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**UNDERSTANDING SOCIAL IMPAIRMENT IN ASD:**

**Investigation of neural correlates and psycho-physiological responses to social interaction in  
virtual environments**

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## INTRODUCTION

### **Social impairment in Autism: from DSM IV-TR to DSM V**

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder involving impairments in social and communication skills, as well as repetitive behaviors or thought process (American Psychiatric Association, 2013). Exact etiology of autism is still unclear, but numbers of new findings clearly show that autism is not a single clinical entity but a behavioral manifestation of many genetic disorders, influenced additionally by environmental factors (Betancur, 2011; Ostatnikova et al., 2015). Abnormal brain structures (the prefrontal lobes, the amygdala, the anterior insular cortex, the anterior cingulate cortex, the cerebellum) have been found in ASD people examined with functional magnetic resonance imaging (Schuman et al., 2009; Amaral et al., 2008).

Due to nature of these symptoms, individuals with ASD often present feelings of social isolation and rejection (Jobe & White, 2007). Social interaction deficits in high functioning autism represent a core characteristic (Volkmar et al., 2014).

The Centers for Disease Control and Prevention (CDC) estimated that 1 in 68 children were diagnosed with an ASD in 2010 (Baio, 2014), a 30% increase from 2008, when the incidence was 1 in 88, and a 60% increase from 2006, when the incidence was reported to be 1 in 110 children. Prevalence estimates have increased dramatically in the last twenty years such that this disorder is now recognized by some to afflict 1 in 88 children nationwide (Baio, 2012). Others estimate the prevalence in the United States to be 1 in 68 (Christensen, 2016).

As a result of the increased incidence and concerns about over diagnosis (Lobar, 2014), in May 2013, new guidelines for identification of ASDs were introduced in the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) by the American Psychiatric Association (APA). This change adds to the notion that ASDs are not discrete disorders under one umbrella term but are on a spectrum of similar disorders with varying presentations and severity of behavior. With this change from categorical description of discrete disorders to the spectrum, diagnosticians were expected to view ASD as a continuum of mild to more severe symptoms (APA, 2013). Furthermore, the DSM-V classified ASD into 3 severity levels, with children classified at level 1 exhibiting higher function and children classified at level 3 having most severe form of ASD.

A change from the DSM-IV-TR to the DSM-V was a reduction in the core symptom domains. The traditional triad of symptom domains (i.e., social, communication, and atypical behaviors) would

be reduced to two domains by combining social and communication symptoms into a single area (Social/Communicative Deficits).

The core symptom domains for ASD were reduced from the previous three to two: impaired social communication and social interaction and restricted, repetitive behaviors, interests, or activities (APA, 2013). Autistic disorder, Asperger syndrome, and pervasive developmental delay were consolidated into a single ASD classification as well. The diagnostic features related to an ASD in the DSM-V have four major criteria: continuous impairment in interaction and communication that are reciprocal and social in nature; patterns of activities, interests, and behaviors that are restricted and repetitive; symptoms that are persistent from early childhood; and symptoms that interfere with everyday functioning (Lobar et al., 2016).

Moreover, since the introduction of Asperger's disorder to the DSM- IV in 1994 there has been debate as to whether it is a separate condition or a form of high functioning autism. Social communication has been described as a major area of concern for children with Asperger disorder, defined as a "higher functioning" type of ASD. The revision of the diagnostic and statistical manual of mental disorders (5th ed.; DSM-5) rejects the delineation of these disorders into distinct categories (APA 2013) and instead proposes a unitary approach whereby autistic disorder (AD). A portion of high-functioning individuals (as recognized by DSM-IV-TR) will not meet the new criteria as they present with less severe social impairments. Authors (Young & Rodi, 2014) suggested that the DSM-5 social-communication domain might be too stringent in requiring an individual to meet all three criteria.

### **Research questions and aims of the thesis**

The overall purpose of the current study was to examine both self-reported emotional and behavioral responses, and the physiological effects of social interaction in persons with ASD, using two versions of Cyberball paradigm, with different level of anthropomorphic avatars.

The first research question is related to the understanding of the social experience in autistic persons. In healthy people, the social interaction has been extensively studied in experimental settings (Williams et al., 2001; de Panflis et al., 2015; Bolling et al, 2011; Eisenberger et al., 2003) and it has been observed that ostracism has immediate, detrimental effects on self-reported emotions. But little is known about how social exclusion or over inclusion is interpreted, experienced or managed by autistic persons.

The second research question is related to the use of virtual environment with high function autism. Many studies pointed out that individuals with ASD are stated to have an affinity to technology and a strong visual memory (Bozgeyikli et al., 2017) and VR provides enriched earning and training

experiences (Weiss et al., 2003; Rizzo & Kim, 2005; Bolte et al., 2010). To respond to this research question we developed and used a 3D version of classical Cyberball paradigm, with more anthropomorphic avatars. Highlighting the power of environmental cues such as anthropomorphism of avatars, this study supported the claim that specific environmental influences can alter biological and psychological responses to social stimuli.

The third research question is related to the use of subjective report with autistic persons. One should be especially wary of the dependability of self-reports from persons with ASD, who may have deficits in processing (i.e., identifying and describing) their own emotions (Hill et al., 2004).

Based on previous research, it was hypothesized that (1) individuals with ASD would be able to detect ostracism when they were excluded from the game; (2) the ASD group would report similar levels of needs threats (sense of belonging, sense of meaningful existence, self-esteem, sense of control) after being ostracized compared with controls; (3) the ASD group would report less negative mood after being ostracized than controls, replicating findings by Sebastian et al. (2009) and (4) individuals with ASD would display similar or increased levels of arousal following ostracism compared with controls, replicating findings by Trimmer et al. (2017).

For the first time with autistic participants, we aimed to (5) investigate the autonomic thermal signature associated with social experiences by facial thermal variation; (6) investigate the effects of virtual environment realism on psycho-physiological responses; (7) test the extreme inclusion (over inclusion on the Cyberball) situation in ASDs, assuming that emotional reactions reflect motivationally relevant outcomes, exclusion should cause emotional distress, and inclusion should cause positive emotional reactions.

## **Overview of the thesis**

To make the writing of this thesis clear and consistent with the predetermined objectives, we decided to dedicate separate sessions for each homogeneous part of the research. More in specific, at the beginning of the manuscript one paragraph explains the social impairment in Autism pointing out the changes from DSM IV-TR to DSM V in diagnosis and symptomatology of the syndrome. The next paragraph focuses on research questions to better understand the aims of the thesis.

Continuing in reading, the first session is dedicated to theoretical framework:

Chapter 1, to account for the framework on virtual reality tools for ASD. The aim of this chapter is to provide an overall view of the studies that used virtual environments in autism spectrum disorder. We explored the virtual reality tools and related methodologies used in training of social skill, 2) or in assessment of social impairment in HF-Autism.

Chapter 2, to account for the evolution of “social pain”, we provided a systematic reviewer of the



studies that investigated results in social pain or social exclusion in persons with ASD: 1) exploring the instruments or methodologies of social exclusion assessment used in experimental studies focused on ASD, 2) examining psycho-physiological responses to the social interaction, in particular social exclusion, in ASD persons, 3) examining the brain responses related to specific social stimuli in ASD.

In the second session it is possible to find chapters dedicated to the experimental phase of the research.

Chapter 3, to account for a validation experiment regarding the fidelity of the CybeballAvatar3D, specifically developed for the next experiment with ASD participants. Highlighting the power of environmental cues such as anthropomorphism of avatars, this study supported the claim that specific environmental influences can alter biological and psychological responses to social stimuli. The overall aim of this study was to validate the functionality of our CyberballAvatar3D. We would obtained this aim by comparing changes in negative emotions, basic needs satisfaction and sense of presence between the 3 steps (inclusion, exclusion and over-inclusion) of original 2D low anthropomorphism Cyberball with our new high-anthropomorphism CyberballAvatar3D in a sample of undergraduate students.

Chapter 4, to describe the experimental design and the used materials and methods. We presented all psychological and physiological measures used to carry out the 3-mixed factors experimental design,  $2(\text{Group: TD vs ASD}) \times 3(\text{Situation: inclusion, exclusion and over inclusion}) \times 2(\text{Condition: 2D vs 3D})$ . The 2 between-subjects factors were the Group and Condition, and the within-subjects factor was the type of game Situation (inclusion, exclusion and over inclusion). Furthermore, we dedicated one paragraph to describe the 20 participants whit HF-Autism and the 22 healthy participants and one more paragraph to introduce the used tools as a thermocamera, EEG and the instrument recording skin conductance and heart rate.

**Chapter 5**, to present the results of psycho-physiological assessment. We examined both self-reported emotional and behavioral responses, and the physiological effects of social interaction in persons with ASD, using two versions of Cyberball paradigm, 2D and 3D. The paragraph of results shows similarities and differences between the two experimental groups in regard to the investigated variables. We have used both well-known and lesser-known measures as Basic Need Threats or Dread of future interactions in order to better understand the effects of social interaction in autism.

**Chapter 6**, to present an explorative study about the neural correlates of social interaction in HF-Autism.

**Chapter 7**, to focus on the relevance of experimental findings. In the Conclusion paragraph we aimed to show the link between all the discovered results in order to present a clear picture of our contribution in the understanding of social interaction effects in autism. This phenomenon were studied and presented under various points of view, psychological and physiological with a specific attention to the brain responses. Furthermore, we describe how these findings can relate to the everyday experience of persons with ASD. The clinical implications of the findings and potential future research directions are also discussed.

## SECTION 1

### THEORETICAL FRAMEWORK OF AUTISM SPECTRUM DISORDER AND SOCIAL EXCLUSION IN VIRTUAL ENVIRONMENTS

#### CHAPTER 1: VIRTUAL ENVIRONMENTS FOR AUTISM SPECTRUM DISORDERS

Recent literature on neuropsychological assessment highlighted the many benefits of using virtual reality environments in the treatment and assessment of social skills and social cognition in ASD. The using of computers might increase motivation, investment in the treatment, and generalization. The flexibility of VR environments, their removal of common stressors of face-to-face interactions may prove to be a more effective platform for enhancing social skills and social cognition in ASD, compared to other therapeutic tools that are more didactic and constrained in their application.

*Aims of the chapter.* The aim of this chapter is to provide an overall view of the studies that used virtual environments in autism spectrum disorders. To achieve this objective we explored the virtual reality tools and related methodologies used in training and assessment of social skills in HF-Autism.

#### 1.1 Virtual Reality Technology for Improved Ecological Validity in Assessment

Recent review of literature (Aresti-Bartolome and Garcia-Zapirain, 2014) brings together different types of technology or applications for autism spectrum disorders. The following division was used: immersive and non-immersive virtual reality applications, dedicated applications, telehealth systems and Robots.

Information and Communication Technologies (ICTs) can compensate and support education of students with special needs, and particularly people with ASD. ICTs make it possible to create controllable predictable environments with multisensory stimulation; they foster or make it possible to work autonomously and develop the capacity for self-control and are highly motivating and reinforcing (Take et al., 2013), encouraging attention and lessening the frustration that may arise from making mistakes (Ingersoll & Wainer, 2013). It is necessary to use correctly ICTs to improve social interaction through their multiple uses and options (Ploog et al., 2013).

In this chapter of the thesis we examine only the non-immersive virtual reality environments.

The term Virtual Reality (VR) refers to a computer-generated environment in which the user can perceive, feel and interact in a manner that is similar to a physical place. The virtual environment is

a computer-generated three-dimensional model of a physical environment, in which the user can navigate and interact with the objects that it contains (Parsons et al., 2017).

VR systems are usually classified as immersive or non-immersive (Bowman et al., 2001). Non-immersive VR systems typically mimic a virtual environment using a standard high-resolution monitor, and feature limited interactive capabilities, for example 3D-videogames and desktop-based 3D modelling applications.

Virtual environments offer a methodology for presenting digitally recreated simulations of real world. These new virtual environment platforms may engender a sense of presence in users that can serve to enhance the representativeness and generalizability of findings from the virtual world to real world social cognitions (Goriri et al., 2011; Diemer et al., 2015).

## **1.2 Using virtual environments for assessment of social skills in HF-Autism**

Virtual Reality (VR) has recently become an increasingly popular form of exposure treatment for various clinical populations. It is an especially useful treatment modality when real-world exposure would be too inconvenient, costly, or dangerous. Furthermore, the recent literature on neuropsychological assessment (Parsons, 2014, 2010, 2007) highlights the many benefits derived from the use of diagnosis supported by technological tools offering greater standardization and ecological environmental (with the use of virtual environments), greater accuracy in the recording of responses (Parsons & Rizzo, 2008a,b). Given that the tools of virtual reality and virtual environments offer better results in the assessment and treatment of ASD compared with normal tools, (Kandalaf et al., 2013; Wallace et al., 2010, Mitchell et al., 2007; Parsons and Mitchell, 2002).

Unlike other therapeutic options, such as role-playing, VR represents real-life experiences in a safe, controllable manner that allow for repeated practice and exposure, which is a key element in treatment. VR can also provide naturalistic environments with unlimited social scenarios and has been shown to replicate social conditions (Wallace et al. 2010).

VR technologies have significant potential for assessment, training and education for individuals with autism (Goodwin, 2008; Parsons & Mitchell, 2002; Trepagnier, 1999), precisely because social scenarios and encounters can be carefully designed and controlled. Furthermore, many studies pointed out that individuals with ASD are stated to have an affinity to technology and a strong visual memory (Bozgeyikli et al., 2017). Several studies indicate that VR provides enriched learning and training experiences (Weiss et al., 2003; Rizzo & Kim, 2005; Bolte et al., 2010). Individuals with ASD are especially thought to benefit from this due to their attribution to strong

visual memory. Individuals with ASD are characterized to learn better when presented with visual spatial information, which can be easily achieved with VR (Meadan et al., 2011).

Moreover, thanks to the repetitive behavior of technology tools, HF-autistic persons do not expect any improvised social reaction like those that occur in the real world with social situations involving a large number of stimuli and variants. These environments produced by researchers are controlled to reduce the participants' stress.

For some authors (Grynszpan et al., 2009) development of a VR system aimed at helping people with ASD to focus on relevant facial cues during social conversation. Their hypothesis was that people with ASD do not recognize the value of paying attention to facial expressions during conversation and, as a result, miss important non-verbal cues that can aid comprehension of meaning. These authors argue that by using eye-tracking technology to identify where participants look during a social conversation with a virtual character (an agent-avatar), they can encourage participants with ASD to shift their attention to the faces of virtual characters by blurring the field outside of the direction of eye gaze.

The highly versatile VR environment can illustrate scenarios which can be changed to accommodate various situations that may not be feasible in a given therapeutic setting because of space limitations, resource deficits, safety concerns, etc. (Parsons and Mitchell, 2002). Parsons and Mitchell (2002) suggest that VEs might be particularly useful for the field of autism research because of the capacity for role playing that allows to practice in realistic settings in the absence of potentially frightening real-world consequences.

However, despite nearly twenty years of research, the potential of VR for autism education still remains an aspiration rather than a reality (Aresti-Bartolome et al., 2014).

### **1.3 Using virtual environments for training of social skills in HF-Autism**

Social impairments, inherent in high-functioning autism (HFA), interfere with the process of building relationships and integrating into the community (Hendricks & Wehman, 2009), all of which are aspects of social functioning that take on a larger role during the transition to adulthood, can be challenging. Recognition of these challenges has led to the development of treatments for adults with HFA that aim to increase functioning by focusing on selected social skills, such as emotion recognition, that in turn may improve social cognition, and social functioning (Kandalaf et al., 2013). For the first time, Strikland in 1997 pointed out that the virtual reality could offer the opportunity to regulate an artificial computer environment to better match the expectations and needs of individuals with special needs (Strikland, 1997).

In response to this need, subsequent studies have investigated the application of advanced interactive technologies to address core deficits related to autism, namely computer technology

(Bernard-Opitz et al., 2001; Moore et al., 2000; Swettenham, 1996), virtual reality environments (Parsons et al., 2004; Strickland et al., 1996; Tartaro and Cassell, 2007), and robotic systems (Dautenhahn and Werry, 2004; Kozima et al., 2009; Michaud and Theberge-Turmel, 2002; Pioggia et al., 2005; Scassellati, 2005). Computer and VR-based intervention may provide an exploratory interaction environment for children with ASD (Moore et al., 2000; Parsons et al., 2004; Strickland et al., 1996).

In specific, Parsons and Mitchell (2002) suggested that the using of computers might increase motivation, investment in the treatment, and generalization. The flexibility of VR environments, their removal of common stressors of face-to-face interactions may prove to be a more effective platform for enhancing social skills and social cognition in ASD, compared to other therapeutic tools that are more didactic and constrained in their application.

According to the most recent definition (APA, 2013), ASD is diagnosed on the basis of pervasive difficulties with social communication and interaction, coupled with restrictive, repetitive, and stereotyped behaviors. VR can provide an effective platform within which social interactions and communication of individuals with ASD can be controlled, explored, examined, and supported. Indeed, collaborative VEs can support the interaction of multiple users (participants, researchers, and confederates) at the same time, and so social interactions can be manipulated and studied; experimental conditions can be replicated for different studies with high fidelity (Blascovic et al., 2002).

These virtual applications could present a limit, the scarce amount of time spent interacting with others outside the autism spectrum. This particularly situation could aggravate the typical closing symptoms the autism spectrum (Aresti-Bartolome, 2014)

Despite this reflection, many authors consider VR a medium well-suited for creating interactive intervention paradigms for skill training in the core areas of impairment for children with ASD (i.e., social interaction, social communication, and imagination). Effectively, VR can offer the benefit of representing abstract concepts through visual means and seamlessly allows for changes to the environment (e.g., changing the color of a ball or making a table disappear) that may be difficult or even impossible to accomplish in a real-world setting (Sherman & Craig, 2003; Strickland, 1997).

#### **1.4 Presence and social presence in virtual environment**

The concept of presence is used to describe the subjective feeling of “being there” within a virtual environment, the concept of immersion refers to the objective degree to which a VR system is able to surround the user with vivid and interactive multi-sensory stimulations (Slater et al., 1997). Several theoretical accounts of presence have been developed, which can be included into two main

perspectives. The first conceptualization sees presence as an experience resulting from the interaction of a user with a given medium. According to this perspective, presence is a psychological state that is shaped, although not completely determined by, the immersive properties of the medium and individual differences among users could also play a role (Parsons et al., 2015). Slater and Wilbur (1997) claim that all computer generated environments, independently on the degree of immersion, control or interaction, can be considered as “virtual” because are perceived by viewers as the “representation of something. Baños et al. (2004) provide evidence that presence is not directly related to immersion and that low-immersive systems, such as desktop monitors, can elicit a strong sense of presence if the computer generated environment is emotionally engaging.

The second perspective considers the sense of presence as an embodied phenomenon, which is linked to the organization and control of action. The concept is strictly related to the subject’s ability to successfully “act there”, in the space where he/she is situated. Central to this conceptualization of presence is Gibson’s notion of affordance, intended as clues in the environment that indicate possibilities for engaging in an intended behavior (Gibson, 1977).

In addition to the characteristics of the users, features of the technology contribute to the sense of presence and are known to influence how participants respond and behave within VEs (Bente et al., 2008). These features include the virtual characters (avatars) within the VE, their anthropomorphism and their behavioral realism (Georgescu et al., 2014; Nowak & Biocca, 2003; Vinayagamorthy, Steed, & Slater, 2005). Riva and Mantovani (2014) emphasized that presence and agency are directly related within experiences of using VEs.

In order to understand how users perceive the presence of other social entities in a virtual environment. The most common methodology is based on the use of self-report questionnaires, which are administered to the participant after he/she has been exposed to the virtual environment.

Research on sense of presence in virtual environment for ASD obtained mixed results. Indeed, some studies (Wallace et al., 2010) suggested that children with ASD do not feel different levels of presence than their typically developed peers in immersive virtual environments and immersive environments can create authentic social situations. At the contrary other studies (Mei et al., 2015) explored effects of customizable avatars in virtual reality on user performance and user experience of adolescents with ASD. Results of the user study with participants with high functioning ASD indicated that customizable virtual avatars yielded improved performance, more motivation and better user experience.

To overcome the limitations of retrospective assessments of presence, more “objective”

measurement methods have been introduced that involve monitoring behavioral or psychophysiological responses during the virtual experience (Sanchez-Vives & Slater, 2005) and there is a growing emphasis upon physiological and behavioral assessment, as well as the relation between immersion and affective responding (Macedonio et al., 2007).

### **1.5 Virtual environment for assessing social exclusion in ASD**

A potential response to the difficulties of ecological validity in social cognitive assessment of persons with ASD is to present stimuli using virtual environments that are being used increasingly in the clinical and social sciences (Bohil, Alicea, and Biocca, 2011; Parsons, 2015). Virtual environments can range from non-immersive 2D presentations to immersive 3D environments presented with head mounted displays. A number of studies have used a 2D virtual Cyberball game (Williams et al., 2000) to assess the impact of social exclusion/ostracism. The virtual Cyberball game is an experimentally controlled social exclusion assessment that elicits affective (Wesselmann, Wirth, Mroczek & Williams, 2012; Williams, 2007), neurobiological (Eisenberger et al., 2012), psychophysiological (Moor, Crone & Van der Molen, 2010; Sijtsema, Shoulberg & Murray-Close, 2011), and hormonal (Geniole, Carré & McCormick, 2011; Zwolinski, 2012) responses. During the Cyberball task, the participant controls an avatar that is either included (inclusion condition) or ostracized (exclusion condition) by two or three other avatars controlled by the experimenter. Telling participants that the avatars are controlled by a computer does not change the effects of ostracism (Zadro, Williams, and Richardson, 2004).

Other studies are emerging that develop the 2D virtual Cyberball paradigm. Andari and colleagues (2016 and 2010) used a variant version of this game by adding photographs corresponding to digital players. Bolling and colleagues (2011) modified the classical version of Cyberball by giving digital players gender and added a comparable game (Cybershape) to assess for rule violations.

While some are simple addition of a fourth “neutral” player to verify the level of understanding of exclusion (Andari et al., 2010; van derMeulen et al., 2016), others have evolved Cyberball into a 3D virtual environment (Mavromihelaki et al., 2014; Venturini et al., 2016). In the 3D virtual environment versions of Cyberball, social information about the confederates’ avatars can be manipulated to ensure a greater identification with (or differentiation from) the player (Bolling et al., 2011; Venturini et al., 2016). Furthermore, Kassener and colleagues (2012) created an immersive virtual environment version that places the participant into a virtual and interactive environment. This change induced an effect sizes medium to large in magnitude of feelings of ostracism. This paradigm could be further implemented by including the variables of gender and age in order to carry out trans-cultural studies.



Immersive 3D Cyberball paradigms may offer enhanced ecological validity to obviate some of the ambiguities found in the literature. While the 2D Cyberball paradigm has been widely used, some of the less robust findings may reflect the fact that 2D versions of the Cyberball task lack the everyday realism and ecological validity that are now available in today's immersive 3D virtual environments. Kassner and colleagues (2012) proposed an advancement in the Cyberball paradigm, an immersive virtual environment version, in which the participants wear a head-mounted display (HMD), through which the virtual environment was displayed (Kassner, et al., 2012). Results revealed that the more immersive virtual environments induced feelings of ostracism in participants. Data from this study suggest that not only does ostracism in this environment have the same negative effects as in other environments, but these effects are powerful. Other virtual reality desktop versions (Bolling et al., 2011; Mavromihelaki et al., 2014; Venturini et al., 2016) have been developed to allow even greater levels of flexibility for manipulation of social information about the participant's interactions with confederate avatars and virtual humans (Wirth et al., 2011). The inclusion of virtual humans enhances the Cyberball paradigm because it allows for additional social information such as non-verbal (e.g., eye-gaze) information that has been found to convey ostracism (Wirth et al., 2010). Furthermore, the immersive virtual environment Cyberball paradigm offers researchers the ability to control aspects (proxemics and non-verbal communication) of the social context that cannot be accomplished in minimalist ostracism paradigms.

## **CHAPTER 2: THE FEELING OF SOCIAL PAIN: UNDERSTANDING THE EFFECTS ON HIGH-FUNCTIONING AUTISM**

Ostracism or social exclusion is an integral part of life, from school years to the work place. The need for affiliation or to belong has long been explored in the literature and it is common knowledge that human beings fear rejection and exclusion and therefore conform, comply or in some way alter their behavior in order to be included or accepted. Williams' model proposes that ostracism threatens four fundamental needs: belonging, self-esteem, control and meaningful existence (Williams et al. 2000)

*Aims of the chapter.* The aim of this chapter is to provide a systematic review of the studies that investigated results in social pain or social exclusion in persons with ASD: 1) exploring the instruments or methodologies of social exclusion assessment used in experimental studies focused on ASD, 2) examining the brain responses related to specific social stimuli in ASD.

### **2.1 Overview of studies on social exclusion in typical development participants**

The significant paradigm of Cyberball is used in several surveys on social exclusion. Currently, more than 5,000 subjects participated to the surveys by employing this paradigm, and we have noticed that just some minutes of ostracism in this context can produce strongly negative emotions (Williams, 2009). The effects of ostracism produced at the hands of the Internet-based cartoonish figures represented in Cyberball are as serious as those caused by ostracism occurring in a face-to-face setting; moreover, enduring ostracism by despised subjects is just as painful as when individuals are ostracized by those who are similar to them (Gonsalkorale & Williams, 2007). Starting from this paradigm, researchers discovered that participants subject to ostracism showed an activation in the same area of the brain which activates when people experience physical pain (Eisenberger et al., 2003).

In healthy people, the ostracism has been extensively studied in experimental settings (Williams et al., 2001; de Panflis et al., 2015; Bolling et al, 2011; Eisenberger et al., 2003) and it has been observed to have immediate, detrimental effects on self-reported emotions.

Consistent with previous research behavioral evidence has demonstrated the negative psychological effects of social exclusion (Sebastian, Viding, Williams, & Blakemore, 2010; van Beest & Williams, 2006; Williams, Cheung, & Choi, 2000; Wirth & Williams, 2009; Zadro, Williams, & Richardson, 2004, 2005) and the immediate negative emotional reactions (Gerber and Wheeler, 2009).

Other studies have revealed that being excluded from ball-tossing reliably evokes increased activation of the dorsal anterior cingulate and anterior insula which correlates with self-reports of

physical pain, (Eisenberger, 2012). While results from a recent meta-analysis suggest that the neural correlates of nociceptive stimuli and social rejection have some distinct patterns of activation, they still share commonalities (Cacioppo et al., 2013). Rotge and colleagues' (2014) meta-analytic review revealed that the virtual Cyberball game activated the dorsal anterior cingulate circuit less than other experimental social pain tasks. Findings suggest that social pain following exclusion in the virtual Cyberball game is less intense than the social pain following more personal forms of social rejection (Eisenberg, 2015).

## 2.2 Psycho-physiological effects of social pain in HF-ASD

Peer rejection is particularly pervasive among adolescents with autism spectrum disorders (ASD). However, how adolescents with ASD differ from typically developing adolescents in their responses to peer rejection is poorly understood.

Persons with high functioning autism (HFA) tend to have a social orienting disturbance wherein children with HFA display a syndrome specific difficulty with the tendency to attend to and process social stimuli, such as faces or the direction of eye gaze. As a result, effective evaluation of social cognitive functioning is an important clinical and public health issue. The social orienting deficits of persons with autism may limit their capacity for social learning at home and in school and also plays a role in their problematic development of social competence and social cognition (Dawson et al., 1998; Mundy and Burnette, 2005). Recent research suggests that the social orienting impairments of autism reflect a disturbance of “social executive” functioning that involves frontal motivation, self-monitoring, and volitional attention regulation. Further, deficits appear to be found in temporal/parietal systems that involve orienting and processing information about the behavior of other persons.

**Table 1: Feelings of Social Exclusion in Studies of Persons with Autism**

Author(s)	Sample(s)	Task(s)	Social interaction	Assessment tools	Self-Report Results	Interpretation
Andari et al (2010)	13 HF-ASD all males Age= n.s. TD= 0	Cyberball +oxytocin “face perception tasks”	Social Exclusion Prosocial behaviour	Item estimating self sentiments of “trust” and “preference” with respect to the fictitious players	Under oxytocin, patients spent more time looking at the face pictures. Under oxytocin patients engaged more often in exchanges with the player who reciprocated strongly, and they exhibited emotional responses congruent with this behavior.	Oxytocin may reduce the fear or anxiety induced by face stimuli in patients. Oxytocin enhanced patients’ ability to process socially relevant cues and acquire their meaning in an interactive context.
Andari et al (2016)	20 ASD 19 males Age Mean= 26.37	Cyberball +oxytocin “face perception	Social Exclusion Prosocial behaviour	Item estimating self sentiments of “trust” and “preference”	In the oxytocin condition, participants reported more trust toward the fair player	The authors speculate that OT administration is likely to remediate

	SD=8.45 TD= 0	tasks”		with respect to the fictitious players	as compared to the unfair player. Oxytocin administration activated appropriate emotional responses for partners' feedback at the behavioral level in ASD, attributing different emotional incentives to the behavior of each partners.	cognitive and neural deficits through mechanisms that are typically engaged by adults without ASD
Bolling et al (2011)	24 ASD 15 male Age 7-17  24 TD 17 male Age 9-17	Cyberball Cybershape	Social exclusion Rule violation	Abbreviated version of Needs Threats Scale Social responsiveness Scale	No differences in exclusion between ASD and TD groups. Following rule violation, ASD participants reporting significantly greater distress than TD participants. Any correlations in ASD between self-reported distress and brain activation to exclusion or rule violation (versus fair play).	The difference in rule violation responses may reflect the inflexible adherence to routines or rituals representing rigid, restricted behavior in ASD.
Krach et al (2015)	16 ASD 16 HC Age > 18	Pictures representing physical pain (56), social pain (40) and neutral (10)	Social pain Physical pain	Item evaluating the intensity of vicarious embarrassment Item estimating the intensity of physical pain	In HC, self-reports on the intensity of their SP experience showed significant coupling with activity of the ACC and AIC ASD did not base their self-report on the embodied representations of affect in this network.	ASD compensate for their reduced ability to access embodied signals by means frigidly learning and memorizing social rules and conventions
Masten et al (2011)	19 HF-ASD 18 males Age Mean=14; SD=2.4 17 TD 15 males Age Mean=13.6; SD=2.5	Cyberball	Social Exclusion	Needs Threats Scale Item of manipulation check	No differences between ASD and controls on self-reported feelings of distress following the exclusion round of Cyberball.	ASD feel just as rejected as their TD counterparts when they are excluded by peers.
McPartland et al (2011)	20 ASD males Age=5-15  34 TD 17 males Age=8-15	Cyberball	Social Exclusion	Needs Threats Scale Scale tapping personal attributions about other players	Both groups reported similar levels of distress in response to social exclusion.	Modifications to the paradigm to enhance realism may have increased investment on the part of participants with ASD, influencing self-

						reported mood.
Paulus et al (2013)	32 ASD (8 HD-ASD, 22 AS and 2 Atypical Autism) Age= young adults	30 vignettes of vicarious embarrassing situations 10 vignettes of neutral situations	Vicarious embarrassment	Items indicating personal experience about the vicarious embarrassment	ASD showed significantly lower vicarious embarrassment when they observe intentional violations of social norms. Both groups reported no differences in response to neutral situations. ASD with high trait of empathy report stronger vicarious embarrassment experiences.	The empathy trait should be responsible of capacity to comprehend vicarious embarrassment experiences in ASD.
Peristeri et al (2014)	10 HFA 8 males Age Mean=9.4; SD=1.9 10 TD 7 males Mean=9.3; SD=1.6  11 HFA 10 males Mean=24.7; SD=10.5 11 TD 10 males Mean=28.2; SD=10.8	Cyberball	Social Exclusion	Needs Threats Scale Item of manipulation check	The ostracism condition affected self-reported mood and three out of four social needs in both healthy groups, while did not modulate overall mood in HFA groups. Indeed, patients failed to interpret appropriately their current emotional state with an impact on understanding affective aspects of emotion words in the self-report questionnaire.	HFA adults have probably responded in a perseverative manner and HFA children's have revealed difficulties with understanding how the ostracism experience affected their current emotional state.
Sebastian et al (2009)	13 ASC (all males) Age Mean=16.9 16 TD (all males) Age Mean=16.9	Cyberball	Social Exclusion	Needs Threats Scale Item of manipulation check STAI-S	Both groups recognized when they are being excluded from a social situation, reporting similar effects of ostracism on anxiety and on need threat. ASC did not report any differences in overall mood following ostracism, while mood was lowered by ostracism in the control group. Self-reported need threat did not differ between groups for	ASC group may have been equally affected by the ostracism condition as TD controls, but might have lacked insight into how the experience affected their current mood.

					self-esteem, belonging and control. In contrast, in the ASC group meaningful existence was higher.	
Trimmer et al (2017)	25 ASD 21 males Age Mean=28 range= 16–61 TD= 26 21 males Age Mean=27 range= 17–50	Cyberball	Social Exclusion	Needs Threats Scale Scale of current mood Item of manipulation check	ASD group self-reported more negative mood responses overall and this was not dependent on whether they had been ostracised.	These findings probably depend on negative social interactions during daily life. Autistic persons are more likely to be rejected and less likely to be accepted by their peers
Twyman et al (2010)	(294 participants) 32 ASD Age Mean=11	Cyberball	Bullying peer victimization ostracism	Reynolds' Bully-Victimization Scale Pilot ostracism scale.	ASD group reported the highest percentages of clinically significant victimization and ostracism experiences	Children who already have difficulty in establishing and maintaining peer friendships such as those with ADHD, ASD, and LD, ostracism may be particularly devastating.

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*Note:* ASD = Autism Spectrum Disorder; HF-ASD = High-Function Autism Spectrum Disorder; HC=Healthy Controls; ASC= Autism Spectrum Conditions; TD = Typically developing

Acknowledgement of these challenges has led to the development of assessments that aim to measure functioning by focusing on social skills, such as emotion recognition. Less often, assessments are based on social cognitive processing, interpretation, and responses to social cues. Assessment of social skills and cognition have been performed using a variety of techniques and measures, and have found mixed levels of success. The most used measures in assessment of social exclusion are the basic needs and the distress following the social exclusion.

Self-reported data suggest that individuals with ASD are as able as controls to recognize when they are being excluded from a social situation. In the studies that used the 2D virtual Cyberball paradigm, both groups reported elevated levels of distress following social exclusion, but there were no group differences. Some studies found differences in mood responses. Peristeri and colleagues (2016; see also Sebastian et al., 2009) provide, as a possible explanation, the perspective that persons with ASD failed to interpret appropriately their current emotional state, which impacted understanding of affective content found in emotion words used for the self-report

questionnaires. Although persons with ASD may feel just as rejected as their TD counterparts when they are excluded by peers, they might have lacked insight into how the experience affected their current mood. The work by Andari and colleagues (2016; 2010) may help to explain these findings. They found some preliminary results that show atypical emotional and behavioral responses can be normalized with intranasal oxytocin administration, by modulating the brain activity of key emotional regions (amygdala and hippocampus) and by reducing their anxiety induced by face stimuli. Indeed, the authors confirmed that oxytocin enhanced the ability of persons with ASD to elaborate socially significant signals and get their meaning within an interactive context.

The degree of self-reported need threat did not differ between groups for self-esteem, belonging, and control. Sebastian and colleagues (2009), however, found greater threat in the ASD group, compared with controls, on the meaningful existence scale. This may be due to the greater likelihood that TD participants would have well-developed support networks that defend against feelings of neglect, meaningless, and/or non-existence during a short virtual episode of ostracism. Meanwhile, the ASD group may have lacked this resource. Furthermore, Bolling et al., (2011) found group differences, using a modified Cyberball game. ASD participants reported significantly greater distress following rule violation and intentional violations of social norms. The difference in rule violation responses may reflect rigid adherence to routines or rituals typical of restricted behavior in ASD (APA, 2000).

Lastly, initial results from Krach and colleagues (2015) suggest that ASD individuals did not base their self-report on the embodied representations of affect, but on memory process. The behavioral response to social pain may dissociate from the neurobiological embodiment of others' affect. This concept recalls clinical observations that people with ASD compensate for their scarce ability to access embodied signals through processes of strict learning and memorization of social rules (Baron-Cohen et al., 2003; Klin et al., 2003). The authors underline the absence of domain-general interference in embodying affect in ASD, but underline a grade regarding the complexity of social situations indeed, in simple circumstances, subjects with ASD are not compromised.

Clinical studies (Twyman et al., 2010) found that participants in the ASD group reported the highest percentages of clinically significant victimization and ostracism experiences in respect to other clinical or healthy populations. They may lack quality friendships to protect them from bullying. Indeed, in individuals with high functioning autism report higher degrees of loneliness than typically developing peers. Furthermore, they present a poorer understanding of the relationship between loneliness and social interaction. Persons with autistic syndrome have difficulties in beginning and maintaining friendships. As a result, ostracism may be experienced as especially difficulty (Bauminger et al., 2003).

In regard to the study about arousal activation, in the Cyberball task, skin conductance and heart rate have been shown to be sensitive to inclusion versus exclusion conditions (Kelly et al. 2012; Kouchaki and Wareham 2015; Iffland et al. 2014). Results from the only study on skin conductance response to the ostracism with HF-autism revealed that individuals with ASD experienced greater arousal than controls during the Cyberball game.

**Table 2: Physiological arousal of Social Exclusion in Studies of Persons with Autism**

Author (s)	Sample(s)	Task(s)	Social interaction	Assessment tools	SCL results	Interpretation
Trimmer et al (2017)	25 ASD 21 males Age Mean= 28 range= 16–61 TD= 26 21 males Age Mean=27 range= 17–50	Cyberball	Social Exclusion	Tool for autonomic measures	Individuals with ASD experienced greater arousal than controls in both conditions. ASD group responded with greater skin conductance level than controls in the exclusion condition	It may be the case that those with ASD were more interested in the game or experienced increased anxiety when engaging in the task.

One likely explanation is because ASD participants were more interested or experienced increased anxiety when engaging in the task. It appears individuals with ASD may not be attuned to their physiological response when they are ostracized. Whilst they experienced a heightened arousal response to being excluded from the game, they did not express this difference in their mood ratings. One more explanation for this seeming dissociation between physiological response and self-reported mood might be the influence of alexithymia traits leading to misattribution of bodily changes and poor emotion regulation (Silani et al. 2008; Bird et al. 2010). Authors highlighted that the neurophysiological mechanisms leading to atypical EDA and HRV pattern related to ASD are still unexplored (Bujnakova et al., 2016).

### 2.3 Neural network of social pain in HF-ASD

Regarding the differences in neuroimaging results, between ASD and TD group, all studies reported group differences. In specific, less activity during exclusion versus inclusion among participants with ASD in brain regions involved in emotion processing, including anterior cingulate cortex and anterior insula.

**Table 3: Neural Correlates of Social Exclusion in Studies of Persons with Autism**

Author(s)	Sample(s)	Task(s)	Social interaction	Assessment tools	Neuroimaging Results	Interpretation
Andari et al (2016)	20 ASD 19 males Age Mean= 26.37 SD=8.45 TD= 0	Cyberball +oxytocin “face perception tasks”	Social Exclusion Prosocial behaviour	fMRI	IN-OT helped ASD to discriminate between different social contexts and social values associated to faces, by modulating the brain activity of key	OT inhibits amygdala activity during the perception of faces and modulates stress responses as a function of positive social interactions. This region is known



					emotional regions (amygdala and hippocampus). During social reciprocity, OT delivery prompted the activity of anterior OFC in individuals with ASD.	for its role in reward value representation.
Bolling et al (2011)	24 ASD N=15 male Age 7-17  24 TD N=17 male Age 9-17	Cyberball Cybershape	Social exclusion Rule violation	fMRI	ASD showed activation to social exclusion only in retrosplenial cortex and left precentral gyrus. ASD showed similar activation to TD peers in dorsomedial and lateral prefrontal cortex and right parietal cortices. Only ASD showed activation to rule violation in bilateral caudate, superior temporal sulcus, and anterior insula.	The finding that posterior insula responds to exclusion in TD participants may reflect a more visceral response to social exclusion. Activation of anterior insula to rule violation in ASD may reflect a more cognitive, conscious emotional response.
Krach et al (2015)	16 ASD 16 HC Age > 18	Pictures representing physical pain (56), social pain (40) and neutral (10)	Social pain Physical pain	fMRI	In ASD, domain-specific decrements in the neurobiological response of vicarious social pain, non-significant for physical pain. Reduced activation of the ACC and AIC as well as by the pupillary responses. In contrast, in the context of vicarious physical pain, no peculiarities were found in any of the neurobiological responses.	ASD use an alternative route to obtain the same behavior and we provide the first evidence for differences in the underlying neurobiological mechanisms that link neural activation with behavior
Masten et al (2011)	19 HF-ASD 18 males Age Mean=14; SD=2.4  17 TD 15 males  Age Mean=13.6;	Cyberball	Social Exclusion	fMRI	ASD display hypoactivation in brain regions involved in emotion processing, including the sub ACC and AI, specifically when performing a range of social	This differential engagement of neural circuitry in response to peer rejection could also be related to a number of qualitative factors related to peer rejection experiences that adolescents with ASD have in their daily lives: may be more

SD=2.5

cognitive tasks. Less activity during exclusion versus inclusion among adolescents with ASD compared to controls in sub ACC and AI, as well as in regions previously shown to be negatively related to distress during exclusion (i.e., VLPFC, VS).

habituated to be rejected or may expect to be rejected when they interact with novel groups of peers.

McPartland et al (2011)	20 ASD males Age=5-15  34 TD 17 males Age=8-15	Cyberball	Social Exclusion	EEG	Temporal dynamics of brain activity associated with social exclusion in ASD reveal a dissociation between reported distress and neural response in ASD. Difference in the temporal course of brain responses between ASD and TD. In contrast to their typical peers, the children with ASD showed differentiation of rejection at an earlier frontal P2 component, although this response also did not associate with their reported experience of rejection.	The absence of differential response to exclusion versus “not my turn” at the LSW suggests that children with ASD might be failing to make critical distinctions based on social context. The temporal course of the early positivity (P2) indicates a role in more basic cognitive processing, such as visual attention in ASD.
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*Note:* ASD = Autism Spectrum Disorder; TD = Typically developing; EEG = electroencephalography; fMRI = Functional magnetic resonance imaging; ACC = anterior cingulate cortex; subACC = subgenual portion of the anterior cingulate cortex; AI = anterior insula; VLPFC = ventrolateral prefrontal cortex; and VS = ventral striatum.

Masten and colleagues (2011) found a positive correlation between sub anterior cingulate and distress in ASD. In particular, a positive correlation was found between the ventrolateral prefrontal cortex and distress. This positive correlation reflects an ineffective attempt to regulate distress resulting from peer rejection in the ASD group. Results from temporal dynamics of brain activity associated with social exclusion and reporting in ASD revealed a dissociation between reported distress and neural responses and differentiation of rejection at an earlier frontal P2 component. This suggests the importance of temporal dynamics in revealing processing strategies in typical and atypical development (McPartland et al., 2011). The rule violation paradigm (Bolling et al., 2011) showed that only participants in the ASD group had activation in bilateral caudate, superior

temporal sulcus, and anterior insula in conjunction with the vicarious social pain paradigm. Moreover, the authors found that the children with ASD showed differentiation of rejection at an earlier frontal P2 component, this reduced amplitude at the P2 for rejection events suggests attenuated engagement of attentional resources during the experience of exclusion in individuals with ASD.

Paulus and colleagues (2013) found that only participants in the ASD group had domain-specific decrements in the neurobiological response for this domain. Andari and colleagues (2016: 2010) demonstrated that oxytocin fosters a first level of prosocial approach by over-turning the main deficit of patients with HFA, namely the lack of eye contact. Moreover, oxytocin enhances affiliation partly by means of the reduction of the fear of social unfaithfulness and the elimination of avoidance behavior. This process reduces the activity of the amygdala, thus leading to a decrease of fear responses (Kirsch et al., 2005; Petrovic et al., 2008). It is possible that patients with autism have hidden social skills and that oxytocin may endorse social engagement behavior by eliminating fear and mistrust.

Krach and colleagues (2015) hypothesized that in complex social situations persons with ASD may fail to intuitively access the embodied representation of affect. This may be particularly apparent in situations requiring representation of another's affective experience necessitates the integration of contextual demands. This hypothesis is supported by their results showing that in healthy controls, self-reports findings of social pain experience match with activity of the anterior cingulate and anterior insula, brain regions associated with embodied affects. In contrast, ASD individuals did not base their self-report on the embodied representations of affect in this network, but exhibited a significant association with the hippocampus, the key region for memory processes.

#### **2.4 Comment on the literature evidences**

In order to understand these results, it is necessary to examine the literature concerning studies on self-report for ASD. Research has revealed that individuals with ASD tend to have limited self-awareness as a component of their disability. The reliability and validity of self-report measures in this population are unclear (Lainhart, 1999). Despite the fact that individuals with ASD are commonly believed to have difficulty processing their own and other people's emotions, authors (Berthoz et al., 2005) have suggested that individuals with ASD more likely show a different approach to processing their emotions, rather than an absence of this processing. Moreover, there is no commonly used self-report measure valid for individuals with ASD (Mazefsky et al., 2011). Some results deriving from the self-report of emotional responses in ASD lead to some doubts on the validity of this approach. Ben Shalom and colleagues (2006) discovered that both TD and ASD

groups obtained similar physiological results from emotional stimuli, but different results in self-reports. Despite the several existing potential interpretations of said results, it may be the case that persons with ASD struggle in identifying their own emotions. The analysis of the self-report tools used in this research suggests that high-functioning individuals with ASD could report their own psychiatric symptoms to a certain level, but this would not be enough for clinical diagnostic purposes (Mazefsky et al., 2011).

In line with previous research, the studies considered in this review show differences in responses to self-report in the two groups (TD and ASD), this may be due to the autistic syndrome being very heterogeneous in symptoms. In some cases we found discrepancy, within the ASD group, between what was reported by self-evaluation and the findings from physiological arousal or brain responses. Indeed, individuals with ASD experience heightened physiological arousal but whilst these individuals reported overall lower mood, this response to ostracism was not expressed as emotionally significant to these individuals, suggesting possible interoceptive difficulties in this population.

It seems clear that the measures of brain function revealed important group differences otherwise undetectable with behavioral methods alone. Persons with ASD showed differentiation of rejection at an earlier frontal P2 component, although this response did not associate with their reported experience of rejection (Bolling et al., 2011). Given this lack of clarity, it is important that focus be placed on more refined experimental paradigms that may reveal the peculiarities in the neural systems of functional architecture in persons with ASD.

Since children with ASD are vulnerable to social exclusion and are exposed to its emotional and psychological consequences (McPartland et al., 2011), this topic requires further discussion. The American Academy of Pediatrics has recognized bullying and victimization as serious problems and has updated a policy statement on youth violence to remark the need for health care providers who can appropriately deal with violence-related problems (AAP, 2009).

## SECTION 2

### DESIGN, DEVELOPMENT AND VALIDATION OF A 3D VIRTUAL ENVIRONMENT FOR SOCIAL INTERACTION RESEARCH IN AUTISM SPECTRUM DISORDER

#### CHAPTER 3: THE VALIDATION STUDY

##### 3.1 Theoretical framework

The traditional Cyberball paradigm (Williams et al., 2000) is a 2D virtual ball-toss game that uses line drawn characters for research on ostracism. Social scientists have started using the virtual gaming task called Cyberball to induce social exclusion in participants. A number of researchers have used the Cyberball game as an experimentally controlled social exclusion assessment that elicits affective (Hartgerink et al., 2015), neurobiological (Eisenberger, 2015), psychophysiological (Gunther Moor et al., 2010; Kelly et al., 2014) and hormonal (Andari et al., 2010) responses. Results from neuroimaging studies have revealed that the experience of being excluded from ball-tossing evokes increased activation of the dorsal anterior cingulate cortex and anterior insula, which correlates with self-reports of physical and social pain (Eisenberger, 2012), however, these results have been put into question in recent publications (Cacioppo et al., 2013). A more recent study (Rotge et al., 2015) found that the Cyberball task activated the dorsal anterior cingulate circuit less than other experimental social pain tasks. These findings are consistent with the suggestion that the social pain that follows from Cyberball is less intense than the social pain that follows from more personal forms of social rejection (Naomi I. Eisenberger, 2015).

Given that the 2D Cyberball was developed purposefully to be minimal (i.e., devoid of most social information), it lacks the ecological validity that many virtual environments now offer (Parsons, 2015). A recent advance in the Cyberball paradigm is an immersive virtual environment version, in which the participants wear a head-mounted display (HMD), through which the virtual environment was displayed (Kassner et al., 2012; Kothgassner et al., 2014). Data from this study suggest that not only does ostracism in this environment have the same negative effects as in other environments, but these effects are powerful. Other virtual reality desktop versions (Mavromihelaki et al., 2014) (Bolling et al., 2011) allow for enhanced flexibility in manipulation of social information about the confederates' avatars, virtual humans, and/or their behaviors (Riva, 1997). Given recent interest in the study of social pain (Bolling et al., 2011), processing of social cues (American Psychiatric Association, 2013), and the need for novel approaches to assessment and treatment of autistic syndrome using virtual environments (Kandalaf, Didehbani, Krawczyk, Allen,

& Chapman, 2013), we developed a non-immersive virtual reality-based Cyberball paradigm (with highly anthropomorphic avatars) that can be used also to assess a sample of adults with high-functioning autism. To investigate the emotional arousal due to social interactions we chose to register sympathetic activity through the skin conductance response (SCR) and the Autonomic Nervous System system by heart rate (HR) (Ekman, 1983). Meehan et al. (2005) reported that changes in physiological activity are evoked by different amounts of presence in stressful VR environments. In general, it is expected that higher physiological activity levels can be associated with greater stress levels (Smith, 1989).

Studies using a virtual ball tossing game, the Cyberball paradigm (Williams et al., 2000), found that Cyberball exclusion in samples with a female majority was associated with higher anger (Zadro et al., 2004) and depression ratings (Zoller et al., 2010). Weik et al. (2010) reported increased anger after Cyberball exclusion in both genders, but increased depression ratings only in females. Conversely a recent work (Seidel et al., 2013) did not show any gender differences in mood and emotion ratings after Cyberball exclusion, but for the whole sample they observed a significant increase of anger and negative mood in general. Furthermore, research has consistently shown that emotional distress created by exclusion is related to enhanced activation of the limbic system (Eisenberger, 2012; Kross, Berman, Mischel, Smith, & Wagner, 2011), which leads to increased sympathetic arousal of the autonomic nervous system (Cavanagh & Allen, 2009).

Other studies suggested that social pain following exclusion in the virtual Cyberball game is less intense than the social pain following more personal forms of social rejection (Eisenberg, 2015). To investigate the role of stimuli in eliciting emotions, mood and social presence feelings during the social interaction, we designed and developed a VR-based social interaction game. This new game was used in the following experiment for exploring psycho-physiological responses to social cues.

### **3.2 Hypothesis and aims of the study**

Highlighting the power of environmental cues such as anthropomorphism of avatars, this study supported the claim that specific environmental influences can alter biological and psychological responses to social stimuli.

The overall aim of this study is to validate the functionality of our game. We would obtained this aim by comparing changes in negative emotions, basic needs satisfaction and sense of presence between the 3 steps (inclusion, exclusion and over-inclusion) of original 2D low anthropomorphism Cyberball with our new high-anthropomorphism CyberballAvatar3D in a sample of undergraduate students.

First hypothesis: the objective was to examine the effectiveness of CyberballAvatar3D in eliciting subjective and psychophysiological feeling of inclusion, exclusion and over-inclusion. We expected

that Cyberball3D elicits highest level of arousal than the 2D version, only in the exclusion situation. The magnitude of negative emotions are usually greater than the positive (Baumeister et al., 2001; Cacioppo et al., 1997; Kahneman & Tversky, 1979), and on that basis, one could predict that rejection would have stronger emotional effects than acceptance (Blackhart et al., 2009).

Second hypothesis: the objective was to understand the participants' sense of presence felt during the game. We expected that the 3D condition elicits greater sense of presence than the 2D one.

Third hypothesis: the objective was to examine whether social interaction elicits more effects on females compared to males on a subjective and objective level. We expected that female group respond with a greater physiological arousal and higher rating on self-report, than male group.

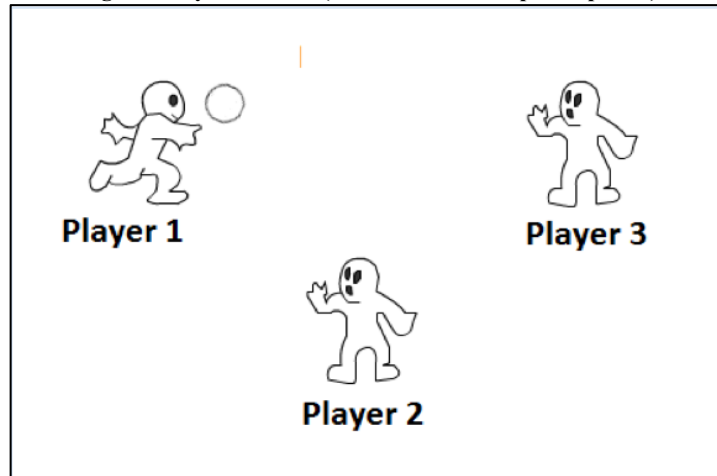
The next aim will be to use the 3D game with a sample of adults with autistic syndrome.

### **3.3 Tools for social interaction**

#### ***3.3.1 Cyberball***

The Cyberball game (Williams, Cheung, & Choi, 2000) is a computer based 'ball tossing' game, Cyberball is an ecologically valid experimental paradigm that can be adopted to evaluate emotional reactions to both social inclusion and exclusion (Fig. 1). Before starting using the game it is possible to set the number of ball tosses and the time between tosses. Furthermore, other features can be adjusted, including the avatars' names, the players' pictures and the presence of a chat. For this experiment we adopted a modified version of the classic Cyberball paradigm that adds an "over-inclusion" condition to the two classic conditions of the game of ostracism and inclusion (see de Panfilid et al., 2015; Leitner et al., 2014; Kawamoto et al., 2012; Van Beest & Williams, 2006; Wesselmann & Williams, 2013; Williams et al., 2000). In the current study, when over-included the actual participant received the ball about 45% of the total launches. In the inclusion condition, throws are distributed evenly to all players throughout the game (33% of tosses). In the exclusion condition, participants receive 4 balls at the beginning of the game and are ignored thereafter (36 tosses). In this study, on-line players were not provided with photos or their names to avoid giving participants any other reason for disliking another player apart from the fact that they were being ostracized by these players.

**Figure 1. Cyberball 2D (low level of anthropomorphism)**



### **3.3.2 CyberballAvatar3D**

The *CyberballAvatar3D* (Fig.2 and Fig.3) is a 3D version of the classic paradigm, developed by Unity 5.0. The avatars are rigged and generated by Autodesk Character Generator (free version). This version of the game features the same operations and the same setting at predetermined blocks. The only aspect that changes regards the characteristics of the environment and the players. In the Avatar3D version, in fact, it has the image of an external environment, a grass in an open field, where 3 avatars (default: left player is a boy and right player is a girl; the participant player is either a boy or a girl) can play with a ball. The dimension of depth and anthropomorphic characteristics of the players make the scene more realistic and immersive.

**Figure 2. Cyberball3D- male participant (high level of anthropomorphism)**





**Figure 3. Cyberball3D- female participant (high level of anthropomorphism)**



In addition, to increase the sense of presence in participant, the corresponding avatar can be customized by choosing his gender (Fig. 3), the color of hair and clothes. Also in this version of the game the three steps of inclusion, exclusion and over-inclusion are programmed with 40 tosses for each one and with the same time between tosses.

### **3.4 Research protocol**

#### ***3.4.1 Participants and procedure***

The experimental sample included 62 students, (males= 37) voluntary recruited from the University of North Texas. Participants were randomly assigned to the two conditions, while engaged in each of the Cyberball paradigms. Before starting the experiment each participant was provided with written information about the study and invited to give written consent for the inclusion. They are told that the researcher is interested in ‘the effects of mental visualization on task performance’. Participants are led to believe they are playing a game of virtual ball-toss with other individuals online, though all tosses are predetermined. They were also told that performance in the game was unimportant, and instead, the game was merely a means for them to engage their mental visualization skills. Starting the experimental session, participants were asked to fill in the scale of mental visualization. This scale was included to emphasize the cover story of the study. This scale had statements such as, “I have vivid dreams” or “When I read a book, I can see the main characters clearly in my mind”.

They were informed that will play with two other individuals stationed in similar laboratories at two other labs. This cover story was augmented by staged phone calls to the other experimenters making sure that their participants were ready to go.

At the end of the game session, instructions inform participants that they had finished, and they can fill out a post-experiment questionnaire

To verify the hypothesis, the study employed a 3 (Inclusionary status: Inclusion vs. Exclusion vs. Over-inclusion) X 2 (Cyberball Type: Cyberball vs CyberballAvatar3D) mixed factorial design, with the first factor (steps of the game) varying within subjects and the second factor varying between subjects.

### **3.4.2 Psychological assessment**

After each step (inclusion, exclusion and over-inclusion) we administered a battery of questionnaires. For each subscale, we reported the value of Cronbach's alpha (mean of the three steps). Following a brief description of the used scales.

The Rejected emotions scale (Buckley, Winkel, & Leary, 2004) assesses sense of rejection during the game (20 items on a 7-point Likert scale). The scale measured the intensity of five emotions: Anger (alpha=.855), Happiness (alpha=.899), Hurt feelings (alpha=.820), Anxiety (alpha=.762), Sadness (alpha=.746).

The Need Threat scale (van Beest & Williams, 2006) measures satisfaction of four basic psychological needs: Belonging (alpha=.857), Self-esteem (alpha=.889), Control (alpha=.680), and Meaningful existence (alpha=.867). These were rated on a 7-point Likert scale from 'not at all' to 'very much'.

Slater-Usch-Steed Presence Questionnaire (SUS) (Slater et al. 1994). The questionnaire (alpha=.899) assesses the level of presence experience during media exposures by asking participants to rate the features of media experience (feeling of being there, realism, involvement) on a 7-point likert scale.

Social presence Questionnaire (Bailenson et al., 2001). The questionnaire (alpha=.752) measured self reported social presence (i.e., the degree to which people report being in the presence of a veritable human being), affect towards the agent, and finally, how willing they would be to perform a series of embarrassing acts in front of the agent. To sub-scale *affect* measured low-high affect towards the agent embodied. To measure social presence, we took a summation score of the first six questions. Higher value indicated high social presence while lower value indicated low social presence. For *willingness to perform an embarrassing act* we took a summation score of the last four questions; Higher value indicated willingness to commit an embarrassing act (i.e., change clothes or tell secrets to the agent) while lower value indicated un-willingness to commit an

embarrassing act.

### ***3.4.3 Physiological assessment***

The final experiment consisted of 46 participants (30 males) with all measures, psychological and physiological, because of technical difficulties occurred with the equipment.

To better understand the effect of social interaction in healthy people, we investigated the change in heart rate (HR) and in skin conductance response (SCR), that are the main physiological metrics used to measure psycho-physiological arousal (Meehan et al. 2005; Andreassi 1995; Guyton 1986; Weiderhold et al. 1998). Indeed, SCR predominantly reflects sympathetic activity and HR reflects parasympathetic activity (Mauss and Robinson 2009).

The indices of HR and SCR were recorded during the baseline condition and during the exposure to the game sessions, in order to obtain objective measures of subjects' emotional activation.

Skin conductance and heart rate were recorded simultaneously with a sampling rate of 1000 Hz. Skin conductance response (SCR) was measured from the distal phalanges of digits II and IV of the non-dominant hand with dry electrodes and HR from a sensor applied in the digit III. Skin conductance signal was converted to microsiemens ( $\mu\text{S}$ ) and heart rate signal to beats per minute (bpm). In

#### SCR

Electrodermal activity consists of two main components - tonic response and phasic response. Tonic skin conductance refers to the ongoing or the baseline level of skin conductance in the absence of any particular discrete environmental events. Phasic skin conductance refers to the event related changes that are caused by a momentary increase in skin conductance (resembling a peak superimposed on tonic skin conductance). For this experiment we chose the tonic response because we analyzed the response during the long time of the game.

#### *Data acquisition and analyses*

Participants' skin conductance response (SCR) and heart rate (HR) were recorded simultaneously using the Biopac MP150 system (Biopac Systems, Goleta, CA) and GSR100C electrodermal activity amplifier at a sample frequency of 1Hz. The SCR and HR data were pre-processed and analyzed using AcqKnowledge 4.1 (Biopac Systems, Inc.), R-waves in the ECG data were identified automatically by the software and converted to BPM. Additionally, a visually artifact inspection was conducted. Skin conductance response (SCR) was measured from the distal phalanges of digits II and IV of the non-dominant hand with dry electrodes and HR from a sensor applied in the digit III. Skin conductance signal was converted to microsiemens ( $\mu\text{S}$ ) and heart rate signal to beats per minute (bpm).

The physiological indices were recorded during the baseline condition and during the exposure to the game sessions, in order to obtain objective measures of subjects' emotional activation. Mean baseline SCR was then subtracted from each of these means to obtain the level of physiological arousal during each session period.

### **3.5 Results**

#### ***3.5.1 Psychological effects***

##### *Analysis strategy*

All the self-report measures were analyzed in separated 3-way mixed ANOVAs, including two between-subjects factors and one within-subjects factor. The between subjects factors were Gender (Males vs Females) and Condition (2D vs 3D), and the within-subjects factor was the type of game Situation (inclusion, exclusion, and over inclusion). Violations of sphericity were assessed using the Mauchly's test, and whenever violations were found we applied the Greenhouse-Geisser (GG) and Huynh-Feldt (HF) corrections for departure from sphericity. Significant interactions of two or more factors were always followed by the analysis of the simple effects (in case of significant 2-way interaction) or of the simple interactions (in case of significant 3-way interaction) of the respective factors. Difference adjusted 95% confidence intervals were computed using the method proposed by Cousineau and Morey for within-subjects factors (Baguley, 2012a), and with the variation of the Goldstein-Healy method for between-subjects factors (Baguley, 2012b), and plotted as error bars in the mean plots, so that non-overlapping error bars (and thus confidence intervals) plots reflect significant differences between pairs of means. Differences between pairs of means were also tested with paired and independent sample t-test, for within-subjects and between-subjects factors respectively.

The social presence and SUS data were analyzed using 2-way between-subjects ANOVAs, including Gender and Condition as within-subject factors. Whenever an ANOVA yielded a significant interaction effect between gender and condition, independent sample t-tests were used to analyze the simple effects.

##### *Description of results*

The results of the analyses of the measures collected after each game session always yielded a significant effect of Situation. Violation of the sphericity assumption were detected for all the dependent variables except the perception of the other players' interest in playing with me, but in all the cases the effect remained significant after the correction. The F statistics and p-values of the

effect of Situations are reported in the plots in figure 1, which display the means of the different measures across the game situations.

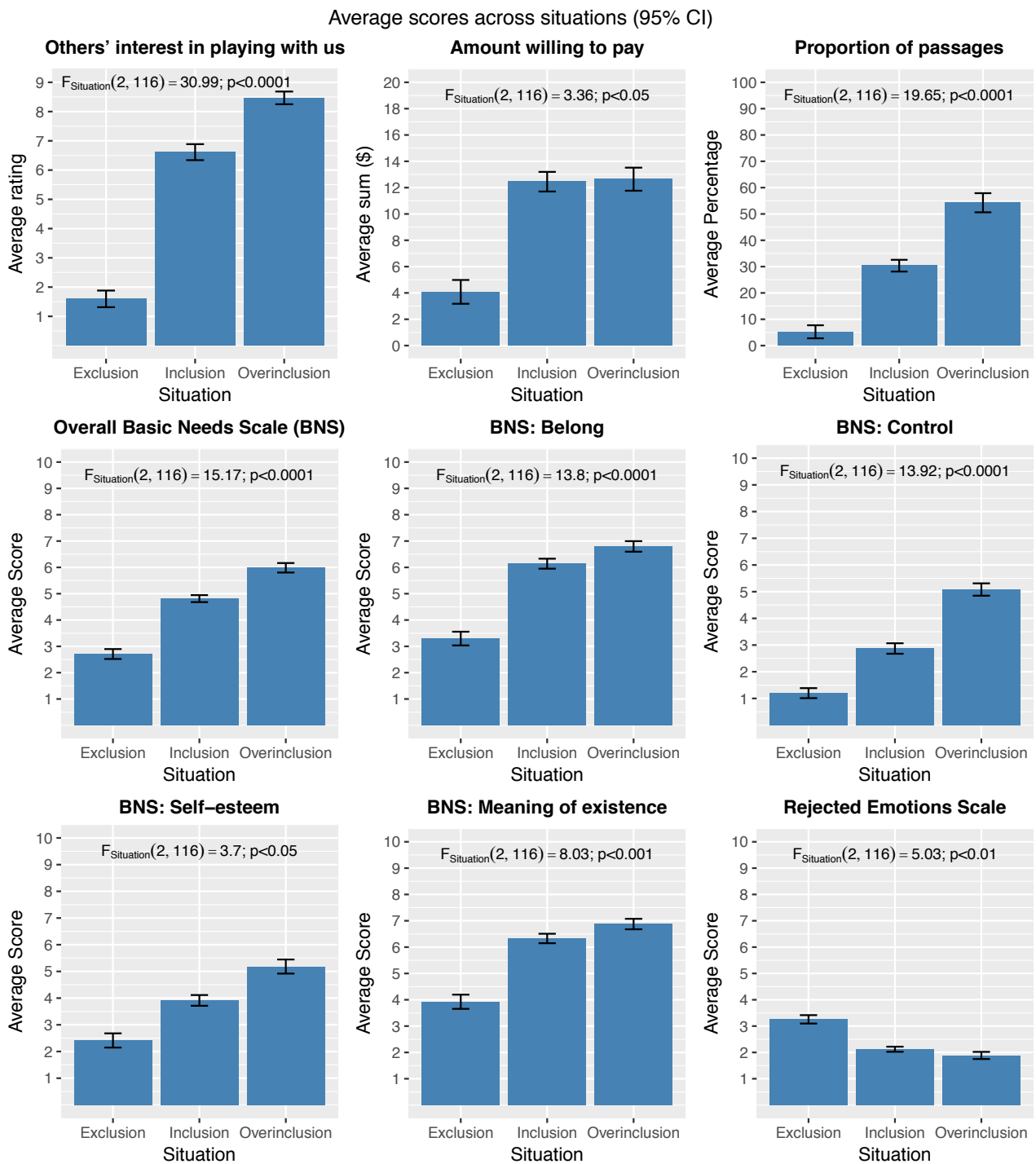
*Items of Manipulation check.* The three items showed that participants accurately perceived the percentage of throws ( $F_{\text{situation}}(2, 116)=19.65; p<0.0001$ ) they received in inclusion ( $M=33.26\%$ ) and exclusion ( $M=9.6\%$ ) situations but underestimated the received throws in over inclusion situation ( $M=59.8\%$ ). Coherently, participants decided to share with other players less money in the exclusion situation, but the same amount in the other two situations ( $F_{\text{situation}}(2, 116)=3.36; p<0.05$ ).

*Self-reported levels of needs and rejected emotions.* The items assessing the four needs (state measures of belonging, control, self-esteem, and meaningful existence) were reverse scored where necessary and the internal consistency of the items assessing each need were examined. At the same time, the items assessing the rejected emotions were reverse scored where necessary.

For each dependent variable, the mean scores in the Exclusion situation were significantly different than in the other two situations. In specific, exclusion was less than in the other two situations for others' interest ( $F_{\text{situation}}(2, 116)=30.99; p<0.0001$ ), Overall Basic Needs ( $F_{\text{situation}}(2, 116)=15.17; p<0.0001$ ) and the subscales of Belong ( $F_{\text{situation}}(2, 116)=13.8; p<0.0001$ ), Control ( $F_{\text{situation}}(2, 116)=13.92; p<0.0001$ ), Self-Esteem ( $F_{\text{situation}}(2, 116)=3.7; p<0.05$ ) and Meaning of Existence ( $F_{\text{situation}}(2, 116)=8.03; p<0.0001$ ). At the contrary, considering the Rejected emotions scale it was possible to find a greater value for exclusion respect to the others situations ( $F_{\text{situation}}(2, 116)=5.03; p<0.01$ ).

A significant difference between the mean in the inclusion situation and in the over-inclusion one was also found for each dependent variable except the amount that participants reported to be willing to pay. Indeed, in this case the amount to pay is similar in the tow situations. No other significant main effect or interaction was found in the ANOVAs, except for the amount participants reported to be willing to pay, that was significantly greater for Females than for Males ( $F(1, 58) = 4.14; p<.05$ ; mean females = 16.6, mean males = 13.2).

**Fig 4. Plots of the means of the further different self-report measures as function of the game situation. Error bars are 95% difference-adjusted confidence interval of the means.**



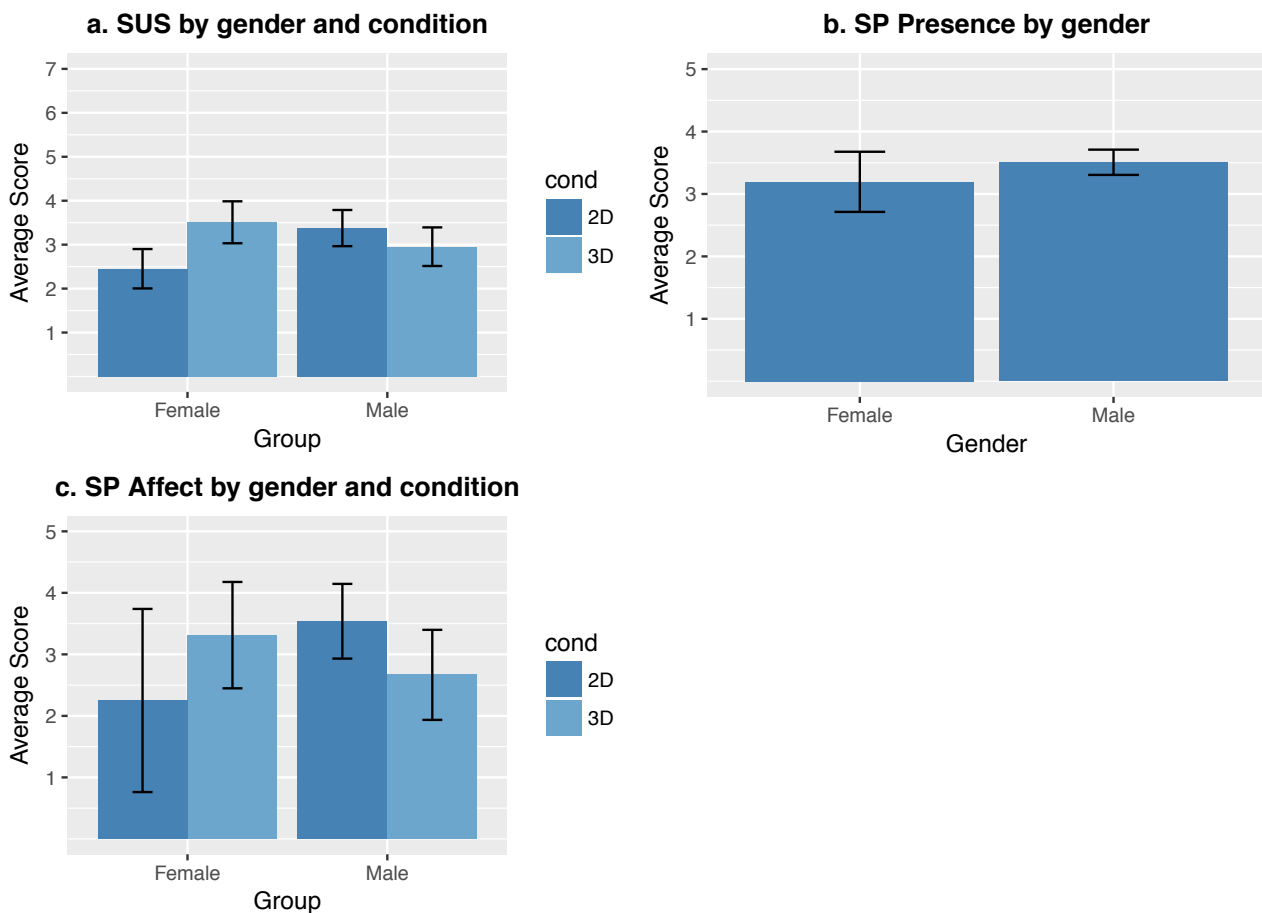
*Self-reported levels of presence*

To test the hypothesis 2 (3D condition elicits greater sense of presence than the 2D one), we compared the means of the two groups of participants assigned to the two different conditions. Following it is possible to find the strategy analysis and results. The analysis of the SUS data revealed a significant interaction of gender X condition ( $F(1, 58) = 4.77; p < .05$ ). We compared the scores across conditions for males and females separately using t-tests to understand the nature of

the interaction. The results revealed that for Females, the SUS scores were significantly higher in 3D than in 2D ( $t(21)=2.57$ ;  $p<.05$ ; mean 3D = 3.51; mean 2D = 2.45), while for Males the pattern of means was reversed, although the scores were not significantly different across conditions ( $t(34.7)=-1.05$ ;  $p=.30$ ; mean 3D = 2.95; mean 2D = 3.38). The mean SUS scores as function of gender and condition are plotted in figure 2a.

The analysis of the three Social Presence sub-scales (Social Presence, Affect toward the agent and Willingness to commit an embarrassing act) did not yield any significant main effect or interaction. For the Social Presence subscale, however, a marginally significant effect of gender was found ( $F(1, 30) = 3.49$ ;  $p = .07$ ), and the pattern of means (figure 5b) revealed higher scores for Males than for Females (mean difference = .87).

**Fig 5.** a) Average scores on the SUS scale as function of gender and condition. b) Average scores on the SP Presence subscale by gender. c) Average scores on the SP *affect toward the agent* subscale as function of gender and condition. Error bars are 95% difference-adjusted confidence interval of the means.



For the Affect subscale, instead, a marginally significant Gender x Condition interaction was found ( $F(1, 30) = 3.38$ ;  $p=.08$ ). Although neither the t-tests of the simple effects of condition nor those of the simple effects of gender were significant, the plot of the means in figure 2c reveals a trend for

females to express higher scores in 3D than in 2D, and males the opposite trend, similar to what found for the SUS scale scores.

### ***3.5.2 Physiological effects***

#### Data analysis strategy

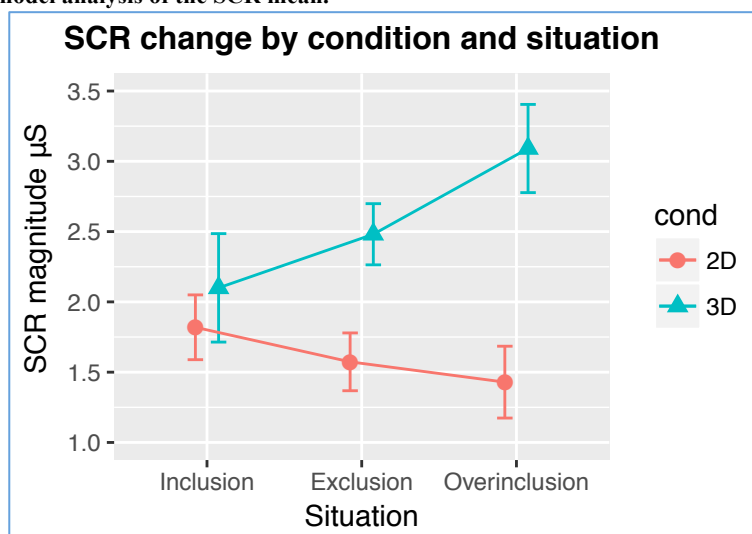
The physiological data were analyzed with linear mixed-effects models, also known as multilevel models. These models are an extension of the linear model behind ANOVA and multiple regression, that allow to model correlations among observation due to one or more grouping factors (factors which can be included as random factors in the model), and thus are suitable for repeated measures designs. Substantially equivalent to ANOVA in case of balanced designs (i.e. same number of observations in each conditions), they have more statistical power than ANOVA in case of missing data (if for a participant we have a missing observation in one of the within-subjects conditions, we do not have to discard all the participant's data as we must do with ANOVA) and greater modeling flexibility (e.g. can be used to model not-normal dependent variables, such as counts, rates or binary variables, and do not require to assume sphericity). The cost of this great power and flexibility is that these models are more difficult to interpret, especially in case of complex multi-factor designs. Being a form of multiple regression, in fact, when a factor has N levels it must be coded with N-1 binary predictors, and this makes interactions substantially more difficult to understand. However, tests of hypotheses analogous to ANOVA type III F tests can be computed for fitted models, using an approximation of denominator degrees of freedom such as the one proposed by Sattethwaite (SAS Institute, 1978). We followed this strategy for each model that we fitted, that included one dummy coded predictor for gender (Female = 0, Male = 1), one dummy coded predictor for condition (2D = 0, 3D = 1), and 3 predictors for Situation each representing a contrast between a game situation and the baseline (i.e Baseline vs Inclusion; Baseline vs Exclusion, and Baseline vs Over Inclusion). A stepwise backward strategy was then followed to identify the best fitting model, starting with the test of the highest order interactions, and proceeding to test further lower effects (interactions or main effects) only if the higher order ones were not significant. All the analyses were conducted using the R language. Mixed-effects models were fitted using the lmer function of the lme4 package (Bates et al. 2015), and approximate F tests and stepwise analysis were conducted using the ANOVA and step functions of the lmerTest package (Kuznetsova et al. 2016).



## Description of results

To test Hypothesis 3 (3D elicits greater arousal), we compared the mean SCLs between conditions during Cyberball. As predicted the means of 3D situations were higher than 2D. Following there is a description of results. Skin conductance level change from baseline was analyzed in a 3-way mixed-effects ANOVA, including game situation as within-subjects factor, and gender and condition (2D vs 3D) as between-subjects factors. The result of the ANOVA did not yield any significant main effect or interaction, although the test of the grand mean was significant ( $F(1, 41) = 6.62$ ;  $p < .05$ ), showing that overall there was indeed a change in the SCR from the baseline. However, when the analysis was repeated including only condition as between-subject factor, the result of the ANOVA showed a significant interaction between condition and situation ( $F(2, 86) = 7.03$ ;  $p < .01$ ), and a marginally significant main effect of condition ( $F(1, 43) = 4.021$ ;  $p = .051$ ). We followed up the significant interaction analyzing first of all the simple effects of situation in the 2D and in the 3D group. No significant effect of situation was found in the data from the 2D group ( $F(2, 46) = 1.55$ ;  $p = .22$ ), while the effect was significant for the 3D group ( $F(2, 40) = 5.54$ ;  $p < .01$ ). As the plot of the mean change score as function of situation and condition in figure 6 shows, for the 3D group the skin conductance response tended to increase from the inclusion situation (minimum) to the over inclusion one (maximum), and paired sample t-tests confirmed that the change score in the over inclusion situation was significantly higher than in both the exclusion ( $t(20) = 2.92$ ;  $p < .01$ ) and the inclusion ( $t(20) = 2.68$ ;  $p < .05$ ) ones. Conversely, the pattern of means for the 2D group shows a slightly decreasing trend, and no significant differences were found in the pairwise comparison between the different situations.

**Figure 6.** Plot of the differences of least squares means for the effect of the Situation x condition interaction, estimated from the linear mixed effects model analysis of the SCR mean.



We also analyzes the simple effects of condition in the different game situations, and the results showed that in the over inclusion situation the means change score for the 3D group were

significantly higher than for the 2D one ( $t(34.17) = 2.71; p < .05$ ), while no differences were found for the inclusion situation ( $t(43.07) = 0.53; NS$ ). In the exclusion situation, moreover, a trend for significance was found in the one tailed paired sample t test comparing the means in 2D and in 3D ( $t(38.71) = 1.49; p = .07$ ).

Given that the analysis of the skin conductance level change only yielded significant results when the gender factor was not included, one could wonder whether the significant interaction effect found in the 2-way ANOVA could be due to gender differences, that when controlled could obscure the effect of the other factors. To better test this hypothesis (hypothesis 4), we also conducted a 2-way ANOVA including gender as the between-subjects factor, and situation as the within-subjects one. The results, however, did not yield any significant main effect or interaction, thus suggesting that the lack of significance in the full factorial ANOVA could have been due to lack of statistical power.

Heart frequency change from baseline data was also analyzed in a 3-way ANOVA, including the same between-subjects and within-subjects factors. No significant main effect of any of the factors, nor of their interaction was found, and even removing the gender factor did not affect the results.

Considering the general heart rate data, we found that females had a significantly higher rate than males ( $F(1, 26.039) = 31.87; p < .0001$ ; mean HR females = 87.2; mean HR men = 69.1).

### **3.6 Discussion**

The ostracism has been extensively studied in experimental settings (Williams et al., 2001; de Panflis et al., 2015; Bolling et al., 2011; Eisenberger et al., 2003) and it has been observed to have immediate, detrimental effects on self-reported emotions.

Consistent with previous research behavioral evidence has demonstrated the negative psychological effects of social exclusion (Sebastian, Viding, Williams, & Blakemore, 2010; van Beest & Williams, 2006; Williams, Cheung, & Choi, 2000; Wirth & Williams, 2009; Zadro, Williams, & Richardson, 2004, 2005) and the immediate negative emotional reactions (Gerber and Wheeler, 2009). In specific, following ostracism participants scored themselves lower on self-esteem, belonging, control, and meaningful existence.

Regarding our results, the ostracism manipulation was perceived accurately by participants, that is, they were aware when they were included versus excluded from the social interaction.

Furthermore, our findings showed that the over-inclusion differed from exclusion situation but also from the inclusion one. Specifically, when we compared the over inclusion situation with the inclusion one, we found that participants felt greater sense of belonging, meaning existence, self-esteem and control. Regarding the emotions of rejection felt during the game, in line with previous

research (de Panfilis et al., 2015), we found that participants experienced the greater degree of rejection in exclusion situation and the minor degree in over-inclusion one. We found the same pattern of results using both versions, 2D and 3D, of Cyberball, suggesting that participants felt similar effects on self reported feeling playing both games. But we found different results assessing physiological indices (see below).

At the best of our knowledge, this is the first study on social interaction in virtual environment, which evaluates all, the effects of inclusionary state, level of realism on psycho-physiological responses and the degree of felt social presence. In addition we evaluated also the gender differences and we found that, considering the subscales of the Social Presence Scale the female group felt a significant greater level of towards the embodied agents in the 3D condition and the male group showed an opposite trend (but this is not significant).

In measuring emotional responses, it is important to consider both subjective and psychophysiological indices to obtain a more complete understanding of the subjective response. Self-report data, when used in isolation, are highly susceptible to extraneous influences (Schwarz, 1999). Therefore, dependent measures based on self-report questionnaires are best used in conjunction with other measures because participants are not always the most accurate judges of their own thoughts and feelings, so they often misreport affective and cognitive responses to stimuli (Bailenson et al., 2004). Psychophysiological indices are less susceptible to demand characteristics and responder bias. Emotional responses are typically thought to be composed of two primary dimensions: valence and arousal.

Previous research showed emotional distress created by exclusion is related to enhanced activation of the limbic system (Eisenberger, 2012; Kross, Berman, Mischel, Smith, & Wagner, 2011), which leads to increased sympathetic arousal of the autonomic nervous system (Cavanagh & Allen, 2009). Kelly and colleagues (2014) proposed that the higher arousal levels observed in the ostracism group are likely to be the result of the stress associated with social pain. There is also supporting evidence to suggest that heart rate slows in response to unexpected social rejection (Gunther Moor et al., 2010). For these reasons, in this study we used skin conductance responses and heart rate as objective indices of arousal.

Partially in line with these cited research, we found greater SCR during the Exclusion situation than the inclusion one, furthermore we found, for the first time, the highest skin conductance response in over-inclusion situation. We found these results only in the 3D condition where the skin conductance response tended to increase from inclusion (minimum) to over-inclusion (maximum). Conversely and in contrast with our hypothesis, the pattern of means for the 2D group showed a slightly decreasing trend, and no significant differences were found in the pairwise comparison

between the different situations.

In summary, we found that the Cyberball 3D elicits the same magnitude in self-reported ratings (basic needs and rejected emotions) in participants but has a greater impact on the skin conductance response, specifically in exclusion and over-inclusion situations. Furthermore, our 3D version of the game elicited in females a greater sense of presence than in 2D Cyberball.

## SECTION 3

### EXPERIMENTAL STUDY. INVESTIGATION OF PSYCHO-PHYSIOLOGICAL RESPONSES TO SOCIAL INTERACTION IN VIRTUAL ENVIRONMENTS

#### CHAPTER 4: AIMS AND METHODS OF THE EXPERIMENTAL STUDY

##### 4.1 Research questions and aims of the study

The first research question is related to the understanding of the social experience in autistic persons. In healthy people, social interaction has been extensively studied in experimental settings (Williams et al., 2001; de Panflis et al., 2015; Bolling et al., 2011; Eisenberger et al., 2003) and it has been observed that ostracism has immediate, detrimental effects on self-reported emotions. But little is known about how social exclusion or over inclusion is interpreted, experienced or managed by autistic persons.

The second research question is related to the use of virtual environments with high-function autism.

Many studies pointed out that individuals with ASD are stated to have an affinity to technology and a strong visual memory (Bozgeyikli et al., 2017), given that VR could provide an opportunity to investigate the power of training experiences (Weiss et al., 2003; Rizzo & Kim, 2005; Bolte et al., 2010) in a controlled setting. Highlighting the power of environmental cues such as anthropomorphism of avatars, this study supports the claim that specific environmental influences can alter biological and psychological responses to social stimuli.

The third research question is related to the reliability of the using of subjective report with autistic persons. One should be especially wary of the dependability of self-reports from persons with ASD, who may have deficits in processing (i.e., identifying and describing) their own emotions (Hill et al., 2004).

In order to respond to these research questions the current study examined both self-reported emotional and behavioral responses, and the physiological effects of social interaction in persons with ASD, using two versions of the Cyberball paradigm, the traditional 2D version and the newly developed 3D version presented in Chapter 3. Both psychological and physiological responses were also analyzed comparing ASD group and TD group.

Based on previous research, it was hypothesized that:

(1) individuals with ASD would be able, as controls, to detect ostracism when they were excluded from the game;

- (2) the ASD group would report similar levels of needs threats (sense of belonging, sense of meaningful existence, self-esteem, sense of control) after being ostracized compared with controls;
- (3) the ASD group would report less negative mood after being ostracized than controls, coherently with findings by Sebastian et al. (2009) and
- (4) individuals with ASD would display similar or increased levels of arousal following ostracism compared with controls, replicating findings by Trimmer et al. (2017).

For the first time with autistic participants, we aim to

- (5) investigate the autonomic thermal signature associated with social experiences by facial thermal variation;
- (6) investigate the effects of virtual environment realism on psycho-physiological responses;
- (7) test the extreme inclusion (over inclusion on the Cyberball) situation in ASDs, assuming that emotional reactions reflect motivationally relevant outcomes, exclusion should cause emotional distress, and inclusion should cause positive emotional reactions.

## 4.2 Materials and methods

### 4.2.1 Participants

22 (female = 2) individuals with High-Functioning Autism (HFA) recruited by clinicians from Istituto Auxologico Italiano and 24 (female = 2) individuals with typical development (TD), matched to the HFA sample in terms of chronological age (range = 17-40) and screened for major psychiatric and neurological illnesses, were recruited/included in for this study.

**Tab. 4 Typical development participants**

GENDER	TOT	AGE MEAN	CONDITION	
			2D	3D
MALE	20	22	11	9
FEMALE	2	22,5	1	1

**Tab. 5 High Functioning-Autistic participants**

GENDER	TOT	AGE MEAN	CONDITION	
			2D	3D
MALE	18	21	10	8
FEMALE	2	22	1	1

Sample with HFA were diagnosed using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and DSM-IV criteria, as well as experienced clinical judgment.

### 4.2.2 Experimental paradigm and procedure

Participants were welcomed in the lab and before starting the session each participant was invited to give written consent to participate in the experiment, according with the Ethical Committee of the Istituto Auxologico Italiano. Then, the experimenter explained test procedure and data treatment method, ensuring the anonymity of data processing.

Participants were also shown and explained how the psychophysiological measures would be used. Participants were then seated in a chair facing a computer screen approximately 75 cm away. At the first, participants were asked to wear the EEG cap in order to install the 64 electrodes. Skin conductance and heart rate recording devices were then attached. Participants were requested to sit quietly and relax to look a black monitor for five minutes to register all autonomic measures at the resting baseline period. All questionnaires and games were computerized versions presented in Medialab (Empirisoft Corporation, Version 2008.1.33).

Just before starting the experimental session, participants were asked to fill in a short demographic questionnaire with questions about age, sex, title of study, current job and residence.

Participants of typical development eligibility were determined via subject-reported medical history, assessed by a team of psychologists. Two participants were excluded from the study.

They were told that performance in the game was unimportant, and instead, the game was merely a means for them to engage their mental visualization skills. Starting the experimental session (Fig. 7), participants were asked to fill in the Scale of Mental Visualization, as typical procedure with Cyberball paradigm (Williams et al., 2000).

**Figure 7. Screen shot of the starting of the experiment**

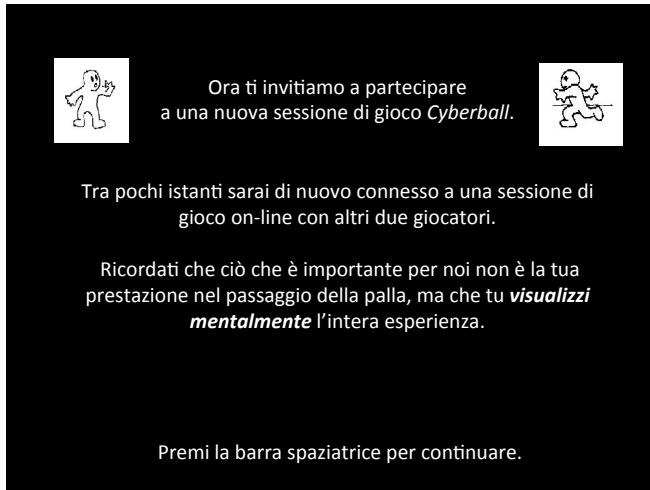


This scale was included to emphasize the cover story of the study. This scale had statements such as, “I have vivid dreams” or “When I read a book, I can see the main characters clearly in my mind”.

In order to make standard the instructions to the participants, these were provided in written form through the MediaLab program. Explanatory figures were included to enrich the

description (Fig. 8). Furthermore, in some slides the program presented the avatars representing other two players (Fig. 9), including that of the participant (Fig. 10).

Figure 8. Screen shot of the game instructions



Participants were randomly assigned to the orders in which condition were presented in Medialab. After the final concurrent measurement was completed, research assistants helped the participant log off, remove the EEG cap. Finally, participants were debriefed and had the chance to comment on the study and ask questions of the researchers.

They were informed that they would play with two other individuals stationed in similar laboratories at two other labs. This cover story was augmented by staged phone calls to the other experimenters making sure that their participants were ready to go.

Figure 9. Screen shot of other two players of Cyb. 2D

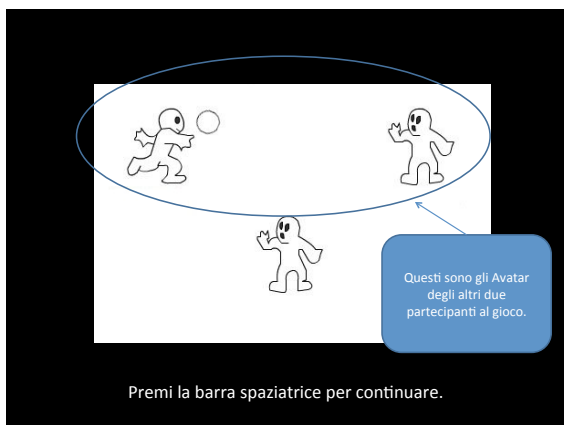
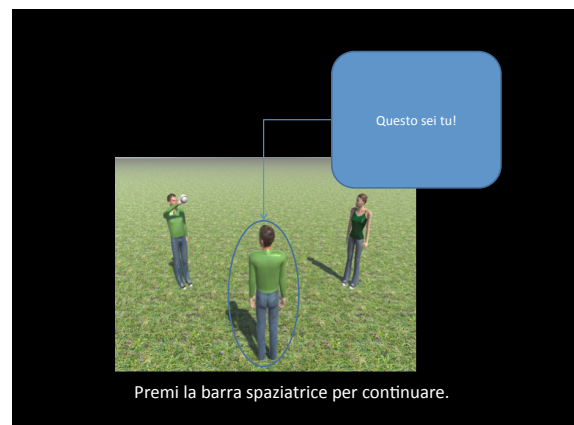


Figure 10. Screen shot of the participant avatar of Cyb. 3D



The experimental design was a 3-mixed factors, 2 (*Group*: TD vs ASD) x 3(*Situation*: inclusion, exclusion and over inclusion) x 2(*Condition*: 2D vs 3D). The 2 between-subject factors were the Group and Condition, and the within-subject factor was the type of game Situation (inclusion, exclusion and over inclusion).

#### 4.2.3 Stimuli for social interaction

##### Cyberball 2D

The Cyberball game (Williams, Cheung, & Choi, 2000) is a computer based 'ball tossing' game,



Cyberball is an ecologically valid experimental paradigm that can be adopted to evaluate emotional reactions to both social inclusion and exclusion (Fig. 1 of chap. 4). Before starting using the game it is possible to set the number of ball tosses and the time between tosses. Furthermore, other features can be adjusted, including the avatars' names, the players' pictures and the presence of a chat. For this experiment we adopted a modified version of the classic Cyberball paradigm that adds an "over-inclusion" condition to the two classic conditions of the game of ostracism and inclusion (see de Panfilid et al., 2015; Leitner et al., 2014; Kawamoto et al., 2012; Van Beest & Williams, 2006; Wesselmann & Williams, 2013; Williams et al., 2000). In the current study, when over-included the actual participant received the ball about 45% of the total launches. In the inclusion condition, throws are distributed evenly to all players throughout the game (33% of tosses). In the exclusion condition, participants receive 4 balls at the beginning of the game and are ignored thereafter (36 tosses). In this study, on-line players were not provided with photos or their names to avoid giving participants any other reason for disliking another player apart from the fact that they were being ostracized by these players.

#### CyberballAvatar 3D

The utility of virtual environments for assessment in ASD has led to a preliminary studies and reviews suggesting that virtual environments may be ideal for the assessment of ASD (Byom & Mutlu, 2013; Parsons & Carlew, 2016).

The *CyberballAvatar3D* (Fig.2 and Fig.3 od chap. 4) is a 3D version of the classic paradigm, specific developed by Unity 5.0, for this project. The avatars are rigged and generated by Autodesk Character Generator (free version). This version of the game features the same operations and the same setting at predetermined blocks. The only aspect that changes regards the characteristics of the environment and the players. In the Avatar3D version, in fact, it has the image of an external environment, a grass in an open field, where 3 avatars (default: left player is a boy and right player is a girl; the participant player is either a boy or a girl) can play with a ball. The dimension of depth and anthropomorphic characteristics of the players make the scene more realistic and immersive. In addition, to increase the sense of presence in participants, the corresponding avatar can be customized by choosing his gender, the color of hair and clothes. Also in this version of the game the three steps of inclusion, exclusion and over-inclusion are programmed with 40 tosses for each one and with the same time between tosses.

#### **4.2.4 Psychological assessment**

In order to acquire changes in psychological response to different social situations, the following psychometric questionnaires were administered to each participant, assessing change in reported

feelings, mood and behaviors, after each session of the game. At the end of the experiment two scales of “presence” were administered, in order to understand the degree/level of sense of presence felt during the game.

#### Manipulation check

Manipulation checks consisted of 3 items administered after each session of the game. The first one (What percentage of throws did you receive during the past session of the Cyberball game?) assessed the perception of throws received. Participants indicated this by typing in their response box, choosing from 0 to 100%.

In the other two items participants were asked to indicate their own feelings felt during the game of *exclusion* and *rejection* in a 10-point Likert scale.

#### Measures of behavior and mood during the game

*Need Threat Index* (van Beest & Williams, 2006) is the short version of the Need Threat Scale (Williams et al., 2000). The index measured satisfaction of four basic psychological needs through 4 items assessing sense of Belonging, Self-esteem, Control, and Meaningful existence. These were rated on a 10-point Likert scale from ‘not at all’ to ‘very much’.

*Scale of Mood* is a section of the Need Threat Scale (Williams et al., 2000). Participants rated how good/bad, happy/sad, friendly/unfriendly and tense/relaxed they were currently feeling, on a scale of 1–10, from ‘not at all’ to ‘very much’. The scale assessed the negative mood, so positive items were reversed and an average mood score was calculated.

*Rejected emotions scale* (Buckley, Winkel, & Leary, 2004) assesses sense of rejection during the game (20 items on a 10-point Likert scale). The scale measured the intensity of five emotions through 5 subscales: Anger, Happiness, Hurt feelings, Anxiety, Sadness. We created two indexes, negative emotions without Happy subscale and with Happy reversed score.

*Behavioral Temptation Scale* (Allen & Leary, 2010) measured two opposite constructs, antisocial behavior (e.g., humiliating other person or slapping him) and social behavior (e.g., smiling at the other person).

This scale (1 = not at all and 10 = very much so) measured participants’ self-reported temptations to engage in the presented behaviors. Just before filling in the scale, participants were reminded that it is not asking whether they would have actually done each behavior, but the degree to which they would have been tempted to do these actions.

*Dread of future interaction* (Waldrup, 2007) assessed participants' level of fear of future interaction with one of the other "supposed" participants. The scale was composed of twelve items (i.e., "I think the interaction with the other participant will be awkward and uncomfortable"), and respondents responded to each statement using a 10-point scale ranging from 1 (strongly disagree) to 10 (strongly agree).

*Self isolation Scale* (adapted from Ren et al., 2016). This measure is composed by three separated items measuring the desire of participants to replay with: Item 1 *alone*, Item 2 *with the same players* and Item 3 *with other players*. This scale was administered after each session of the game to investigate the effect of exclusionary status in the participants' desire to replay alone, with the same players or alternatively with different players.

#### Single items during the game

*The Faces Pain Scale* (Bieri et al., 1990) is a self-report measure of the perceived pain intensity through a close linear relationship with visual analog pain scales, in a 5 points likert scale (Fig. 10).

**Figure 10. The face pain scale**



*The Inclusion of Other in the Self Scale (IOS)* (Aron et al., 1992). The item is a pictorial measure of the psychological overlap between the self and the other. We adapted this item for the project purposes. The participants were asked to express their degree of connection with other participants, indicating how close they felt with the two other players during the past session. Higher scores indicate a higher degree of social connection.

*Amount to Pay* is a single item created for this research project. The participant says he has 30 euros and that he can decide to share or not with the other players. Participant were asked to indicate the amount of Euros that he decided to give to other participants, from 0 to 15 euros.

### Measures of presence after the game

*Social presence Questionnaire* (Bailenson et al., 2001). The questionnaire measured self reported social presence (i.e., the degree to which people report being in the presence of a veritable human being), affect towards the agent, and finally, how willing they would be to perform a series of embarrassing acts in front of the agent. The sub-scale *affect* measures negative-positive affect towards the agent embodied. To measure social presence, we took a summation score of the first six questions. Positive numbers indicate high social presence while negative numbers indicate low social presence. *Willingness to perform an embarrassing act*. We took a summation score of the last four questions; positive numbers indicate willingness to commit an embarrassing act (i.e., change clothes or tell secrets to the agent) while negative numbers indicate un-willingness to commit an embarrassing act.

*Slater-Usuh-Steed Presence Questionnaire (SUS)* (Slater et al. 1994). The questionnaire assesses the level of presence experience during media exposures by asking participants to rate the features of media experience (feeling of being there, realism, involvement) on a 7-point likert scale.

### **4.3 Physiological assessment**

Several physiological signals, judged as a favorable approach (Bethel et al., 2007), are examined in this experimental project. The set of signals consists of various cardiovascular, electrodermal, and skin temperature signals, all of which have been extensively investigated in psychophysiology literature (Bradley, 2000).

Emotional engagement given by simulations has been measured by physiological arousal, that is considered a byproduct of the responses to stressful and fearful events (Cacioppo et al. 1993, Wiederhold et al. 2002, Wilhelm et al. 2005, Fox et al. 2012). In particular, heart rate (HR) and skin conductance level (SCL) can be considered reliable indexes of arousing involvement during exposure to virtual representation of real environments (Pallavicini et al., 2013; Gorini et al., 2010; Groenegrass et al. 2010; Kotlyar et al. 2008; Slater et al. 2006; Krantz et al. 2004). The autonomic nervous system (ANS) is responsible for maintaining homeostasis, adaptability and physiological flexibility of the organism. Physiologically, both the sympathetic and parasympathetic systems work at dynamic balance at rest as well as in response to stress.

Evidence of structural and functional abnormalities in central nervous system in ASD persons may be associated with autonomic dysregulation (Bujnakova et al., 2016). Recent research has focused on parasympathetic activity, due to its potential role in regulating emotional and behavioral

function (Porges et al. 2013). Regarding sympathetic activity quantified by electrodermal activity (EDA), existing findings in ASD are mixed, such as increased (Kushki et al. 2013, O'Haire et al. 2015) and unaltered (Levine et al. 2012, Legiša et al. 2013) basal skin conductance, as well as atypical EDA reactivity to faces (Hirstein et al. 2001) or eye contact (Kylliäinen and Hietanen 2006) and greater arousal in response to ostracism (Trimmer et al., 2017).

#### **4.3.1 Data acquisition and analyses**

The SCR and HR indices were registered using the NeXus-4 equipment, with the BioTrace+ software for recording signals, developed by Mind Media. The module gets similar data coming from different physiological sensors and, after conditioning and digitalizing, sends them to a host Personal Computer (PC) using a wireless connection.

Physiological data were pre-processed and analyzed using AcqKnowledge 4.2 (Biopac System, Inc), R-waves in the ECG data were identified automatically by the software and converted to bpm. Additionally, a visual artifact inspection was conducted and the average score of the tonic SCR were calculated.

Thermal IR imaging was performed by means of a digital thermal camera FLIR A655sc (IR resolution: 640 X 480 pixels; thermal sensitivity/NETD: < 30 mK @ 30°C). The acquisition frame rate was set to 5 Hz (5 frames/sec). Before the experimental session, each participant was left to acclimatize for 15 minutes in the experimental room, to allow the skin temperature to stabilize. The recording room was set at standardized temperature (26°C), humidity (50–60%) and without direct sunlight, ventilation, airflow. The distance between the subject and the camera was set to 1 m (Cardone & Merla, 2017).

Skin conductance and heart rate were recorded simultaneously with a sampling rate of 1000 Hz. Skin conductance response (SCR) was measured from the distal phalanges of digits II and IV of the non- dominant hand with dry electrodes and HR from a sensor applied in the digit III. Skin conductance signal was converted to microsiemens ( $\mu\text{S}$ ) and heart rate signal to beats per minute (bpm).

All physiological indices were recorded during the baseline condition and during the exposure to the game sessions, in order to obtain objective measures of subjects' emotional activation.

#### **4.3.2 SCR and SCL**

The skin conductance response (SCR) is a method of measuring psychological or physiological arousal. Electrodermal activity can be considered also a measure of emotional arousal by measuring the activity of the sympathetic nervous system (SNS) (Ravaja 2004). When someone becomes emotionally aroused, their skin becomes more conductive due to increased sweat gland secretions.

Thus, skin conductance serves as a measure of the SNS activity that has been associated with increased emotional arousal (Dawson et al., 2005). In order to evaluate the trend during the time session (situation of the game: inclusion, exclusion and over-inclusion), we calculated the level of skin conductance (SCL) splitting the duration of session in epochs of 40 seconds. In order to calculate the arousal value, the mean value of the two minutes of baseline was subtracted at each epoch (Kelly et al., 2014; Trimmer et al., 2017).

#### **4.3.3 Heart Rate**

The Heart Rate (HR) was used to evaluate the autonomic nervous system response. It is known that in stressful situations this index tends to increase from baseline (Meehan et al., 2005). SNS activation increases cardiovascular activity, and so researchers commonly use heart-rate (HR) as an additional measure of emotional arousal (Rooney et al., 2012). Following Hubert and de Jong-Meyer (1990), HR was converted to a measure of beats-per-minute and the score average of the BPM was calculated.

#### **4.3.4 Facial thermal responses**

To preserve the ecological nature of the experimental setting, we employed the functional infrared imaging (fIRI) technique (Paoletti et al., 2016) fIRI is a non-contact, non-invasive method which estimates variations in autonomic activity reflected by cutaneous temperature modulations by means of recording the thermal infrared signals spontaneously released by the human body (Merla & Romani, 2007). Under emotional or physical stressors, heat variations involving skin tissue, inner tissue, local vasculature, and metabolic activity are observable. Sympathetic neural control of skin blood-flow includes the noradrenergic vasoconstrictor system and a sympathetic active vasodilator system.

Thermal signals have been extracted through the use of tracking software, developed with homemade Matlab algorithms (The Mathworks Inc., Natick, MA). The tracking algorithm is based on the 2-D cross-correlation between a template region, chosen by the user on the initial frame, and a similar region of interest (ROI) in a wider searching region, expected to contain the desired template in each of the following frames. In this way it is possible to automatically extract the thermal signals in defined regions of interest during the whole experiment. Thermal signal was extracted on one specific ROI: nose tip.

The mean temperature inside the ROI was evaluated over the four experimental sessions. Then, the average value has been calculated for each subject and each experimental phase.

In order to acquire information about the dynamic evolution of the signals during the game (situation of the game: inclusion, exclusion and over-inclusion), we calculated the temperature

variation by splitting the duration of session in epochs of 40 seconds. In order to calculate the arousal value, the mean value of the two minutes of baseline was subtracted at each epoch (Kelly et al., 2014; Trimmer et al., 2017).

At least to the best of our knowledge, nothing is known about facial temperature variations in autistic persons, not only when they are exposed to ostracism, but not in any other experimental situation.

## CHAPTER 5: PHYSIO-PSYCHOLOGICAL RESPONSES TO 2D AND 3D SOCIAL SITUATIONS IN HIGH-FUNCTIONING AUTISM. RESULTS.

### 5.1 Psychological responses

Data were entered into Microsoft Excel and analyzed using SPSS 21 (IBM® SPSS® Chicago, Illinois) and R software.

In order to assess changes in mood and feelings of social inclusion-exclusion, we administered more than one questionnaire (see chapter 4). Following the description of results and related analyses.

#### 5.1.1 Correlations of the self-report measures

For each group, we computed the pairwise Pearson correlations among all the self-report measures in each different game situation. The correlation matrixes are plotted in figures 11-13 as correlograms (Friendly, 2002). Each plot presents a correlation matrix, with ellipses representing correlations between pair of variables. The strength of the correlation is represented by the aspect ratio of the ellipses (more like a circle = weaker correlation, more like a line = stronger correlation) and simultaneously by the lightness of the color (higher lightness = weaker correlation, lower lightness = stronger correlation). The direction of the correlation is represented by the orientation of the ellipses (from bottom-left to top right = positive correlation, from top-left to bottom-right = negative correlation) and by their hue (blue = positive, red = negative). Insignificant correlations (i.e. correlations with  $p\text{-value} > .05$ ) are flagged by “X”, and when it was not possible to compute the correlation between two variables, ellipses are replaced by the question mark symbol (“?”). That was only the case for all the correlations of the ratings for pain in the exclusion situation for the ASD group, because the value reported for this measure was always 1, so that it was not possible to estimate the correlation with the other measures.

To test the difference between the correlation matrices for each game situation we used the approach proposed by Larntz and Perlman (1985). The results of the tests showed that for each game situation the correlation matrix of the TD group was not equal to the one of the ASD group ( $p < .0001$ ).

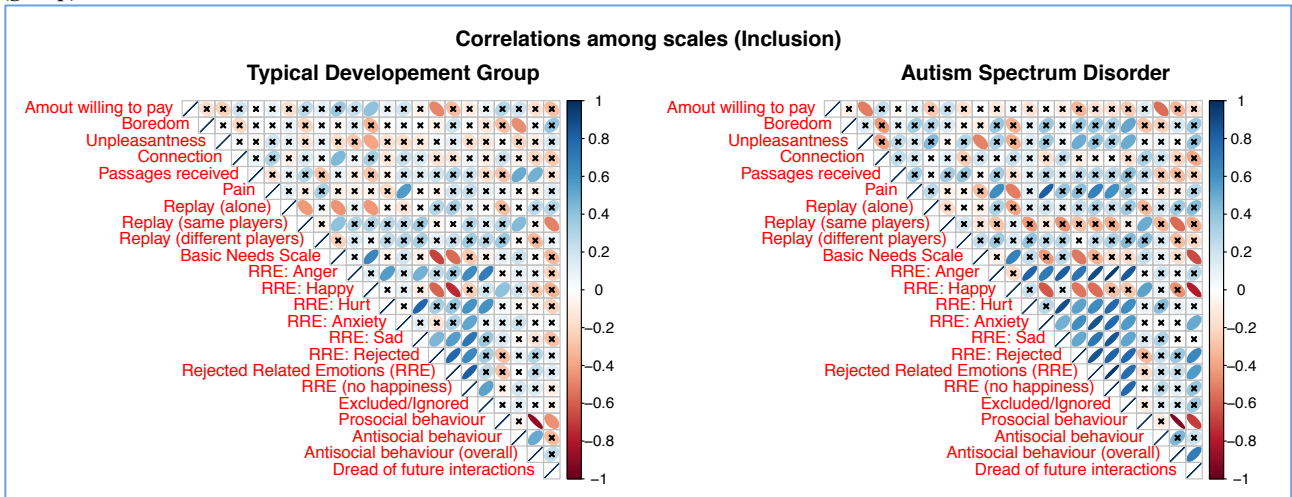
The strength and direction of the bivariate correlation are represented by lightness, aspect ratio, hue and orientation. Black X represents insignificant correlations. See below for instructions on how to read these plots.



Inclusion situation

Regarding the *Inclusion* situation there were more significant correlations for the ASD group then for the TD group (Fig. 11). We can see this more clearly looking at Unpleasantness, which for the ASD is negatively correlated to the overall basic needs index, and positively correlated to Rejected and to Excluded/Ignored, whilst for the TD is only negatively correlated to Anger.

**Figure 11. Plots of the correlations among self-report measures in the Inclusion situation for the TD (left) and the ASD (group).**



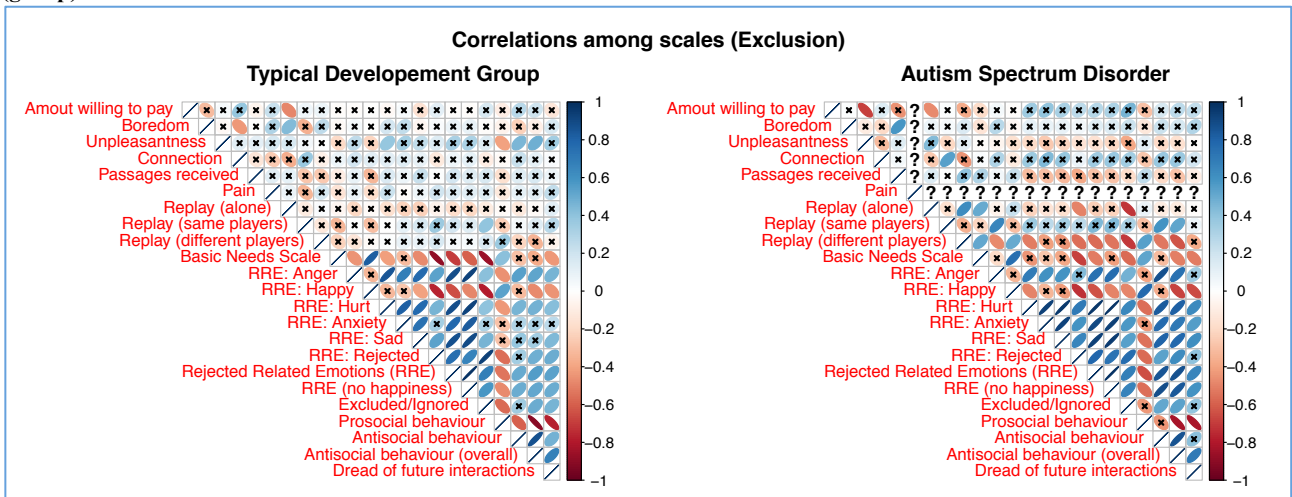
This is even more evident for Pain, which only for the ASD is negatively correlated to Happiness, and positively to Anger, and Rejected Related Emotions scale and to Anxiety, higher than TD group. Moreover, in general there are more significant correlations among the various RRE subscales for the ASD, and these correlations are also stronger. In specific, the more significant correlations it is possible to find considering the *Happiness* subscale that correlates with the variable “Dread of future interaction” only in the ASD group. At the same time, the scale “Dread of future interaction” shows higher correlations in ASD group, Prosocial behavior, Happy and Basic Needs. The excluded index positively correlates, only in ASD group, with unpleasantness and boredom variables.

Exclusion situation

The ratings for pain for the ASD group were constant so it was not possible to estimate the correlation between this variable and the other ones, so question marks are placed over the corresponding ellipses. See below for instructions on how to read these plots.

The 3 items of the *Self isolation Scale* presented any significant correlations into the TD group and at the contrary showed more than one correlations into the ASD group. In specific, *Replay with different players* positively correlated with Basic Needs, Happy and Prosocial Behavior and negatively with all negative subscales of Rejected Emotions and the Excluded index.

**Fig. 12. Plots of the correlations among the self-report measures in the Exclusion situation for the TD (left) and the ASD (group).**

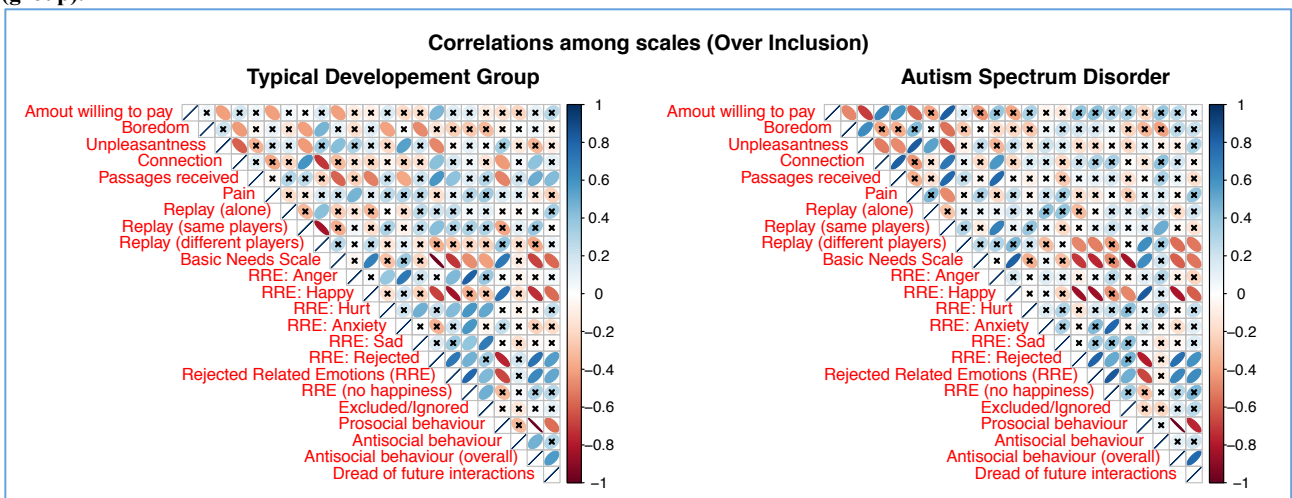


The item *Replay with the same players* positively correlated with Anger and Antisocial Behavior. The item *Replay alone* positively correlated with Basic Needs and negatively with Sad and Excluded/Ignored.

Over-inclusion situation

Also in this case, the items of the *Self isolation Scale* presented more correlations in ASD group than in the TD group. In specific, the item *Replay with different players* positively correlated with Prosocial behavior and negatively with *Antisocial behavior* and *Dread of future interactions*. The item *Replay alone* correlated positively with Anger and Antisocial behavior. The Anger value correlated, in ASD group, positively with Replay with same players, Passage received and degree of Connection. Only for the TD group Anger positively correlated with Rejected emotions.

**Fig.13. Plots of the correlations among the self-report measures in the Over inclusion situation for the TD (left) and the ASD (group).**



## **Analysis strategy of ANOVAs**

The data collected using the self-report questionnaires were always analyzed using a series of 3-way mixed ANOVAs, including 2 between-subjects factors and 1 within-subjects factor. The between-subject factors were *Group* (TD vs ASD) and *Condition* (2D vs 3D), and the within-subjects factor was the type of game *Situation* (inclusion, exclusion and over inclusion). A separate analysis was conducted for each self-report scale, subscale or measure as the dependent variable. Violations of sphericity were assessed using the Mauchly's test, and whenever violations were found we applied the Greenhouse-Geisser (GG) and Huynh-Feldt (HF) corrections for departure from sphericity. Significant interactions of two or more factors were always followed by the analysis of the simple effects (in case of significant 2-way interaction) or of the simple interactions (in case of significant 3-way interaction) of the respective factors. Difference adjusted 95% confidence intervals were computed using the method proposed by Cousineau and Morey for within-subject factors (Baguley, 2012a), and with the variation of the Goldstein-Healy method for between-subject factors (Baguley, 2012b), and plotted as error bars in the mean plots, so that non-overlapping error bars (and thus confidence intervals) plots reflect significant differences between pairs of means. Differences between pairs of means were also tested with paired and independent sample t-test, for within-subjects and between-subjects factors respectively.

In the following sections we report the results of all the analyses we conducted on the self-reports.

### **5.1.2 Manipulation check**

#### *Estimates of the passages received*

The analysis of the estimates provided by participants of the proportion of times they were passed the ball during the game yielded only a significant main effect of Situation ( $F(2, 80) = 50.22$ ;  $p < .0001$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means and the 95% CIs (fig. 4) revealed that the participants were fairly accurate in their estimate of the percentage of times they received the ball in the inclusion situation (mean=33.9%; 95% CI: 31.4%–36.3%), and well as in the exclusion (mean=8.1%; 95% CI: 4.8%–11.4%), and in the over inclusion (mean=58.7%; 95% CI: 54.8%–62.7%) ones. No other significant main effects or interactions were found.

### **5.1.3 Effect of Cyberball situations**

#### *Amount that participants were willing to share with other players.*

The ANOVA conducted on the amount of money participants reported they were willing to pay yielded only a significant main effect of Situation ( $F(2, 80) = 16.55$ ;  $p < .0001$ ). A violation of sphericity was found, but the effect of situation remained significant with both the types of

corrections (GG and HF). The pattern of means (fig. 4) revealed that the amount participants were willing to pay in the inclusion (mean=9.4 €) and in the over inclusion (mean=10.2 €) situations was significantly higher than in the exclusion situation (mean=2.9 €). No other significant main effects of group or condition were found, and neither any significant interactions.

#### *Boredom during the game*

The analysis of the ratings of the degree of boredom felt during the game revealed significant main effects of Group ( $F(1, 40) = 8.57$ ;  $p < .01$ ) and Situation ( $F(2, 80) = 15.56$ ;  $p < .0001$ ), a significant Group x Situation interaction ( $F(2, 80) = 4.01$ ;  $p < .05$ ) and a significant Condition x Group x Situation interaction ( $F(2, 80) = 3.18$ ;  $p < .05$ ). A violation of sphericity was found, but all the effects remained significant with both types of corrections (GG and HF), except the 3-way interaction, which after the correction was only marginally significant ( $p = .06$ ).

The boredom ratings for the TD group (mean=5.5) were significantly higher than for the ASD group (mean=2.7). The pattern of means across situations (fig. X), moreover, revealed that the ratings in the exclusion situation (mean=6) were significantly higher than in the other situations, and that ratings in the inclusion situation (mean=4) were significantly higher than in the over inclusion situation (mean=3.2). However the analyses of the simple effects of situation that we conducted to follow up the significant interaction revealed that only for the TD group the ratings in the inclusion situation were significantly higher than in the over inclusion situation ( $t(25) = 1.94$ ;  $p < .05$ ), while they were not significantly greater for the ASD group ( $t(17) = 1.41$ ;  $p = .17$ ). See figure 6 for the plot of the average ratings for boredom as function of group and situation.

#### *Excluded/Ignored*

The analysis of the ratings of the feeling of exclusion felt during the game revealed a significant main effect of *Situation* ( $F(2, 80) = 74.04$ ;  $p < .0001$ ), a significant *Group x Situation* interaction ( $F(2, 80) = 3.3$ ;  $p < .05$ ) and a marginally significant effect of *Group* ( $F(1, 40) = 2.93$ ;  $p = .09$ ). A violation of sphericity was found, but the effect of Situation remained significant with both types of corrections (GG and HF), while the 2-way interaction after the correction was only marginally significant ( $p = .06$ ).

The pattern of means and their CIs across situations (fig. 5), revealed that the ratings in the exclusion situation (mean = 7.9) were significantly higher than in the other situations, and that in the inclusion situation (mean=1.7) they were also slightly but significantly higher than in the over inclusion one (mean = 1.1). However, the analyses of the simple effects of situation that we conducted to follow up the significant interaction revealed that only for the TD group the sense of

exclusion was significantly higher ( $t(50) = 3.62$ ;  $p < .001$ ) in the inclusion situation (mean = 1.9) than in the over inclusion one (mean = 1.04), while that was not true ( $t(34) = .86$ ;  $p = .4$ ; mean inclusion = 1.5; mean over inclusion = 1.17) for the ASD group. See figure 5 for the plot of the average ratings for Excluded/Ignored as function of group and situation.

#### *Unpleasantness of the experience*

The analysis of the ratings of the unpleasantness of the experience yielded only a significant main effect of Situation ( $F(2, 80) = 7.64$ ;  $p < .001$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means and the 95% CIs (fig. 4) revealed that the ratings of the unpleasantness in the exclusion situation (mean=2.9) were significantly higher than in the other situations, but that there were no significant differences between inclusion (mean=1.27) and over inclusion (mean=1.25). No other significant main effects or interactions were found.

#### *Degree of connection with the other players*

The analysis of the ratings of the degree of connection with the other players yielded only a significant main effect of Situation ( $F(2, 80) = 15.84$ ;  $p < .0001$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means and the 95% CIs (fig. 4) revealed that the ratings of degree of connection in the over inclusion situation (mean=5.6) were significantly higher than in the inclusion situation (mean=4.8), and that in turn in these two situations ratings were significantly higher than in the exclusion situation (mean=2.2). No other significant main effects or interactions were found.

#### *Painfulness of the experience*

The analysis of the ratings of the degree of pain felt during the experience yielded only a significant main effect of Situation ( $F(2, 80) = 7.64$ ;  $p < .001$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means and the 95% CIs (fig. 4) revealed that the ratings of the unpleasantness in the exclusion situation (mean=2.9) were significantly higher than in the other situations, but that there were no significant differences between inclusion (mean=1.27) and over inclusion (mean=1.25). No other significant main effects or interactions were found.

#### *Play again alone, with same players or different players*

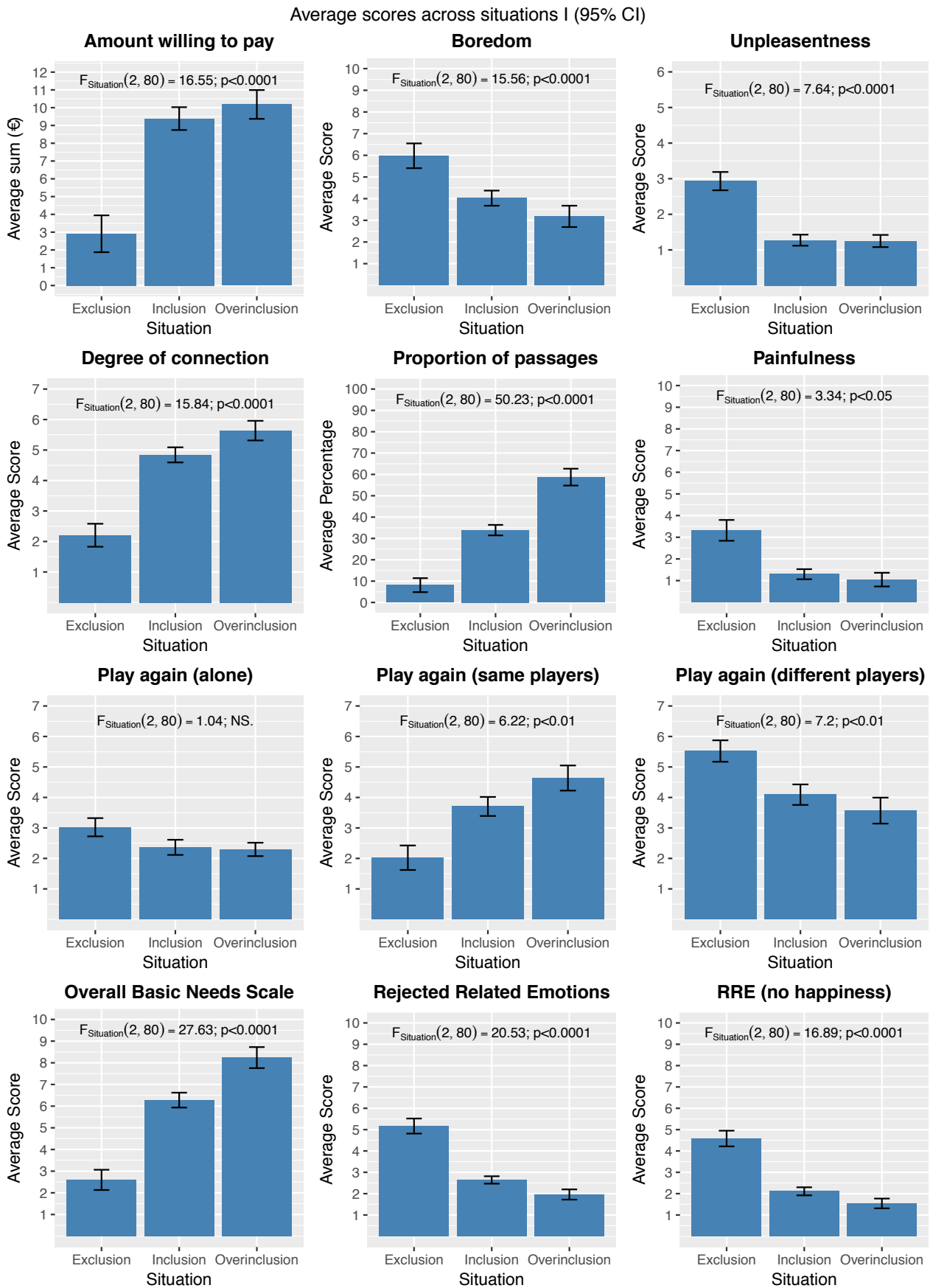
NO significant effects of any of the factors was found in the analysis of the intention to play alone ( $F(2, 80) = 1.04$ ; NS). The analysis of the intention to replay with same players or different players yielded only a significant main effect of *Situation*: With same players ( $F(2, 80) = 6.22$ ;  $p < 0.01$ );

with different players ( $F(2, 20) = 7.2; p < 0.01$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means and the 95% CIs (fig. 4) revealed that the ratings of the intention to play with different players in the exclusion situation (mean=5.5) were significantly higher than in the other situations, but that there were no significant differences between inclusion (mean=4.1) and over inclusion (mean=3.6). The pattern of means and the 95% CIs (fig. 4) revealed that the ratings of the intention to play with the same players in the exclusion situation (mean= 2.02) were significantly lower than in the other situations, and that in turn the ratings in the inclusion situation (mean = 3.7) were significantly lower than in the over inclusion situation (mean = 4.64). No other significant main effects or interactions were found on the ratings of the intention to replay with the same or with different players.

#### *Basic Needs Index*

The analysis of the ratings of the Basic Needs felt during the game revealed a significant main effect of Situation ( $F(2, 80) = 27.63; p < .0001$ ), a significant Group x Condition interaction ( $F(1, 40) = 4.15; p < .05$ ) and a marginally significant effect of Condition ( $F(1, 40) = 2.96; p = .09$ ). A violation of sphericity was found, but the effect of Situation remained significant with both types of corrections (GG and HF). The pattern of means across situations (fig. 5), revealed that the ratings in the exclusion situation (mean=2.6) were significantly lower than in the other situations, and that in turn ratings in the inclusion situation (mean=6.3) were slightly but significantly lower than in the over inclusion situation (mean=8.2). The analyses of the simple effects of group following the significant interaction also revealed that in the 2D condition there were no significant differences ( $F(1, 21) = 0.67; p = .51$ ) between ASD (mean = 5.6) and TD group (mean = 6), while on the contrary, in 3D condition the ratings considered needs for the ASD group (mean = 6.4) were significantly higher ( $t(19) = 2.16; p < .05$ ) than for the TD group (mean = 5). The plot in figure 6 shows an opposite trend for ASD and TD group, in specific the basic needs rating increases for ASD from 2D to 3D condition and decreases for TD from 2D to 3D condition.

**Fig 14. Plots of the means of the some of the different self-report measures as function of the game situation. Error bars are 95% difference-adjusted confidence interval of the means.**



### *Rejected Emotions Scale*

The analysis of the Rejected Emotions Scale yielded only a significant main effect of Situation ( $F(2, 80) = 20.53$ ;  $p < 0.0001$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means and the 95% CIs (fig. 4) revealed that the emotions of rejection felt during the game in the exclusion situation (mean=5.2) were significantly higher than in the other situations, furthermore there were significant differences between inclusion (mean=2.6) and over inclusion (mean=2). No other significant main effects or interactions were found on the overall emotion of rejection.

The analysis of the subscales Anger ( $F(2, 80) = 11.5$ ;  $p < 0.0001$ ), Sadness ( $F(2, 80) = 6.85$ ;  $p < 0.01$ ), and Hurt ( $F(2, 80) = 3.43$ ;  $p < 0.05$ ) and Rejected ( $F(2, 80) = 56.15$ ;  $p < 0.0001$ ) yielded only a significant main effect of Situation. In each case a violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means (fig. 5) revealed that these emotions felt during the game in the exclusion situation (means of Anger=4.3; Sadness=3.6; Hurt=3.5; Rejected= 7.8) were significantly higher than in the other situations. For Rejected, moreover, the scores in the inclusion situation (Rejected=3.4) were also significantly higher than in the over inclusion one (Rejected=2), while for the other ones there were no significant differences between inclusion (means of Anger=1.5; Sadness=1.5; Hurt=1.4,) and over inclusion (mean of Anger=1.3; Sadness=1.1; Hurt=1.2). No other significant main effects or interactions were found in the analyses of these emotions.

The analysis of the Anxiety subscale did not reveal a significant effect of Situation ( $F(2, 80) = 1.69$ ;  $p = .19$ ), although the pattern of means revealed that the degree to which this emotion was felt during the game in the exclusion situation (mean=3.6) was significantly higher than in the other situations, and that it was also significantly higher in the inclusion (Anxiety=2.8) and over inclusion (Anxiety=2.2).

The analysis of the subscale *Happiness* revealed significant main effects of *Situation* ( $F(2, 80) = 16.91$ ;  $p < 0.0001$ ) and *Group* ( $F(1, 40) = 8.2$ ;  $p < .01$ ), and a significant 2-way *Condition* x *Group* interaction ( $F(2, 40) = 5.86$ ;  $p < .05$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means across situations revealed that this emotion was felt more strongly the game in the over inclusion situation (mean = 6.9) than in the other situations, and in turn that it was stronger in the inclusion situation (mean = 5.7) than in the exclusion one (mean = 2.9). This emotion was also stronger for the ASD group (mean=6.2) than for the TD group (mean=4.5). The further analyses conducted to follow-up on the significant interaction revealed that in 2D condition there were no significant differences ( $t(21) = .64$ ;  $p = .5$ ) between ASD (mean=5.9) and TD group (mean=5.4), at the contrary, in 3D



condition the happiness ratings for the ASD group (mean=6.5) were significantly higher ( $t(19)=4.08$ ;  $p<.001$ ) than for the TD group (mean=3.4). The plot in figure 6 shows an opposite trend for ASD and TD group, in specific the happiness emotion increases for ASD from 2D to 3D condition and decreases from TD from 2D to 3D condition.

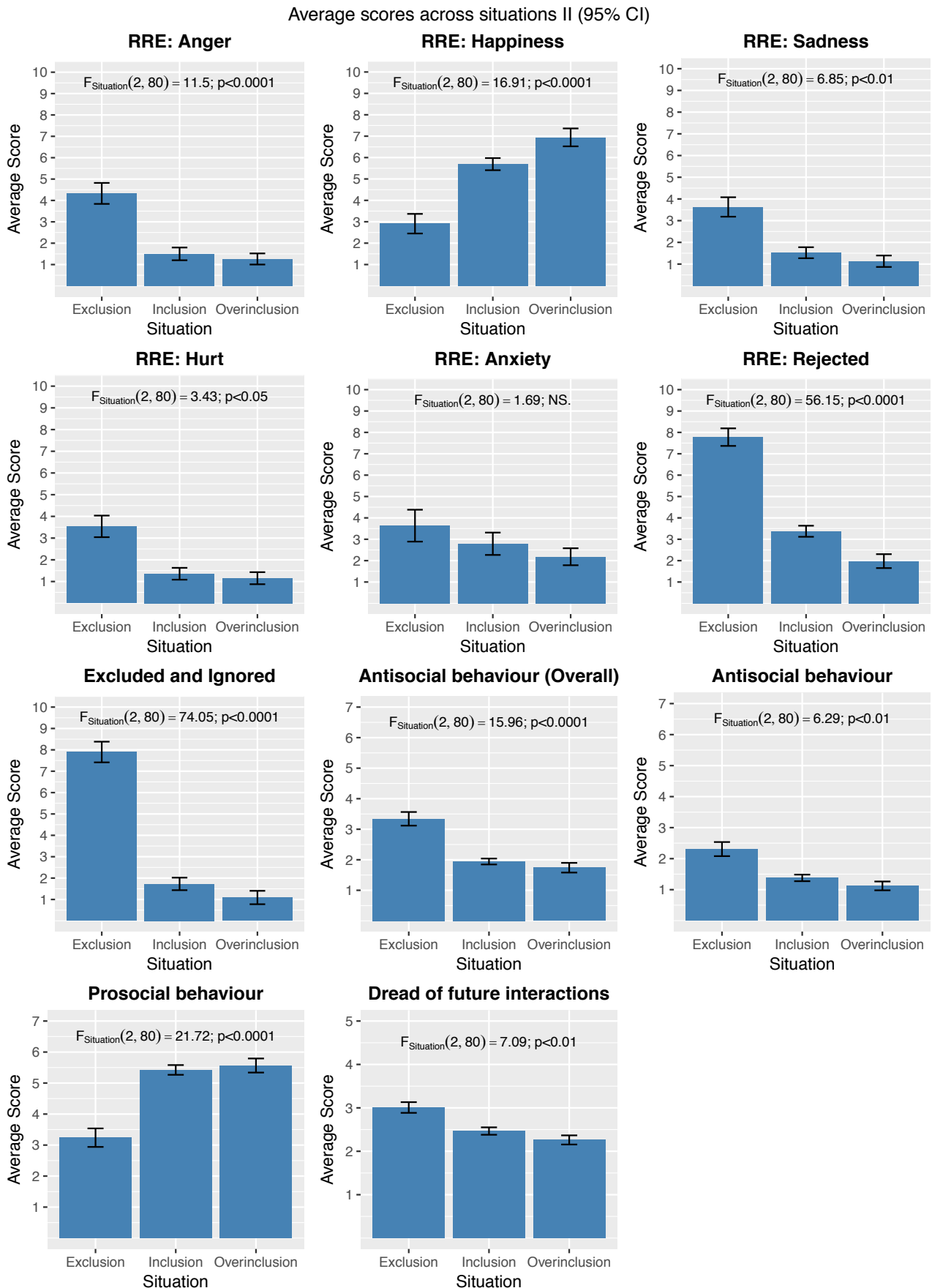
#### *Aggressive temptation Scale: Prosocial Behavior and Antisocial Behavior*

The analysis of the scales of Aggressive Temptation Scale with the subscales *Prosocial Behavior* ( $F(2, 80) = 21.72$ ;  $p<0.0001$ ) and *Antisocial Behavior* ( $F(2, 80)=15.96$ ;  $p<0.0001$ ) yielded only a significant main effect of Situation. A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means revealed that these intentions of behavior in the exclusion situation were respectively significantly lower (mean of Prosocial = 3.2) and significantly higher (Antisocial = 3.3) than in the other situations, but there were no significant differences between inclusion (mean of Prosocial = 5.4 and Antisocial = 1.9) and over inclusion (mean of Prosocial = 5.6 and Antisocial = 1.6) situations.

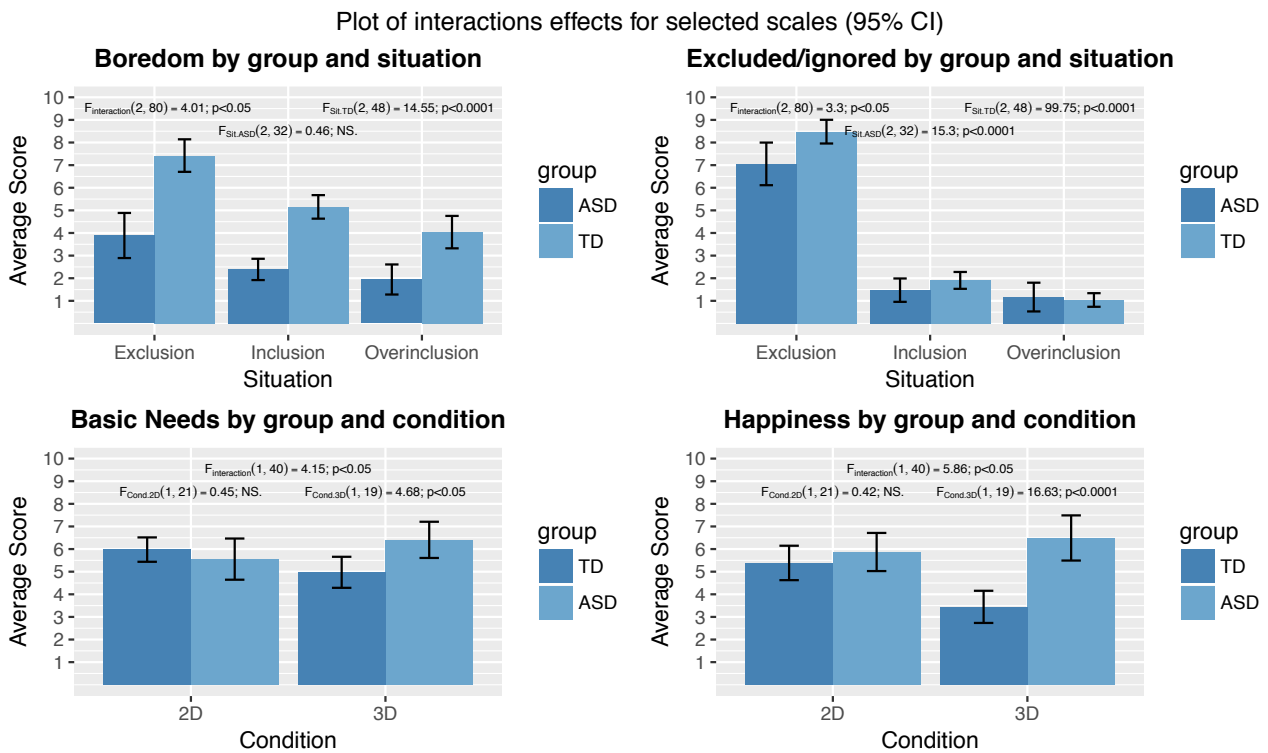
#### *Dread of future interactions*

The analysis of the scale *Dread of future interactions* yielded only a significant main effect of Situation ( $F(2, 80) = 21.72$ ;  $p<0.0001$ ). A violation of sphericity was found, but the effect of situation remained significant with both types of corrections (GG and HF). The pattern of means revealed that the dread feeling of future interactions with the other two players in the exclusion situation (mean = 3) were significantly higher than in the other situations, and there were, also, significant differences between inclusion (mean =2.5) and over inclusion (mean =2.3).

**Fig 15. Plots of the means of the further different self-report measures as function of the game situation. Error bars are 95% difference-adjusted confidence interval of the means.**



**Fig 16. Plots of the significant 2-way interactions revealed by the ANOVAs conducted on the self-report measures. Error bars are 95% difference-adjusted confidence interval of the means.**

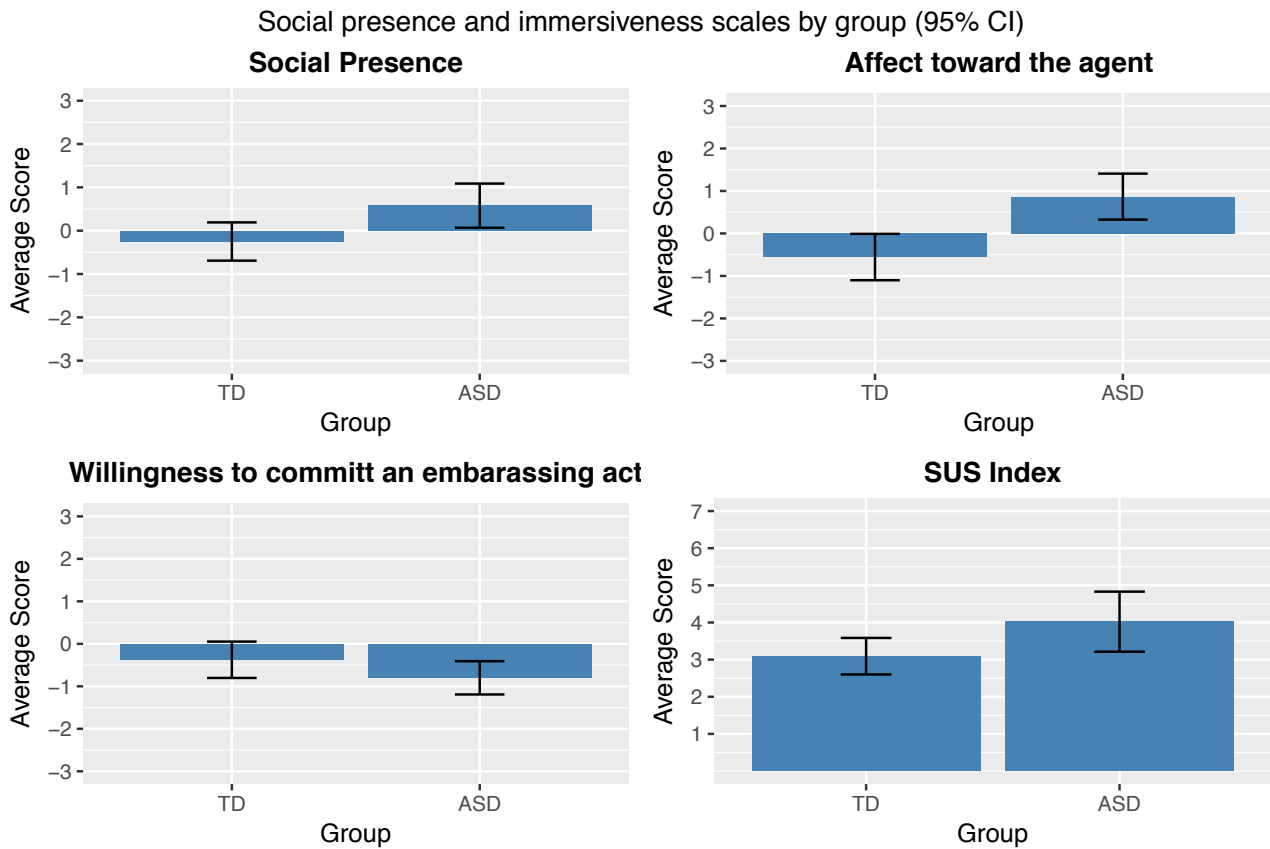


### 5.1.4 Effect on Social presence

Social presence (as measured by the three Social Presence subscales: Social presence, Affect toward the agent and willingness to commit an embarrassing act) and sense of presence in the virtual environment (as measured using the SUS questionnaire) were analyzed using 2-way, between-subjects ANOVAs, including group (TD vs ASD) and condition (2D vs 3D) as factors, and the ratings scores in the different scales or subscales as dependent variables (in different analyses). Significant interactions were always analyzed by conducting independent samples t-tests.

The analysis on the social presence subscales revealed a significant main effect of *Group* ( $F(1, 29)=7.9; p<.01$ ) for the degree of Affect toward the agent, with higher scores for the ASD group than for the TD group (difference = 1.5). For the social presence subscale the effect of group was only marginally significant ( $F(1, 29)=3.6; p=.07$ ) and again the mean was higher for the ASD group than for the TD group (difference = 0.86). The means of all the scales as function of group are present in figure 7. No other significant effect of *group*, *condition* or their interaction was found on the other social presence scale or on the SUS scale.

**Fig 17.** Average scores on two social presence subscales: *social presence* (left) and *affect toward the agent* (right) as function of group. Error bars are 95% difference-adjusted confidence interval of the means.



We also analyzed the correlations among the scores in the social presence scales and participants' ratings of Basic Needs and Happiness in the exclusion situation. The results revealed a moderate significant correlation between the Basic Needs Index and two Social Presence subscales (social presence:  $r=.36$ ;  $p<.05$ ; affect toward the agent:  $r=.35$ ;  $p<.05$ ), and also between happiness and social presence ( $r=.38$ ;  $p<.05$ ).

## 5.2 Physiological responses

### Analysis strategy

To analyze the physiological measure two different techniques were generally used.

On the one hand, as we did for the self-reports, for each measure we first conducted a 3-way mixed ANOVAs, including 2 between-subjects factors and 1 within-subjects factor. The between subject factors were *Group* (TD vs ASD) and *Condition* (2D vs 3D), and the within-subjects factor was the type of game *Situation* (inclusion, exclusion and over inclusion). Violations of sphericity were again assessed using the Mauchly's test, and whenever violations were found we applied the Greenhouse-Geisser (GG) and Huynh-Feldt (HF) corrections for departure from sphericity. Significant interactions of two or more factors were always followed by the analysis of the simple effects (in case of significant 2-way interaction) or of the simple interactions (in case of

significant 3-way interaction) of the respective factors. Difference adjusted 95% confidence intervals for the means were computed using and plotted as error bars in the plots of the means, so that non-overlapping error bars (and thus confidence intervals) plots reflect significant differences between pairs of means. Differences between pairs of means were also tested with paired and independent sample t-test, for within-subjects and between-subjects factors respectively.

Given that data collection was faulty for some participants in some conditions, leading to data loss, we decided to complement the repeated measures ANOVAs with linear mixed-effects model analyses, also known as multilevel models. These models are an extension of the linear model behind ANOVA and multiple regression, that allow to model correlations among observation due to one or more grouping factors (factors which can be included as random factors in the model), and thus are suitable for repeated measures designs. Substantially equivalent to ANOVA in case of balanced designs (i.e. same number of observations in each conditions), they have more statistical power than ANOVA in case of missing data (if for a participant we have a missing observation in one of the within-subjects conditions, we do not have to discard all the participant's data as we must do with ANOVA) and greater modeling flexibility (e.g. can be used to model not-normal dependent variables, such as counts, rates or binary variables, and do not require to assume sphericity). The cost of this great power and flexibility is that these models are more difficult to interpret, especially in case of complex multi-factor designs. Being a form of multiple regression, in fact, when a factor has N levels it must be coded with N-1 binary predictors, and this makes interactions substantially more difficult to understand. However, tests of hypotheses analogous to ANOVA type III F tests can be computed for fitted models, using an approximation of denominator degrees of freedom such as the one proposed by Satterthwaite (SAS Institute, 1978). We followed this strategy for each model that we fitted, that included one dummy coded predictor for group (TD = 0, ASD = 1), one dummy coded predictor for condition (2D = 0, 3D = 1), and 3 predictors for Situation each representing a contrast between a game situation and the baseline (i.e Baseline vs Inclusion; Baseline vs Exclusion, and Baseline vs Over Inclusion). All the analyses were conducted using the R language. Mixed-effects models were fitted using the *lmer* function of the *lme4* package (Bates et al. 2015), and approximate F tests were conducted using the *anova* function of the *lmerTest* package (Kuznetsova et al. 2016).

Prior to fitting the models, we inspected the distribution of the data to check for deviation from normality using box plot and histograms, and when we detected possible variations we applied to

log transformation. In every case we applied the transformation, further inspection showed that the transformed data were clearly more normal.

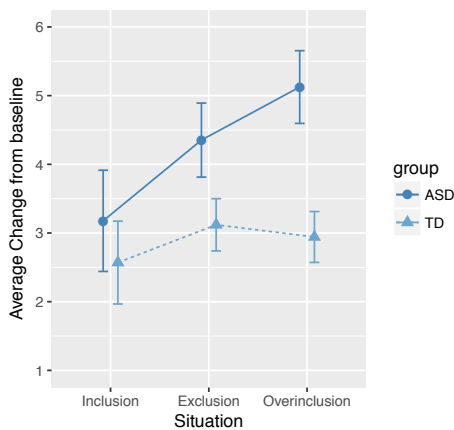
### 5.2.1 Effects on Skin Conductance Response

The figure 18b shows two different trends for ASD group and TD group. For the first one there is less activation in inclusion situation to more activation in over inclusion situation. For the TD group we found similar trend for inclusion and exclusion but an opposite value in over inclusion situation, indeed this situation presented less activity in skin conductance response.

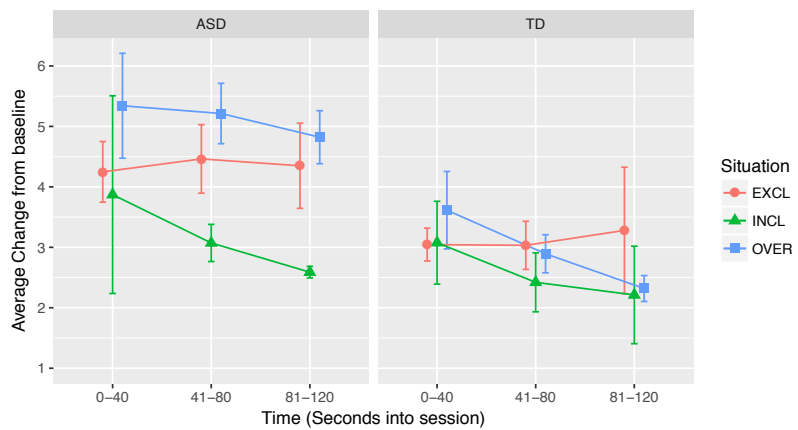
Changes in skin conductance levels (SCL) were quantified for each subject, for each epoch, as the difference between the mean value obtained for the 2-minute baseline period and the mean value occurring for each 40 seconds epoch throughout the game (Fig. 18a).

**Figure 18. Skin conductance level (SCL)**

**a) SCL change by group and situation**



**b) SCL change across time by situation and group**



A trend toward significance was found for phase in the ASD group ( $F(2, 6)=5.08$ ;  $p=.051$ ). The following analysis showed non significant differences between first and second phase ( $t(3)=1.244$ ;  $p=.30$ ), whilst there is a marginal significance between the first phase and the third one ( $t(3)=2.711$ ;  $p=.073$ ,  $\text{diff} = .57$ ), and between the second and the third one ( $t(3)=2.638$ ;  $p=.078$ ;  $\text{diff} = .33$ ).

For TD group, moreover, we found a trend toward significance for the phase by situation interaction ( $F(4, 20)=2.65$ ;  $p=.063$ ). There is any significant effect of phase for the exclusion situation. Conversely there is significant effect of the phase for Inclusion ( $F(2, 12) = 4.64$ ;  $p<.05$ ) and for over inclusion ( $F(2, 12) = 12.72$ ;  $p<.01$ ). Both situations showed a decreasing trend over time. In specific, the mean value in the first phase of inclusion is higher than in the second one ( $t(6)=2.602$ ;  $p<.05$ ;  $\text{diff} = .82$ ) and third one ( $t(6) = 2.158$ ;  $p = .074$ ;  $\text{diff} = 1.22$ ). The over inclusion situation showed mean value higher in the first phase than in second one ( $t(5)=3.209$ ;  $p<.05$ ;  $\text{diff} =$

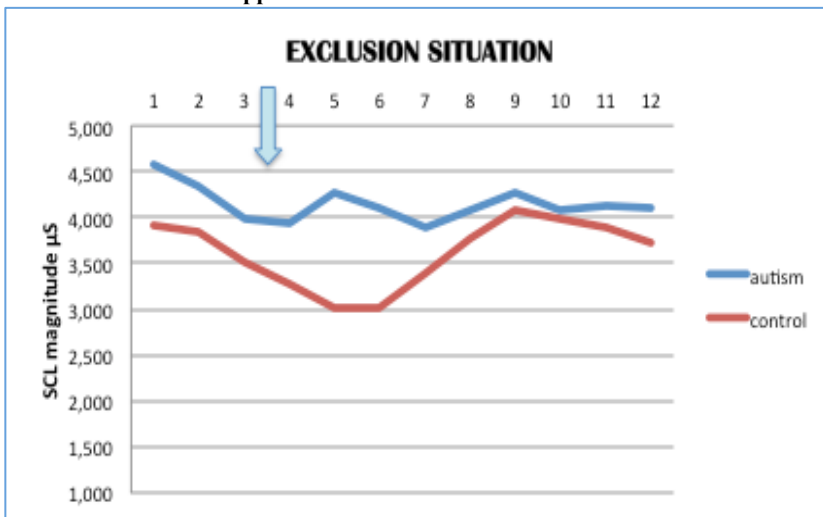
.72) and third one ( $t(5)=3.635$ ;  $p<.05$ ;  $\text{diff} = 1.3$ ), and also a higher value in second situation than in third one ( $t(5)=3.936$ ;  $p<.05$ ;  $\text{diff} = .57$ ).

The analyses of the simple effects of group revealed that only in the over inclusion condition the mean change scores were significantly higher for the ASD group than for the TD one ( $t(7.99)=2.15$ ;  $p<.05$ ), while in the other situations the mean score for the two groups were not significantly different (Inclusion:  $t(10.36)=0.002$ ; NS; Exclusion:  $t(10.34)=0.37$ ; NS).

We calculated also 12 epochs of 10 seconds each to investigate the change in skin conductance level over time of session. We found differences only in exclusion (fig. 19) and over-inclusion situations (Fig. 20).

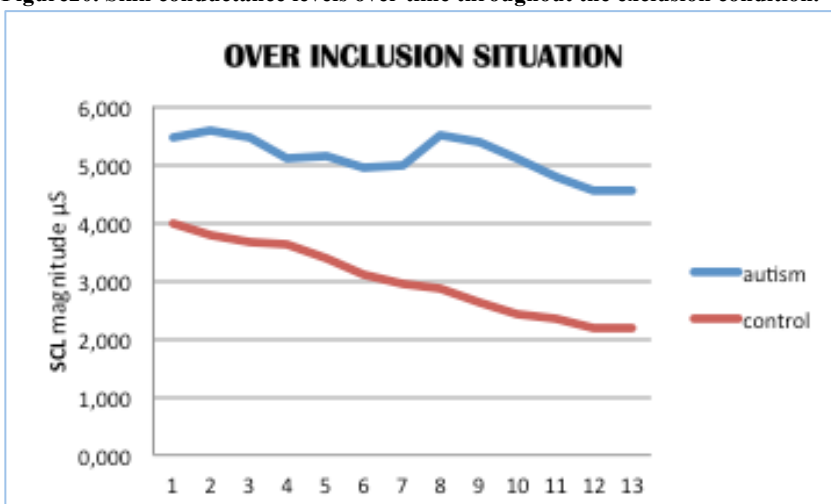
The trends of exclusion situation showed a partial difference between groups (Fig. 19).

**Figure.19. Skin conductance levels over time throughout the exclusion condition. Blue arrow indicates approximate time of exclusion.**



During the over inclusion situation (Fig. 20) the trend was different between groups. For controls the skin conductance level gradually decreased over time and conversely for ASDs early decreased and the showed a pick approximately for 30 seconds and then decreased again.

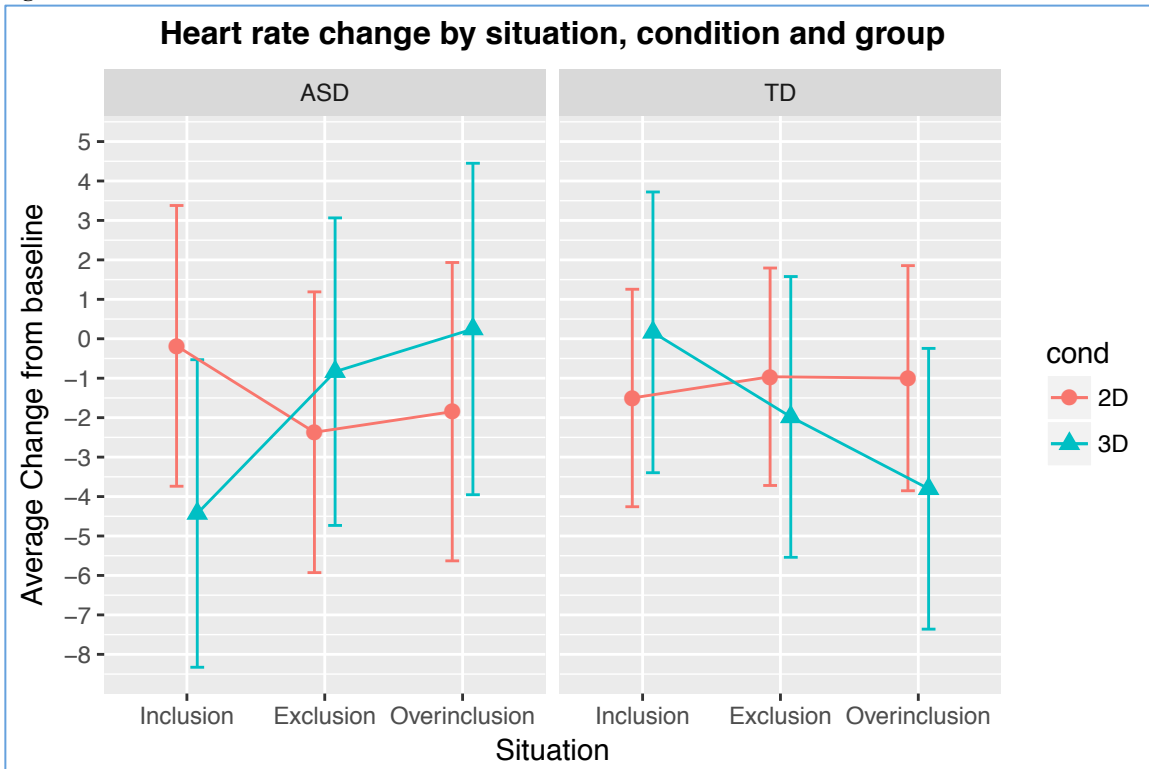
**Figure20. Skin conductance levels over time throughout the exclusion condition.**



### 5.2.2 Effects on Heart Rate

The analysis of the Heart Rate data, change from baseline (Fig.21) revealed only a significant 3 way interaction between situation, condition (2D vs 3D) and group ( $F(2, 40) = 5.69; p < .01$ ). As it can be seen in the plot in figure 21, the meaning of this interaction seems to be related to the 3D condition: for the TD group, in fact, the change score was highest in the Inclusion situation, and lowest in the over inclusion one, while the opposite pattern is present for the ASD group.

Figure 21. Main effect of the heart rate



Follow up tests of the simple interactions in the two groups, however, did not reveal for neither groups a significant 2-way, situation x condition interaction, although a trend for significance was found for the ASD group ( $F(2, 14) = 3.58; p = .06$ ). It likely that given the limited number of data point used for this analysis, since for several subjects the recording device did not work properly, we simply do not have enough statistical power to reveal significant effects in these follow up analyses.

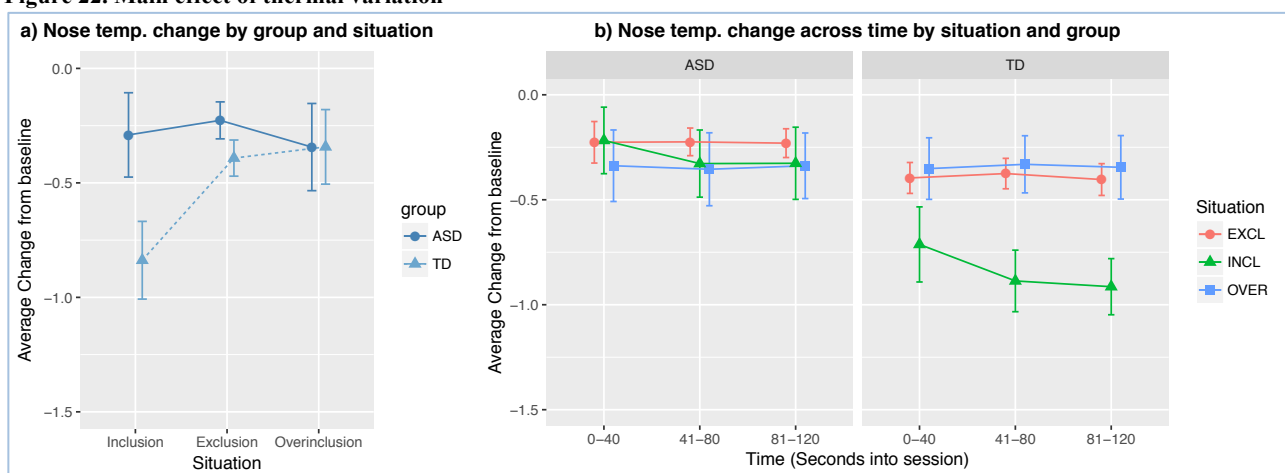
### 5.2.3 Effects on thermal response

The results of the 3-way, mixed, ANOVA conducted on the temperature change from the baseline, and including game situation and time within a session as within-subjects factors, and group as between-subjects factor, revealed only a significant interaction between group and situation ( $F(2,$



54) = 3.98;  $p < .05$ ). Follow-up tests revealed a significant effect of situation only for the TD group ( $F(2, 30) = 8.24$ ;  $p < .01$ ), but not for the ASD one ( $F(2, 24) = 0.32$ ; NS). As it can be seen by the plot of the means as function of group and situation in figure 22a, for the ASD group the change scores did not vary across situations, while for the TD group the mean in the Inclusion situation was significantly lower than in the other situations. Moreover, independent sample t-test showed that in the inclusion situation the change scores varied significantly across groups, being lower for the TD group than for the ASD one ( $t(27.22) = 2.77$ ;  $p < .01$ ), but in the other situations there were no significant differences between groups (exclusion:  $t(22.99) = 1.05$ ;  $p = .31$ ; over inclusion:  $t(24.63) = -0.003$ ;  $p = .997$ ).

**Figure 22. Main effect of thermal variation**



For the TD group, moreover, the analysis revealed also a significant main effect of time ( $F(2, 30) = 3.41$ ;  $p < .05$ ) and a significant time x situation interaction ( $F(4, 60) = 6.48$ ;  $p < .001$ ). As it can be seen the plot in figure 22b, for the TD group the change score varied across time only in the Inclusion situation, showing a decreasing trend, and remained basically constant in both the Exclusion and in the Over inclusion situations.

### 5.3 Discussion

Peer rejection is particularly pervasive among adolescents with autism spectrum disorders. Symes and Humphrey (2010) found that pupils with ASD were more likely to be rejected and less likely to be accepted by their peers than children with other forms of learning disorders and typically developing children (Symes & Humphrey, 2010). However, how adolescents with ASD differ from typically developing adolescents in their responses to peer rejection is poorly understood, and even less in adults with HF autism.

In healthy people, the ostracism has been extensively studied in experimental settings (Williams et al., 2001; de Panflis et al., 2015; Bolling et al, 2011; Eisenberger et al., 2003) and it has been

observed to have immediate, detrimental effects on self-reported emotions.

Consistent with previous research behavioral evidence has demonstrated the negative psychological effects of social exclusion (Sebastian, Viding, Williams, & Blakemore, 2010; van Beest & Williams, 2006; Williams, Cheung, & Choi, 2000; Wirth & Williams, 2009; Zadro, Williams, & Richardson, 2004, 2005) and the immediate negative emotional reactions (Gerber and Wheeler, 2009). In specific, following ostracism participants scored themselves lower on self-esteem, belonging, control, and meaningful existence. In the present research project the focus was on social interactions of high function autistic persons. Some studies underlined that trough self-reported data individuals with ASD are as able as controls to recognize when they are being excluded from a social situation (Bolling et al., 2011; Masten et al., 2011; Sebastian et al., 2009, Trimmer et al., 2017). In the studies that used the 2D virtual Cyberball paradigm, both groups reported elevated levels of distress following social exclusion, but there were no group differences. The degree of self-reported need threat did not differ between groups for self-esteem, belonging, and control. Sebastian and colleagues (2009), however, found greater threat in the ASD group, compared with controls, on the meaningful existence scale. Trimmer and colleagues (2017) found that mood was rated as more negatively by ASDs overall. Consistent with these studies, our findings suggested that ASDs are as able than controls to identify when they were being ostracized from an online ball-toss game. Both ASD participants and controls were correctly able to identify the approximate percentage of throws given when included or excluded and, for the first time with an HF autistic participants, when they were over included. This means that the manipulation was successful. Participants reported feeling more “ignored and excluded” when ostracized and perceived receiving fewer throws than when they included. Due these findings we can argue that participants were cognitively aware of their inclusionary status. The awareness of being excluded from the other players motivated both groups to share a minimum amount of money with them. Conversely, after inclusion and over inclusion all participants decided to fairly share the amount of money with other players. We found similar findings in regard to the degree of social connection felt during the past session of Cyberball.

In summary, examining the psychological effects we found that exclusionary status affected negatively the degree of connection with the other players, the basic needs, the feeling of happiness and the prosocial behavior. In these mentioned cases all the three game situations were differently perceived, indeed we found a trend from exclusion (minimum value) to over-inclusion (maximum value). Conversely, the magnitude of feelings increased during the exclusion situation considering the perception of boredom, unpleasantness, anti-social behavior and painfulness. In this case, the over inclusion affected only the feelings of boredom and the antisocial behavior. Similar to

previous research (de Panfilis et al., 2015) ASDs, as their counterpart, experienced higher level of overall rejected emotions during the exclusion situation. In particular for anger, sadness, hurt and anxiety we found that exclusion differed from the other two conditions but participants felt the same magnitude of emotions during inclusion and over inclusion situations. Only for sense of rejection participants reported significant lower level of rejection in over inclusion in respect to other two conditions. We used, for the first time, the Self Isolation Scale with a sample of autistic persons and found similar results in comparison to control group. In specific, results showed that after the exclusion situation, participants preferred play again with different players or, alternatively, alone and with a lower magnitude with the same players. However, the desire to play alone is always very low, regardless of the gaming situation. These results indicated that the ostracism manipulation significantly influenced participants' preference for the next game. Indeed, ostracized participants expressed a stronger desire to get away from the people who have excluded them and try to play again with new players. Conversely, the desire to replay with the same players is greater after the over-inclusion situation.

One similar study (Ren et al., 2016) with students of college found that ostracized participants had more desire not only to distance themselves from the source of ostracism but also to be alone than included participants. It is important to consider other factors, social and personal, that could lead to the desire to be alone and the costs and benefits that seeking solitude affords. To better understand the effects of ostracism or extreme inclusionary situation in participants with autistic syndrome, we studied also the dread of future interaction after the specific social interactions. This notion point out in individual differences in reactions to negative social pain experiences, underling the idea that certain individuals dread continued interactions with members of the group that have excluded them (Geller, Goodstein, Silver, Sternberg, 1974). Our findings suggested that, overall the feeling of dread was weak and that ostracism affected the dread sense of interactions with other players, in autistic participants as well as in their counterpeers. Indeed, after the exclusion situation, the magnitude of dread is higher than in the other two situations. Moreover, the over inclusion showed the lowest level of this feeling.

We highlight as most interesting findings of our research the differences occurred between groups. In specific, we found that ASDs felt lower level of boredom than TDs and furthermore, didn't show any difference between social interactions. Conversely, the TDs felt the greatest sense of boredom during the exclusion situation than during inclusion and even less over inclusion. At the same time, ASDs felt lower level of exclusion than the TDs during the exclusion situation and we registered for both groups low degree of exclusion during the inclusionary interactions. In addition our research studied also the effect of the virtual environment, for the first time with a sample of autistic

persons. We found that ASDs when played in the 3D environment felt than TDs a greater level of basic needs, so more control, self-esteem, meaning of existence and belonging. At the same time, they felt happier than TDs. These results are in contrast with previous research (Sebastian et al., 2009) that found the ASD group self-reported more negative mood responses overall and this was not dependent on whether they had been ostracized. For these authors, it can reflect the lower mood in general in the ASD group or lower insight into their own physiological mood responses. This disparity may depend on the experimental sample. Adolescents may be more susceptible to decreased mood and negative feelings following social exclusion. Our study examined these effects primarily in adults, the negative feelings produced after ostracism may have been less intense due to greater emotional maturity.

In order to better understand the effect of virtual environment in ASDs we administered two questionnaires assessing presence and social presence felt during the game. We didn't find any difference by condition (2D or 3D) but overall ASDs felt a greater sense of social presence and a greater "affect" toward the agent. These results are in line with the extensive literature that demonstrated the greater effectiveness of virtual training for autistic persons. Many studies pointed out that individuals with ASD are stated to have an affinity to technology and a strong visual memory (Bozgeyikli et al., 2017) and VR provides enriched learning and training experiences (Weiss et al., 2003; Rizzo & Kim, 2005; Bolte et al., 2010).

In summary, ASDs self-reported feelings showed that this group was as able as controls to identify when they were being ostracized and that does not differ in most of the investigated variables from their counterpart. The ASDs differed from their counterpart for greater happiness and a lesser sense of boredom at each stage of the game. Furthermore, during the exclusionary situation they felt a lower degree of exclusion than controls. Previous research with healthy participants and more immersive virtual environment found a greater magnitude of exclusionary condition, using a more realistic environment we didn't find this result. But we found that autistic persons were happier than controls to play with the Cyberball3D and in this condition they felt their basic needs more fulfilled than controls. Moreover, the ASDs experienced a greater social presence during the interactive game and a greater affect toward the virtual agent.

#### *Physiological responses to social interaction*

In measuring emotional responses, it is important to consider both subjective and psychophysiological indices to obtain a more complete understanding of the subjective response. Self-report data, when used in isolation, are highly susceptible to extraneous influences (Schwarz,

1999). Therefore, dependent measures based on self-report questionnaires are best used in conjunction with other measures because participants are not always the most accurate judges of their own thoughts and feelings, so they often misreport affective and cognitive responses to stimuli (Bailenson et al., 2004). Psychophysiological indices are less susceptible to demand characteristics and responder bias.

Previous research showed emotional distress created by exclusion is related to enhanced activation of the limbic system (Eisenberger, 2012; Kross, Berman, Mischel, Smith, & Wagner, 2011), which leads to increased sympathetic arousal of the autonomic nervous system (Cavanagh & Allen, 2009). Kelly and colleagues (2014) proposed that the higher arousal levels observed in the ostracism group are likely to be the result of the stress associated with social pain. There is also supporting evidence to suggest that heart rate slows in response to unexpected social rejection (Gunther Moor et al., 2010). With the focus on autistic persons, it is particularly interesting to evaluate the physiological arousal because they could have deficits in processing their own emotions (Hill et al., 2004). One recent research (Trimmer et al., 2017) underlined a discrepancy in ASDs between a heightened arousal response to being excluded from the game and any evidence in their mood ratings.

For these reasons, in this study we used skin conductance responses and heart rate as objective indices of arousal. Furthermore, for the first time with autistic syndrome we used a measure of variations in autonomic activity reflected by cutaneous temperature modulations.

Consistent with previous research we found any differences in self-reported basic needs and rejected emotions ratings between ASDs and TDs, and conversely we found different physiological arousal between groups. In particular, considering the skin conductance response we found two opposite trends for groups. The group with autistic syndrome felt less activation in inclusion situation to more activation in over-inclusion one. Healthy participants showed a similar trend for inclusion and exclusion but an opposite value in over-inclusion situation, with lower response of SCR. The situation of extreme inclusion showed a significant higher response for ASDs than TDs. Overall, ASDs presented a greater value of SCR during the game and a significant higher value in over-inclusion. This result is in line with Trimmer and colleagues (2017) research finding that individuals with ASD experienced greater arousal than controls in both conditions, inclusion and exclusion. For these authors, it may be the case that those with ASD were more interested in the game or experienced increased anxiety when engaging in the task. Due our findings in which ASDs felt happy and only slightly excluded engaging the game, we supposed that our experimental sample were very interested in the game and being in relationship with other players. Regarding the heart rate investigation, it likely that given the limited number of data point used for this analysis, since for several subjects the recording device did not work properly, we simply do not have

enough statistical power to reveal significant effects in these analyses. We found only a marginal effect of situation x condition interaction, for the ASD group, showing a greater activation in 3D condition.

On the contrary, for the thermal variation we found significant effect of situation only for the TD group, where the value of the inclusion situation was significantly lower than in the other two. ASDs showed a higher overall temperature (1 degree) than controls but we found a significant difference only for the inclusion situation with lower value for TDs. At the best of our knowledge, this is the first investigation of thermal variation during social interactions in ASDs.

In conclusion, based on results from both psychological and physiological measures, and partially consistent with previous research, it appears that individuals with ASDs may not be attuned to their physiological response when they are ostracized or extremely included. Whilst they experienced a heightened arousal response to being excluded and over included from the game, they did not express this difference in their self-reported ratings. Furthermore, only ASDs experienced an overall higher frequency of heart rate in 3D than in 2D condition and an overall higher temperature than controls with a significant difference in inclusion situation.

Peristeri and colleagues (2016; see also Sebastian et al., 2009) provided, as a possible explanation, the perspective that persons with ASD failed to interpret appropriately their current emotional state, which impacted understanding of affective content found in emotion words used for the self-report questionnaires. Although persons with ASD may feel just as rejected as their TD counterparts when they are excluded by peers, they might have lacked insight into how the experience affected their current mood, and an impairment in emotion regulation (Mazefsky et al. 2013).

## SECTION 4

### NEURAL CORRELATES OF SOCIAL INTERACTION IN HIGH-FUNCTIONING AUTISM

#### CHAPTER 6: AN EXPLORATIVE INVESTIGATION

##### 6.1 Theoretical framework

Peer rejection is particularly pervasive among adolescents with autism spectrum disorders (ASD). However, how adolescents with ASD differ from typically developing adolescents in their responses to peer rejection is poorly understood (Masten et al., 2011).

The Cyberball paradigm has been used in functional magnetic resonance imaging (fMRI) studies with healthy participants. Behavioral evidence has demonstrated the negative psychological effects of social exclusion (Boyes & French, 2009; Sebastian, Viding, Williams, & Blakemore, 2010; van Beest & Williams, 2006; Williams, Cheung, & Choi, 2000; Wirth & Williams, 2009; Zadro, Williams, & Richardson, 2004, 2005). In addition, neuroimaging studies have begun to identify neural correlates of this negative response to ostracism, including activations in anterior insula, anterior cingulate cortex, and right ventrolateral prefrontal cortex (Bolling et al., 2010, in press; Eisenberger, Lieberman, & Williams, 2003; Masten et al., 2009, 2011; Onoda et al., 2009, Sebastian et al., 2010). All of which have been linked to the emotional response to exclusion. Furthermore, previous studies have shown that activation to social exclusion in anterior cingulate cortex (ACC) is correlated with self-reported distress (Eisenberger et al., 2003; Masten et al., 2009). These neuroimaging studies have reported that social pain involves some regions pertaining to the so-called physical pain matrix (Peyron et al., 2000). This matrix is a complex neural circuit activated when people experience the unpleasant sensation caused by a nociceptive stimulus (Eisenberger et al. 2003, 2007). Recent literature revision confirmed that the neural network of social pain consists of brain structures such as the dorsal anterior cingulate cortex (dACC), right ventral prefrontal cortex (RVPFC), inferior frontal gyrus, posterior superior temporal sulcus (pSTS), temporo-parietal junction (TPJ), amygdala, and anterior insula (Sebastian, Viding, Williams, & Blakemore, 2010). Moreover, Cristofori and colleagues (2012) investigated the of theta-band oscillations during the Cyberball tasks. Theta signals, compared with more cognitive cerebral waves such as gamma or beta (Buzsaki 2002), are known to be associated with emotional feelings and physical pain (Liu et al. 2010; Schulz et al. 2011), and empathy for pain (Jeon et al. 2010).

Results of this research revealed that time–frequency analyses increased in power of theta-band oscillations during exclusion in the anterior insula (AI) and posterior insula, the subgenual anterior cingulate cortex (sACC), and the fusiform “face area” (FFA). Interestingly, the AI showed an initial fast response to exclusion but the signal rapidly faded out. The significant theta effect was present in some major regions of the social–physical pain matrix.

The Cyberball paradigm has been used also in assessing social pain in ASD persons. To our knowledge, very few studies have used an experimental design to test brain responses to the experience of social rejection in individuals with ASD, in comparison to typically developing individuals. Masten and colleagues (2011) found a positive correlation between sub anterior cingulate cortex and distress in ASD. In particular, a positive correlation was found between the ventrolateral prefrontal cortex and distress. This positive correlation reflects an ineffective attempt to regulate distress resulting from peer rejection in the ASD group. Results from temporal dynamics of brain activity associated with social exclusion and reporting in ASD revealed a dissociation between reported distress and neural responses and differentiation of rejection at an earlier frontal P2 component. This suggests the importance of temporal dynamics in revealing processing strategies in typical and atypical development (McPartland et al., 2011). Moreover, Bolling and colleagues (2011) found that in the right insula region, compared to TD participants, children with ASD showed hypoactivation during social exclusion and hyperactivation during rule violation. More in specific, posterior insula responds to exclusion in TD participants, reflecting a more visceral response to social exclusion, while the activation of anterior insula to rule violation in ASD participants may reflect a more cognitive, conscious emotional response.

Because brain regions activated during Cyberball (e.g., ACC, VLPFC, insula) are also implicated in the neuropathology of ASD (Barnea-Goraly et al., 2004; Di Martino et al., 2009), examination of their functional integrity is of direct relevance to understanding the neuropathology of ASD and in meaningfully defining sub-groups (McPartland et al., 2011). Furthermore, the heterogeneity of behavioral manifestation of autism spectrum disorders (ASDs) requires a model which incorporates understanding of dynamic differences in neural processing between ASD and typically developing populations. Understanding neural bases of autism requires analysis methods that capture dynamic differences in processing across heterogeneous participants and group (Malaia et al., 2016). To better understand the neural basis of autism one interesting method is represented to the mirror neurons system analysis. fMRI studies in humans have implicated an extensive network of brain regions whose activity is assumed to reflect some aspect of mirror functioning. Studies that incorporated visual images of actions and/or a requirement to execute motor actions. This analysis yielded significant activation clusters in the inferior frontal gyrus, ventral premotor cortex and



inferior parietal lobule. Also yielded significant activation clusters in the superior parietal lobule and dorsal premotor cortex (Molenberghs et al., 2012).

## **6.2 Aims of the study**

The present study aimed to explore the neural correlates of a core deficit in Autism Spectrum Disorders (ASDs), social impairment, during social exclusion in a group of subjects with HFA as compared to their typically developing counterparts.

Theta signals, compared with more cognitive cerebral waves such as gamma or beta (Buzsaki 2002), are known to be associated with emotional feelings and primitive sensations (Knyazev 2007), physical pain (Liu et al. 2010; Schulz et al. 2011), and empathy for pain (Jeon et al. 2010). For this reason we decided to investigate the theta signal.

Due the results of previous study (Cristofori et al., 2012) we expected differences in theta signal between groups.

## **6.3 Material and method**

For the experimental procedure see chapter 4.

### **6.3.1 Participants**

Sample of 22 (female = 2) individuals with High-Functioning Autism (HFA) recruited by clinicians from Istituto Auxologico Italiano and sample of 24 (female = 2) individuals with typical development (TD) matched to the HFA sample in terms of chronological age (mean = 22, DS=6) and screened for major psychiatric and neurological illnesses. Sample with HFA were diagnosed using the Autism Diagnostic Observation Schedule (ADOS; Lord et al., 2000) and DSM-IV criteria, as well as experienced clinical judgment. Four participants from the ASD group were excluded due to a malfunction of the instrument and 3 from the TD group.

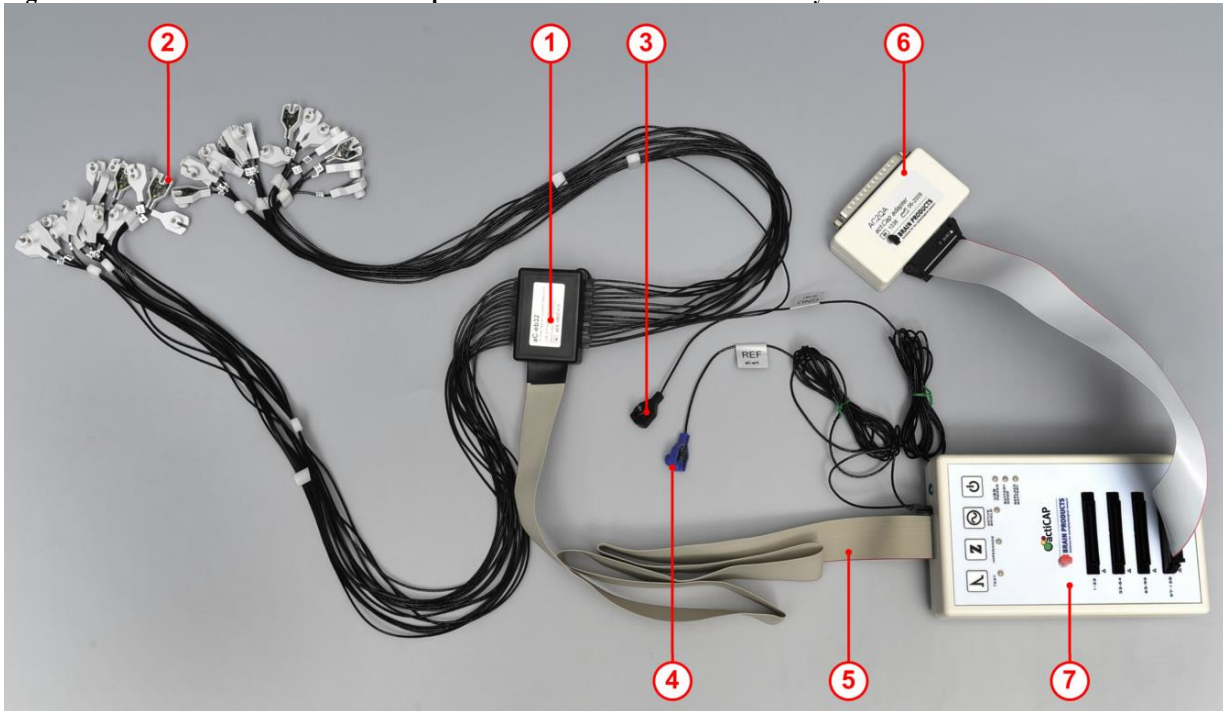
### **6.3.2 EEG equipment**

The Brain Products EEG is composed of one amplifier, four different measures caps and 128 electrodes. The amplifier equipped with a number of noise reduction techniques such as active noise cancellation and active electrodes.

Electroencephalography is the neurophysiological measurement of electrical activity in the brain as recorded by electrodes placed on the scalp or, in special cases, subdurally or in the cerebral cortex. The resulting traces are known as an electroencephalogram (EEG) and represent a summation of

post-synaptic potentials from a large number of neurons. In conventional scalp EEG, the recording is obtained by applying electrodes to the scalp using a conductive gel or paste, usually after preparing the scalp area by light abrasion to reduce electrode-scalp impedance.

**Figure 23. Overview of the hardware component the actiCAP active electrode system with Version I of the ControlBox**

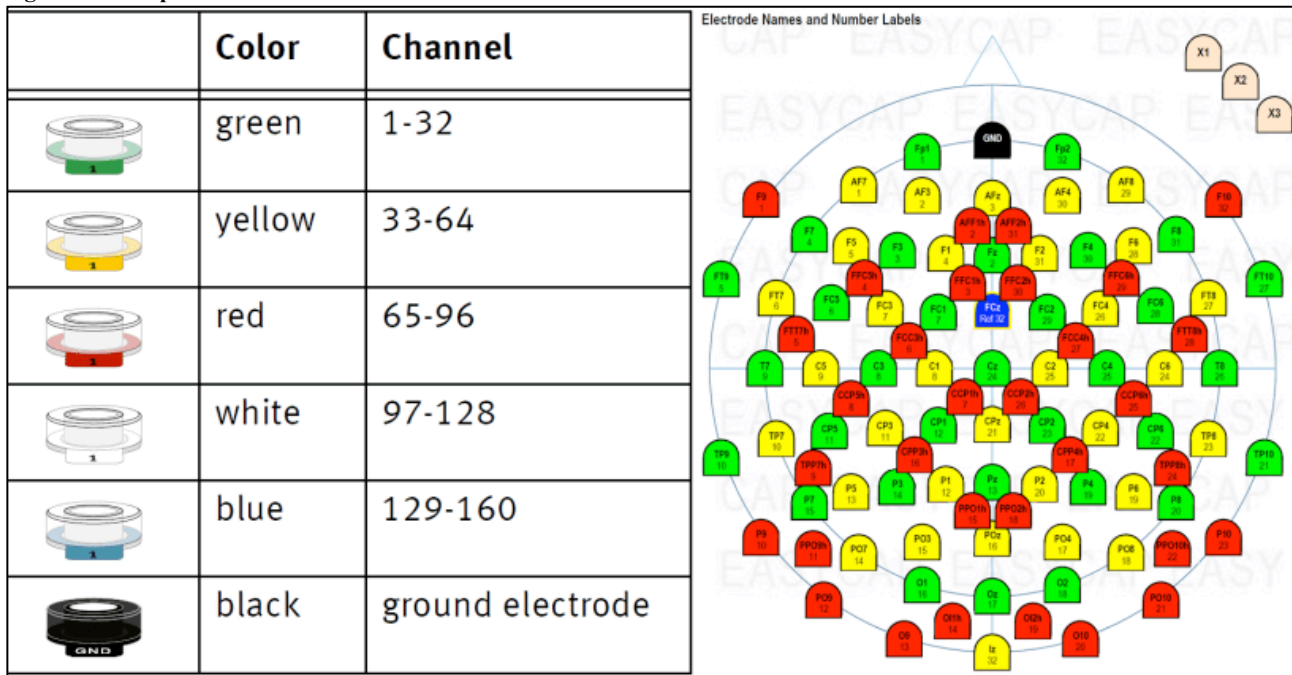


### 6.3.3 Data acquisition

EEG recordings allow to measure electrical brain activity recording several brain wave frequencies such as theta (4-8 Hz), alpha (8-12 Hz), Beta (12-30), and Gamma (30-100+ Hz). In this study we considered only theta activity, specifically differences in amplitude, because it appears to be involved in the mediation of emotional processing such as empathy for pain in the anterior cingulate cortex, prefrontal cortex and insula (Mu, Fan, Mao, & Han, 2008; Cristofori et al., 2012).

Scalp EEG was recorded from 64 Ag/AgCl electrodes mounted in a pre-cabled electrode cap with an average mastoid reference. Electrodes were positioned according to the standard 10–20 system. A pair of bipolar electrodes was used to record vertical eye movements. Electrode impedances were maintained below 10 k during recording. The EEG analog signal was digitized at a 500-Hz sample rate. The electrode cap montage required approximately 30 minutes and was well tolerated by subjects. Electrode impedances were kept less than 5 K $\Omega$ . Data were collected with a sampling rate of 500 Hz.

Figure 23. Template of the actiCAP 128 channels



#### 6.3.4 Pre-processing

Data were analyzed in the EEGLAB environment (<http://www.scn.ucsd.edu/eeGLab/index.html>) a collection of scripts running under Matlab 7.7.0 R2010a (Mathworks Inc., Natick, MA). At the beginning of preprocessing pipeline, EEG data were filtered between 1 Hz to 100 Hz. After visual inspection and manual elimination of paroxysmal artifact periods, artifact non-cerebral source activities (eye blinks and movements, saccadic, cardiac and muscle/electromyographic activity) were identified and rejected using a semiautomatic procedure based on Independent Component Analysis (Porcaro et al., 2009).

#### 6.3.6 Processing

##### *Electrical Source Imaging (ESI)*

To compute the intracerebral electrical sources underlying EEG activity recorded at the scalp we used the exact low resolution brain electromagnetic tomography (eLORETA) software (<http://www.uzh.ch/keyinst/loreta.htm>). Computations were made in a realistic head model (Fuchs et al., 2002), using the Montreal Neurological Institute (MNI; Montreal, Quebec, Canada) MNI152 template, with the three-dimensional solution space restricted to cortical gray matter and hippocampi, as determined by the probabilistic Talairach atlas (Lancaster et al., 2000). The intracerebral volume (eLORETA inverse solution space) is partitioned in 6239 voxels at 5 mm spatial resolution (i.e., cubic elements of  $5 \times 5 \times 5$  mm). Anatomical labels as Brodmann areas are also reported using MNI space, with correction to Talairach space (Brett et al., 2002). We

calculated eLORETA images corresponding to the estimated neuronal generators of brain activity within each band (Frei et al., 2001). The ranges of the frequency bands were as follows: delta ( $\delta$ ), 1.5-4 Hz; theta ( $\theta$ ), 4-8 Hz; alpha ( $\alpha$ ), 8-12 Hz; beta 1 ( $\beta_1$ ), 12-20 Hz; beta 2 ( $\beta_2$ ), 20-30 Hz; gamma ( $\gamma$ ), 30-80 Hz.

### *Statistics*

eLORETA software package was used to perform statistical analyses. The methodology used was non-parametric randomization statistics (Statistical non-Parametric Mapping, SnPM) (Nichols et al., 2002). A second level of non-parametric analysis, the exceedance proportion tests evaluated the significance of activity based on its spatial extent, obtaining clusters of supra-threshold voxels.

Between-group comparisons of the eLORETA current density distribution were performed using a statistical analysis based on voxel-by-voxel log of F ratio test with 5000 randomizations. The results correspond, for each band, to maps of log-F-ratio statistics for each voxel, for corrected  $p < 0.05$ . Significant activations at the exceedance proportion tests with a  $p$  value  $< 0.01$ , F value over 2 z-score and a minimum cluster of voxels major than 27 (an intracerebral volume cube with an edge of 15 mm) within a hemisphere for single Brodmann Area (BA) were accepted.

## **6.4 Preliminary results and discussion**

In order to assess the differences in Theta activity between ASD and TD during the exclusion session of the Cyberball game, we performed a between-group comparisons of the eLORETA current density distribution. The figure 26 shows the first and preliminary results of the wider study, that is still in progress.

The comparison between ASDs and TDs (Fig. 26) shows in Brodmann area 6 (Fig. 24), Middle frontal Gyrus and Frontal Lobe (Fig. 25) a lower Theta activity of ASDs compared to TDs.

The Brodmann area 6 (see Fig.24) is apart of the frontal cortex in the human brain. Situated just anterior to the primary motor cortex, it is composed of the premotor cortex and medially, the supplementary motor area. This large area of the frontal cortex is believed to play a role in the planning of complex, coordinated movements. In the human brain, it is located on the portions of the precentral gyrus and it extends onto the caudal portions of the superior frontal and middle frontal gyri.

Figure 24 Brodmann areas

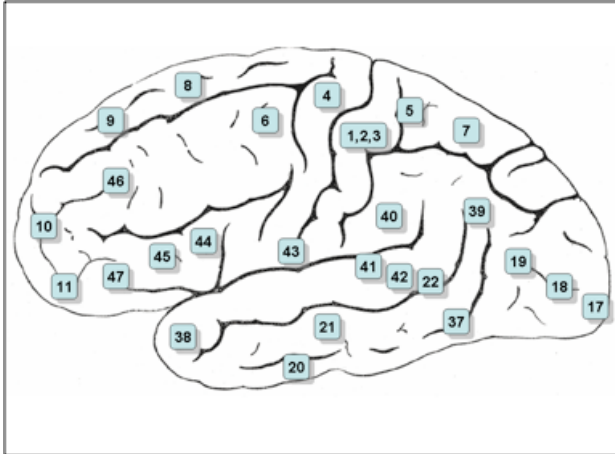
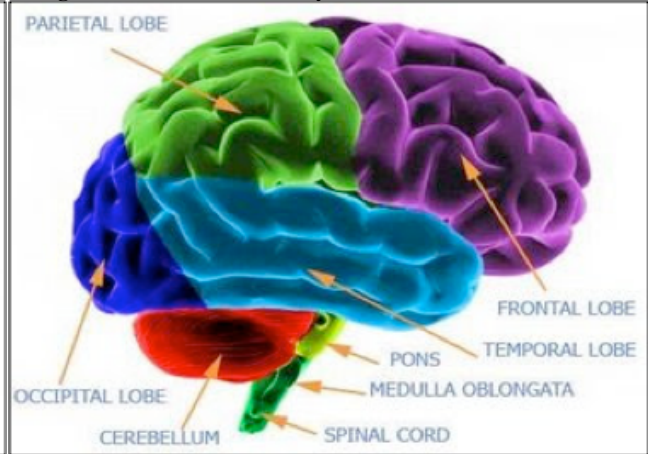


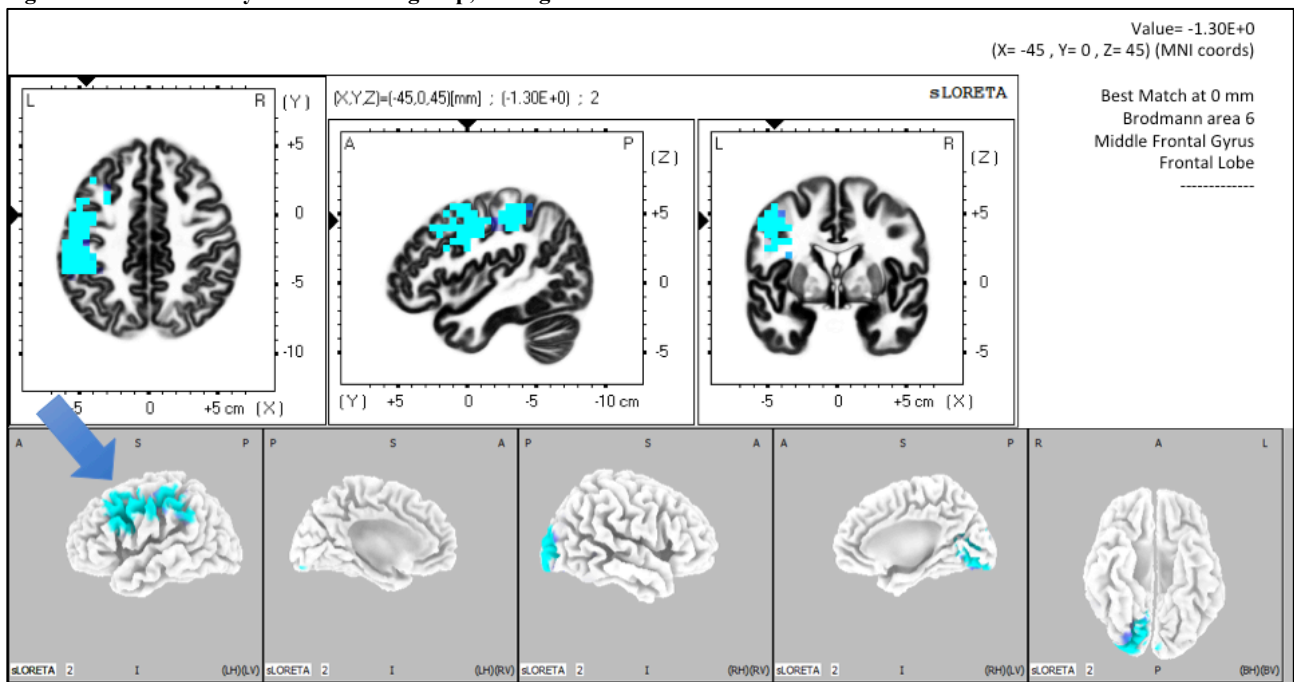
Figure 25 Cerebral anatomy



Free images from the web

It extends from the cingulate sulcus on the medial aspect of the hemisphere to the lateral sulcus on the lateral aspect. It is bounded rostrally by the granular frontal region and caudally by the gigantopyramidal area 4 (Brodmann, 1904).

Figure 26. Theta activity of ASD vs TD group, during exclusion situation



The areas activated during the exclusion session of the game are partially included in the physical pain matrix (Peyron et al., 2000, Eisenberger, 2003). Results of Cristoforis' research (2012) revealed that time–frequency analyses increased in power of theta-band oscillations during exclusion in the anterior insula (AI) and posterior insula, the subgenual anterior cingulate cortex (sACC), and the fusiform “face area” (FFA). We found that, during the exclusion condition the ASD group showed a less theta band activity in the primary motor cortex and frontal lobe. This

area showed in fig. 26 and pointed out by a blue arrow is included in the mirror neurons system. As it is known, dysfunction of the mirror neuron system in individuals with autism spectrum disorder causes difficulties in social interaction and communication. To our knowledge, it was the first time that researchers assessed a hypoactivation of the mirror system in autism during a situation of social exclusion.

## SECTION 5

### CONCLUSION

#### CHAPTER 7: GENERAL DISCUSSION

##### 7.1 Main results

The present study used various techniques, both psychological and physiological, to inform our understanding of the experience of social interaction, both in 2D and 3D environments, in persons with ASD. Following a brief description of the carried out path to answer the research questions and the main obtained results.

On the first step, we examined the effectiveness of CyberballAvatar3D in eliciting subjective and psychophysiological feelings of inclusion, exclusion and over-inclusion. We expected that Cyberball3D elicits highest level of arousal than the 2D version, only in the exclusion situation. Partially in line with our hypothesis, we found that the Cyberball 3D elicits the same magnitude in self-reported ratings (basic needs and rejected emotions) in participants but has a greater impact on the skin conductance response, specifically in exclusion and over-inclusion situations. Furthermore, we expected that the 3D condition would have elicited greater sense of presence than the 2D one in participants and that female group would have responded with a greater physiological arousal and higher rating on self-report, than male group. In this case, the results partially confirm our hypothesis, indeed our 3D version of the game elicited in females only a greater sense of presence than in 2D Cyberball, but we found any significant results in physiological responses.

Keeping on the research project we carried out the main experiment with both ASDs and TDs participants

ASDs self-reported feelings showed that this group was as able as controls to identify when they were being ostracized and that does not differ in most of the investigated variables from their counterpart. The ASDs differed from their counterpart for greater happiness and a lesser sense of boredom at each stage of the game. Furthermore, during the exclusionary situation they felt a lower degree of exclusion than controls. Previous research with healthy participants and more immersive virtual environment found a greater magnitude of exclusionary condition, using a more realistic environment we didn't find this result. But we found that autistic persons were happier than controls to play with the Cyberball3D and in this condition they felt their basic needs more fulfilled than controls. Moreover, the ASDs experienced a greater social presence during the interactive game and a greater affect toward the virtual agent.

Furthermore, based on results from both psychological and physiological measures, and partially consistent with previous research, it appears that individuals with ASDs may not be attuned to their physiological response when they are ostracized or extremely included. Whilst they experienced a heightened arousal response to being excluded and over included from the game, they did not express this difference in their self-reported ratings. Furthermore, only ASDs experienced an overall higher frequency of heart rate in 3D than in 2D condition and an overall higher temperature than controls with a significant difference in inclusion situation.

Authors provided, as a possible explanation, the perspective that persons with ASD failed to interpret appropriately their current emotional state, which impacted understanding of affective content found in emotion words used for the self-report questionnaires. Although persons with ASD may feel just as rejected as their TD counterparts when they are excluded by peers, they might have lacked insight into how the experience affected their current mood, and an impairment in emotion regulation (Mazefsky et al. 2013).

To better understand the social impairment in HF-Autism we investigated the brain responses to the exclusionary situation. In line with previous study with healthy persons, we confirmed our first hypothesis about the theta signal as a neural code for social pain and for the first time with autistic persons we discovered, during the exclusion condition, a lower theta band activity in the primary motor cortex and frontal lobe. In addition, our results a hypoactivation of the mirror system in autism during a situation of social exclusion.

## **7.2. Clinical implications**

There are numerous reasons why a VR-based intervention system may be particularly relevant for persons with ASD. The strength of VR technology for ASD intervention includes malleability, controllability, reduced sensory stimuli, individualized approach, safety, and a reduction of human interaction during initial skill training (Strickland, 1997). VR does not necessarily include direct human-to-human interaction, which may work well for an initial intervention to remove the difficulties common in ASD related to mere human interaction that is part of a typical intervention setting involving a child and a clinician .VR has also shown the capacity to ease the burden, both time and effort, of trained clinicians in an intervention process as well as the potential to allow untrained personnel to aid a participant in the intervention.

Technology can therefore give important support in therapy and diagnosis of persons with ASD and may even help to obtain objective values which enable us to understand autism a bit more and what people with autism feel in their day to day. This helps professionals to adapt therapies to each person, and families to work from their homes and gain a better understanding of their children's



behavior and needs (Aresti-Bartolome et al., 2014).

Authors (Zhu et al., 2011) described a novel interdisciplinary study program to improve mirror neuron functioning in autistic individuals by using a brain–computer interface (BCI) system with virtual reality technology. The BCI system can give a real-time view of the relative suppression of the mu rhythm (8–13 Hz) which is considered as an index of the mirror neuron activity. Although, the mu rhythm suppression is suggested to be involved only in the high-function autism but not in the low-function autism. We therefore believe that whether the mu rhythm is suppressed or not during an ASD individual observing human actions can be considered as a criterion for estimating the grade of autistic symptom. Playing the therapeutic computer games are of special interest for autism children. These rules-based environments can provide children with easy, safe and fun vehicle for interventions to improve their mirror neuron mechanism. It would be a promising approach to improving the human mirror mechanism of the autism by learning social communication in a human–computer interaction (HCI) environment.

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