxiety in the general population, considering its multiple dimensions.

The 3-factor structure of the Portuguese version of the AQ

To attain the objectives of this thesis, it was also critical to assure an adequate measurement of autism characteristics in the general population. In this regard, since the AQ is a brief and accessible instrument, and possibly the most used to measure autism traits in the general population (English et al., 2020; Ruzich et al., 2015), it was considered a suitable candidate for such assessment. However, some of its originally

proposed subscales have previously shown poor reliability (e.g., Broadbent et al., 2013; English et al., 2020; J. L. Stevenson & Hart, 2017) and studies exploring AQ's factor structure have observed contradictory results (e.g., English et al., 2020; Russell-Smith et al., 2011). Therefore, to better understand the AQ's factor structure, we extended its studies to the general Portuguese population.

As expected, results suggested that the original 5-factor model presented poor adjustment (Barros, Figueiredo, & Soares, 2022). Therefore, EFA and CFA analyses were conducted as distinct approaches to explore and support the factor structure of the AQ with an adequate level of robustness. Similarly to the observed by previous studies (e.g., English et al., 2020; Palmer et al., 2015; Russell-Smith et al., 2011), results provided empirical support for a 3-factor structure composed of two correlated social factors, namely Social Skills (10 items) and Communication factors (seven items), and one non-social dimension, Restricted Interests and Detail Orientation dimension (RIDO; six items). Goodness-of-fit indices evidenced acceptable fit and the reliability analyses also suggested adequate internal consistency for the three dimensions (Barros, Figueiredo, & Soares, 2022).

The final solution was composed of 23 items (46% of the original items), following the exclusion of 27 items due to low factor loadings, cross-loadings, and/or theoretical criteria (Barros, Figueiredo, & Soares, 2022). In general, the 23 items loaded in factors where they were expected to load, consistently with previous studies (e.g., English et al., 2020). Yet, some concerns are worth noting. First, a direct comparison of our results with other studies may be limited. The number of items composing our final structure is, indeed, reduced in comparison with the original model and even with other studies that observed a 3-factor structure (e.g., 26 items - Austin, 2005; 28 items - English et al., 2020; Russell-Smith et al., 2011). Nevertheless, it is important to note that almost all studies exploring the psychometric properties of the AQ found factor structures composed of fewer items than the originally proposed, and even one of them observed a structure very similar to ours (namely a 3-factor structure with similar items retained in the factors, and the same number of items; Palmer et al., 2015). Furthermore, many items were found to be problematic across studies, possibly due to their ambiguity or inadequacy, which may lead to distinct interpretations (English et al., 2020; Palmer et al., 2015). The culture was also pointed out as a possible explanation for the variability

in retained items for some dimensions (e.g., Hurst et al., 2007). Yet, studies performed in the same culture have led to distinct results (e.g., Russell-Smith et al., 2011). It is also relevant to mention that our results, aligned with the previous literature, emphasize the role of Social Skills in the understanding of autism traits, considering that this factor accounts for a significant portion of explained variance and the items loading in this dimension are very consistent across studies (see Barros, Figueiredo, & Soares, 2022).

One could also argue that a reduced number of items, in comparison with the structure that was originally proposed, may limit the ability of the AQ to represent the core features and variability of autism traits. Even so, our model of two correlated social factors and one non-social factor seems to align with the key diagnostic criteria for autism diagnosis found in the last DSM editions (even though from the DSM-5 onwards the social dimensions were collapsed in one; American Psychiatric Association, 2013). The AQ may, therefore, at least reflect the core characteristics of autism, although other features that characterize the spectrum, such as altered attention-switching and sensory processing, are not directly assessed by this instrument. The fact that our sample size was relatively small (N=292) may have also impacted results regarding the factorial structure of the AQ, by limiting the full representation of autism traits in our sample. Nevertheless, interestingly, in the study of Palmer et al. (2015), which was conducted with a much larger sample of the general adult population (N=2343), the results were very similar to the present study in terms of factor structure and number of items of the final solution.

As a last main result, the fact that the social dimensions correlated moderately with each other but did not correlate with the non-social dimension at all provided further support for the "fractionated" nature of autism (Happé & Frith, 2020; Happé & Ronald, 2008; Warrier et al., 2019). This also highlights the need to explore the unique and separated contributions of these dimensions to understand how distinct patterns of autism traits impact the strengths, difficulties, and general functioning of the autism spectrum, which was our rationale for the second part of Study 2 and for Study 3.

Alexithymia mediates the relationship between autism traits and trait anxiety

High levels of anxiety were previously shown to be associated with autism traits in the general population (Bothe et al., 2019; Kanne et al., 2009; Liss et al., 2008). Importantly, the literature has been suggesting that anxiety symptomatology is positively associated with alexithymia (e.g., Karukivi et al., 2010; Liew et al., 2015; Rosbrook & Whittingham, 2010), a personality trait that is also significantly and positively correlated with autism traits (e.g., Aaron et al., 2015; Bothe et al., 2019; Gökçen et al., 2016; Liss et al., 2008). A few studies further observed that alexithymia mediates the relationship between autism and anxiety symptomatology (Maisel et al., 2016; Morie et al., 2019), although they did not evaluate how the distinct dimensions of these multidimensional constructs relate to each other. The second part of Study 2 aimed to extend this research, while also further exploring the nomological net of the AQ.

Results of a path analysis gave further support for the role of alexithymia as a mediator of the relationship between autism traits and trait anxiety in the general population (Barros, Figueiredo, & Soares, 2022). The model presented an acceptable fit and explained 37.9% of the variance of trait-cognitive anxiety and 26.6% of trait-somatic anxiety, suggesting that the indirect effect of autism traits through alexithymia on anxiety symptomatology was higher for cognitive than for somatic anxiety. Furthermore, while a significant positive correlation between the Social Skills dimension of the AQ and anxiety was previously observed in past studies (Bothe et al., 2019; Liss et al., 2008), as well as in the present study (please refer to Table S2 of the Supplementary Material of Study 2), when assessing the multivariate pattern of relationships between these variables and when controlling for alexithymia, a direct effect of autism traits in anxiety symptomatology was not observed. This emphasizes the need to consider alexithymia when addressing anxiety symptomatology in people with high levels of autism traits (Barros, Figueiredo, & Soares, 2022).

The Social Skills and RIDO dimensions of the AQ had a significant and moderate impact on DIF, while the Communication dimension of the AQ had a moderate impact on the remaining dimensions of alexithymia. This may reflect a relationship between impaired emotional processing and difficulties in social interaction

(Poquérusse et al., 2018; Scheerer, Curcin, et al., 2021), as well as an impact of attentional alterations, often described in the context of alexithymia (see Luminet et al., 2021; G. J. Taylor et al., 1997), in the ability to identify feelings and emotions (Barros, Figueiredo, & Soares, 2022). Furthermore, the fact that alexithymia significantly predicted anxiety symptoms seems to be consistent with the previous literature observing an association between these two variables (e.g., Bothe et al., 2019; Liss et al., 2008). These results also extended this literature by showing that, while the dimension of alexithymia corresponding to difficulties in identifying feelings had a large impact on both trait anxiety dimensions, the other dimensions of alexithymia (DDF and EOT) did not present a significant impact in any of the dependent variables. This was particularly interesting since the DDF dimension of alexithymia receives influence from the three dimensions of autism traits, but still did not show a significant impact in any of the anxiety dimensions. Although intriguing, these results are partially in line with previous evidence showing that the DIF is the alexithymia dimension that is more markedly associated with current anxiety symptomatology (e.g., Oakley et al., 2020).

Finally, the role of alexithymia (specifically difficulties in identifying feelings) in the relationship between autism traits (specifically social difficulties and patterns of restrictive interests and orientation to details) in the tendency to present high trait anxiety, especially trait-cognitive anxiety, was emphasized by the imposed model (Barros, Figueiredo, & Soares, 2022). A possible interpretation is that having greater difficulties in identifying feelings, as well as more social difficulties and an altered pattern of restricted interests and orientation to details, may be reflected in a more negative appraisal of social situations, for instance, which may favor the tendency to display cognitive anxiety (Barros, Figueiredo, & Soares, 2022; Sharma et al., 2014).

In sum, Study 2 provided a better comprehension of the mechanisms underlying the relationship between autism traits, alexithymia, and anxiety, considering the multiple dimensions composing these constructs. Importantly, results emphasized the need to include alexithymia, especially concerning difficulties in identifying feelings and emotions, as an important part of the interventions targeting anxiety symptomatology in people with a high manifestation of autism traits (Albantakis et al., 2020; Barros, Figueiredo, & Soares, 2022; Kinnaird et al., 2019; Milosavljevic et al.,

2016). Future studies should also explore the efficacy of interventions targeting alexithymia, including the assessment of their ability to reduce anxiety symptomatology and improve quality of life and well-being (Barros, Figueiredo, & Soares, 2022). In the same vein, the results of Study 2 support the view that adopting a broader, multidimensional assessment of these profiles of symptomatology is critical to adequately identify the needs for further assessment, intervention and prevention of psychopathology (Barros, Figueiredo, & Soares, 2022). These results, together with the results obtained in the psychometric study of the AQ, emphasize the importance of considering the distinct dimensions of autism and related variables when exploring their impact on the cognitive, emotional, and behavioral functioning of the spectrum.

Autism traits and trait anxiety are significant predictors of olfactory abilities

Although anxiety symptomatology was previously shown to be associated with alterations in sensory processing in autism (e.g., Ben-Sasson et al., 2008; MacLennan et al., 2020; Mazurek et al., 2013; Uljarević et al., 2016), research assessing specifically odor detection, odor discrimination and odor identification abilities did not account for the impact of individual differences in anxiety symptomatology. Study 3 extended this literature by assessing the multivariate relationship between autism characteristics, trait anxiety and olfactory abilities in the general population. The results of three hierarchical multiple regressions – one for each olfactory ability – showed that the model regarding odor discrimination was the only one achieving statistical significance (Barros et al., 2021). Specifically, the model for odor discrimination explained 13.5% of the variance and indicated that being a woman, having more attention to detail and less trait-somatic anxiety was associated with poorer odor discrimination. These results differ from previous evidence that did not observe a relationship between autism traits and olfactory abilities, in the general population (A. E. Robertson, 2012; Stafford et al., 2017). However, it is important to note that a total score of the AQ was used instead of the scores of the distinct autism dimensions in these studies. Our results, therefore and in line with Study 2, support the relevance of exploring the separated effect of several autism characteristics in olfactory perception (Barros et al., 2021).

When looking at studies with clinical samples, the pattern of results is very inconsistent. On the one hand, Ashwin et al. (2014) observed an association between autism traits and olfactory abilities, specifically regarding olfactory detection, in adults with autism. However, they did not evaluate how the expression of autism traits was associated with olfactory abilities in the typically developing group. Other studies that did not explore the expression of autism traits observed inconsistent results across olfactory abilities and developmental groups. For instance, they mostly observed normal odor detection abilities in adults (e.g., Galle et al., 2013; Tavassoli & Baron-Cohen, 2012) but impaired abilities in children (e.g., Dudova et al., 2011; Muratori et al., 2017), although several outer studies point to opposite results (e.g., Ashwin et al., 2014; Koehler et al., 2018; Sweigert et al., 2020). Regarding odor identification, although most studies suggest impaired abilities both in children (e.g., Bennetto et al., 2007; Sweigert et al., 2020) and adults with autism (e.g., Galle et al., 2013; Koehler et al., 2018), there is also evidence suggesting normal abilities (e.g., Dudova et al., 2011; Xu et al., 2020). Finally, previous studies observed normative odor discrimination in children (Muratori et al., 2017) and adults with autism (Galle et al., 2013), but the evidence is still too scarce to draw conclusions. This mixed pattern of results may be mostly due to differences in the methodology employed to assess olfactory abilities (tests and odor stimuli used), as well as in the recruited samples, which differed in age and possibly in other characteristics that were not properly assessed and controlled (e.g., anxiety). Nevertheless, our results are not directly comparable with studies involving clinical samples. Future studies should further explore the association between autism symptoms, olfactory abilities and individual differences in variables that may be relevant in these contexts, such as anxiety, in samples composed of individuals with and without autism. Longitudinal studies are also needed to further investigate how age influences olfactory processing in autism (e.g., May et al., 2011; Sweigert et al., 2020).

As argued in Study 3 (Barros et al., 2021), the fact that higher attention to detail was associated with higher odor discrimination provides further empirical support for the impact of attentional processes in sensory processing (e.g., Pessoa et al., 2003; see also Marco, 2011). The relationship between altered attentional processes and sensory alterations in autism was also discussed by previous studies (Baron-Cohen et al., 2009; Liss et al., 2006; Marco et al., 2011). The implications of having higher attention to

detail and, consequently, better odor discrimination remain to be explored in future research. We hypothesize that, while this pattern of characteristics may comprise an advantage in contexts that require an effective scanning of stimulus properties (e.g., for instance, it may be crucial to distinguish between food that is good to eat from spoiled food; Barros et al., 2021), it may confer a disadvantage in other contexts that require to "smell the big picture", such as in social contexts (see, for instance, Happé & Frith, 2006; Hill et al., 2014). In fact, previous studies also hypothesized an association between olfaction and social difficulties in autism (e.g., Bennetto et al., 2007; Endevelt-Shapira et al., 2018; Sweigert et al., 2020; Thye et al., 2018). In the real world, we are faced with different odors around us, which are frequently complex or masked, coming from a variety of sources namely from people, animals, objects, flowers, or food. These odors give us important information, which is integrated with cues from other senses to allow a proper and adaptive response (e.g., Pause, 2012; R. J. Stevenson, 2010). Possibly, having increased attention to detail and increased odor discrimination can make it difficult to appropriately select and prioritize important information in these contexts, and may even lead to a feeling of "sensory overload" (e.g., R. S. P. Jones et al., 2003 see Barros et al., 2021; Strömberg et al., 2022). Marco et al. (2011) further provided a useful discussion that is compatible with this line of thought, by suggesting that attentional abilities, particularly the abilities to properly select and filter sensory inputs and change the attentional focus from one feature to another, may be compromised in autism, which may pose difficulties to attend to the environment in a flexible way.

It is interesting that we did not observe a relationship between olfactory abilities and the social skills dimension, similarly to other studies with clinical population (e.g., Bennetto et al., 2007; Del Valle Rubido et al., 2020; Sweigert et al., 2020). Notably, the observed relationships involved the odor identification ability specifically, but these studies did not evaluate olfactory discrimination. Moreover, they are limited to clinical samples, and most were performed with children. Even when considering the general population, a previous study observed a relationship between mentalizing skills (a social dimension) and odor discrimination in women (Lübke et al., 2022). Yet, this study used a distinct test to assess odor discrimination, and the mentalizing abilities were assessed through a specific behavioral task; importantly, the social skills dimension of the AQ

used in the present study may not strongly reflect this social cognitive ability, which explains the absence of a relationship between odor discrimination and social skills in Study 3. Therefore, future research should extend these results to the whole autism spectrum and further investigate how olfactory abilities – in all dimensions, including the sniff response, for instance (e.g., Rozenkrantz et al., 2015) – and individual differences regarding autism-related variables are associated with social functioning and other relevant outcomes.

Our results also emphasize the role of trait anxiety in olfactory processing, specifically trait-somatic anxiety (Barros et al., 2021). This is partially consistent with previous studies since, while they found an association between olfaction and anxiety, studies exploring the relationship with odor discrimination specifically found opposite results (Havlíček et al., 2012; Krusemark & Li, 2012). Yet, once more, due to critical differences between studies (e.g., one measured neuroticism and the other measured state anxiety) direct comparisons cannot be made. Although the pattern of results is inconsistent, an association between high levels of anxiety and improved olfactory performance is often observed (e.g., D. Chen & Dalton, 2005; Havlíček et al., 2012; Krusemark & Li, 2012; La Buissonniere-Ariza et al., 2013; see Barros et al., 2021 for an overview). Previous evidence also observed a relationship between trait anxiety and sensory processing, particularly hypersensitivity, in the general population (e.g., Engel-Yeger & Dunn, 2011). Nevertheless, as argued in Study 3, anxiety may impair performance in tasks that require higher levels of working memory (Eysenck et al., 2007; e.g., Eysenck & Calvo, 1992), which is likely the case of olfactory discrimination (e.g., Hedner et al., 2010; Hummel et al., 1997; La Buissonniere-Ariza et al., 2013; Wilson & Stevenson, 2003; see Barros et al., 2021). Particularly, the self-reported physiological activation associated with anxiety symptomatology may play a particular role in this process, as suggested by our results (Barros et al., 2021).

While our results allowed a better understanding of the relationship between autism characteristics and olfactory processing, two aspects are worth discussing here (as also noted in Barros et al., 2021); first, Study 3 used the original dimensions of the AQ instead of the dimensions resulting from the analyses of Study 2. Yet, the reliability of the original subscales was analyzed before proceeding with the regression analyses, and only the dimensions showing adequate reliability were used – namely, the Social

Skills and Attention to Detail subscales. Therefore, our results are limited to these dimensions in particular. Lastly, a few participants presented more extreme values of autism traits; while this would be expected in a sample of the general population, this may have limited our analyses and, consequently, our conclusions about the relationship between autism traits and olfactory performance.

Olfaction as a critical piece to understand autism, sensory processing, and social behavior

Although sensory processing has been extensively studied in autism, olfaction has been less explored, despite its role in social functioning. Existent reviews about olfactory processing are mostly focused on olfactory abilities (Larsson et al., 2017; Martin & Daniel, 2014; Tonacci et al., 2015) and in the perception of common odors (Tonacci et al., 2015), which seem to be abnormal in autism. Yet, an integrated approach to what we know about olfaction in autism and how odors could influence social cognition and behavior in this condition was missing, and it could bring to light critical opportunities and challenges for future investigation – these were the main purposes of the last study of this thesis (Study 4).

In the first part, we briefly presented an overview of social cognition in autism, with a particular emphasis on social attention, especially attention to faces, and face processing (Barros & Soares, 2020). The focus on these two domains of social cognition was justified by the fact that both atypical social attention and face processing are observed since early development in children with an autism diagnosis and children at risk for autism (e.g., Chawarska et al., 2013; Dawson et al., 2004; Falck-Ytter et al., 2013; W. Jones et al., 2008). Plus, attending less to faces may influence the typical course of face processing development (Dawson et al., 2005). Importantly, impairments in these abilities may also be related to broader social difficulties, such as joint attention (e.g., Dawson et al., 2004) and theory of mind (e.g., Baron-Cohen, 1991), which highlights the need to understand the nature and extent of these social cognitive impairments, as well as the need to develop interventions that are able to tackle these difficulties while also providing long-term and real-life improvements (Barros & Soares, 2020).

This discussion allowed us to address some of the aspects of social attention and face processing that are still little understood, despite being extensively studied in autism (e.g., Chita-Tegmark, 2016; Guillon et al., 2014; Harms et al., 2010; Uljarevic & Hamilton, 2013; Weigelt et al., 2012); importantly, it was also put forward as an opportunity to show how and in which contexts olfaction could help to mitigate the difficulties observed in autism. To this end, in the second part of Study 4, some of the particularities of olfaction that have the potential of making it a privileged channel of socioemotional information, especially in the context of autism features, were presented; these particularities essentially include:

1) the overlap of cerebral structures involved in olfactory processing with structures of the limbic system (e.g., Gottfried, 2006). Some of these structures, such as the amygdala, were previously reported as abnormal in autism during face processing tasks, for instance (Schultz, 2005); yet, it is possible that socioemotionally salient olfactory cues are able to more easily recruit these areas;

2) the fact that olfactory information can reach the olfactory cortex directly, without passing through the thalamus (e.g., Merrick et al., 2014), a structure that was shown to be abnormal in autism (e.g., Nair et al., 2013);

3) the subliminal nature of olfaction (e.g., Smeets & Dijksterhuis, 2014), which could be critical in a population that often presents verbal language and behavioral problems;

4) the ability of olfaction to facilitate visual attention and processing, including face perception, which is also altered in autism (e.g., Leleu, Demily, et al., 2015; see Barros & Soares, 2020).

At this point, the following question arises: Does olfaction indeed improve social attention and facilitate visual processing in autism, and face processing in particular? Before directly addressing this question, it was important to contextualize the current knowledge about olfactory processing in autism. In line with previous reviews (Addo et al., 2017; Martin & Daniel, 2014; Tonacci et al., 2015), it was observed that olfactory abilities are either normal, enhanced or decreased in autism, and this mixed pattern of sensory processing may be due, at least in part, to the high

heterogeneity across studies regarding their samples and methodology. On the other hand, important gaps were also noted in this literature. Particularly, evidence regarding the processing of social (i.e., body odors) and non-social odors is very scarce and mostly focused on specific developmental groups (e.g., children, in the case of non-social odors), which precludes conclusions about the nature, extent and impact of altered olfactory processing in autism (Barros & Soares, 2020). Notwithstanding potential alterations in olfactory processing in autism and their impact on social, emotional and overall functioning (see Tonacci et al., 2015), previous research also suggested that olfactory stimuli may reduce social difficulties in children with autism (e.g., Parma et al., 2013, 2014), while also having the potential to facilitate other dysfunctional behaviors, such as eating behavior (e.g., Luisier, Petitpierre, Clerc Bérod, et al., 2019). However, studies supporting the beneficial role of olfactory stimuli in autism are still too scarce to understand the short- and long-term impact of olfaction on the behavior and functioning of this population, as well as the associated mechanisms.

In the last part of Study 4, some opportunities and potential hindrances associated with future research in olfactory processing and multisensory integration in autism were discussed, and the manuscript moved forward with concrete examples of how this could be operationalized – for instance, by integrating olfactory stimuli in previously studied visual attention and face perception paradigms (Barros & Soares, 2020). Furthermore, in the context of olfaction research in autism, it would be of value to explore if both social and non-social odors are able to influence socioemotional processing and behavior and if the extent of their impact is similar; although both types of odors were previously shown to be able to influence face perception in the general population, for instance (e.g., Rocha et al., 2018; Seubert et al., 2010), this is not well-understood in autism (Endevelt-Shapira et al., 2018). Endevelt-Shapira et al. (2018) explored the role of fear body odors in the processing of fearful facial expressions. Results suggested no behavioral effect of these fear body odors; however, it is worth noting that only prototypical fearful facial expressions were used in this study, and the effect of fear body odors was compared only with empty pads. It is possible that with more variable stimuli (e.g., other facial emotions), as well as with more dynamic and/or ambiguous stimuli the differences between groups at the baseline would be more evident – and, therefore, according to the principle of inverse effectiveness (Holmes &

Spence, 2005; Meredith & Stein, 1986), multisensory integration of fearful faces and fear body odors could be more pronounced.

Notwithstanding the relevance of studying distinct processes in isolation – namely visual and olfactory processing separately – the need to explore more complex and ecologically valid approaches was also emphasized in Study 4, since previous research suggests that differences between people with autism and their typically developing peers are more evident in more complex and realistic contexts (e.g., Chita-Tegmark, 2016). For this, multisensory paradigms would be useful, although challenging, since previous evidence suggests that multisensory integration processes may be generally impaired in autism (Baum et al., 2015; Feldman et al., 2018). However, this is particularly true when considering the combination of senses other than olfaction (e.g., visual and auditory stimuli); regarding visuo-olfactory integration, there is evidence supporting similar performances between adults with and without autism (Stickel et al., 2019). Yet, research exploring the integration of olfactory information with cues from other sensory modalities in autism is also lacking, whereby future studies should further explore these mechanisms.

The need to measure and control for the characteristics of the spectrum that can modulate differences in olfactory performance (as also discussed in Martin & Daniel, 2014) was another point discussed in Study 4. In this thesis, the role of anxiety was emphasized, given its high comorbidity with autism (e.g., Hollocks et al., 2019), its previously shown association with altered sensory processing in autism (e.g., Ben-Sasson et al., 2008; MacLennan et al., 2020; Mazurek et al., 2013; Uljarević et al., 2016), and its role in olfactory perception in the general population (e.g., Havlíček et al., 2012; Takahashi et al., 2015). As previous research did not evaluate the role of anxiety on olfactory abilities of the autism spectrum – particularly considering the expression of autism traits in the general population – this was the aim of Study 3 (Barros et al., 2021). As also discussed in Study 4, studying autism from a dimensional perspective would be useful to extend our understanding of the subclinical manifestation of autism and its relationship with the clinical side of the spectrum (e.g., Ingersoll & Wainer, 2014; see also Barros and Soares, 2020). In addition, it would allow to assess the characteristics of autism taking into account their full range of variability, since in the general population people may only present some of the key-features more markedly,

and distinct profiles of symptoms may occur (e.g., Palmer et al., 2015). This would be critical to understand the heterogeneity observed in autism, as well as the contribution of distinct autism components to the several alterations reported in this population (Happé & Frith, 2020; Landry & Chouinard, 2016) – which is the reason why a dimensional perspective was also considered in the current thesis.

4.2. Implications

The results provided by the four studies included in this thesis pave the way for research that intends to further extend the knowledge about autism and olfactory processing, particularly when considering a dimensional perspective. Many studies have observed a continuous distribution of autism characteristics in the general population (e.g., Constantino & Todd, 2003; Hoekstra et al., 2007; Ingersoll & Wainer, 2014; A. E. Robertson & Simmons, 2013). Furthermore, accumulating evidence from past studies has been providing further support for the association between these subclinical autism traits and many of the alterations described in clinical autism, such as sensory processing problems (e.g., Horder et al., 2014), anxiety and depression (e.g., Kanne et al., 2009), and atypical facial emotion processing (e.g., English et al., 2017). The results of Study 2 and Study 3 also provide empirical support for the relationships between autism traits, anxiety, alexithymia and olfactory alterations (Barros et al., 2021; Barros, Figueiredo, & Soares, 2022). Improving the multidimensional assessment of autism-related features, such as autism traits and anxiety symptoms, are important steps to further understand the BAP and autism from a dimensional perspective, as well as to understand the previously observed mixed findings regarding olfactory processing in autism. Providing accessible, brief, and accurate instruments, capable of reliably measuring individual differences considering distinct dimensions, is critical for this purpose, and was the aim of Study 1 and Study 2 (Barros, Figueiredo, & Soares, 2022; Barros, Figueiredo, Brás, et al., 2022).

Increased anxiety levels are not only often observed in autism, but are also frequently observed in the general population as well (e.g., Castaldelli-Maia & Bhugra, 2022; Steel et al., 2014). Importantly, anxiety disorders have been associated with many physical and psychological problems (e.g., Celano et al., 2016; Goldstein-Piekarski et

al., 2016; Johnson, 2019; Lamers et al., 2011), as well as with negative outcomes, such as poor quality of life and social functioning (e.g., Saris et al., 2017; Wilmer et al., 2021). Although self-report is an accessible and brief means of assessing anxiety symptomatology, some of the available instruments present limitations; for instance, regarding their abilities to assess multiple anxiety dimensions, and to distinguish anxiety from depression (e.g., Kennedy et al., 2001). The STICSA has the potential of overcoming (or partially mitigating at least) some of these limitations, by assessing anxiety symptomatology in four distinct dimensions, namely state-cognitive, state-somatic, trait-cognitive and trait-somatic anxiety (Ree et al., 2008). Instruments like the STICSA, i.e., that assess changes in multiple dimensions of anxiety symptomatology, can bring substantial benefits for both research and clinical practice by providing a better characterization of the distinct anxiety profiles presented by different individuals, which can be critical to understand the clinical presentation of anxiety across distinct populations, and to provide an accurate assessment, intervention and monitoring (e.g., Elwood et al., 2012; Ree et al., 2008; Schouten et al., 2020; Schwartz et al., 1978; see also Barros et al., 2022). Considering the often-observed association between trait anxiety and olfactory processing (e.g., La Buissonniere-Ariza et al., 2013; Takahashi et al., 2015), as also corroborated by our results (Barros et al., 2021), the STICSA can be especially useful in future studies that further explore these relationships in the Portuguese context.

Instruments able to reliably assess and capture the variability of autism traits in the general population – as well as in the whole spectrum – are also needed to further explore the BAP and its relationship with the cognitive, emotional, and behavioral functioning of the spectrum. The work developed in Study 2 provided psychometric support for the AQ; furthermore, these analyses were conducted for the first time in a sample of the Portuguese population (Barros, Figueiredo, & Soares, 2022). This work allowed us not only to extend the existing knowledge about the factor structure and nomological network of the AQ, but also to provide a useful tool for future research in this area, especially for future investigations developed in the Portuguese context. Apart from its potential in research, the AQ may also be useful for screening and referral. For instance, the AQ total score was previously observed to be highly correlated with a measure of autism symptoms, namely the SRS-A, in a sample of adults referred for

autism assessment (Bezemer et al., 2021). Importantly, in the same study, the AQ demonstrated an acceptable ability to predict an autism diagnosis, performing better than the SRS-A (Bezemer et al., 2021). Nevertheless, future studies should extend this knowledge by further exploring the psychometric properties of the AQ and its utility and predictive validity in clinical settings.

Study 2 may also bring additional implications for intervention and future research. On the one hand, its results suggested that including alexithymia, and especially difficulties in identifying feelings and emotions, in assessment and intervention plans that aim to reduce anxiety symptomatology in people with high levels of autism traits may be critical (Albantakis et al., 2020; Barros, Figueiredo, & Soares, 2022; Kinnaird et al., 2019). Yet, future research should further investigate the efficacy of such interventions, as well as their impact on functioning and well-being as this was not explored in the present work (see Cameron et al., 2014). Furthermore, it is important to note that the multivariate relationship between autism traits, anxiety and alexithymia was explored exclusively in the general population, whereby generalizations for the clinical spectrum cannot be made based on the present work. On the other hand, Study 2, together with Studies 1 and 3, provide further support for the multidimensional nature of the assessed constructs and a differential pattern of relationships between the subconstructs (Barros et al., 2021; Barros, Figueiredo, & Soares, 2022; Barros, Figueiredo, Brás, et al., 2022). This emphasizes the need to use a multidimensional approach when studying (or screening for) anxiety, alexithymia, autism symptomatology and olfactory abilities, to better understand the mechanisms underlying these relationships and their impact on the variables of interest. It is also equally important to keep this in mind when planning the assessment of these symptoms and when planning interventions adjusted to people's patterns of needs and strengths.

Research in the BAP has also been proven useful to understand the relative contribution of distinct autism dimensions to some of the observed alterations in the spectrum (e.g., Barros et al., 2021; J. Davis et al., 2017). This objective is more difficult to accomplish if we look at autism from a categorical and “exclusively clinical” perspective because there is decreased variability in the manifestation of the different autism key characteristics in people with the diagnosis (see Barros, Figueiredo, & Soares, 2022; Landry & Chouinard, 2016). Furthermore, barriers in the research with

clinical populations are often encountered, including the difficulty in recruiting sex and gender-balanced samples, as well as difficulties in controlling for certain variables that are very prevalent in autism. Investigating the BAP is, thus, critical to understand autism, including the relationship between the clinical and nonclinical presentations of autism traits (Barros, Figueiredo, & Soares, 2022; Landry & Chouinard, 2016). Although research about the BAP has been increasing exponentially in the last years, much remains to be understood, including sensory processing – and olfactory processing in particular. Unlike most previous studies, Study 3 addressed the relationship between autism traits, anxiety and olfactory in a multidimensional perspective, while using a psychophysical method for olfactory evaluation. This adds to previous research that used self-report instruments to assess sensory processing (e.g., Horder et al., 2014; Mayer, 2017; A. E. Robertson & Simmons, 2013), as well as to previous evidence that, despite also using the Sniffin' Sticks to evaluate olfactory abilities, did not explore the impact of distinct autism dimensions (and of other variables, such as anxiety; e.g., A. E. Robertson, 2012). Moreover, many studies performed in clinical samples gave higher emphasis to odor identification and odor detection abilities, with very few assessing odor discrimination (Galle et al., 2013; Muratori et al., 2017). Although the results of Study 3 cannot be generalized for the clinical spectrum, one could argue that the often-observed inconsistent results found in the literature of olfactory processing may be due, at least in part, to individual differences in anxiety symptomatology – and possibly in other variables that have not yet been explored. Therefore, Study 3 provides important information for future studies further exploring how the olfactory abilities are related with variations in autism symptoms, trait anxiety and other relevant variables across the whole spectrum (Barros et al., 2021).

Altered sensory processing is nowadays recognized in the formal diagnostic criteria of autism (American Psychiatric Association, 2022). These alterations are present throughout development (e.g., Crane et al., 2009; DuBois et al., 2017) and influence these people's lives and well-being. For instance, sensory alterations in general, such as hypo- and hypersensitivity to sensory stimuli, seem to be associated with behavioral problems (e.g., regarding sleep behaviour; Reynolds et al., 2012), mental health conditions (e.g., anxiety; MacLennan et al., 2020), social difficulties (e.g.,

Kojovic et al., 2019; Lundqvist, 2015), poorer adaptive function (e.g., Kojovic et al., 2019), and increased levels of repetitive behaviors (e.g., Glod et al., 2019; Wigham et al., 2015) in people with autism (see also Glod et al., 2015 for a review). Sensory alterations, and especially taste and olfactory hypersensitivity, also seem to play a significant role in food selectivity and eating behavior in autism (Nimbley et al., 2022; Zulkifli et al., 2022). Yet, the nature and impact of olfactory alterations, especially in other areas of functioning (e.g., social functioning), is still poorly understood. As argued by Endevelt-Shapira et al. (2018, p. 111), "social dysosmia", i.e., distorted perception of social chemosignals, "could be devastating" in autism, due to its potential role in broader emotional and social functioning.

Therefore, as stated throughout this thesis and especially in Study 4 (Barros & Soares, 2020), extending the research on sensory processing in autism, particularly in olfactory processing, should be a research priority and may entail important clinical and research implications. Study 4 hopefully presented an innovative and integrated overview of the relevant literature about social cognition and olfactory processing in autism, going further with the discussion of several proposals about how olfactory processing and visuo-olfactory processing can be addressed and how this research can impact research and practice on social behavior, eating behavior, and emotional functioning in this neurodevelopmental condition (see Barros & Soares, 2020). The potential of olfaction to modify behavior and improve emotional, social, and behavioral functioning in autism has also been discussed in Study 4, as well as in previous research (e.g., Endevelt-Shapira et al., 2018; Hrdlicka et al., 2011; Tonacci et al., 2015). For instance, body odors may play a particularly relevant role in the social context, due to their differential processing and potential to activate the social and emotional centers of the brain (e.g., Lundström et al., 2008). And, in fact, maternal body odors were previously shown to facilitate social behavior in children with autism (e.g., Parma et al., 2013). Outside the social context, previous research also showed the potential of common odors to mitigate food difficulties in autism (Luisier, Petitpierre, Clerc Bérod, et al., 2019). However, research about the influence of (altered) olfactory perception and odors in the cognitive, emotional, and behavioral functioning of the spectrum is still very scarce. It would be important to extend previous studies and explore how different types of odor stimuli (e.g., social vs. non-social, familiar vs. non-familiar) are processed

throughout development and considering individual differences (e.g., severity of symptoms/autism traits, alexithymia; Barros & Soares, 2020).

Another notable implication of extending this research is the potential of olfaction to be a non-invasive, inexpensive marker assisting and improving clinical assessment and diagnosis (Barros & Soares, 2020; Tonacci et al., 2015). Despite the efforts of researchers to identify reliable diagnostic markers in autism (e.g., Anderson, 2015; Ecker, 2017; Frye et al., 2019), the autism diagnosis is still currently based on clinical observation and assessment of behavioral symptoms (e.g., Hus & Segal, 2021; McPartland et al., 2020). Methodological limitations of research investigating autism, difficulties inherent to the conceptualization of the condition, as well as its complexity and heterogeneity are some of the factors hindering the establishment of markers with adequate performance (e.g., Anderson, 2015; Frye et al., 2019; Klin, 2018; McPartland et al., 2020; Walsh et al., 2011). Stratification markers could be particularly critical to define more "biologically homogeneous" subgroups of autism, which would allow us to learn more about autism in general and its heterogeneity (Anderson, 2015; Lord et al., 2022; Loth et al., 2016). Olfactory dysfunction has been often associated with neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease (e.g., Rahayel et al., 2012), and has been proposed as an early marker of these disorders (Haehner et al., 2009; Murphy, 2019). Previous studies also suggested that olfactory dysfunction could constitute a biomarker for autism (e.g., Hrdlicka et al., 2011; Muratori et al., 2017). However, the nature of olfactory alterations and the mechanisms underlying the often-observed heterogeneity across studies need to be further understood to evaluate the potential of olfactory alterations to assist a more objective assessment, diagnosis and understanding of autism.

I conclude this line of thought with the question posed by Fletcher-Watson and Happé (2019, p. 62): "If a biomarker for autism was found (…) Would people with real needs be denied help because they don't meet this new biological criterion for autism?". The results of Study 2 may be used as an example that helps to answer this question; this study suggested that people with higher levels of autism traits – especially concerning social skills and/or restrictive interests and detail orientation – and increased difficulties in identifying feelings, may present elevated levels of anxiety, especially cognitive anxiety (Barros, Figueiredo, & Soares, 2022) – apart from their potential

struggles with social situations and their attentional particularities. This means that, even without a formal diagnosis of autism or anxiety, this profile of symptoms may cause substantial suffering and may impact these people's well-being and quality of life. Therefore, even without a formal diagnosis or a "biological criterion for autism", there is a need to intervene and adopt preventive and mental health promotion strategies for people expressing high levels of autism traits (Barros, Figueiredo, & Soares, 2022). Although there is a need to invest in tools and mechanisms that assist an early and correct identification of autism from a clinically significant perspective, we believe that these should be used as a complement to clinical evaluation and a multidimensional assessment (e.g., Frye et al., 2019), with a focus on the well-being and quality of life of the individuals.

4.3. Strengths and limitations

Across four studies, the present work sought to extend the current understanding of the relationships between autism, emotion-related variables – namely anxiety and alexithymia – and olfactory processing, considering a multidimensional perspective. Hopefully, adopting this approach and studying these variables in the general population allowed a broader and complementary comprehension of the phenomena and paved the way for future investigation that wishes to extend this knowledge to the clinical spectrum. Although research about the BAP, and particularly the expression of autism traits in the general population, has increased to a great extent in the last years, the relationship between autism traits and several areas of functioning is still poorly understood – as is the case of olfactory processing. This study proposed to contribute to the understanding of this area, investigating autism in a dimensional perspective from multiple points of view, starting with the understanding of the measurement of autism traits, and finishing with an integrative review that tackles some important gaps in the literature and proposes concrete new ideas of research. To ensure an adequate, reliable, and comprehensive measurement of the constructs of interest, instruments assessing these constructs multidimensionally were selected and further analyzed. In Study 1, we went further by providing a detailed psychometric study of the STICSA which included the analysis of STICSA-Trait predictive validity; this is a dimension of the instrument's

validity that is often underexplored. Notwithstanding the limitations in the interpretation of the results of Study 1, this information can be very useful, for instance, for future research exploring how people with distinct anxiety profiles respond in specific emotional situations. Moreover, studies addressing the predictive validity of the STICSA are lacking (Ree et al., 2008). Finally, while Study 4 intended to provide an overview of the current knowledge about olfactory processing in autism, it hopefully also provided a step forward by presenting concrete proposals for advancing research in the area.

Notwithstanding the considered strengths, the present work is not exempt from limitations. As some of them were specific to the different studies and were already discussed in the respective manuscripts – as well as in the main results section – here we will emphasize the major limitations and especially those that were common across studies. First, the sample size may be a limitation, at least in some of the studies; for instance, in the second part of Study 1, the sample recruited to explore the relationship between the STICSA-Trait scores and HRV may have limited the variability in anxiety profiles and, therefore, may have influenced our results (Barros, Figueiredo, Brás, et al., 2022). Similarly, the samples recruited for Study 2 (especially the psychometric study of the AQ) and Study 3 were also small, and although we did not lack statistical power for the performed analyses, it is possible that we did not capture all the variability in the expression of autism traits (Barros et al., 2021; Barros, Figueiredo, & Soares, 2022). In fact, only a few participants scored in the extremes of the scale, although this would be expected in a sample of the general population (e.g., Baron-Cohen et al., 2001). It can be sometimes challenging to recruit people scoring at the lower and high extremes of the autism traits scale, which frequently limit the interpretability of results; furthermore, the actual context of the COVID-19 pandemic posed extra hindrances, which limited our ability to recruit larger samples and, consequently, of obtaining higher variability and exploring other variables and analyses.

Finally, we did not evaluate the expression of autism traits across the whole spectrum, which prevents us from generalizing our conclusions to the clinical population. Some studies observed distinct patterns of relationships in clinical and nonclinical samples; for instance, Albantakis et al. (2020) observed that the pattern of relationships between autism traits, alexithymia and trait anxiety differed in the clinical

and nonclinical groups. Furthermore, Mayer et al. (2017) also observed slightly distinct relationships between autism traits and sensory processing patterns in the clinical and nonclinical groups. These differences may be due to the combined presentation of symptoms observed in clinical autism, and/or to other features that are not as observed in the general population as in the clinical group – or at least in the same level of expression (Barros, Figueiredo, & Soares, 2022). Yet, these relationships should be further explored in future research to see if the pattern of results is consistent across the spectrum and, in case it is not, which variables possibly underly these differences.

4.4. Future studies

Starting with the last point discussed in the previous section, it would be important to extend this research to the clinical population. This would be critical to understand if the pattern of results found in Study 2 (path analysis) and Study 3 is consistently observed across the spectrum, or if distinct relationships are observed when a diagnosis is present. Extending the psychometric analyses performed in Study 1 and Study 2 to clinical samples would be critical for this purpose and to reliably compare clinical and nonclinical groups in the future. Although the STICSA seems to be an adequate instrument to measure the state-cognitive, state-somatic, trait-cognitive, and trait-somatic dimensions of anxiety in the general population, this instrument is not yet validated for other conditions and populations where unique patterns of anxiety symptoms may be present, including autism. Anxiety assessment across medical conditions is frequently based on instruments validated for the general, neurotypical population, which does not allow us to understand if these instruments are interpreted in an equivalent way across groups and if they are able to reach the complex manifestation of anxiety in certain populations (see Kerns et al., 2014; Kerns & Kendall, 2014). In the context of the present thesis, and since individuals with autism often present co-occurring anxiety disorders and have an atypical manifestation of anxiety (e.g., Kerns & Kendall, 2012, 2014), future studies should further explore the STICSA's usefulness and adequacy to measure this symptomatology in autism – and, if necessary, the use of the STICSA should be complemented with other instruments designed to measure the specificities of anxiety in autism (e.g., Rodgers et al., 2020). Furthermore, as self-report

may be often problematic in autism (e.g., S. L. Bishop & Seltzer, 2012; Mazefsky et al., 2011), the use of more objective measures of anxiety (such as physiological indicators) would be crucial to complement the information gathered by self-report, parent-report (in the case of children and adolescents) and the clinician's observation (e.g., Kerns & Kendall, 2012; Vasa et al., 2018). For this, it is crucial to develop and test contactless, non-invasive and inexpensive instruments, to surpass difficulties often encountered due to sensory hypersensitivities and communication problems; studies exploring the adequacy of radar-based systems in this context may be useful (e.g., Gouveia et al., 2020).

Future studies should also recruit larger samples with a broader representation of autism traits and should use standardized methods to explore olfactory abilities in the general population. This, hopefully, will allow us to understand previously observed inconsistent results in the area. Furthermore, it is also critical to explore the role of other variables that were not controlled or explored in previous studies and that may also help to understand the complex pattern of olfactory processing in autism, such as cognitive abilities (e.g., Hedner et al., 2010) and odor awareness (e.g., Arshamian et al., 2011). A step forward in this research could also include the analysis of the relationship between autism traits and the processing of common and social odors. In fact, studies exploring odor's pleasantness and intensity in autism are very scarce and heterogeneous regarding their samples (e.g., children vs. adults), odors used (e.g., different types of common odors), assessed dimensions (e.g., pleasantness vs. intensity), and methods to measure the response (e.g., subjective response vs. psychophysiological response; Addo et al., 2017; Galle et al., 2013; Hrdlicka et al., 2011; Legisa et al., 2013; Rozenkrantz et al., 2015; Sweigert et al., 2020; Wicker et al., 2016; Xu et al., 2020). Similarly to the proposed for olfactory abilities, exploring additional variables that may have an impact on these mixed results may also be useful. For instance, as previous research observed that alexithymia influences psychophysiological responses to common odors of different valences, and that distinct dimensions of alexithymia are related to distinct aspects of olfactory processing (e.g., performance in the olfactory tests, reaction time in the rating task and psychophysiological response; Cecchetto et al., 2017), future research should explore how distinct aspects of alexithymia, together with autism characteristics and other relevant variables, influence olfactory processing in autism.

Finally, it would be interesting to further explore both the consequences of altered olfactory functioning and the role of odors of distinct socioemotional nature in the social and emotional functioning of the autism spectrum (Barros & Soares, 2020; see also Endevelt-Shapira et al., 2018; Rozenkrantz et al., 2015; Tonacci et al., 2015). As several concrete examples of future studies and how to operationalize them were already described both in Study 4 and in the main results section, here it is emphasized the need to test and provide empirical support for the formulated hypotheses in order to deconstruct the complex picture of olfactory perception in autism. Studying how odors impact social cognitive processes (e.g., social attention, biological motion processing, facial emotion processing) in function of the expression of autism traits would also be of value to understand the influence of the distinct facets of autism in the potential role of olfaction as a social facilitator in autism.

4.5. Conclusion

Throughout four studies, the present research contributed to a broader understanding of autism, considering a dimensional perspective, olfactory processing, anxiety, and alexithymia. Study 1 provided empirical support for the adequacy and utility of the STICSA to measure anxiety considering trait-cognitive, trait-somatic, state-cognitive, and state-somatic anxiety, in the general population. In addition, the results of Study 2 supported a 3-factor structure of the AQ, in line with previous evidence and consistent with the core features of autism. The results of Study 2 also denoted the role of alexithymia as a mediator of the relationship between autism traits and trait anxiety. In this regard, a full mediation model was supported and highlighted the role of DIF (alexithymia) in the relationship between autism traits (Social Skills and RIDO) and trait anxiety (especially trait-cognitive anxiety). Study 3 also provided support for the role of sex, attention to detail and trait-somatic anxiety as significant predictors of olfactory discrimination, emphasizing the need to explore the distinct dimensions of these variables in the evaluation of olfactory function. Lastly, Study 4 provided an integrative view of olfactory processing in autism, illuminating possible paths for future research in the field of social cognition and behavior, as well as multisensory integration in autism. Hopefully, these results will be useful to inform

future steps on the challenging road that leads to a better understanding of autism, and that provides adequate, timely, innovative, and inclusive solutions for the needs of these people.

CHAPTER 5 – BIBLIOGRAPHY

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CHAPTER 6 – APPENDIX

Supporting Information – Study 1

Cardiac signal processing

- Considering that the HRV evaluates the variation inherent to inter-beat intervals, the first step on the algorithm was the R-peak detection.
    - R-peaks were detected using a widely known algorithm (Kathirvel et al., 2011), with a Random Sample Consensus (RANSAC) window size of 5.
    - The cut-off frequencies of the band pass filter were 5 Hz and 15 Hz, respectively.
- The RR intervals were obtained by the consecutive differences between two R-peaks.
- A pre-processing step was performed to guarantee robustness of the data; in this step, outliers were removed by ignoring R-R differences below 300 ms or above 2000 ms, according to the best practices described by Inbar and colleagues (1994).
- Furthermore, ectopic beats were removed using the process described by Kamath and colleagues (1995), with the Malik method (Malik, 1995), considering a removing rule of 0.2.

Calculation of the HRV indexes

- The HRV metrics were calculated over the normal-to-normal beats.
- All the frequency domain features were computed using the Welch method, considering a sampling frequency of 7 Hz, a linear interpolation, an LF band between 0.04 Hz and 0.15 Hz and a HF band between 0.15 Hz and 0.40 Hz.
    - This process was performed both in the baseline cardiac signal, and in the signal recording during emotional induction.
- The chosen HRV metrics were calculated over a window of at least 5 minutes of duration to allow the method to infer the individual variability; the analysed baseline had 5 minutes of duration, and the emotional segment of cardiac signal had approximately 30 minutes of duration.
    - **Note:** The duration of the segments may influence the evaluation and limit comparisons between segments with distinct durations, since the information and its variability may be affected by external events. Nevertheless, in this study all the emotional segment (30 minutes) was used to describe the full emotional process.
- The code underlying all these analyses was implemented in Python 3.6 using the numpy and hrvanalysis libraries. Finally, and as expected (e.g., Castaldo et al., 2015), the HRV measures did not meet the assumption of normality and were thus log-transformed (log10) after inspecting skewness and kurtosis.

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S1 Fig. Cardiac signal processing and calculation of the HRV indexes.

S2 Table. Means and standard deviations regarding psychophysiological and self-report measures, considering trait-cognitive anxiety groups.

		LowCG (N=41)				HighCG (N=33)			
		Baseline		Emotion		Baseline		Emotion	
Measure	Condition	M	SD	M	SD	M	SD	M	SD
LF	Fear	823.94	705.07	930.15	539.20	830.47	702.73	941.69	577.65
	Neutral	744.99	427.57	994.43	566.85	772.22	608.58	1132.24	729.42
	Happy	719.11	428.33	1139.23	612.66	741.16	700.67	994.72	601.99
HF	Fear	537.49	648.04	525.83	506.13	464.42	390.11	444.93	318.18
	Neutral	466.94	351.60	428.10	294.90	483.57	395.87	540.17	489.04
	Happy	545.98	520.77	556.30	445.94	416.45	339.60	425.30	340.50
LF/HF	Fear	2.77	2.82	2.93	2.43	2.67	1.82	2.76	1.63
	Neutral	2.30	1.53	3.15	1.99	2.52	2.11	3.07	1.81
	Happy	2.54	2.29	3.16	2.02	2.36	1.65	3.12	1.73
Happy	Fear	50.66	25.33	25.93	25.17	46.33	19.21	27.67	25.08
	Neutral	51.79	23.72	45.31	26.97	46.84	21.66	45.94	25.57
	Happy	47.62	25.62	73.84	19.22	39.21	24.95	66.19	20.03
Fear	Fear	5.20	13.79	37.99	34.04	6.88	13.62	37.76	31.40
	Neutral	4.40	14.13	0.44	1.72	11.72	16.64	4.99	10.88
	Happy	5.76	15.55	0.34	0.88	7.34	13.98	3.47	9.61
Arousal	Fear	35.82	30.67	78.78	23.10	30.22	25.54	69.30	27.18
	Neutral	34.25	28.40	26.59	24.75	36.14	26.71	35.35	28.52
	Happy	31.12	25.39	72.74	20.33	38.78	26.35	63.19	25.73

S3 Table. Means and standard deviations regarding psychophysiological and self-report measures, considering trait-somatic anxiety groups.

		LowSG (N=42)				HighSG (N=32)			
		Baseline		Emotion		Baseline		Emotion	
Measure	Condition	M	SD	M	SD	M	SD	M	SD
LF	Fear	734.60	661.05	874.66	489.28	947.93	739.33	1014.87	625.73
	Neutral	681.77	441.59	1068.01	662.84	856.04	585.61	1039.97	627.28
	Happy	667.16	382.75	1065.04	592.59	810.03	733.54	1087.58	637.16
HF	Fear	557.91	641.85	535.45	496.46	435.33	385.32	429.77	326.17
	Neutral	525.36	382.95	526.20	409.054	407.42	345.45	414.92	371.41
	Happy	593.48	523.28	580.89	458.67	350.05	286.39	388.93	294.23
LF/HF	Fear	2.34	2.47	2.51	2.07	3.24	2.27	3.30	2.09
	Neutral	1.91	1.38	2.67	1.53	3.04	2.09	3.68	2.18
	Happy	2.20	2.27	2.86	1.92	2.80	1.61	3.51	1.81
Happiness	Fear	49.75	24.53	24.55	26.20	47.40	20.51	29.53	23.36
	Neutral	49.51	24.36	41.05	26.53	49.69	20.97	51.54	24.86
	Happy	41.66	26.25	69.15	21.47	46.78	24.59	72.12	17.63
Fear	Fear	5.00	13.61	40.38	32.40	7.19	13.80	34.50	33.23
	Neutral	6.52	15.00	0.85	2.13	9.16	16.54	4.59	11.14
	Happy	5.41	15.05	0.57	1.47	7.84	14.43	3.26	9.75
Arousal	Fear	33.21	30.88	76.97	26.03	33.49	25.38	71.38	24.28
	Neutral	32.81	29.20	23.99	24.26	38.09	25.20	39.03	27.65
	Happy	28.09	25.50	67.57	24.75	43.01	24.34	69.67	21.39

S4 Table. Results of the repeated measures ANOVA, regarding differences between the baselines of the three emotional conditions.

	F	**p**	**η^2**	**Post-hoc tests**
LF	0.189	.809	.003	NA
HF	0.502	.606	.007	NA
LF/HF	0.910	.405	.012	NA
Happiness	3.352	.043	.044	All n.s.
Fear	0.375	.688	.005	NA
Arousal	0.142	.845	.002	NA

Note. NA: Not applicable; n.s.: nonsignificant.

S5 Table. Results of the repeated measures ANOVA, regarding differences between groups considering the three emotional conditions at the baseline moment.

Variables		Condition				Group				Condition x Group			
		F	**p**	**η^2**	**Post-hoc tests**	**F**	**p**	**η^2**	**Post-hoc tests**	**F**	**p**	**η^2**	**Post-hoc tests**
Cognitive Groups	**LF**	0.223	.781	.003	NA	0.014	.905	.000	NA	0.562	.571	.008	NA
	HF	0.464	.630	.006	NA	0.047	.829	.001	NA	0.208	.813	.003	NA
	LF/HF ratio	0.962	.384	.013	NA	0.122	.727	.002	NA	0.195	.823	.003	NA
	Happiness	3.536	.037	.047	All n.s.	1.513	.223	.021	NA	0.419	.658	.006	NA
	Fear	0.531	.589	.007	NA	2.026	.159	.027	NA	1.296	.277	.018	NA
	Arousal	0.246	.758	.003	NA	0.068	.796	.001	NA	1.922	.150	.026	NA
Somatic Groups	**LF**	2.94	.745	.004	NA	1.672	.200	.023	NA	1.219	.298	.017	NA
	HF	0.318	.728	.004	NA	1.773	.187	.024	NA	1.339	.265	.018	NA
	LF/HF ratio	0.814	.445	.011	NA	7.513	.008	.094	HighSG>LowSG	0.396	.674	.005	NA
	Happiness	2.837	.068	.038	NA	0.041	.840	.001	NA	1.252	.289	.017	NA
	Fear	0.376	.688	.005	NA	0.935	.337	.013	NA	0.006	.994	.000	NA
	Arousal	0.269	.742	.004	NA	1.856	.177	.025	NA	2.407	.094	.032	NA

Note. NA: Not applicable; n.s.: nonsignificant; LowCG: Low trait-cognitive anxiety group; HighCG: High trait-cognitive anxiety group.

S6 Table. ANOVA's results regarding the psychophysiological measures, considering trait-cognitive anxiety groups.

		LF				HF				LF/HF Ratio			
		F	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**
Main effects	**Condition**	0.785	.458	.011	NA	0.136	.873	.002	NA	0.177	.838	.002	NA
	Moment	96.052	p<.001	.572	Pre<Post	6.165	.015	.079	Pre<Post	72.038	p<.001	.500	Pre<Post
	Group	0.006	.937	<.001	NA	0.049	.826	.001	NA	0.105	.747	.001	NA
Second-order interaction effects	**Condition x Moment**	2.850	.061	.038	NA	1.275	.281	.017	NA	8.974	p<.001	. 111	Pre: No ≠ across conditions Post: F<H, p=.049 Pre<Post across conditions, p<.001
	Condition x Group	1.514	.224	.021	NA	0.943	.392	.013	NA	.177	.838	.002	NA
	Moment x Group	0.046	.831	.001	NA	0.007	.934	<.001	NA	.114	.736	.002	NA
Third-order interaction effects	**Condition x Moment x Group**	1.303	.275	.018	NA	1.730	.181	.023	NA	.101	.904	.001	NA

Note. NA: Not applicable; Pre: evaluation before the emotional induction (baseline); Post: evaluation after the emotional induction (emotion condition); F: Fear condition; H: Happy condition.

S7 Table. ANOVA's results regarding the self-report measures, considering trait-cognitive anxiety groups.

		Happiness				Fear				Arousal			
		F	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**
Main effects	**Condition**	34.978	p<.001	.327	F<N<H	59.138	p<.001	.451	F>N, F>H	31.853	p<.001	.307	F>N, F>H
	Moment	0.054	.818	.001	NA	30.229	p<.001	.296	Pre<Post	127.321	p<.001	.639	Pre<Post
	Group	0.905	.345	.012	NA	1.670	.200	.023	NA	0.072	.789	.001	NA
Second-order interaction effects	**Condition x Moment**	82.991	p<.001	.535	Pre: No ≠ across conditions Post: F<N<H, p<.001 Pre>Post in F, Pre<Post in H, p<.001	60.848	p<.001	.458	Pre: No ≠ across conditions Post: F>N, F>H, p<.001 Pre<Post in F, Pre>Post in N and H, p<.01	54.709	p<.001	.432	Pre: No ≠ across conditions Post: F>N, H>N, p<.001 Pre<Post in F and H, p<.001
	Condition x Group	1.292	.278	.018	NA	1.052	.352	.014	NA	2.601	.078	.035	NA
	Moment x Group	1.419	.238	.019	NA	0.147	.703	.002	NA	1.321	.254	.018	NA
Third-order interaction effects	**Condition x Moment x Group**	0.300	.742	.004	NA	0.174	.840	.002	NA	3.414	.036	.045	In N condition: the LowCG had Pre>Post, p=.025; the HighCG had Pre=Post

Note. NA: Not applicable; Pre: evaluation before the emotional induction (baseline); Post: evaluation after the emotional induction (emotion condition); F: Fear condition; H: Happy condition; N: Neutral condition; LowCG: Low trait-cognitive anxiety group; HighCG: High trait-cognitive anxiety group.

S8 Table. ANOVA's results regarding the psychophysiological measures, considering trait-somatic anxiety groups.

		LF				HF				LF/HF Ratio			
		F	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**
Main effects	**Condition**	0.620	.540	.009	NA	0.062	.940	.001	NA	0.294	.746	.004	NA
	Moment	96.523	p<.001	.573	Pre<Post	6.064	.016	.078	Pre<Post	72.716	p<.001	.502	Pre<Post
	Group	0.770	.383	.011	NA	2.169	.145	.029	NA	6.390	.014	.082	LowSG<HighSG
Second-order interaction effects	**Condition x Moment**	3.015	.052	.040	Pre: No ≠ across conditions Post: F<H, p=.027 Pre<Post across conditions, p<.001	1.499	.228	.020	NA	8.595	p<.001	.107	Pre: No ≠ across conditions Post: F<H, p=.027 Pre<Post across conditions, p<.01
	Condition x Group	1.430	.243	.019	NA	1.454	.237	.020	NA	0.327	.721	.005	NA
	Moment x Group	5.132	.026	.067	In both groups, Pre<Post, p<.001 No ≠between groups in Pre and Post	0.001	.982	.000	NA	6.765	.011	.086	In both groups, Pre<Post, p<.001 In Pre and Post: HighSC > LowSG, p=.05
Third-order interaction	**Condition x Moment x Group**	0.710	.493	.010	NA	0.441	.644	.006	NA	0.204	.816	.003	NA

Note. NA: Not applicable; Pre: evaluation before the emotional induction (baseline); Post: evaluation after the emotional induction (emotion condition); F: Fear condition; H: Happy condition; LowSG: Low trait-somatic anxiety group; HighSG: High trait-somatic anxiety group.

S9 Table. ANOVA's results regarding the self-report measures, considering trait-somatic anxiety groups.

		Happiness				Fear				Arousal			
		F	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**	**F**	**p**	**η^2**	**Simple effects**
Main effects	**Condition**	36.327	p<.001	.335	F<N<H	57.825	p<.001	.445	F>N, F>H	31.831	p<.001	.307	F>N, F>H
	Moment	0.093	.762	.001	NA	29.467	p<.001	.290	Pre<Post	125.671	p<.001	.636	Pre<Post
	Group	0.778	.381	.011	NA	0.303	.584	.004	NA	1.889	.174	.026	NA
Second-order interaction effects	**Condition x Moment**	82.372	p<.001	.534	Pre: No ≠ across conditions Post: F<N<H, p<.001 Pre>Post in F, Pre<Post in H, p<.001	59.280	p<.001	.452	Pre: No ≠ across conditions Post: F>N and F>H, p<.001 Pre<Post in F, Pre>Post in N and H, p<.01	53.141	p<.001*	.425	Pre: No ≠ across conditions Post: F>N and H>N, p<.001 Pre<Post in F and H, p<.01
	Condition x Group	0.398	.672	.005	NA	1.127	.327	.015	NA	3.053	0.50	.041	LowSG: F>N, H>N HighSG: F>N, H>N LowSG = HighSG in F, N, H
	Moment x Group	2.218	.141	.030	NA	0.718	.400	.010	NA	0.514	.476	.007	NA

Third-order interaction effects	**Condition x Moment x Group**	1.485	.230	.020	NA	0.870	.421	.012	NA	3.102	.048	.041	Pre: No ≠ across conditions for both groups Post: F>N and H>N, p<.001 for both groups Pre ≠ Post in all conditions for LowSG Pre ≠ Post in all conditions except in the Neutral condition for HighSG HighSG > LowSG in post-induction of neutral emotion HighSG > LowSG in pre-induction of happiness

Note. NA: Not applicable; Pre: evaluation before the emotional induction (baseline); Post: evaluation after the emotional induction (emotion condition); F: Fear condition; H: Happy condition; N: Neutral condition.

Supplementary Material – Study 2

Table S1. Sample's demographic information (psychometric study).

		Total sample (n=292)		Full-time students (n=194)		Community individuals (n=98)		Women (n=178)		Men (n=114)	
Age		M= 24.22	SD= 4.45	M=22.76	SD=3.90	M=27.12*	SD=4.07	M= 24.13*	SD=4.78	M=24.35	SD=3.92
		N	%	N	%	N	%	N	%	N	%
Sex	Female	178	61	121	62.4	57	58.2	178	100	-	-
	Male	114	39	73	37.6	41	41.8	-	-	114	100
Ethnicity	Caucasian	270	92.5	179	92.3	91	92.9	168	94.4	102	89.5
	Other	3	1	3	1.5	-	-	2	1.1	1	0.9
	No answer	19	6.5	12	6.2	7	7.1	8	4.5	11	9.6
Literacy	Secondary education	95	32.5	86	44.3	9	9.2	66	37.1	29	25.4
	Higher education	197	67.5	108	55.7	89	90.8	112	62.9	85	74.6
Occupation	Students	194	66.4	194	100	-	-	121	68%	73	64
	Active employment	82	28.1	-	-	82	83.7	47	26.4	35	30.7
	Both student and employee	11	3.8	-	-	11	11.2	6	3.4	5	4.4
	Unemployed	5	1.7	-	-	5	5.1	4	2.2	1	0.9
Psychiatric problems	Yes	37	12.7	21	10.8	16	16.3	29	16.3	8	7
	No	255	87.3	173	89.2	82	83.7	149	83.7	106	93

Note. *data from one participant is missing.

Table S2. Means, standard deviations (SD) and Pearson correlations regarding key variables (N=244).

	Mean	SD	1	2	3	4	5	6	7
1. AQ-SS	20.41	5.21	-	-	-	-	-	-	-
2. AQ-COM	13.66	3.30	.25***	-	-	-	-	-	-
3. AQ-RIDO	13.36	3.44	-.05	.05	-	-	-	-	-
4. TAS-Identifying	16.20	6.21	.28***	.18**	.25***	-	-	-	-
5. TAS-Describing	14.66	4.86	.28***	.34***	.17**	.58***	-	-	-
6. TAS-Thinking	19.66	4.52	.09	.31***	.05	.20**	.32***	-	-
7. STICSA-Cognitive	20.70	6.21	.24***	.04	.16*	.60***	.35***	.06	-
8. STICSA-Somatic	17.45	5.04	.09	-.003	.11	.50***	.22**	.05	.65***

Note. AQ-SS: Social skills dimension of the AQ; AQ-COM: Communication dimension of the AQ; AQ-RIDO: Restricted Interests and Detail Orientation dimension of the AQ; The levels of significance considered were *p<.05, **p<.01, ***p<.001.